COVER PAGE

Title: A randomized, double-blind, placebo-controlled, clinical trial of a 3-dose, 28-day regimen of PfSPZ Vaccine in healthy, adult participants to determine safety, tolerability and efficacy against heterologous *Plasmodium falciparum* controlled human malaria infection conducted 3 or 12 weeks after immunization

NCT Number: NCT05604521

Date: 21 September 2022

A randomized, double-blind, placebo-controlled, clinical trial of a 3-dose, 28-day regimen of PfSPZ Vaccine in healthy, adult participants to determine safety, tolerability and efficacy against heterologous *Plasmodium falciparum* controlled human malaria infection conducted 3 or 12 weeks after immunization

Protocol Number: USSPZV7

ClinicalTrials.gov identifier (NCT number): pending

IND Sponsor: Sanaria Inc.

Funding:

(1) NIAID 1U44AI167783 - 01

(2) Sanaria

Version Number: V2.0

21 September 2022

Collaborating Sites (clinical)	Kirsten E. Lyke, MD University of Maryland, Baltimore, Center for Vaccine Development and Global Health 685 W. Baltimore Street HSF I, Rm 480, Baltimore, MD 21201 Telephone: (410) 706-5328/0462 *klyke@som.umaryland.edu*

Summary of Changes from Previous Version:

1. Protocol revised for a single site.
2. Clarified that study participants are allocated to 3-week or 12-week CHMI, then randomized within the allocated group to vaccine or placebo.
3. Update Schedule of Activities to align with the text.
4. Update Table 4.
5. Additional information provided on prepatent periods with CHMI. Terminal treatment of uninfected study participants removed.
6. Allow inclusion of participants in CHMI with only 1 or 2 immunizations at discretion of the study team.
7. Addition of a test of understanding to the inclusion criteria.
8. Addition of a history of malaria in the past 2 years to the exclusion criteria.
9. Removal of severe allergic reaction or anaphylaxis to other vaccines as an exclusion criterion.
10. Removal of the requirement for a letter to document a previous sterilization procedure.
11. Clarification of HIV and HCV lab test results for exclusion criteria.
12. Change of visit 6 (1 week after 2nd immunization) to an in-person visit, removal of original visit 7 (2 weeks after 2nd immunization) and addition of a new visit 9 as a telephone follow up visit.
13. Changes to the timing of immunology lab collections to simplify lab collection.
14. Inclusion of serum pregnancy testing in lieu of urine testing.
15. Removal of terminal antimalarial treatment.
16. Compensation tables are updated for the site.
17. Edits made throughout to improve clarity of information presented or procedures described.

Rationale for changes: This study was originally planned for 2 study sites – this has now been condensed to a single site. Adjustments have been made to compensation and other language in the protocol to conform to the usual practices for this site which has considerable experience in the conduct of malaria vaccine trials. Terminal antimalarial treatment was removed to conform with previous CHMI studies conducted in the US and EU. Adjustments were made to either clarify inclusion/exclusion criteria, or add/remove criteria that are relevant or no longer relevant to the execution of the trial. The date of one safety visit was changed (from 2 weeks after the second dose to 1 week after the second dose) to better reflect following solicited adverse events and corresponding lab events. A new telephone safety visit was added after the 3rd dose for safety follow -up. Immunology lab collections have been simplified, improving the logistics to execute the study and reduce the burden of blood draws on the study participants. A new cellular immunology lab has been substituted for Dr. Seder's lab.

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines and the following:

- 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; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

SIGNATURE PAGE

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

Principal Investigator UMB CVD: _____

Signed: _____ Date: _____

1. PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:

Full title: A randomized, double-blind, placebo-controlled, clinical trial of a 3-dose, 28-day regimen of PfSPZ Vaccine in healthy, adult participants to determine safety, tolerability and efficacy against heterologous *Plasmodium falciparum* controlled human malaria infection conducted 3 and 12 weeks after immunization

Sponsor's Protocol Number: USSPZV7

Study Description and Rationale:

USSPZV7 is a randomized, double-blind, placebo-controlled trial of Sanaria® PfSPZ Vaccine administered on Days 1, 8 and 29 by direct venous inoculation (DVI) to assess safety, tolerability, immunogenicity, and vaccine efficacy (VE) against heterologous controlled human malaria infection (CHMI) with the 7G8 clone of *Plasmodium falciparum* (Pf) conducted at 3 or 12 weeks after the third immunization. The trial is designed to determine if individuals living in a non-malaria endemic area such as the United States (US) are protected against heterologous CHMI conducted at these time points. Enrollment will target at least 50% women due to the importance of collecting data relevant to women of child-bearing potential in Africa, for whom Pf malaria is a serious cause of morbidity and mortality during pregnancy. A companion trial taking place in malaria-naive Indonesian soldiers will assess the same regimen for safety, tolerability, immunogenicity, and VE against naturally transmitted Pf malaria in Indonesian West New Guinea. Together, the two trials will provide a direct bridge between VE against CHMI and VE against naturally transmitted malaria in malaria-naive individuals, a first in the history of malaria vaccine development.

The regimen for the USSPZV7 trial is based on a prior study (MAVACHE) where the day 1, 8 and 29 immunization regimen protected 5/6 non-malaria immune German adults against heterologous CHMI at 3 weeks after the last vaccination and 5/6 different German adults against heterologous CHMI 9-10 weeks after the last vaccination and 6-7 weeks after an initial homologous CHMI. The use of a heterologous strain for CHMI (heterologous meaning different from the vaccine strain) increases the stringency of the CHMI by introducing variant antigens and epitopes. When naturally transmitted malaria in Mali, and CHMI in the US, were used to measure VE, protection in the field over 24 weeks was as good as or better than protection against heterologous CHMI conducted at 24 weeks using exactly the same regimen [1], indicating the stringency of heterologous CHMI and its ability to conservatively estimate field protection (Silva, *Nature Communications*, 2022).

[1] In the first pair of bridging studies (Warfighter 1 trial in the US and MLSPZV1 trial in Mali), the regimen was $2.7x10^5$ PfSPZ administered by DVI weeks 0, 4, 8, 12 and 20; in the second pair of bridging studies (Warfighter 2 trial in the US and MLSPZV2 trial in Mali), the regimen was $1.8x10^5$ PfSPZ administered weeks 0, 8 and 16. In these paired studies, VE over 24 weeks of surveillance was as good or better in Mali (field transmission) than in the USA (24 week heterologous CHMI using Pf7G8). References: Warfighter 1 [1], MLSZPV1 [2], Warfighter 2 [3], MLSPZV2 [4].

To provide additional data on VE to support PfSPZ Vaccine licensure in the US, EU, Indonesia and African countries, several companion trials are assessing VE against naturally transmitted malaria in Indonesia (see above), African women of child-bearing potential (MLSPZV4), African children 6 to 9 years of age (MLSPZV5) and African women who are pregnant (MLSPZV6). In combination, these several trials are designed to provide data supporting the use of PfSPZ Vaccine to protect three important vulnerable groups: malaria-naive travelers, children and pregnant women. In the latter case, vaccine can be administered prior to pregnancy (MLSPZV4) or during pregnancy (MLSPZV6). The pregnancy indication includes malaria-naive women as well as malaria-exposed women, as the former have limited options for chemoprophylaxis when traveling to malaria-endemic areas.

The TravSPZV trial replaces the 9-10 week interval to CHMI used in the MAVACHE trial in Germany with two intervals (3 weeks, 12 weeks), to represent a duration of travel for a typical traveler to a malaria endemic area. Twelve weeks is an interval that encompasses most of the international travel to such areas. The Warfighter 3 trial is being conducted in the US at the current time and is providing preliminary estimates of VE against heterologous CHMI at 2, 6 and 10 weeks; USSPZV7 will be adding two additional timepoints to these data; 3 and 12 weeks.

USSPZV7 will be conducted at the University of Maryland at Baltimore's Center for Vaccine Development and Global Health (UMB CVD).

Objectives:

Primary Objective:

1. To measure the combined vaccine efficacy (VE) of PfSPZ Vaccine against Pf malaria (parasitemia) following heterologous parenteral CHMI with PfSPZ Challenge (7G8) administered 3 and 12 weeks after last dose of vaccine, in the modified intention-to-treat (mITT) population.
2. To assess the safety and tolerability of PfSPZ Vaccine administered as 3 doses of 9.0×10^5 PfSPZ on days 1, 8 and 29.

Secondary Objective:

1. To measure VE separately at each CHMI timepoint (3 and 12 weeks after V3).
2. To measure antibody responses to PfCSP following vaccination and determine if they are correlated with protection.

Exploratory Objectives:

1. To compare VE between groups with CHMI 3 or 12 weeks after V3.
2. To measure VE in the ITT and ATP populations.
3. To measure VE using time-to-event analysis.
4. To measure antibody responses to other Pf antigens and to PfSPZ and determine if they are correlated with protection.
5. To measure the ability of antibody responses to inhibit sporozoite invasion of hepatocytes and determine if the percent inhibition is correlated with protection.
6. To measure cellular immune responses following immunization and determine if they are correlated with protection.
7. To analyze innate and adaptive immune system states before and at early time-points after vaccination by using: 1) RNA-seq profiling of whole blood/PBMC; 2) immune phenotyping of single cells using flow cytometry

and CITE-seq; and 3) profiling of circulating proteins and metabolites. The objective of this exploratory goal is to search for biomarkers of vaccine induced humoral and cellular immune responses and protection.

8. To deep sequence the peripheral immune repertoire of cells to assess the cell receptor frequencies.

Endpoints:

Primary Endpoint:

1. VE computed as one minus the estimated risk ratio for Pf malaria (parasitemia) detected by PCR beginning 7 days after CHMI up to 28 days in the modified intention to treat population (proportional efficacy analysis) combining data across the two CHMI timepoints.
2. The differences in proportions of vaccinees compared to controls experiencing related moderate, severe, or serious solicited and unsolicited adverse events and laboratory abnormalities after vaccination.

Secondary Endpoints

1. VE as above, for each timepoint.
2. Antibody levels to PfCSP measured by ELISA comparing vaccinees to controls, and protected (no parasitemia occurring post CHMI) to non-protected (parasitemia occurring post CHMI) vaccinees.

Exploratory Endpoints:

1. VE as above.
2. VE as above.
3. VE computed as one minus the estimated hazard ratio.
4. Investigation of any differences in outcome based on gender, race or ethnicity.
5. Antibody levels to whole PfSPZ by automated immunofluorescence assay (IFA) and/or by other assays to be further defined, which may include assays to assess responses to multiple Pf antigens, antibody subclass assays and functional assays, comparing vaccinees to controls, and protected (no parasitemia occurring post CHMI) to non-protected (parasitemia occurring post CHMI) vaccinees.
6. Capacity to inhibit sporozoite invasion of hepatocytes (HC-04 cells) in vitro by inhibition of sporozoite invasion (ISI) assay comparing vaccinees to controls, and protected (no parasitemia occurring post CHMI) to non-protected (parasitemia occurring post CHMI) vaccinees.
7. Cellular immune responses to whole PfSPZ and synthetic peptides from selected Pf antigens by intracellular cytokine staining (ICS)/ flow cytometry and/or other assays to be defined comparing vaccinees to controls, and protected (no parasitemia occurring post CHMI) to non-protected (parasitemia occurring post CHMI) vaccinees.
8. RNA transcriptome quantification as detected by RNA-seq and protein levels in serum, and serum analyte analysis, comparing vaccinees to controls, and protected (no parasitemia occurring post CHMI) to non-protected (parasitemia occurring post CHMI) vaccinees.
9. Deep sequencing of immune receptor genes.

Methodology This will be a randomized, double-blind, placebo-controlled trial conducted at a single site as described above. Participants will be randomized to PfSPZ Vaccine

(vaccinees) or normal saline (NS) placebo (controls) in a 3:1 assignment ratio. PfSPZ Vaccine and NS injectates are prepared as clear, non-viscous, odor-free solutions that cannot be distinguished by the administrator or by the recipient, facilitating a double-blind design. Randomization will be done online using software provided by data management.

There will be 45 vaccinees and 15 controls. The reasons for the 3:1 imbalance are to: 1) collect more information on adverse events associated with vaccine administration, and 2) optimize the numbers to assess vaccine efficacy (VE) in the CHMI model. Participants who complete all immunizations will receive a total of 2.7 x 10^6 PfSPZ. In prior studies, single doses of 2.7 x 10^6 PfSPZ have been well tolerated in non-immune adults and there has not been any evidence that repeat dosing leads to increasing side effects. Therefore, 3 doses of 9.0x10^5 PfSPZ has been shown to be very safe and well tolerated.

Table 1. USSPZV7 Study Design

Groups	n	PfSPZ Vaccine dose (# PfSPZ)	Total PfSPZ over 3 doses (# PfSPZ)	Immunization (Days)			Weeks to CHMI	PfSPZ Challenge (7G8) dose (# PfSPZ)
				1	8	29	3, 12	
1 -PfSPZ Vaccine	45	9.0 x 10^5	2.7 x 10^6	x	x	x	x	3.2 x 10^3
2 -NS* controls	15	0	0	x	x	x	x	3.2 x 10^3
Total # of participants: 60								

* normal saline placebo

VE will be assessed 3 and 12 weeks after last immunization by exposure to PfSPZ Challenge (7G8), which is composed of aseptic, purified, cryopreserved infectious PfSPZ cloned from a Pf strain isolated in Brazil, and thus heterologous to the vaccine, which is derived from strain PfNF54 originating in West Africa. As with immunization, the PfSPZ will be administered by direct venous inoculation (DVI). Participants will be followed intensively for 4 weeks after CHMI to determine CHMI outcome and then for an additional 4 weeks until last study visit. Parasitemia following CHMI will be measured by highly sensitive quantitative polymerase chain reaction (qPCR). Protection will be defined as absence of parasitemia as detected by qPCR during the 28 days following injection of 3.2x10^3 PfSPZ of PfSPZ Challenge (7G8).

Statistical Methods

The primary outcome for these studies will be VE measured as [1 – risk ratio] x100. The outcomes of both CHMI's will be combined. Sample sizes of 45 and 15 for the vaccine and placebo groups were selected to be able to show with 83% power, if there are six drop-outs from the vaccine group and two from the control group prior to CHMI, that an infection rate of 92.3% in the controls (12/13 developing parasitemia) and 51% in the vaccinees (20/39 developing parasitemia) is statistically significantly different (α=0.05, 2-tailed) (VE=44.4%). As we expect VE to be higher than this, the sample size should be adequate.

Regarding safety, the study provides the following power to identify adverse outcomes:

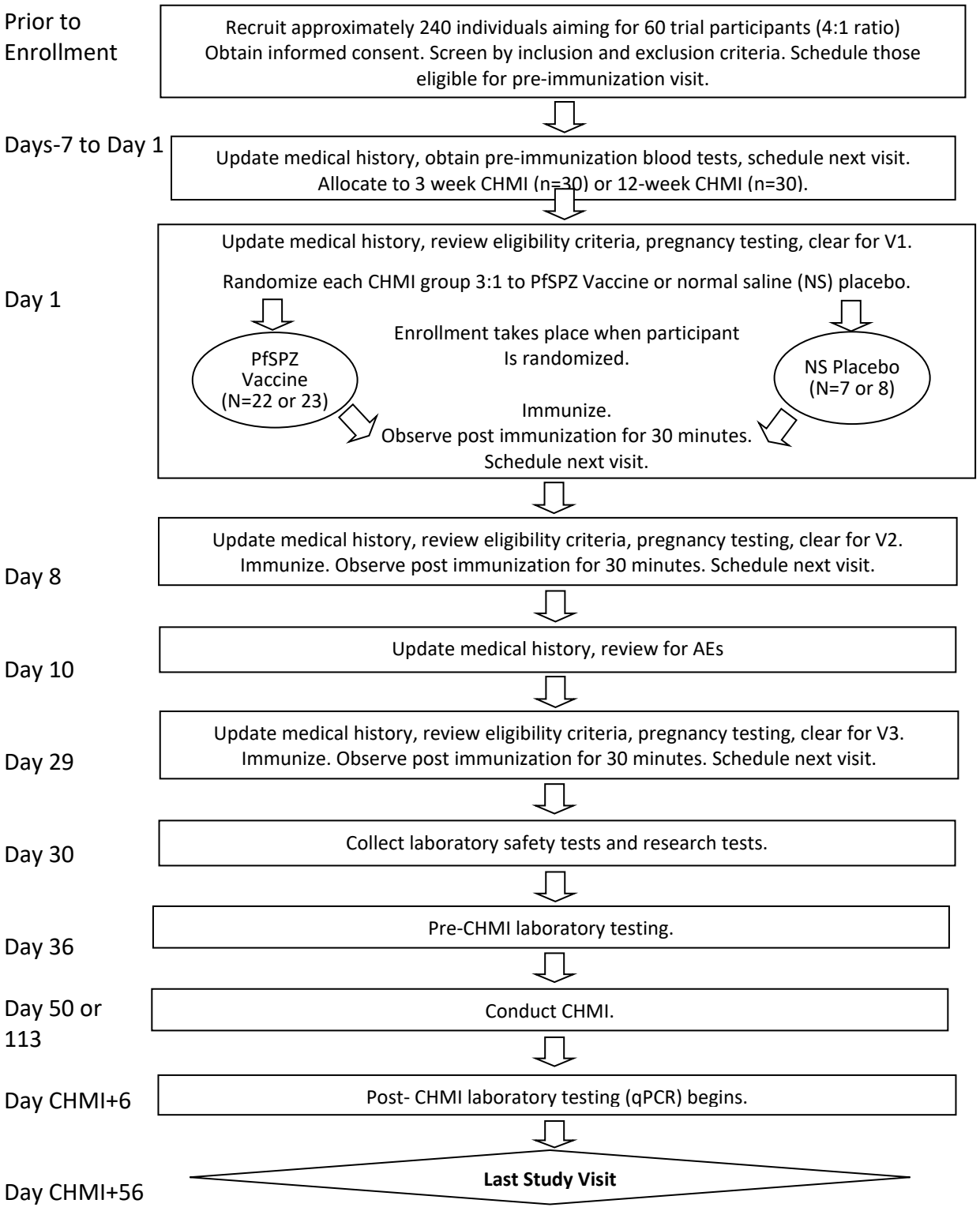
1. If no PfSPZ Vaccine-related SAEs are observed, the sample size of 45 provides 95% confidence that the true rate of SAEs caused by immunization is less than 6.7%, 90% confidence that the true rate of SAE's is less than 5.0%, 85% confidence that the true rate of SAEs is less than 4.2%, and 80% confidence that the true rate of SAEs is less than 3.5%.

With regard to the ability to make comparisons between vaccinees and placebo recipients in the rates of grade 2 and grade 3 adverse events, where significant differences might indicate poor tolerability, a sample size of 45 vaccinees and 15 placebo recipients will show a statistically significant difference ($p<0.05$, two-tailed) between 10/45 vaccinees experiencing poor tolerability on the one hand, and 0/15 controls on the other hand.

Phase:	Phase I
Estimated Number of Participants Screened:	240 (4:1)
Estimated Number of Participants Enrolled:	60 healthy adults (males or non-pregnant females), 18-50 years of age (45 vaccinees, 15 placebo controls); a target of 50% women
Description of Sites/Facilities Enrolling Participants:	1. University of Maryland at Baltimore's Center for Vaccine Development and Global Health
IND Sponsor:	Sanaria Inc.
Name of Investigational Product:	Vaccine test product: Sanaria® PfSPZ Vaccine (NF54) Product used to conduct CHMI: Sanaria® PfSPZ Challenge (7G8)
Description of Study Intervention:	The vaccine consists of radiation-attenuated, aseptic, purified sporozoites (SPZ) cryopreserved in liquid nitrogen vapor phase (LNVP) at -150^{o} to -196^{o}C. PfSPZ Vaccine is diluted in phosphate buffered saline (PBS) with human serum albumin (HSA) to achieve the correct dosage and is administered by DVI. PfSPZ Challenge (7G8) is similar but has not been attenuated by radiation and is therefore infectious. PfSPZ Vaccine is composed of PfSPZ derived from the NF54 strain of Pf, which is thought to be from West Africa, and PfSPZ Challenge (7G8) is composed of PfSPZ derived from the 7G8 clone of Pf, which is from Brazil.

Safety Monitoring	Solicited and unsolicited adverse events will be collected at specified intervals during the trial. In addition, laboratory testing (complete blood count, creatinine level, alanine aminotransferase level) will be conducted periodically during the trial. Serious adverse events (SAEs) will be recorded from the time of first vaccination to the end of the trial. Halting rules are defined in the protocol (e.g., the occurrence of an SAE deemed related to the vaccine). There will be a Safety Monitoring Committee (SMC) to provide independent safety oversight. The SMC will consist of senior physicians with expertise in the conduct of clinical trials including a Local Safety Monitor. Membership will be restricted to individuals without conflicts of interest. The SMC will be scheduled to meet prior to the start of the study or early in the course of the trial to review the SMC Charter and the details of the clinical trial, again at the time of study close-out. Other SMC meetings will be scheduled on an "as needed" basis to address any safety concerns that may arise.
Study Duration:	The entire study will take approximately 12 months to complete, including 3 months of recruitment. The period of follow-up for each immunized participant and placebo control is through 8 weeks post-CHMI.
Participant Duration:	Participation will last up to 24 weeks (not including screening) for those undergoing CHMI at 12 weeks and 15 weeks (not including screening) for those undergoing CHMI at 3 weeks.
Study Period:	Estimated date first participant screened: October, 2022. Estimated date first participant immunized: November, 2022. Estimated date last study visit of last study participant: July, 2023.

1.2 SCHEMA



Note: Additional visits will be included to collect samples for exploratory objectives 4-7.

1.3 SCHEDULE OF ACTIVITIES

The detailed schedule of events outlining the procedures to be performed at each study visit and participant contact is provided in **Table 3**.

Table 3. Schedule of Activities

3 week CHMI																														
Study Day		Screen	Day -7	1	8	10	15	29	31	38	43	50	57	58	59	60	61	62	63	64	65	66	67	68	70	72	75	78	106
Windows		Day -90 to Day -1	+/- 7	+/-3	+/-3	+/- 5	+/- 2	+/-3	+2	+/- 2	+/- 5	+/- 5	-	-	-	-	-	-	-	-	-	-	-	-	+/- 2	+/- 2	+/- 2	+/- 7	+/- 10
Visit Number		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Time Interval		Scr[1]	-7	Imm1	Imm2	+2	+7	Imm3	+2	+7	+14	CHMI	+7	+8	+9	+10	+11	+12	+13	+14	+15	+16	+17	+18	+20	+22	+25	+28	+56
Informed Consent		X																											
Eligibility Criteria Review		X	X	X	X			X				X																	
Medical History/Update		X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Examination[2]		X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs (BP, HR, temperature)		X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CBC with differential	EDTA	4	4	4			4		4		4	4																4	
Serum chemistry[3]	SST	8.5		8.5			8.5				8.5	8.5																8.5	
HIV ELISA, HbSAg, anti-HCV	SST	3.5																											
COVID-19 PCR (if clinically indicated)			X																										
EKG		X																											
Urine β HCG[4]		X		X	X			X				X																	
Immunization				X	X			X																					
Provision /Review of Memory Aid				X	X	X	X	X	X	X	X	X	X																
Telephone Follow Up										X																			
CHMI												X																	
qPCR[5]			2										2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
Adverse Events / Clinical Events				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
SAEs				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
IMMUNOASSAYS																													
Serum (antibodies)	SST		25	0			25		0		25	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	
Immune regulation	PAXgene		1						1				1																
PBMCs (cellular immunology, immune regulation)	EDTA		60	0			50		50		50	50	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Daily volume (mL)		16	92	12.5	0	0	75	0	55	0	87.5	87.5	33	2	2	2	2	2	2	2	2	2	2	2	2	2	2	39.5	0
Cumulative blood volume (mL)		16	108	121	121	121	196	196	251	251	338	426	459	461	463	465	467	469	471	473	475	477	479	481	483	485	487	526	526

[1] Screening Visit also includes Assessment of Understanding, Cardiovascular Risk Assessment, height, weight and BMI measurements

[2] Physicals will be performed during screening. All other physicals will be performed if indicated by medical history

[3] Screening serum chemistry: glucose, creatinine, ALT, AST; serum chemistry at all other time points: ALT and creatinine

[4] Serum β HCG may be substituted

[5] If a subject is diagnosed with malaria infection, the following samples will be drawn prior to initiation of treatment: CBC w/diff (4mL), Chemistry (5mL), sample for TBS (2mL), sample for UWash PCR (2mL). This will add an additional 13 mL to the cumulative blood volume. qPCR will be performed 28 days post CHMI if subject remains malaria negative. Blood draw for qPCR will be discontinued upon malaria diagnosis and treatment.

12 week CHMI																														
Study Day		Screen	Day -7	1	8	10	15	29	31	38	43	113	120	121	122	123	124	125	126	127	128	129	130	131	133	135	138	141	169
Windows		Day -90 to Day -1	+/- 7	+/-3	+/-3	+/- 5	+/- 2	+/-3	+2	+/- 2	+/- 5	+/- 5	-	-	-	-	-	-	-	-	-	-	-	-	+/- 2	+/- 2	+/- 2	+/- 7	+/- 10
Visit Number		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Time Interval		Scr[1]	-7	Imm1	Imm2	+2	+7	Imm3	+2	+7	+14	CHMI	+7	+8	+9	+10	+11	+12	+13	+14	+15	+16	+17	+18	+20	+22	+25	+28	+56
Informed Consent		X																											
Eligibility Criteria Review		X	X	X	X			X				X																	
Medical History/Update		X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Examination[2]		X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs (BP, HR, temperature)		X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CBC with differential	EDTA	4	4	4			4		4		4	4																4	
Serum chemistry[3]	SST	8.5		8.5			8.5				8.5	8.5																8.5	
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Urine β HCG [4]		X		X	X			X				X																	
Immunization				X	X			X																					
Provision /Review of Memory Aid				X	X	X	X	X	X	X	X	X	X																
Telephone Follow Up										X																			
CHMI												X																	
qPCR[5]			2										2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
Adverse Events / Clinical Events				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
SAEs				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
IMMUNOASSAYS																													
Serum (antibodies)	SST		25	0			25	0	0		25	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	
Immune regulation	PAXgene		1						1				1																
PBMCs (cellular immunology, immune regulation)	EDTA		60	0			50	0	50		50	50	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Daily volume (mL)		16	92	12.5	0	0	75	0	55	0	87.5	87.5	33	2	2	2	2	2	2	2	2	2	2	2	2	2	2	39.5	0
Cumulative blood volume (mL)		16	108	121	121	121	196	196	251	251	338	426	459	461	463	465	467	469	471	473	475	477	479	481	483	485	487	526	526

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2. INTRODUCTION

2.1 BACKGROUND

2.1.1 BACKGROUND: MALARIA

The World Health Organization's annual World Malaria Reports [5-9] highlight the urgent need for new tools for prevention, control, and elimination of malaria. The 2017 report states the following: "*..after an unprecedented period of success in global malaria control, progress has stalled."* [6]. In 2018 there were an estimated 228 million cases of malaria - an increase of about 16 million cases over 2015. Deaths were 405,000, only 25,000 fewer than in 2015 [6]. In 2019, the situation did not improve [9] and in 2020, the numbers of cases and deaths increased by 14 million and 69,000, respectively [10]. More than 98% of all deaths from malaria are caused by Pf, and 95% of cases and 96% of deaths occurred in sub-Saharan Africa [10]. To put this in context, more people die every 10 days from malaria than the 11,310 who died during the 2013-15 Ebola epidemic [11]. Others believe the numbers of deaths are at least 50% higher [12, 13].

While nearly all cases and deaths occur in endemic areas, malaria is also a serious concern for travelers. In 2019, 8,638 confirmed malaria cases were reported in the EU/EEA [14], the highest number of annual cases in the past 5 years. 99.8% were travel-related, and 88.2% were due to Pf, most of these imported from Africa. The situation is similar in the US, with gradually increasing numbers of cases of malaria imported each year, 61.9% Pf, and most of these imported from Africa [15]. Each imported case has the capacity to lead to autochthonous transmission, as the *Anopheles* vectors remain present in many areas [15].

Malaria has an enormous economic impact in the developing world, especially sub-Saharan Africa [10, 16]. It also adversely affects cognitive development and educational achievement in childhood due to pregnancy malaria, anemia during childhood and absenteeism from school [17-19]. Taking into account these ongoing burdens on individual and public health, there is an urgent unmet medical need for a malaria vaccine that prevents infection with Pf, and which can be deployed for elimination in mass vaccination programs. The highest priority and the focus of our efforts is a vaccine to prevent Pf infection in Africa and in travelers to Africa.

Malaria parasites have a complex life cycle consisting of several stages of development [20] during which both human and insect hosts are required. The infectious form of the parasite (sporozoite) is transmitted to humans when an infected female *Anopheles* mosquito bites a human. The sporozoites are deposited into the skin and migrate to the vasculature or are deposited directly into the vasculature [21], transit to the liver and within 30 minutes invade hepatocytes where they develop into schizonts, each containing approximately 10,000-60,000 merozoites. The merozoites rupture from the hepatocytes and are released into the bloodstream where they invade red blood cells. Merozoites reproduce asexually in the red blood cells, ultimately causing the cells to rupture, and increase in number until the density is sufficient to trigger the onset of clinical malaria – fever, chills, headache, myalgia that can progress to prostration, multi-

system organ failure and death. Some merozoites develop into gametocytes, which are imbibed as the mosquito takes a blood meal. The parasites develop in the mosquito and, after about two weeks, sporozoites are produced which make their way to the mosquito's salivary glands where they are ready for injection into a susceptible host, thus initiating the next round of the life cycle. From a vaccinology perspective, interrupting the parasite life cycle at the sporozoite and liver stages is ideal, as it would prevent both clinical disease and transmission, both of which result exclusively from blood stage malaria.

Development of insecticide resistance in the mosquito and drug resistance in the parasite contribute to the international crisis. A vaccine would be the ideal preventative measure for malaria.

2.1.2 BACKGROUND: PFSPZ VACCINE

The development of the *Plasmodium falciparum* (Pf) sporozoite (SPZ) vaccine PfSPZ Vaccine is based on the promising results from mosquito-administered PfSPZ [22, 23]. In these now classic studies dating back to the early 1970s, Pf-infected mosquitoes were irradiated, and then allowed to bite malaria-naïve humans who subsequently underwent controlled human malaria infection (CHMI) to assess efficacy. It was shown that >90% of study participants were protected against homologous CHMI (homologous meaning that the challenge strain was the same as the vaccine strain) when first conducted within 10 weeks, if the total number of infectious bites was 1000 or greater, and the majority of protected individuals were still protected when re-challenged up to 10 months after immunization [24]. The biopharmaceutical company Sanaria Inc. was established in 2003 to develop a vaccine based on this model and quickly developed the capacity to manufacture aseptic, purified, cryopreserved PfSPZ in compliance with current Good Manufacturing Practices (cGMPs) and suitable for parenteral injection [25]. Sanaria's first vaccine product, PfSPZ Vaccine, is composed of aseptic, purified, live (metabolically active), radiation-attenuated, PfSPZ suspended in phosphate buffered saline and human serum albumen. There is no adjuvant and as a result the vaccine is minimally reactogenic. The vaccine is cryopreserved in liquid nitrogen vapor phase (LNVP) at - 150° to -196°C and appears stable over many years.

The first-in-humans clinical trial of radiation-attenuated PfSPZ (PfSPZ Vaccine) was conducted in 2009 – 2010 at NMRC and the University of Maryland. Whether administered to humans as radiation-attenuated SPZ (PfSPZ Vaccine) or as non-attenuated SPZ (PfSPZ Challenge), the PfSPZ have been safe and well tolerated, with the PfSPZ themselves inducing almost no measurable side effects. Thousands of vials of PfSPZ Vaccine that meet regulatory standards of purity, sterility, safety, and potency have now been manufactured [26]. 2,021 participants aged 5 months to 61 years in the US, 2 countries in Europe, 1 country in Asia and 6 countries in Africa have safely received 6,377 doses of PfSPZ Vaccine (up to $2.7x10^6$ PfSPZ per dose).

Because the PfSPZ are eukaryotic cells, they must be stabilized at very cold temperatures, the same requirement as for human eggs and sperm. To use the vaccine, vials of PfSPZ are removed from the LNVP, thawed in a controlled way, diluted with phosphate buffered saline and human serum albumen, and injected by direct venous inoculation (DVI). By conducting an extensive stability program using PfSPZ stored in LNVP, it has been shown that the PfSPZ are extremely stable over the four years of testing

mandated for each lot [Sim, unpublished data]. It has also been shown that the cost and logistics of distributing cryopreserved whole PfSPZ vaccines in the field are favorable, due to the fact that electricity is not needed to maintain the cold chain [27].

2.1.3 BACKGROUND: PFSPZ CHALLENGE

CHMI can now be done by one of two methods: by mosquito bite or by DVI of fully infectious PfSPZ, called PfSPZ Challenge. We have opted to use the PfSPZ Challenge model to standardize dose delivery and to allow flexible timing for CHMI (no need to generate a precisely timed batch of infected mosquitoes). Using DVI to inject 3,200 SPZ of PfSPZ Challenge (NF54), 81/81 (100%) malaria-naive participants undergoing first CHMI have been infected [28, 29][Wildfire unpublished), whereas the corresponding numbers for a heterologous parasite strain, also manufactured by Sanaria (heterologous meaning different from the vaccine strain), PfSPZ Challenge (7G8), are 27/28 (96.4%) [30-32]. In contrast to mosquito bite challenge, the dosage for DVI of PfSPZ Challenge is tightly controlled and, because cryopreservation allows a vial of infectious sporozoites to be thawed at any time, clinical studies have fewer restrictions in timing and design. We plan to use heterologous PfSPZ Challenge (7G8) in order to assess the capacity of PfSPZ Vaccine to generate cross-strain protection. Because Pf7G8 is more divergent from the vaccine strain, PfNF54 (which originates from West Africa), than any African parasite, protection against PfSPZ Challenge (7G8) CHMI is a strong predictor of protection in the field [33].

The manufacturing process for PfSPZ Challenge is identical to that of PfSPZ Vaccine, with the exception that the PfSPZ of PfSPZ Challenge are not attenuated by irradiation and are cryopreserved at lower PfSPZ numbers per vial compared to PfSPZ Vaccine.

Like PfSPZ Vaccine, PfSPZ Challenge contains PfSPZ purified from the salivary glands of *Anopheles stephensi* mosquitoes raised under aseptic conditions. Like PfSPZ Vaccine, the PfSPZ are formulated in cryoprotectant to maintain potency in LNVP at - 150° to -196°C for an extended period. Immediately prior to use, a vial is thawed and brought to the appropriate dose concentration with additional diluent (PBS and HSA).

1,157 participants in 12 countries have undergone CHMI with PfSPZ Challenge (NF54) (n=1070) or PfSPZ Challenge (7G8) (n=135) or both (48 individuals have received both in separate CHMIs).

2.1.4 PRECLINICAL INVESTIGATIONS OF PFSPZ VACCINE AND PFSPZ CHALLENGE

2.1.4.1 TOXICITY STUDIES OF PFSPZ VACCINE

The safety and immunogenicity of PfSPZ Vaccine administered by subcutaneous (SC), intradermal (ID), and intravenous (IV) routes was demonstrated in New Zealand White (NZW) rabbits and mice. Three preclinical toxicology studies in rabbits, giving 5 to 7 doses of $1.35x10^5$ radiation-attenuated PfSPZ Vaccine by the ID, SC and IV routes were performed. PfSPZ Vaccine was safe and well tolerated in all 3 studies. The vaccine was also safe and well tolerated in the mouse study. Details are available in the PfSPZ Vaccine Investigator's Brochure (IB).

2.1.4.2 BIODISTRIBUTION STUDIES OF PFSPZ VACCINE

Formal biodistribution studies were performed, which examined the distribution and persistence in mouse tissues of Pf DNA following SC, ID, or IV inoculation of a single $1.35x10^5$ 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 PfSPZ in these studies was equivalent to the highest dose administered in the first 2 Phase 1 clinical trials of PfSPZ Vaccine, a dose that has now been shown to be safe and well tolerated in adult and pediatric participants administered PfSPZ Vaccine by SC, ID, and/or IV routes. More details are available in the Investigator's Brochure (IB).

2.1.4.3 REPRODUCTIVE TOXICOLOGY

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% 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 US FDA in support of a planned study of PfSPZ Vaccine in pregnant women, to be conducted this year in Mali.

2.1.4.4 PRECLINICAL STUDIES OF PFSPZ CHALLENGE

The toxicity and biodistribution studies described above for PfSPZ Vaccine administered SC, ID and IV supported the use of PfSPZ Challenge in Phase I testing. No additional pre-clinical studies of PfSPZ Challenge were done prior to proceeding to clinical trials.

2.1.5 CLINICAL INVESTIGATIONS OF PFSPZ VACCINE AND PFSPZ CHALLENGE

2.1.5.1 CLINICAL INVESTIGATIONS OF PFSPZ VACCINE

Twenty-two 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, 6 countries in Africa (Tanzania, Kenya, Mali, Burkina Faso, Gabon, Equatorial Guinea) and in Indonesia. In these trials, as of 18JUL2022, 6255 doses of PfSPZ Vaccine (over 5.7 billion PfSPZ) have been administered to 1981 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. A series of important milestones along the development pathway are described below. Further details of each trial including safety data are included in the PfSPZ Vaccine IB and in the section on Risks and Benefits.

1. *First clinical trial of PfSPZ Vaccine*: Starting in 2009, PfSPZ Vaccine was administered ID or SC in a first-in-humans Phase 1 clinical trial in healthy, malaria-naïve adults at the Naval Medical Research Center and UMB CVD [34]. PfSPZ Vaccine was safe, fully attenuated by irradiation (no breakthrough blood stage infections) and well tolerated in human participants but immunogenicity and VE were sub-optimal. Based on studies in non-human primates, it became clear that IV administration of the vaccine would be required to induce protective immune responses.
2. *100% protection achieved after IV administration*: This vaccine trial which began in October 2011, demonstrated that 4 or 5 doses of 1.35×10^5 PfSPZ administered through an IV catheter were safe, well-tolerated and provided VE of 67% (6/9) in participants who received 4 doses and 100% (6/6) in participants who received 5 doses at 3 weeks after the final immunization [35].
3. *VE extends to heterologous parasite strains, lasts >1 year, can be achieved after 3 doses, and can be achieved using condensed regimens*: Other than the trial shown below as VRC314, the subsequent trials using PfSPZ Vaccine in 17 additional Phase 1 or 2 trials have used the direct venous inoculation (DVI) route, where the vein is punctured directly and nearly painlessly with a 25-gauge needle and the vaccine in 0.5 mL of diluent is injected over a few seconds, a procedure that in most recipients is well tolerated and causes no AEs.

Table 4. Chronological Listing of Trials of PfSPZ Vaccine

Study Identifier (Clinicaltrials.gov) **Start Date**	**Study Design Summary**	**PfSPZ Immunization Schedules Evaluated** (Dose, Route, Number of administrations)	**Vaccine groups and Numbers of Volunteers**
1. NMRC/UMB CVD (NCT01001650) May 2009 *(completed)*	Phase 1, open-label, dose-escalation with CHMI in USA **(ID, SC only)**	$7.5x10^3$ SC x 4; $7.5x10^3$ ID x 4; $3x10^4$ SC x 4; $3x10^4$ ID x 4; $1.35x10^5$ SC x 4 or 6; $1.35x10^5$ ID x 4 or 6	Malaria-naïve adults: 80
2. VRC 312 (NCT01441167) Oct 2011 *(completed)*	Phase 1, open-label, dose-escalation with CHMI in USA **(IV only)**	$2x10^3$ IV x 2; $7.5x10^3$ IV x 4 or 6; $3x10^5$ IV x 4 or 6; $1.35x10^5$ IV x 4 or 5	Malaria-naïve adults: 40
3. VRC 314 (NCT02015091) Dec 2013 *(completed)*	Phase 1, open-label, dose-escalation, regimen comparison with CHMI in USA **(IV or IM)**	$2.2x10^6$ IM x 4; $1.35x10^5$ IV x 4 + $4.5x10^5$ IV boost; $2.7x10^5$ IV x 3 or 4; $2.7x10^5$ IV x 2 + $4.5x10^5$ IV x 2; $9.0x10^5$ 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
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

Study Identifier (Clinicaltrials.gov) **Start Date**	**Study Design Summary**	**PfSPZ Immunization Schedules Evaluated** (Dose, Route, Number of administrations)	**Vaccine groups and Numbers of Volunteers**
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: 71
11. Warfighter 2 (NCT02601716) Apr 2016 *(completed)*	Phase 2, open-label, regimen comparison with CHMI in USA	$4.5x10^5$ x 5 (Days 1, 3, 5, 7 and week 16); or $9x10^5$ x 3 (Weeks 1, 9, 17); or $1.8x10^6$ x 3 (Weeks 1, 9, 17); or $2.7x10^6$ x 1 + $9x10^5$ x 2 (Weeks 1, 9, 17)	Malaria-naive adults: 60
12. KSZPV1 (NCT02687373) Jul 2016 *(completed)*	Phase 1 dose escalation followed by Phase 2 double-blind, randomized, placebo-controlled* with field efficacy in Kenya	Ph 1 - Older children: $4.5x10^5$ x 1; then $9x10^5$ x 2; then $1.8x10^6$ x 2 Ph 1 - Younger children, infants: $1.35x10^5$ x 1; then $2.7x10^5$ x 1; then $4.5x10^5$ x 1; then $9x10^5$ x 2; then $1.8x10^6$ x 2, all Ph 2 - Infants: $4.5x10^5$, $9x10^5$, or $1.8x10^6$, all x 3	Malaria-exposed children: 64 infants: 352
13. MAVACHE (NCT02704533) Sep 2016 *(completed)*	Phase 1 dose escalation, regimen-condensation and dose number reduction with CHMI in Germany	$9x10^5$ x 3 (Days 1, 8, 29); then $1.8x10^6$ x 2 (Days 1, 8); then $2.7x10^6$ x 2 (Days 1, 8)	Malaria-naive adults: 40
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: 32** 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: 15
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: 140
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: 133
18. EGSPZV3 (NCT03590340) *(completed)*	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: 84

Study Identifier (Clinicaltrials.gov) **Start Date**	**Study Design Summary**	**PfSPZ Immunization Schedules Evaluated** (Dose, Route, Number of administrations)	**Vaccine groups and Numbers of Volunteers**
19. MLSPZV4 (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: 200
20. Warfighter 3 (NCT04966871) *(ongoing)*	Phase 1 double-blind, randomized, placebo-controlled* with heterologous CHMI in Seattle, US	$9x10^5$ x 3 (Days 1, 8 and 29)	Malaria-naive adults: 42
21. MLSPZV5 (NCT04940130) *(ongoing)*	Phase 2 double-blind, randomized, placebo-controlled* with field efficacy in Mali	$9x10^5$ x 3 (Days 1, 8 and 29)	Malaria-exposed 6- to 10-year-olds: 134
22. IDSPZV1 (NCT03503058) *(ongoing)*	Phase 2 double-blind, randomized, placebo-controlled* with field efficacy in Indonesia	$9x10^5$ x 3 (Days 1, 8 and 29)	Malaria-naive adults: 172***
*The placebo control used in all trials is normal saline; ** 20 additional adult volunteers in EGSPZV2 received PfSPZ-CVac; *** 172 additional volunteers in IDSPZV1 received PfSPZ-CVac			
In these trials, dosing schedules have evaluated various intervals between immunizations 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).			

The four trials after VRC 312 in malaria-naive participants (studies #3, 6, 11, and 13, Table 4) demonstrated several important findings:

A. *The degree of protection depends on the dose of PfSPZ administered*: **There has been a clear dose response seen up to doses of about $9.0x10^5$ PfSPZ per injection. As individual doses were increased above about $9.0x10^5$ PfSPZ, further improvements were not always seen. For example, in the Warfighter 2 Trial (#11, Table 4), doubling 3-dose regimens from $9.0x10^5$ PfSPZ per dose to $1.8x10^6$ PfSPZ per dose did not improve VE against heterologous CHMI conducted at 3 or 6 months.**

B. *The number of doses affects protection*: **This was illustrated by the MAVACHE trial in Germany, where two attempts at immunizing with 2 doses showed only 50-67% protection against homologous CHMI, while 3 doses were more protective [32]. The difference between 3 and 5 doses has been less marked than the difference between 2 doses and 3 doses, and for this reason 3 doses has been selected as the standard regimen for further development, based on practicality and lower cost of goods.**

C. *The protection induced by PfSPZ Vaccine extends to heterologous parasites*: **In the field, most of the parasites transmitted by mosquitoes are heterologous, differing antigenically from the vaccine strain; therefore, demonstrating heterologous protection by CHMI is important. In**

the WRAIR 2080 trial [1], a 5-dose regimen similar to that providing 100% protection in VRC 312 protected 4/5 (80%) volunteers against heterologous CHMI at 3 weeks; the CHMI used mosquito-bite inoculation of Pf7G8, a clone of a Brazilian Pf strain that is antigenically divergent from PfNF54, indicating that potent cross-strain immunity is induced [1]. In the MAVACHE trial in Germany, 10/12 (83.3%) volunteers were protected against PfSPZ Challenge (7G8) administered by DVI 3 or 10 weeks after immunization, confirming the WRAIR 2080 results [32].

D. *PfSPZ Vaccine gave protection lasting > 1 year*: In the VRC 314 trial (conducted at VRC and UMB), 5/5 (100%) volunteers protected against homologous CHMI at ~20 weeks after immunization were sterilely protected against repeat homologous CHMI at 59 weeks [36]. In addition, 5/6 (83%) volunteers protected against homologous CHMI at 18 weeks were sterilely protected against heterologous (7G8) CHMI at 33 weeks [37].

E. *Condensed regimens with a multi-dose prime were highly protective*: A Day 1, 3, 5, 7 multi-dose prime regimen with a 16-week boost in Warfighter 2 (#11 in **Table 4**) gave the best protection results yet seen in the development of PfSPZ Vaccine – 40% sterile protection against a stringent mosquito bite Pf7G8 CHMI conducted at 13 weeks after immunization. This was twice as high as the comparator 3-dose regimens administered over the standard 0, 8 and 16 weeks, despite a lower total dose of PfSPZ [37]. The MAVACHE trial in Germany then showed similarly favorable results for a condensed regimen using a 2-dose multi-dose prime (Days 1 and 8): 14/17 (78.8%) volunteers were protected against 3-week or 10-week homologous CHMI following 3 doses of $9.0x10^5$ PfSPZ administered on Days 1, 8 and 29 [32].

F. *Antibody levels to Pf circumsporozoite protein (CSP) measured by ELISA have correlated with protection against CHMI*: An additional finding from VRC314 and WRAIR 2080 (# 3 and 6, **Table 4**) was that antibody levels to the main surface protein of PfSPZ, the Pf circumsporozoite protein (PfCSP), as measured by ELISA, correlated with protection in malaria-naïve individuals immunized with PfSPZ Vaccine [1, 36].

4. *PfSPZ Vaccine is also safe, well tolerated and protective in malaria-exposed African adults, and safe and well tolerated in African children and infants:* Eleven completed trials in malaria-exposed Africans including adults, teenagers, children and infants (studies # 4, 5, 7, 8, 9, 10, 12, 14, 15, 16 and 18 in **Table 4**) have demonstrated several additional important findings:

A. *PfSPZ Vaccine induces durable protection against naturally transmitted malaria in Africa*: A randomized, double-blind, placebo-controlled trial conducted in Mali (#4 in **Table 4**) using 5 doses of vaccine showed 52% vaccine efficacy (VE) against Pf infection as measured by microscopic examination of thick blood smears (TBS) during a 24-week follow-up period by time-to-event analysis, and 29% VE by proportional analysis [2]. Antibody titers induced by PfSPZ Vaccine were lower than when the same dose was administered to malaria-naïve adults in the US, a result that has been reproduced in other trials in African adults. Two follow-up trials in Mali and one in Burkina Faso have demonstrated that 3-dose regimens can be as effective as a 5-dose regimen (#9, 19 and 10, respectively, in **Table 4**) [4]; Diawara and

Healy, unpublished; Sirima and Laurens, unpublished]. The VE results of these four field trials are summarized in **Table 5**. In all four of these studies, trial participants were cleared of parasitemia prior to the first and last doses of vaccine, to avoid the immunosuppressive effects of blood stage malaria on vaccine take, and then followed by active and passive case detection for 24 weeks. In studies where clearance was not performed, VE was not statistically significant (#16 and #17 in **Table 4**) [Sissoko and Healy unpublished].

B. *PfSPZ Vaccine twice achieved 100% protection against short term homologous CHMI in Africa, and once on repeat CHMI 21 weeks later*: 100% protection has been demonstrated in Bagamoyo, Tanzania (#5 and 8 in **Table 4**) [38, 39] and in Mali (#9 in **Table 4**) [Sissoko and Healy, unpublished]. More recently, a study of PfSPZ Vaccine was conducted in HIV- and HIV+ adults in Tanzania (#15 in **Table 4**). Although not protective against CHMI, the vaccine was safe and well tolerated in all HIV+ study participants [Jongo, unpublished data].

C. *PfSPZ Vaccine is safe and well tolerated in African infants and children*: Three studies have been completed in African children and infants (#8, #12, #14 in **Table 4**) [40-42] [Jongo, unpublished]. Participants in Kenya were followed over six months for incident malaria, and did not show statistically significant protection compared to controls over this period, although protection was significant during the first 90 days after immunization in the highest dose group [42]. A fourth pediatric study is underway in 1-12-year-old Gabonese children (#17 in **Table 4**).

Table 5. PfSPZ Vaccine VE in Field Studies with Clearance of Parasitemia prior to First and Last Dose

Trial ID NCT Number	PfSPZ Vaccine Doses				Follow-up period (weeks)	No. of Vaccinees/ Controls	Pf Incidence Rate in Controls	VE by Type of Analysis			
	Timing	No.	Number of PfSPZ x10^6					Time to Event (1-hazard ratio)	P value	Proportional (1-risk ratio)	P value
	Weeks		Per dose	Total							
MLSPZV1 14-I-N010[a] NCT01988636	0, 4, 8, 12, 20[b]	5	0.27	1.35	24	42/44 (malaria) 44/44 (clinical malaria)	92.5%	52% (95% CI: 21.9 to 70.6)	0.005	29% (95% CI: 9.0 to 46.4)	0.004
MLSPZV2 16-I-N004[c] NCT01988636	0, 8, 16[d]	3	1.8	5.4	24	57/55 (TTE) 55/54 (P)	77.8%	51% (95% CI: 20 to 70)	0.004	24% (95% CI: 2 to 41)	0.031
BFSPZV1 15-0001[e] NCT02663700	0, 8, 16	3	2.7	8.1	24	39/41	57.5%	48% (95% CI: -2.9 to 73.6)	0.061	38% (95% CI: 6.8 to 68.7)	0.017
MLSPZV4 19-I-N113[f] NCT03989102	1, 8 and 29 days	3	0.9	2.7	24	56%	73%	41% (95% CI: 14 to 59)	0.005	23% (95% CI: 3 to 40)	<0.001
	1, 8 and 29 days	3	1.8	5.4	24	47%	73%	57% (95% CI: 37 to 71)	<0.001	36% (95% CI: 16 to 51)	<0.001

[a] [2].

[b] When this same dosage regimen was assessed for long term protection against heterologous CHMI in the US, protection at 24 weeks was only 8% [1]. Thus, protection for 24 weeks in the field against intense transmission of heterogeneous Pf parasites was higher than it was against heterologous CHMI with Pf7G8 in the US.

[c] Sissoko and Healy, unpublished.

[d] When this same dosage regimen was assessed for long term protection against heterologous CHMI in the US, protection at 24 weeks was only 23% [3]. Thus, protection for 24 weeks in the field against intense transmission of heterogeneous Pf parasites was higher or at least as high as it was against heterologous CHMI with Pf7G8 in the US.

[e] Sirima and Laurens, unpublished.

[f] Diawara and Healy, unpublished.

5. *Down-selection of the final vaccine regimen for PfSPZ Vaccine and ongoing studies*: The Day 1, 8 and 29 day regimen shown in the MAVACHE trial in Germany to provide >80% protection against heterologous CHMI at 3 and 10 weeks after immunization has been selected as the optimal regimen, combining high-level protection with a practical 3-dose, 4-week administration schedule. All the most recent trials – #16 to #22 in **Table 4** – assess this regimen. The results from the EGSPZV3 trial are recently available and indicate that the Day 1, 8 and 29 regimen was the most protective of the four regimens tested [43]. The study underway in 6-10-year-old Malian children (#21 in **Table 4**) and the study underway in Indonesian soldiers (#22 in **Table 4**) are using the same Day 1, 8 and 29 regimen, and are measuring VE against naturally transmitted malaria. Likewise, the Warfighter 3 trial in Seattle (#20 in **Table 4)** is testing the same regimen against CHMI, with results expected soon.
6. *Rationale for the USSPZV7 trial*: The USSPZV7 trial is designed to help link the results of these various trials. Together with Warfighter 3, USSPZV7 will allow bridging of VE data between malaria-naive and malaria-exposed individuals as illustrated in **Table 6**.

Table 6. Linked studies using the Day 1, 8 and 29 regimen

Population	Malaria-naive adults		Malaria-exposed adults
VE measurement	CHMI	naturally transmitted Pf	naturally transmitted Pf
Gender	50% male / 50% female	100% male (soldiers)	100% female (women of child-bearing potential)
Trial location	US	Indonesia	Mali
Name	USSPZV7	IDSPZV1	MLSPZV4

A comparison of USSPZV7 and IDSPZV1 will tell us how protection against CHMI translates into field efficacy in the same malaria-naive population (relying on males), and a comparison between TravSPV1 and MLSPZV4 will tell us how protection against CHMI in a malaria-naive population translates into field efficacy in a malaria-exposed population (relying on females). The Warfighter 3 trial will provide similar information using different intervals to heterologous CHMI (2, 6 and 10 weeks) compared to USSPZV7 (3 and 12 weeks).

2.1.5.2 CLINICAL INVESTIGATIONS USING PFSPZ CHALLENGE (NF54)

Initial trials with PfSPZ Challenge were done using PfSPZ Challenge (NF54) administered via ID and IM routes [28, 44-48]. Once it became apparent that optimal administration would likely be achieved with the IV route, a dose-finding trial of PfSPZ Challenge (NF54) was completed and it was determined that 3,200 PfSPZ administered by DVI was sufficient to infect all malaria-naive research participants [49]; this was confirmed in another Phase I trial [28]. The clinical safety profile of PfSPZ Challenge indicates that PfSPZ Challenge is safe and well tolerated when administered by the IV route. 3,200 PfSPZ administered by DVI is confirmed as a 100% infectious dose for first CHMIs in malaria-naive individuals (79/79 infected).

Once the use of PfSPZ Challenge for CHMI was standardized as 3,200 PfSPZ administered by DVI, subsequent trials of PfSPZ Challenge have focused on three broad research objectives.

1. *Studies of host-pathogen biology and innate and acquired immunity*: A study in Gabon evaluated how sickle cell trait and natural acquired immunity impact the prepatent period during CHMI, demonstrating that both conditions prolong time to parasitemia and impact parasite multiplication rates [50]. Additional studies also

aimed at understanding innate and acquired immunity have been conducted in The Gambia [51] and Kenya [52]. Most recently, a fourth study examining the acquisition of immunity following repeated CHMI's was conducted in Gabon [Zinsou and McCall, unpublished].

2. *Studies using PfSPZ Challenge to immunize against malaria (PfSPZ-CVac approach)*: There are ten trials either completed or in progress assessing the PfSPZ-CVac approach. This consists of the administration of PfSPZ Challenge under anti-malarial chemoprophylaxis, which attenuates the parasites in vivo [29, 39, 53]. Two trials completed at NIAID and a recent trial in Mali are evaluating chloroquine and pyrimethamine as the prophylaxis components [[54]; Sagara unpublished]. These trials have demonstrated very promising efficacy results and have shown PfSPZ-CVac regimens to be safe, well-tolerated and more potent than PfSPZ Vaccine for the induction of protective immunity [54].
3. *Studies using PfSPZ Challenge to assess vaccine efficacy*: There have been 17 trials wherein PfSPZ Challenge (NF54) has been used to assess VE. These assessed the VE of PfSPZ-CVac, the vaccine candidate GMZ2, and PfSPZ Vaccine (**Table 4**: Trials # 5, 8, 9, 13, 14, 15, 18) [4, 31, 32, 38, 40]. Using PfSPZ Challenge to assess efficacy is convenient because timing is not dependent on the development of a batch of infected mosquitoes, the dose is standardized, and the cost is considerably less than mosquito bite CHMI. These features have allowed the first modern CHMI's in Africa, and to date these have been performed in six African countries.

The clinical trials described above used Pf Challenge (NF54), which is composed of the same strain of Pf as PfSPZ Vaccine. PfSPZ Challenge (Pf7G8) has been developed more recently to provide heterologous CHMI, a more stringent assessment of VE. PfSPZ Challenge (7G8) contains a cloned parasite from Brazil which is heterologous (genetically and antigenically) from PfNF54. The trials with PfSPZ Challenge (Pf7G8) are discussed below.

2.1.5.3 CLINICAL INVESTIGATIONS USING PFSPZ CHALLENGE (7G8)

1. MAVACHE Trial: This was a Phase 1 sequential dose and schedule optimization trial primarily designed to assess the safety, tolerability and efficacy of PfSPZ Vaccine. However, a component of the trial consisted of a small dose escalation study of PfSPZ Challenge (7G8), performed in healthy, malaria-naïve participants, to establish the minimum 100% infective dose. DVI of 3,200 PfSPZ was fully infective in this small trial, while lower doses were not. Thus PfSPZ (Challenge 7G8) infectivity appeared similar to that of PfSPZ Challenge (NF54). PfSPZ Challenge (7G8) was subsequently used in the MAVACHE trial to assess heterologous protection induced by PfSPZ Vaccine [32].
2. NIAID sponsored trial 14-0040 at University of Maryland: This randomized, double-blind Phase 1 trial of DVI of PfSPZ Challenge (7G8), with PfSPZ Challenge (NF54) used as a comparator, had a similar objective to the 7G8 component of the MAVACHE trial: dose escalation of PfSPZ Challenge (7G8) to establish the minimum 100% infective dose. As with MAVACHE, the trial was done in non-immune adults and assessed the safety and reactogenicity of PfSPZ Challenge (7G8) administered by DVI. The CHMI was safe and well tolerated and yielded the following infectivity results: 800 PfSPZ: 3/7 infected; 1600 PfSPZ: 4/7 infected; 3,200 PfSPZ: 8/9 infected; 4800 PfSPZ: 2/2 infected [30]. On the basis of the results of MAVACHE and NIAID 14-0040, 3,200 PfSPZ was selected as the standard dose, the same as for PfSPZ Challenge (NF54), although there was one participant in the University of Maryland trial who did not become positive at this dose.
3. Protocol 17-I-0067 trial in the US and TÜCHMI-003 trial in Germany: Subsequent to these two dose optimization trials, PfSPZ Challenge (7G8) has been used to assess VE in two trials in addition to the MAVACHE trial: the 17-I-0067 PfSPZ-CVac (pyrimethamine) trial at the NIH Clinical Center [54], and the

TÜCHMI-003 trial in Tübingen, Germany [31]. The product was safe and well tolerated in all research participants injected, and as with MAVACHE, all controls inoculated with 3,200 PfSPZ became infected. In total, 27/28 malaria-naive individuals undergoing CHMI with PfSPZ Challenge (7G8) using 3,200 PfSPZ administered by DVI have turned positive.

In summary, PfSPZ Challenge (7G8) is available as a standardized method for CHMI that is safe, well tolerated, consistently infectious, and allowing flexibility with respect to the timing of inoculation. When used to assess the efficacy of PfSPZ Vaccine, it provides a heterologous CHMI, increasing stringency and better representing the heterogeneous population of parasites found in nature, almost all of which will be different from NF54. In two comparisons where precisely the same regimen was tested in Malian adults against naturally transmitted malaria over a 24 week surveillance period and in US adults against PfSPZ Challenge (7G8) CHMI conducted 24 weeks after immunization, protection was as high or higher in the field than following heterologous CHMI [33]. CHMI with PfSPZ Challenge (7G8) should thus provide a conservative estimate of "real world" protection in the field.

2.2 RISK/BENEFIT ASSESSMENT

2.2.1 KNOWN POTENTIAL RISKS

2.2.1.1 RISK ASSOCIATED WITH PFSPZ VACCINE

2.2.1.1.1 LOCAL REACTIONS

PfSPZ Vaccine will be administered by DVI. In the DVI procedure, a small-bore (25 gauge) needle is inserted into the vein, the syringe plunger is withdrawn slightly to demonstrate blood flashback, and the vaccine injected directly, a procedure lasting <10 seconds once the skin has been prepped with alcohol. The procedure has been extremely well tolerated. This is likely due to the small needle (the trauma of penetration is minimized) and the fact that with intravascular injection there is no depot of fluid forced into a tissue space as with other routes of injection.

The local risks of PfSPZ Vaccine administered by DVI include pain, tenderness, erythema, swelling, bruising, pruritus and induration. Additional, less common risks associated with venipuncture are listed in Section 2.2.1.4 'Risk Associate with Venipuncture'.

2.2.1.1.2 SYSTEMIC REACTIONS

PfSPZ Vaccine has been exceptionally safe and well tolerated in malaria-naïve adults in the US and Europe, and in malaria-exposed adults, children and infants in Africa. In these trials, as of 18 July 2022, 6255 doses of PfSPZ Vaccine (over 5.7 billion PfSPZ) have been safely injected into 1981 participants 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 [55]. 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 [36]. 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. 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. There also did not appear to be an increase in adverse events with repeated dosing (i.e., the rate of adverse events after second or third doses compared to the rate after first dose). In addition, there have been no allergic reactions clearly linked to the vaccine. The overall side effect profile appears to be nil, with no differences identified between vaccinees and normal saline controls in eight randomized, double-blind, placebo-controlled trials with respect to solicited adverse events, unsolicited adverse events, or laboratory abnormalities. PfSPZ Vaccine, which is formulated without an adjuvant, has appeared to be one of the least reactogenic vaccines ever assessed in clinic trials.

One safety question not yet fully resolved is the adverse event profile of condensed regimens. For example, if the second dose of vaccine is given 7 days after the first dose, rather than the 4 to 8 weeks after the first dose, is there increased reactogenicity? Data from the MAVACHE trial in Germany indicate that this might be the case at the higher doses tested in that trial. In the group (n=6) that received $9x10^5$ PfSPZ per dose on Days 1, 8 and 29, these systemic reactions were not observed. However, higher doses of the condensed regimens were associated with systemic symptoms when the second dose was given on Day 8. In the group receiving $1.35x10^6$ PfSPZ on Days 1 and 8, 4/6 participants had systemic symptoms following the second immunization of the vaccine. Symptoms occurred approximately 10-12 hours after administration on the same day in three volunteers and approximately 24 hours after administration on the next day in another volunteer. These symptoms included fever, chills, sweats and fatigue that were Grade 1 in severity. One participant had additional symptoms of Grade 1 diarrhea, arthralgia, myalgia and abdominal pain. All four participants experienced headache (Grade 1 in two participants and Grade 2 in two participants). In the group receiving $2.7x10^6$ PfSPZ on Days 1 and 8, 1/6 participants experienced Grade 3 fever, chills, sweating, myalgia, fatigue and vomiting the evening of the day of the second immunization, symptoms which lasted about one day.

A frequent laboratory observation in the MAVACHE trial was lymphopenia one day following immunization. There were transient decreases one day after the second dose in all groups. For the group which received 3 doses ($9x10^5$ PfSPZ on Days 1, 8 and 29), lymphopenia was also observed after the third dose (Grade 1 to 2 in severity). The lymphopenia resolved within approximately one day. Although not well understood, it is hypothesized that the transient lymphopenia may reflect white blood cells migrating to target organs such as the liver to respond to invading parasites.

The dose of PfSPZ planned for USSPZV7 – $9.0x10^5$ PfSPZ, is lower than the doses in MAVACHE where adverse events were observed after the second immunization. Such adverse events have not been observed in the pediatric trial in Gabon where the same vaccination regimen as planned for USSPZV7 was used in children age 1 to 12 years. Although the data are still blinded, the combined (vaccine + placebo) adverse event data do not

indicate any concerns. **Table 7** and **Table 8** provide data on solicited adverse events and laboratory abnormalities, respectively, from the LaSPZV1 trial in Gabon.

Table 7. Solicited adverse events recorded days 0-7 post-all immunizations.
Participants received three doses weeks 0, 1 and 4 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 (38-38.4°C)	3	1	3
	Moderate (38.5-38.9)	1	4	1
	Severe (≥39.0°C)	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 aged 1-6 were scored with regard to inability to eat, drowsiness and irritability /fussiness; there were no instances of drowsiness or irritability/fussiness.
Children aged 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 8. 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 are 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

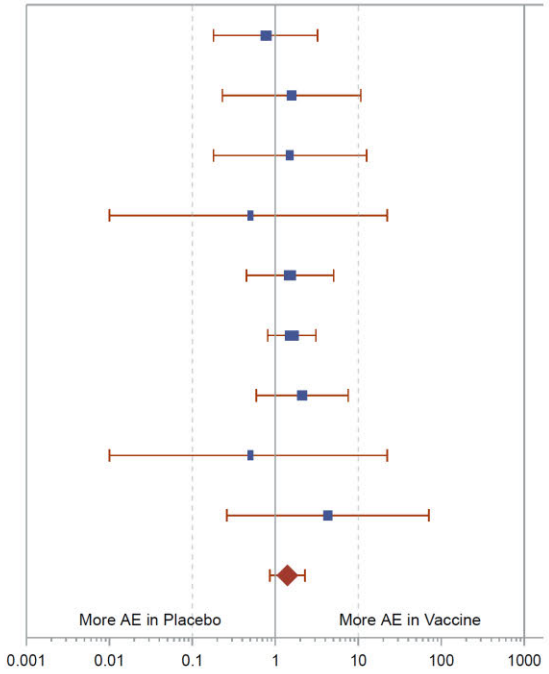
These findings in pediatric studies are illustrative of the findings in all trials of PfSPZ Vaccine, including all twelve randomized, double-blind, normal saline placebo-controlled trials that have been completed and unblinded. None of these trials has shown any differences in the rates of any AEs between vaccine recipients and controls after unblinding that have achieved statistical significance (#4, 5, 7-10, 12, 14-16, 18-19 in **Table 4**) [2, 4, 38-43, 56, 57] except for one study where there was an excess of myalgia in the vaccine group (#10 in **Table 4**). 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 [40, 41]. It is thus not clear that PfSPZ Vaccine has consistently caused *any* adverse reactions, other than the anecdotal data from the high doses administered in the MAVACHE trial.

2.2.1.1.3 META-ANALYSIS OF ADULT AND PEDIATRIC SAFETY

A meta-analysis of AE data is shown for adults and children using forest plots of total solicited AEs in vaccinees and placebos in the randomized, double-blind, placebo-controlled trials analyzed to date (**Figure 2).** In all cases, 95% confidence intervals cross a ratio of 1, indicating no differences between vaccinees and controls.

Figure 2. Effect of Vaccine on Incidence of any Solicited Systemic Adverse Event (AE) per Study Participant in Randomized, Double-Blind, Placebo-Controlled Trials of PfSPZ Vaccine in Adults Age ≥ 18 Years in Sub-Saharan Africa

Trial	Country	Year	# Vac. (# AE)	# Plac. (# AE)	Risk Ratios	Lower 95% C.I	Upper 95% C.I.	Study Weight* (%)
MLSPZV1	Mali	2014	46 (3)	47 (4)	0.77	0.18	3.24	13
BSPZV1	Tanzania	2014	46 (9)	8 (1)	1.57	0.23	10.73	11
EGSPZV1	EG	2015	20 (3)	10 (1)	1.50	0.18	12.65	9
BSPZV2	Tanzania	2015	12 (0)	6 (0)	0.50	0.01	22.25	7
MLSPZV2	Mali	2016	60 (6)	60 (4)	1.50	0.45	5.05	14
BFSPZV1	Burkina Faso	2016	39 (15)	41 (10)	1.58	0.81	3.08	16
EGSPZV2	EG	2016	32 (15)	9 (2)	2.11	0.59	7.56	12
BSPZV3a	Tanzania	2018	12 (0)	6 (0)	0.50	0.01	22.25	7
EGSPZV3	EG	2018	84 (9)	20 (0)	4.29	0.26	70.88	11
Overall			351 (60)	207 (22)	1.40	0.88	2.28	100



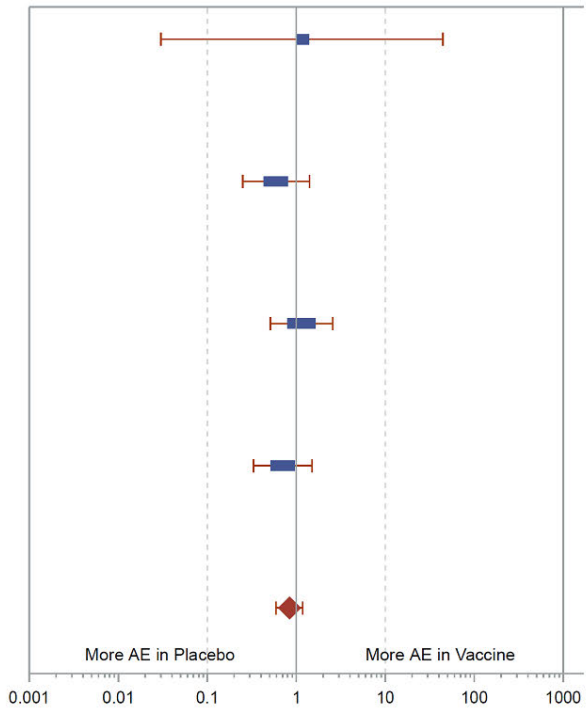
- Weighting = Modified Variance
- I^2 = 50% (0-77)
- P-value overall = 0.18

#Vac. is the number of participants enrolled in the vaccine arm in each study; # Plac. is the number of participants enrolled in the NS control arm of each study. Numbers in parentheses are the number of participants experiencing any solicited adverse event (AE) after any of the injections administered. Solicited local AEs were collected for three days (BSPZV2, BSPZV3, EGSPZV2 and EGSPZV3) or seven days (BSPZV1, EGSPZV1, BFSPZV1 (DMID protocol ID 15-0001), MLSPZV1 and MLSPZV2 (DMID protocol ID 16-I-N004)) after each dose, while solicited systemic AEs were collected for seven days after each dose, except for MLSPZV1, for which they were collected for 28 days after each dose. Depending on the trial, solicited local AEs included pain, tenderness, erythema, swelling, induration and pruritus; solicited systemic AEs included fever (measured), fever (subjective), headache, fatigue/malaise, arthralgia, myalgia, rash/pruritus/urticaria, chills/rigors, nausea/vomiting, abdominal pain, diarrhea, chest pain, palpitations, shortness of breath, bradycardia/tachycardia, hypertension/hypotension.

Figure 3. Effect of vaccine on incidence of any solicited systemic adverse events per study participant 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 participants enrolled in the vaccine arm in each study; # Plac. is the number of participants enrolled in the NS control arm of each study. Numbers in parentheses are the number of participants 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**

2.2.1.1.4 HUMAN SERUM ALBUMEN

PfSPZ Vaccine is formulated with human serum albumin. The manufacturer CSL Behring states in the package insert: "AlbuRx® 5, Albumin (Human) 5% solution is made from human plasma. Products made from human plasma may contain infectious agents, such as viruses, that can cause disease. The risk that such products will transmit an infectious agent has been extremely reduced by screening plasma donors for prior exposure to certain viruses, by testing for the presence of certain current virus infections, and by inactivating and/ or removing certain viruses through alcohol fractionation and through heat treatment of the product in the final container for 10 hours at 60°C. Despite these measures, such products can still potentially transmit disease. A theoretical risk for transmission of Creutzfeldt-Jakob Disease (CJD) is considered extremely remote. No cases of transmission of viral diseases or CJD have ever been identified for Albumin (Human). There is also the possibility that unknown infectious agents may be present in such products."

2.2.1.1.5 SERIOUS ADVERSE EVENTS

Against this background of favorable safety data from double-blind, placebo-controlled trials, two serious adverse events (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 with a plausible, but not definitive, pathophysiological link identified. Both SAEs occurred in a trial in Equatorial Guinea (#14 in **Table 4)** 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.

1. In the first case, 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 pregnancies end in spontaneous abortion, it is unclear whether the administration of PfSPZ was related to the loss of this embryo. Until first studies in pregnant women are completed (planned for later in 2021), avoidance of pregnancy remains an important inclusion criterion for trials of PfSPZ Vaccine.
2. In the second 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. A work-up showed that the boy had a normal head CT scan and a normal head MRI. However, an electroencephalogram (EEG) revealed abnormalities consistent with generalized epilepsy. Consulting neurologists posited that the general inflammatory response to the vaccine may have lowered the seizure threshold in this individual, whose EEG is consistent with a predisposition to seizures, although the precise mechanism that could explain the link is not clear. At this point, it is not possible to know whether the PfSPZ non-specifically affected the seizure threshold in this boy, or whether the timing of the seizure was coincidental. No other vaccine recipient in any trial has experienced a seizure with timing suggesting an association with PfSPZ Vaccine administration. This includes the 400 infants receiving PfSPZ Vaccine

in the KSPZV1 trial in Kenya (#12 in **Table 4**). Although many infants experience febrile seizures in association with malaria or other infections in that trial, these febrile seizures were expected adverse events not linked to immunization. It was noted that epilepsy was diagnosed in several children during the KSPZV1 trial, at a frequency not statistically different from the known background rate of epilepsy in the same population. Until larger numbers of children have received PfSPZ Vaccine, Sanaria recommends excluding any individuals with a history of non-febrile seizures from participation in clinical trials of PfSPZ-based products, although with further clinical experience this restriction may be lifted. This exclusion applies to the USSPZV7 trial.

Although the data indicate that PfSPZ Vaccine does not cause adverse reactions at the doses planned for this study, nevertheless the consent form lists the following reactions any vaccine might theoretically cause, including: objective fever (oral temperature > 100.4° F), subjective fever, chills, headache, fatigue, malaise, myalgia, arthralgia, rigors, and systemic reactions that might indicate allergic responses (rash, urticaria, pruritus, and edema); these will constitute the list of solicited adverse events which are assess after each immunization. Additional risks are abnormal laboratory tests, including elevated liver function tests and transient changes in blood counts. Because the vaccine is administered by DVI into a superficial vein, the participant is also at risk of having a vasovagal response.

2.2.1.2 RISK ASSOCIATED WITH PFSPZ CHALLENGE

2.2.1.2.1 LOCAL REACTIONS

As with PfSPZ Vaccine, DVI is used for administration of PfSPZ Challenge. Potential risks include: pain, tenderness, erythema, bruising, swelling and induration. Other potential risks, which have not been observed, but are theoretically possible include local inflammatory reactions, pruritus, large local reactions involving the whole forearm, lymphangitis and lymphadenopathy. Additional risks associated with venipuncture are listed below.

2.2.1.2.2 SYSTEMIC REACTIONS

Like the administration of PfSPZ Vaccine, administration of PfSPZ Challenge has been very well tolerated. Prior to the potential onset of malaria (6 or more days post administration of PfSPZ Challenge), potential risks include systemic allergic reactions to the sporozoites or the diluent (including anaphylaxis), and other reactions typical of vaccines, including objective fever (oral temperature >100.4°F), subjective fever, chills, sweats, malaise, fatigue, headache, myalgia, arthralgia, rigors, chest pain, abdominal pain/tenderness, nausea, vomiting and diarrhea. Additional risks are abnormal laboratory tests, including elevated liver function tests and transient changes in blood counts. Because it is administered via DVI, there is also the risk of a vasovagal response. The incidence of any of these potential risks has been comparable to the risks after the administration of NS in randomized, double-blinded, placebo-controlled trials.

PfSPZ Challenge is formulated with human serum albumin like PfSPZ Vaccine, and carries the same theoretical risks associated with HSA as mentioned for PfSPZ Vaccine.

2.2.1.3 RISK ASSOCIATED WITH *P. FALCIPARUM* PARASITEMIA

Participants undergoing CHMI may be expected to develop malaria, and may experience signs and symptoms of malaria which include subjective fever, objective fever (>100.4°F), chills, rigors, headache, vomiting, nausea, dizziness, malaise, insomnia, diarrhea, fatigue, sweats, myalgia, neck ache, arthralgia, abdominal pain, tachycardia, abnormal laboratory tests, (e.g. decreased leukocytes, platelets, mild anemia) and, rarely, enlarged liver or spleen. In study participants from the US and Europe, parasitemia presents between days 7 and 17 in both vaccinees and normal saline controls. No parasitemias have been detected beyond 17 days; study participants who remain negative by qRT-PCR on day 28 do not progress to patent parasitemia and have been safely managed without terminal antimalarials in the most recent CHMI studies (NCT02601716, NCT02704533, NCT04966871), The clinical outcomes of participants post-CHMI are discussed in detail in the published literature [58].

Complications can occur during malaria when diagnosis and treatment are delayed and high parasite densities develop in the blood. In uncontrolled circumstances, malaria infections can lead to kidney, liver, or brain injury (seizures, coma) and death. Under the carefully controlled conditions and implementation of early diagnosis and antimalarial treatment in this study, the chance of such complications is low and the risk of death from malaria infection is very small. There have been no cases with complications resulting in severe disability or death in study participants undergoing CHMI. Participants are monitored closely and treatment is initiated immediately upon identification of parasitemia.

Cardiac events have been reported following CHMI in clinical trials in the Netherlands at Radboud University Nijmegen Medical Center (RUNMC). In all cases these events followed CHMI from the bites of infected *Anopheles* sp. mosquitoes. Similar events have not been seen following the administration of PfSPZ Challenge at this or other clinical trial sites.

1. In 2008, a 20-year-old healthy female participant developed retrosternal chest pain 2 days after treatment with artemether/lumefantrine for parasitemia. A diagnosis of acute coronary syndrome with limited myocardial necrosis of the inferior wall was based upon the pain, < 1 mm ST segment elevation EKG findings and cardiac enzyme analysis. A cardiac MRI was negative for evidence of atherosclerotic disease. The participant recovered quickly and her follow-up was uneventful, with no re-occurrence of similar symptoms during the 1-year follow-up period. It is unclear if this participant's AE was the result of the experimental vaccine, malaria infection, anti-malarial medication, or an unknown cause [59].
2. A second cardiac event occurred at this same site in the Netherlands in a participant undergoing CHMI administered with chloroquine prophylaxis as a "chemoprophylaxis vaccine" approach. The SAE occurred in the participant who underwent CHMI (without chloroquine) using five Pf NF54-infected mosquitoes, 60 days after he had received the last injection of PfSPZ (with chloroquine) for immunization. On Day 9 post-CHMI the participant had a sore throat. On Day 11 post-CHMI, the participant's thick film was positive for malaria, and treatment with Malarone® (atovaquone-proguanil) was initiated. The participant was asymptomatic, but due to elevated troponin T levels (maximum: 299 ng/L), which are routinely assessed at RUNMC on a daily basis, the participant was hospitalized on Day 13 after CHMI. On Day 14 after CHMI, the participant experienced chest pain for 10 minutes and received sublingual nitroglycerin spray. He was diagnosed with myocarditis based on

a magnetic resonance imaging (MRI) and minor repolarization disturbances on electrocardiogram. The participant had no further symptoms on follow-up, and the troponin T levels returned to normal by Day 28 post CHMI. The participant was discharged on Day 17, and the electrocardiogram was normal on Day 31 post CHMI. A follow-up cardiac MRI, performed approximately 5 months after the start of the SAE, demonstrated good left ventricular function with mild hypokinesia in a few segments and some remaining mid-wall delayed enhancement. An etiology was not established, but the participant's throat swab was positive for rhinovirus 9 days after CHMI. Furthermore, 14 days after the third immunization with PfSPZ Challenge, the participant received immunizations for diphtheria, tetanus, polio, typhoid, hepatitis A and hepatitis B in preparation for international travel. Whether or not these cardiac events are related to CHMI itself remains unclear [60].

3. Two additional (unpublished) cardiac events took place at RUNMC in study participants enrolled in "chemoprophylaxis vaccine" trials.
    a. In November 2014, a 23-year-old male participant had an asymptomatic high sensitive (hs)-troponin-T elevation ten days after the first immunization with bites from 15 NF54 infected mosquitoes under chloroquine prophylaxis. The participant was admitted to the hospital for cardiac monitoring for two days, after which he was discharged in good clinical condition. During the 48-hour observation period, the participant remained asymptomatic and both EKG and cardiac monitoring showed no abnormalities, but the hs-troponin-T was elevated at 168 ng/l (normal value <14 ng/l). Urine toxicology was positive for cannabis and a cardiac MRI showed findings that may have been consistent with a myocarditis or with artifact due to movement. It was concluded the elevated hs-troponin-T concentrations and cardiac MRI findings likely represented a mild myocarditis in an apparently healthy male participant 10 days after a first immunization per protocol. After thorough study of the event, the Data Safety and Monitoring Board (DSMB) for this trial and the Central Committee on Research Involving Human Subjects (CCMO) in the Netherlands approved the continuation of the clinical trial on December 8th, 2014.
    b. In 2020, a 23-year-old female trial participant presented with acute retrosternal pain 10 days after she had received her first inoculation with the bites of 15 Pf-infected mosquitoes and one day after completion of presumptive antimalarial treatment. She had an unremarkable medical history except analysis for tachycardia (including Holter and echocardiography) at ±17 years of age, which was concluded to represent sinus tachycardia for which no further follow-up was indicated. She had no other known cardiac risk factors. On day 7 post-CHMI she had developed a fever to 39.5°C with grade 1 headache, fatigue, myalgia, dizziness and nausea and was treated for malaria. Upon presentation with chest pain on day 10, she had elevated cardiac biomarkers (troponin T maximally 331ng/L, normal range <14ng/L; CK maximally 230 U/L, normal range <145 U/L) and the ECG showed minimal changes (negative T-wave in III on one reading, which thereafter normalized), defining an acute coronary syndrome (ACS). She was admitted to acute cardiac care and later to the cardiology department. The chest pain resolved after a single 10mg dose of metoprolol and repeated sublingual nitroglycerin, and cardiac biomarkers decreased. She was discharged 6 days after

admission. Cardiac MRI showed no conclusive evidence regarding the pathophysiology or origin.

It remains unclear whether any of these events are related to Pf parasites or the treatment of Pf parasitemia. While there are confounding factors associated with each of these cardiac events, including the peculiar fact that these events have occurred only at one center following CHMI by the bite of infected *Anopheles* sp. mosquitoes, and never following PfSPZ Challenge inoculations by DVI or PfSPZ inoculations by mosquito bite performed elsewhere, nevertheless a causal relationship with CHMI cannot be excluded and therefore the design of the USSPZV7 trial will incorporate safeguards to minimize to the extent possible the risk of cardiovascular events in study participants. Enrolled participants will be at low risk of cardiovascular events due to their age, absence of known cardiovascular disease, absence of known diabetes mellitus, and documented normal EKG, systolic and diastolic blood pressure at screening. During the study, participants will be closely followed for the occurrence of cardiovascular-related signs or symptoms. Monitoring will include questioning about chest pain, palpitations and shortness of breath starting on day 7 after administration of PfSPZ Challenge (7G8). Signs or symptoms of an event suggestive of a cardiac etiology will prompt a cardiology work-up.

Relapsing malaria is not an issue since Pf has no hypnozoite stage and therefore does not relapse after appropriate treatment.

2.2.1.4 RISK ASSOCIATED WITH VENIPUNCTURE

At specified points throughout the study, blood samples will be collected from the participant for safety laboratories and qPCR. There are risks associated with blood drawing that include discomfort, swelling, bruising around the vein, lightheadedness/fainting, anemia following repeated blood draws, and, rarely, infection at the blood-drawing site or clinically significant hematoma. Infection could lead to more generalized symptoms such as limitation of arm movement, lymphadenopathy, fever, chills, headache, malaise, myalgia, joint pain or sepsis.

2.2.1.5 RISK ASSOCIATED WITH ANTI-MALARIAL MEDICATIONS

Malarone® (atovaquone/proguanil), a generally well tolerated and highly effective treatment regimen, will be used as the first line anti-malarial treatment for all participants. Second-line treatment will be Coartem® (artemether/lumefantrine). The study team will discuss these medications and their possible side effects in detail as part of the informed consent process and prior to initiation of treatment for participants who are infected with malaria.

Side effects of atovaquone/proguanil (Malarone®) include: nausea, vomiting, abdominal pain, anorexia diarrhea, headache, cough and, rarely, anemia, oral ulcerations, insomnia, fever, edema, and alopecia.

Side effects of artemether-lumefantrine (Coartem®) for those participants who cannot tolerate Malarone® include:

Common side effects: abdominal pain, nausea, vomiting, anorexia, chills, cough, heart palpitations, fever, headache, myalgia, sore throat, nasal congestion, dyspnea on exertion, dizziness/vertigo, insomnia, hepato- or splenomegaly, elevated AST, pruritus/rash, easy bruising, fatigue and weakness.

Serious side effects: bleeding significant enough to cause melena and hematuria, dysuria, changes in hearing, chest pain, seizures, hypersensitivity reaction, QT prolongation and bullous dermatitis.

2.2.1.6 PREGNANCY

Risks to unborn babies are unknown at this time; pregnant females will be excluded from this study. Study participants should not become pregnant for 2 months after CHMI. Sexually active females, unless surgically sterile or post-menopausal, must use an effective method to avoid pregnancy (including oral or implanted contraceptives, intrauterine device, female condom, diaphragm with spermicide, cervical cap, abstinence, use of a condom by the sexual partner or surgical sterilization of sexual partner). If post-menopausal, female participants must have experienced at least 1 year of amenorrhea.

2.2.1.7 RISKS ASSOCIATED WITH SARS-COV-2

Screening for participants is anticipated to begin in October, 2022. Although a significant proportion of the population is vaccinated, a risk of SARS-CoV-2 acquisition exists beginning during screening and persisting throughout the study period. The trial will be conducted using standard operating procedures (SOPs) to assure the safety of research participants and members of the clinical team. Measures include mask wearing, social distancing, restrictions of interactions to rooms with frequent good air circulation, disinfection of surfaces, hand-washing, etc. These policies and procedures will be based on the most up-to-date information available and consistent with recommendations from the US Centers for Disease Control and Prevention. If the prevalence of SARS-CoV-2 dictates, participants may be screened by PCR/antigen prior to vaccination 1.

2.2.2 KNOWN POTENTIAL BENEFITS

Although there is no direct benefit to participants in this study they may gain a potential indirect benefit of increased personal knowledge about their health status from their medical history, physical examination, and laboratory testing. Participation is voluntary and participants may withdraw at any time without penalty or loss of benefits to which the participant is otherwise entitled. Society as a whole may benefit from information learned through this study.

2.2.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

The acute need for a vaccine to protect travelers and military personnel against malaria when deployed to malaria-endemic areas and the enormous global burden of morbidity and mortality due to Pf malaria in endemic areas provide the impetus for continued efforts to develop a vaccine to control and eliminate this disease. Studies of PfSPZ Vaccine to date have demonstrated a favorable safety profile, and promising

VE. Serious adverse events have only rarely been associated with PfSPZ Vaccine and causality has not been established. The study is considered greater than minimal risk for participants.

Overall precautions:

Only healthy adults will be enrolled following a prescribed medical history and physical examination, screening clinical laboratories, and an electrocardiogram. Inclusion/exclusion criteria will be adhered to. Study doctors will be available 24 hours a day, 7 days a week to provide care if needed. Emergency equipment and supplies, including injectable epinephrine, oral antihistamines and corticosteroids, will be readily available to treat any acute allergic symptoms that might occur.

To minimize the risks, immunizations and PfSPZ Vaccine and injections with PfSPZ Challenge will be administered in a carefully cleaned and disinfected area of the upper extremity via a sterile 25-guage tuberculin needle and syringe by a skilled medical provider using aseptic technique, thus minimizing any chance of extravasation or infection.

Precautions to reduce risk associated with immunization:

Participants will be monitored closely for the occurrence of adverse events, including the measurement of vital signs and laboratory tests, and the collection of solicited symptoms (to be assessed for seven days after each immunization). Both unsolicited symptoms and SAEs will be monitored.

To monitor for breakthrough blood stage infection (which has never happened with the thousands of doses of PfSPZ Vaccine already administered), participants will be informed about potential signs and symptoms of breakthrough malaria infection and will be told to contact research personnel if any such symptoms develop. If symptoms are present, a blood sample can be obtained outside the window of the daily sample and will be examined for the presence of parasites.

Precautions to be taken to reduce risk to participants associated with CHMI:

The WHO Consensus Document for the Standardization of Design and Conduct of *P. falciparum* Sporozoite Challenge Trials [61] will be utilized to help ensure the safety of all participants from the day of CHMI until completion of all post-CHMI follow-up.

The clinical study team will review each participant's adherence to the schedule and safety follow-up to date. This review will be done to identify any likelihood that the participant may be unreliable or non-compliant with study visits post-challenge. A participant who has been non-compliant with prior study visits may be excluded from the CHMI phase and followed for safety. Prior to CHMI, at least 1-2 emergency contact numbers will be confirmed and verified as authentic for each participant.

Study participants will be counseled at the time of challenge about the signs and symptoms of malaria and given 24 hour/day contact information should any signs or symptoms develop. Participants will have daily PCR done beginning 7 days after PfSPZ Challenge until 18 days post-CHMI. If symptoms are present, a blood sample for PCR may be obtained outside the window of scheduled samples. Those participants that do not develop parasitemia will have follow-up visits on +20, +22, +25 and +28 days post CHMI. A

confirmatory negative PCR will be documented on all participants at Day 28 post CHMI. All participants will be seen for their final visit on 56 days post CHMI.

As soon as malaria infection is documented, the participant will be treated; prompt treatment minimizes the risk of developing a serious complication due to the malaria infection. Complaints such as chest pain, palpitations, or shortness of breath are not uncommon in individuals with malaria. Nonetheless, the report of these symptoms by a participant will prompt a clinical evaluation by one of the investigators, and may include the consultation of a cardiologist. If a participant's symptoms are consistent with or suspicious for a cardiac etiology, patient care will be expedited and will be in accordance with the current American College of Cardiology/ American Heart Association clinical practice guidelines.

Participants will receive instructions not to travel outside of the clinical center area from the day of PfSPZ Challenge injection to 4 weeks after CHMI. If a participant must travel and is at risk of developing malaria, he/she may be presumptively treated for malaria infection and proper arrangements will be made to ensure adequate post-treatment follow-up, or if a qualified physician is available to provide monitoring and care at a distant site (and a suitable plan is pre-approved by the IRB), ongoing follow-up without treatment may be considered.

Participants will be counseled to use methods that will reduce risk of exposure to mosquitoes beginning +7 days after CHMI until +28 days post-CHMI (if remaining without parasitemia). However, individuals developing Pf malaria do not generate gametocytes until several days after the infection becomes patent, making transmission extremely unlikely even in the absence of precautions against mosquito bites.

Precautions taken to reduce risks associated with blood drawing:

Phlebotomy will be performed by trained staff using aseptic technique in a designated area, to reduce the risks of complications. Participants will be counseled to return to the clinical center if infection or any other unexpected outcome is suspected.

The amount of blood collected will be monitored closely. Throughout this study, the amount of blood collected will be no more than 5 mL/kg (250 mL for a 50 kg participant) during a 24 hour period and 10.5 mL/kg (525 mL for a 50 kg participant) in any 8-week period following the guidelines of the American Association of Blood Banks.

Precautions taken to reduce risks associated with anti-malarials:

Participants will be educated about potential side effects of anti-malarials and instructed to contact study staff with all questions and concerns.

Precautions taken to reduce risks associated with participant confidentiality:

To decrease the risk of a breach of participant confidentiality, all study procedures will be conducted per GCP guidelines, and every effort will be made to protect participant privacy and confidentiality to the fullest extent possible. No personal identifiers will be used in any publication or communication used to support this research study. Coded and unique study numbers will identify specific details related to all

participants. 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 participants' 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 improve medical care.

3. OBJECTIVES AND ENDPOINTS

3.1 ESTIMAND FRAMEWORK

Target Population: This study is being conducted to support the proposed indication of prophylaxis of Pf malaria in US adults traveling to Africa. This population of travelers is generally healthy and malaria-naive, and without exception, highly susceptible to Pf infection with the potential for severe disease and death if parasitemia is not promptly diagnosed and treated. The relative exception is recent immigrants to the US from malaria endemic countries with a history of repeated episodes of malaria prior to emigration. While such individuals also travel to Africa, often to visit friends and relatives, their prior exposure may provide a degree of protection against high density parasitemia and severe disease that may persist for several years after moving to malaria-free countries [62-66]. However, protection fades with time and immigrants from Pf-endemic countries, like life-long US residents, are susceptible to malaria infection and clinical illness, even if the risk of severe or fatal disease is reduced and need protection during travel to endemic areas. Even fully semi-immune African adults living in endemic areas are not protected against reinfection by their acquired immunity, as shown by cleared cohort studies where African adults cleared of parasitemia with curative drug regimens readily develop new infections detectable by thick blood smear (TBS) [2, 67, 68].

To achieve a study population representative of the traveler population, the enrollment criteria for this trial aim to exclude only those adults in poor health, at risk of poor compliance with study-related procedures or who might be on drugs with antimalarial properties or receive other vaccines that could interfere with PfSPZ Vaccine take. Otherwise, minimal restrictions are placed on enrollment and the participants in this trial should be representative of the target population including migrants as well as life-long residents.

Treatment: The active treatment is three immunizations of $9.0x10^5$ PfSPZ of PfSPZ Vaccine administered on days 1 8 and 29 by DVI. The alternative treatment is immunization with normal saline, which is indistinguishable from the test article. Treatment with PfSPZ Vaccine is the only intervention in trial that might affect the near universal susceptibility of US adult residents to Pf infection after administration of the standard dose of 3,200 PfSPZ of PfSPZ Challenge (Pf7G8), which has to date infected 96.4% (27/28) malaria-naive research participants in four different trials conducted in the US or EU.

Variable of Interest: The primary variable of interest is whether trial participants develop Pf malaria parasitemia following CHMI. This is a dichotomous outcome – infected or not infected, and can be definitively determined during the 28-day follow-up for CHMI because all individuals, except in the rare case of a failed inoculum (currently a 1 in 28 likelihood, or 3.6%), will develop detectable Pf parasitemia if not protected by the vaccine. The risk of failed inoculum will be equivalent in vaccine and placebo recipients, since the study is double-blind and all injections will be done without the possibility of bias based on knowledge of the treatment received. A failed inoculum should occur with equal frequency in

both study groups and therefore should not affect the trial outcome, and the sample size has been calculated anticipating that some controls will not develop parasitemia.

Estimator: Malaria parasitemia will be detected by quantitative polymerase chain reaction (qPCR) which will be performed daily starting on day 7 post CHMI. This is a highly sensitive and dependable assay and because Pf parasitemia will increase in density by about 10-fold every two days, all non-vaccine protected individuals will become positive. In case an individual becomes sick with a malaria-like illness and PCR is negative, thick blood smear (TBS), the gold standard for the clinical diagnosis of malaria, will be available, and in addition, individuals will undergo a work-up for other infectious diseases. All qPCR positive (or TBS positive) individuals will be immediately treated with a curative antimalarial regimen. In addition, blood specimens will be saved for the performance of a confirmatory qPCR at the laboratory of Dr. Sean Murphy, University of Washington. This assay has been established as a qualified biomarker by the US FDA (see FDA's website *https://www.fda.gov/drugs/biomarker-qualification-program/fda-reviews-qualified-biomarker-plasmodium-18s-rrnardna*).

Estimate (or Summary Measure): Once the CHMI outcome is known for each participant and the study is unblinded, vaccine efficacy, expressed as a percent, will be calculated as:

((IRU-IRV)/IRU) x 100, which is equivalent to
(1 – (IRV/IRU)) x 100, which is equivalent to
(1 – risk ratio) x 100

Where IRU = infection rate of unvaccinated participants (# qPCR positive/ # undergoing CHMI) and IRV = infection rate of vaccinated participants (# qPCR positive/ # undergoing CHMI).

The results of both CHMI's (at 3 and 12 weeks) will be combined to provide an average measure of protection over the twelve-week period as the primary outcome (VE at each timepoint is a secondary outcome).

Intercurrent Events: Because three injections according to a defined schedule are presumed to be required to induce an optimal immune response, failure to fully immunize according to schedule constitutes an important intercurrent event potentially affecting the outcome variable. This can happen through a partial immunization (IV administration of less than 80% of the intended 0.5 mL dose), administration of one or more doses outside the specified immunization windows or by missing one or more immunizations. At this point, we have no data to indicate that missing any one of the three planned doses has a significantly different effect than missing any of the other doses, or that immunization outside of the protocol specified immunization windows is significantly different than immunization according to the immunization windows. Consequently, there are only three strata with respect to this intercurrent event: receiving three doses per protocol, receiving three doses but not per protocol, or receiving only two doses but remain in the trial and, in the judgement of the investigative team, may proceed to CHMI (see below and also see the Statistics section for population definitions).

Another intercurrent event that can affect the primary protection outcome is an inadvertent switch in treatment (from vaccine to placebo or vice versa due to clinical or pharmacy error). This has never occurred in a CHMI study of PfSPZ Vaccine, although it has occurred rarely in field studies. However, should it occur, any individual designated as a placebo recipient who inadvertently receives PfSPZ Vaccine

will be included in the vaccine population, following an "as treated" approach, even if only one dose of PfSPZ Vaccine is received.

Another variable that could influence outcome is the administration of drugs with antimalarial properties; for this reason, concomitant medications are carefully monitored, and participants are educated regarding the importance of communicating to the study staff all drugs taken outside the context of the study. Although surreptitious use of antimalarial drugs has occurred in the past, historically this has been a rare event.

Another group of intercurrent events include participants who drop out or are lost to follow-up which may preclude measurement of the primary protection outcome variable, protection against CHMI. Those dropping out or lost to follow-up after receiving CHMI but before the requisite 28 days of observation are completed are a particular case that is discussed further in the Statistics section; briefly, if the participant is qPCR positive prior to dropping out, or drops out and remains qPCR negative later than the last day that any other research participant turns positive, inclusion in the mITT analysis dataset is possible.

None of these intercurrent events are likely to be related to treatment assignment. This is because of the data reviewed earlier showing that there are no differences in adverse event rates between PfSPZ Vaccine and normal saline administration. Thus, neither participants nor study staff will be able to intuit treatment assignment with accuracy. As a result, dropout rates are unlikely to differ between the two groups. Blinding and randomization should therefore remain effective at balancing variables between the two study groups.

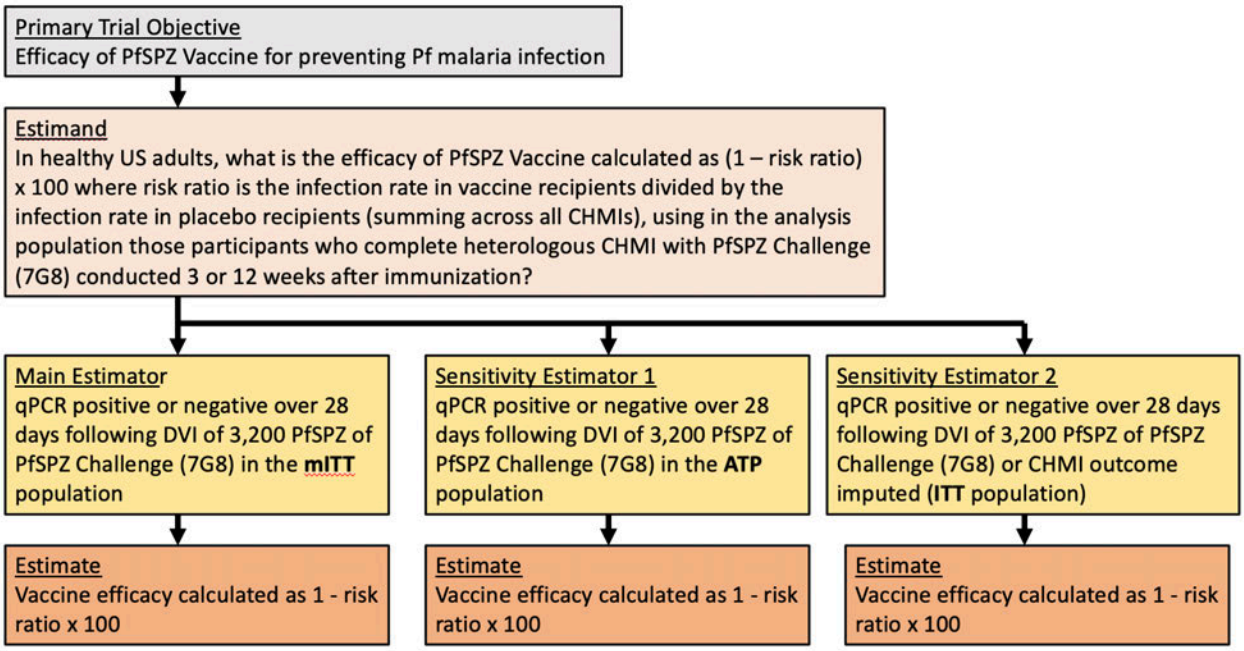
Development of an Estimand: The travel population to be immunized with PfSPZ Vaccine following licensure will not always receive all three immunizations with PfSPZ Vaccine. Some individuals will report to a travel clinical at a point where travel is imminent, and a four-week immunization schedule is not feasible. These individuals may receive just one or two immunizations, and (based on available data on the two-dose regimens tested in the MAVACHE trial) will not receive the full degree of protection afforded by a three-dose regimen. It is thus of practical interest to know both VE in the general travel population reporting to a travel clinic (and thus potentially not completing a three-dose series) and VE in those receiving a correctly administered three-dose series. To explore the effect of these important "intercurrent events", the study population is defined at three levels: the intention-to-treat (ITT) population includes all individuals receiving at least one immunization (receiving at least one immunization is required for enrollment into the study). The modified intention-to-treat (mITT) population includes all individuals receiving three immunizations, even if outside allowable windows, as long as at least 20% of each dose is administered. The according to protocol (ATP) population is those receiving immunization regimens compliant with protocol procedures (three doses of at least 80% of the injectate within allowed time windows). Based on advice from regulatory agencies, the mITT population will be the primary analysis population, and ITT and ATP populations will be analyzed as part of the sensitivity analysis of the study. The ITT analysis will include all participants receiving at least one dose of PfSPZ Vaccine, with the outcome of CHMI imputed when VE cannot be directly measured. All members of the mITT and ATP populations will have CHMI outcomes. With these considerations in mind, the following estimand is used for the study:

> In healthy US adults, what is the efficacy of PfSPZ Vaccine calculated as (1 – risk ratio) x 100 where risk ratio is the infection rate in vaccine recipients divided by the infection rate in placebo recipients (summing across both CHMIs), using in the analysis population those participants who

complete heterologous CHMI with PfSPZ Challenge (7G8) conducted 3 or 12 weeks after immunization?

This estimand, along with the planned sensitivity analyses examining ATP and ITP participant populations, is illustrated in **Figure 4**.

Figure 4. Estimand Framework



With this as background, the following Objectives and Endpoints are established for this study:

3.2 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To measure the combined vaccine efficacy (VE) of PfSPZ Vaccine against Pf malaria in adults living in the US following heterologous controlled human malaria infection (CHMI) with PfSPZ Challenge (7G8) administered 3 or 12 weeks after last dose of vaccine, in the modified intention-to-treat (mITT) population.	VE computed as one minus the estimated risk ratio for Pf infection (parasitemia) detected by qRT-PCR beginning 7 days after CHMI and censoring participants at 28 days in the "modified Intention to Treat" (mITT) population, combining data across the three CHMI timepoints.	Complete protection against infection during CHMI (i.e., no parasitemia) is a primary measure of PfSPZ Vaccine VE.
To assess safety and tolerability of 3 doses of $9.0x10^5$ PfSPZ of PfSPZ Vaccine administered by direct venous inoculation (DVI) on days 1 (V1), 8 (V2) and 29 (V3).	The differences in proportions of vaccinees compared to controls experiencing related solicited and unsolicited adverse events and laboratory abnormalities after vaccination.	The number and nature of solicited and unsolicited adverse events, SAEs and abnormal laboratory values will provide an objective measure of safety and tolerability of the investigational product (IP).
Secondary		
To measure VE separately at each CHMI timepoint (3 and 12 weeks after V3).	VE measured as above, for each timepoint.	Determination of VE at each of the two CHMI timepoints will provide information useful for prescribing PfSPZ Vaccine.
To measure antibody responses to PfCSP 2 weeks after V2 and V3, on the day of CHMI and 4 weeks post-CHMI and determine if antibody responses are correlated with protection.	Antibody levels to PfCSP measured by ELISA comparing vaccinees to controls and protected (no parasitemia occurring post CHMI) and non-protected (parasitemia occurring post CHMI) vaccinees.	Antibody studies are important measures of the immunogenicity of the vaccine and have shown an association with protection in prior studies.
Tertiary/Exploratory		
To compare VE between groups with CHMI 3 or 12 weeks after V3.	VE measured as above.	Determination of VE across the span of a 12-week travel period will provide information useful for prescribing PfSPZ Vaccine
To measure VE in the ITT and ATP populations.	VE measured as above.	This constitutes an important sensitivity analysis regarding the robustness of the primary VE analysis using the mITT population.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
To measure VE using time-to-event analysis.	VE measured as 1 minus the estimated hazard ratio.	Including delay in the onset of parasitemia (prolonged prepatent period) is a measure of partial protection.
To compare safety and VE outcomes between male and female participants and between racial and ethnic groups to explore gender, race and ethnicity effects.	The differences in proportions of males and females in the vaccine group experiencing particular safety, tolerability or VE outcomes; similar outcomes relating to race and ethnicity.	These investigations are mandated by NIH guidelines[2].
To measure antibody response to other Pf antigens and to PfSPZ and determine if they are correlated with protection.	Antibody levels to whole PfSPZ by automated immunofluorescence assay (aIFA) and/or by other assays to be further defined, which may include assays to assess responses to multiple Pf antigens, antibody subclass assays and functional assays, comparing vaccinees to controls, and protected (no parasitemia occurring post CHMI) and non-protected (parasitemia occurring post CHMI) vaccinees.	These antibody studies are important measures of the immunogenicity of the vaccine.
To measure the ability of sera from immunized participants to inhibit sporozoite invasion of hepatocytes *in vitro* and determine if the level of inhibition *in vitro* is correlated with protection.	Capacity to inhibit sporozoite invasion of hepatocytes (HC-04 cells) *in vitro* by inhibition of sporozoite invasion (ISI) assay comparing vaccinees to controls, and protected (no parasitemia occurring post CHMI) and non-protected (parasitemia occurring post CHMI) vaccinees.	These functionally inhibitory responses are important measures of the immunogenicity of the vaccine.

[2] "If the proposed research includes an NIH-Defined Phase III Clinical Trial, the "Inclusion of Women and Minorities" attachment must address plans for how sex/gender, race, and ethnicity will be taken into consideration in the design and valid analysis of the trial" (*https://grants.nih.gov/grants/how-to-apply-application-guide/forms-f/sbir-sttr-forms-f.pdf*)

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
To measure cellular immune responses following immunization and determine if they are correlated with protection.	Cellular immune responses to whole PfSPZ and synthetic peptides from selected Pf antigens by intracellular cytokine staining (ICS)/ flow cytometry and/or other assays to be defined comparing vaccinees to controls, and protected (no parasitemia occurring post CHMI) and non-protected (parasitemia occurring post CHMI) vaccinees.	Cellular responses are thought to be the primary mechanism of protection, although key cellular effectors present in the liver generally do not appear to be detectable in the periphery.
To analyze innate and adaptive states before and at early time-points after vaccination by using: 1) RNA-seq, profiling of whole blood/PBMC; 2) immune phenotyping of single cells using flow cytometry and potentially CITE-seq; and 3) potentially profiling of circulating proteins and metabolites. The objective of this exploratory goal is to search for biomarkers of vaccine induced humoral and cellular immune responses and protection.	RNA transcriptome quantification as detected by RNA-seq and protein levels in serum, and serum analyte analysis, comparing vaccinees to controls, and protected (no parasitemia occurring post CHMI) and non-protected (parasitemia occurring post CHMI) vaccinees.	The innate immune responses may be an important measure of the immunogenicity of the vaccine.
To deep sequence the peripheral immune repertoire of cells to assess the cell receptor frequencies.	Deep sequencing of immune receptor genes.	Studying the peripheral immune receptor repertoire of cells will contribute to correlates of protection analyses.

4. STUDY DESIGN

4.1 OVERALL DESIGN

Hypothesis: This trial is designed to test whether it is possible to reject the null hypothesis that there will be no significant difference between the vaccine group and the control group with regard to the proportion or research participants developing parasitemia following heterologous CHMI conducted 3 and 12 weeks after immunization with PfSPZ Vaccine, and that any observed difference is due to sampling or experimental error. Please see the Statistics section for additional discussion.

Phase of the trial: TBD[3].

Design features:

1. Randomized, double-blind, placebo-controlled clinical trial enrolling healthy adult participants 18-50-years-old living in the US.
2. The trial is designed to measure safety, tolerability, immunogenicity and VE against heterologous controlled human malaria vaccine conducted at 3 or 12 weeks after vaccination.
3. Participants will be randomized to two study groups, vaccine and placebo, in a 3:1 ratio. Treatment assignment will be double blind; however, whether a given participant will be receiving CHMI at 3 or 12 weeks will not be blind.
4. Bias will be minimized by the randomized, double-blind design and by the inability to distinguish vaccine or placebo based on appearance, tolerability or other characteristics discernable by clinical staff or study participants. Syringe preparation will be done by pharmaceutical operations teams that do not interact with participants or make any follow-up assessments.
5. The study will take approximately 6 to 8 months to complete, not including 2-3 months of recruitment. The period of follow-up for each immunized participant and placebo controls is through 8 weeks post-CHMI.
6. Study participation by individuals will last 4 to 6 months (not including screening) depending upon group assignment.
7. Screening will be done within 90 days of enrollment. If clinical laboratories expire (meaning they were obtained greater than 90 days prior to planned enrollment), specific safety labs will be repeated for screening purposes. Enrollment occurs with first administration of PfSPZ Vaccine or normal saline placebo.
8. A study participant target of ~50% female study participants.
9. Safety evaluation includes an electrocardiogram (ECG) performed at screening. Participants with abnormal cardiovascular symptoms or findings that appear clinically significant will be excluded and if appropriate, referred to a cardiologist for further evaluation; individuals with history of non-febrile seizures will also be excluded.
10. For the second and third immunizations, allowable windows are assigned (see Section 1.3 'Schedule of Activities').

[3] FDA recommendation will be sought.

11. Solicited adverse events will be collected for 7 days after each immunization. The first six days after CHMI, solicited AEs will be assessed for a potential relationship to PfSPZ Challenge; beginning 7 days after CHMI solicited AEs will be assessed for a potential relationship to malaria infection if individuals develop parasitemia. Unsolicited adverse events will be collected from the first immunization (V1) until 21 or 28 days after the third immunization (V3), depending on the timing of CHMI, and for 28 days after CHMI. Physical exams and medical history will be assessed repeatedly as indicated. Safety laboratories will be collected at screening, day of V1, 7 days after V2, 14 days after V3, day of CHMI and 28 days after CHMI.
12. Surveillance for SAEs will occur for the duration of the study.
13. Follow-up of adverse events (including SAEs) and other clinical events will occur until resolution or stability.
14. Participants will be observed for 30 minutes following each dose of PfSPZ Vaccine or PfSPZ Challenge.
15. Clinical laboratory testing will be conducted by certified laboratories.
16. A Local Safety Monitor (LSM) will be assigned to the site, and a Safety Monitoring Committee (SMC) will provide safety oversight.
17. External monitoring will be the performed.
18. Electronic case report forms (eCRFs) will serve as the repository of source documents and other relevant data for each study participant. Only information that cannot be collected initially into the CRF (for instance, EKGs and AE medical records) will first be collected onto separate source documents before transcription into the CRF. Laboratory results will be entered from source documents directly into the database.
19. Detection of parasitemia post CHMI will be based upon qPCR. Participants who have 2 positive qPCR results separated by greater than or equal to 12 hours or a single qPCR result reading > 250 estimated parasites/mL will be treated with anti-malaria therapy.
20. Anti-malarial therapy will consist of Malarone® (first line) or Coartem® (second line).

4.2 EXECUTION PLAN

The study will be performed at the University of Maryland at Baltimore, Center for Vaccine Development and Global Health, with 45 vaccinees and 15 controls in total. Twenty-two or 23 vaccinees and 7 or 8 controls will be randomized to CHMI at 3 weeks, with the remainder of the vaccinees and controls randomized to CHMI at 12 weeks.

4.3 SCIENTIFIC RATIONALE FOR STUDY DESIGN

4.3.1 SCIENTIFIC RATIONALE FOR STUDY GROUPS (DOSE, DOSE NUMBER, DOSE INTERVALS)

Dose: Data from clinical trials of PfSPZ Vaccine indicate that $9.0x10^5$ PfSPZ is the optimal dose for administration to malaria-naïve adults and that higher doses may be less protective. For example, the higher doses tested in the Warfighter 2 Trial ($1.8x10^6$ PfSPZ administered as 3 doses at 8-week intervals and $2.7x10^6$ PfSPZ followed at 8-week intervals by 2 doses of $9.0x10^5$ PfSPZ) were less protective when assessed by heterologous CHMI at 24 weeks (23% VE and 21% VE, respectively) than a lower dose assessed by heterologous CHMI at 12 weeks (40% VE). In the BSPZV2 trial in Tanzania, three doses of $9.0x10^5$ PfSPZ were more protective than three doses of $1.8x10^6$ PfSPZ [39]. In the MAVACHE trial in Germany, $9.0x10^5$ PfSPZ of PfSPZ Vaccine administered on Days 1, 8 and 29 days protected approximately 80% of research participants against both homologous and heterologous strains of malaria at 3 and 10 weeks after V3 (14/18 or 78.8% against homologous CHMI and 10/12 or 83.3% against heterologous CHMI) [32].

Dose number and intervals: The MAVACHE trial regimen of 3 doses on Days 1, 8 and 29 was recently compared against three other regimens in adults in Equatorial Guinea, with homologous CHMI performed approximately 6 weeks after CHMI as the measure of VE. The comparator groups included the best regimen from Warfighter 2 (Days 1, 3, 5, 7 and week 17), the same regimen with the boost at Day 29 (Days 1, 3, 5, 7 and 29), and the same regimen without a boost (Days 1, 3, 5 and 7). Three doses of $9.0x10^5$ PfSPZ on days 1, 8 and 29 provided 51% protection, compared to 40%, 11% and 30% for the other three regimens, respectively [43]. While the trial was not powered to show superiority or inferiority, this regimen appeared at least as good if not better than the other regimens, as it was the only regimen to show statistically significant protection. This regimen also entails fewer doses (3, compared to 4 or 5) and is completed in four weeks, providing logistical and cost of goods advantages.

4.4 SCIENTIFIC RATIONALE FOR CHMI SCHEDULE

This trial is designed to estimate overall VE during a 12 week trip to a malaria endemic area by conducing heterologous CHMI at 3 and 12 weeks after the final immunization. As described in the background section, in two separate comparisons of the same regimen in malaria-naive and malaria-exposed adults, heterologous CHMI provided a reasonably conservative estimate of protection in the field. The Warfighter 3 trial is testing a complementary CHMI schedule: three CHMIs at 2, 6 and 10 weeks.

The primary outcome will be VE calculated by combining data from both CHMI timepoints, in order to provide an average VE over a 12-week period that would cover most journeys to a malaria-endemic area. VE at each individual CHMI timepoint will also be assessed as a powered objective (see Statistics section).

4.5 SCIENTIFIC RATIONALE FOR PLACEBO GROUP

The randomized, double-blind assignment of research participants to treatment with PfSPZ Vaccine or normal saline is pivotal to the study. As described earlier in this document, PfSPZ Vaccine appears to cause very few, if any, adverse events at the doses that will be tested in this trial. For this reason, an inert comparator is required to accurately measure background rates of adverse events in the population, and thus distinguish any side effects induced by PfSPZ Vaccine that stand out against background rates. Because PfSPZ Vaccine is minimally reactogenic, use of another vaccine as a comparator would, through its own side effect profile, make it impossible to measure any adverse events caused PfSPZ Vaccine.

4.6 SCIENTIFIC RATIONALE FOR AGE RANGE

CHMI can cause physiological stress in research participants in the unusual circumstances that individuals develop chills, rigors, sweats and fever. In addition, as described in the Background section, there have been cardiac events associated with CHMI at one study center – Radboud University in the Netherlands, all in association with mosquito-bite inoculation of sporozoites and subsequent administration of antimalarial drugs. Although these events have not occurred in association with CHMI at any other center, until these events are explained, participation in the CHMI is limited to those individuals without evidence or significant risk factors for coronary artery disease and restricted to adults age 50 years or less.

4.7 END OF STUDY DEFINITION

End of Study: A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit and all scheduled procedures as shown in Section 1.3 'Schedule of Activities'.
Early termination: A participant will be considered an early termination if he or she withdraws from the study or is withdrawn from the study by the site PI, or is lost to follow- up, prior to the last visit and a study termination form has been completed.

5. STUDY POPULATION

5.1 INCLUSION CRITERIA

To be eligible to participate in this study, an individual must meet all of the following criteria:

- Healthy adults (male or non-pregnant female) 18 to 50 years of age.
- Able and willing to participate for the duration of the study.
- Able and willing to provide written informed consent and satisfactorily complete a test of understanding with a passing score ≥ 80%.
- Physical examination and laboratory results without clinically significant findings.
- Women of childbearing potential must agree to use effective means of birth control (e.g. oral or implanted contraceptives, IUD, female condom, diaphragm with spermicide, cervical cap, abstinence, use of a condom by the sexual partner or sterile sexual partner) during the entire study. Women with a history of surgical or chemical sterilization (e.g. tubal ligation, hysterectomy, other).
- Willing to refrain from blood donation for 3 years following CHMI.
- Agree not to travel to a malaria endemic region during the trial.

5.2 EXCLUSION CRITERIA

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

- Unable to provide informed consent including inability to pass the test of understanding, which is written in English for the US-based study sites.
- Receipt of a malaria vaccine in a prior clinical trial.
- History of a splenectomy or sickle cell disease.
- History of a neurologic disorder (including non-febrile seizures or complex febrile seizures) or formal history of migraine headache.
- Current use of systemic immunosuppressant pharmacotherapy.
- Receipt of a live vaccine within 4 weeks of first immunization or of 3 or more non-live vaccines within 2 weeks of first immunization.
- Women who are breast-feeding, pregnant or planning to become pregnant during the study period.
- Known allergy to atovaquone-proguanil (Malarone®), artemether-lumefantrine (Coartem®), or any component of the investigational products.
- A history of malaria in the 2 years prior to screening.
- Participation in any study involving investigational vaccine or drug within 4 weeks prior to enrollment that in the estimation of the site PI might adversely affect the individual's safety or

the quality of data to be collected.

- Evidence of increased cardiovascular disease risk; defined as >10% five-year risk by non-laboratory method (Gaziano, 2008) [69].
- Plan to participate in another investigational vaccine/drug research during the study.
- Plan for major surgery between enrollment until 28 days post-CHMI.
- Use or planned use of any drug with anti-malarial activity that would precede or coincide with malaria challenge or vaccination.
- Anticipated use of medications known to cause drug reactions with atovaquone-proguanil or artemether-lumefantrine such as cimetidine, metoclopramide, antacids, and kaolin.
- Positive HBsAg or positive HIV or HCV testing consistent with active infection.
- An abnormal electrocardiogram, defined as one showing pathologic Q waves and significant ST-T wave changes; left ventricular hypertrophy; any non-sinus rhythm including isolated premature ventricular contractions, but excluding isolated premature atrial contractions; right or left bundle branch block; or advanced (secondary or tertiary) A-V heart block; or other clinically significant abnormalities on the electrocardiogram as determined by the consulting cardiologist.
- Any clinically significant deviation from the normal range in biochemistry or hematology tests measured at screening and not resolving.
- Any medical, psychiatric, social, behavioral or occupational condition or situation (including active alcohol or drug abuse) that, in the judgment of the site PI, impairs the volunteer's ability to give informed consent, increases the risk to the participant of participation in the study, affects the ability of the participant to participate fully in the study, or might negatively impact the quality, consistency, integrity or interpretation of data derived from their participation in the study.

5.3 SCREEN FAILURES

Screen failures are defined as potential participants who were screened for study participation but found not to be eligible based on inclusion/exclusion criteria. Individuals who do not meet the criteria for participation in this trial because of an acute illness may be rescreened once this has resolved. Rescreened participants will be assigned the same participant number as for the initial screening. Those dropping out prior to enrollment (marked by first receipt of investigational product) will be considered screening failures. Screening labs need to be repeated if more than 90 days elapse before first DVI of investigational product.

5.4 STRATEGIES FOR RECRUITMENT AND RETENTION

Longitudinal compliance with study visits and procedures will be encouraged through the provision of a study calendar to participants and reminders about study visits. It is critical that study staff be able to reach participants at short notice post-CHMI. Besides provision of participant contact information, participants will provide phone numbers of friends and relatives to serve as emergency contacts.

6. STUDY INTERVENTION

6.1 STUDY INTERVENTION DESCRIPTION

After signing an informed consent, participants will undergo screening, and if determined to be eligible, will be notified that they may participate. Participants who agree to participate will be assigned to a group (CHMI at 3 weeks or CHMI at 12 weeks) and then randomized to receive the vaccine or placebo in a 3:1 ratio. Receipt of PfSPZ Vaccine or placebo will constitute enrollment. Participants will receive DVI injections on Days 1, 8 and 29 with follow-up visits as indicated for each group in Section 1.3 'Schedule of Activities'. CHMI will be done by the injection of PfSPZ Challenge (7G8), followed by monitoring for parasitemia from day 7 to 28 post CHMI. If a participant becomes positive as detected by PCR, he/she will receive a full treatment course of Malarone (atovaquone/proguanil) or another suitable antimalarial, using directly observed therapy (DOT). The final study visit will be +56 days after CHMI; however, unscheduled study visits may continue for participants with adverse events which have not resolved and require continued follow-up.

6.2 DOSING AND ADMINISTRATION

The dose of PfSPZ Vaccine will be $9x10^5$ PfSPZ. PfSPZ Vaccine and normal saline are dispensed in identical 1 mL syringes in a 0.5 mL volume. Each syringe has a fixed 25-gauge needle. Each product is a clear, non-viscous solution that is visually indistinguishable and has no perceptible color or odor. While PfSPZ Vaccine and normal saline will be shipped by Sanaria to the study site in distinguishable packaging and labeling, the final constituted test article and the normal saline placebo are identical in appearance and once the syringe is prepared by the Pharmaceutical Operations team, neither the clinical team nor the study participants will be able to ascertain the treatment assignment.

The syringes are prepared by an unblinded Pharmaceutical Operations team following SOPs. The preparation room will not be accessible to 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 team preparing the injections does not interact with study participants. The maximum allowable time between thawing of a vaccine vial and injection is 30 minutes. The time of thaw and the time of injection are recorded.

DVI is performed according to SOP. A superficial vein in the arm or hand is located and 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 rapidly 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 participant will then proceed to the observation area.

PfSPZ Challenge is prepared and administered in the same way as PfSPZ Vaccine, at a dose of $3.2 x10^3$ PfSPZ, also in 0.5 mL of diluent (PBS with HSA). The timepoint for administering PfSPZ Challenge after the final PfSPZ Vaccine injection is according to group assignment as per Section 1.3 'Schedule of Activities'. Samples will be collected daily for PCR (2 mL) starting 7 days post-CHMI and continuing to 18 days post-CHMI. Samples will then be collected 20, 22, 25 and 28 days post-CHMI.

There is a permissible time window around each immunization and the day of CHMI as specified in Section 1.3 'Schedule of Activities'. There are no restrictions on administration of PfSPZ Vaccine, PfSPZ Challenge or normal saline with respect to time of day or meal consumption.

6.3 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.3.1 ACQUISITION AND ACCOUNTABILITY

PfSPZ Vaccine and Diluent (containing PBS and HSA) will be provided by Sanaria. Sanaria will transport PfSPZ Vaccine and PfSPZ Challenge to the study sites in liquid nitrogen vapor phase (LNVP) in dry shippers and Diluent in temperature-controlled containers (Crēdo Cube Pelican BioThermal) at ambient temperature. The site PI and/or her/his designee will ensure that all product is inventoried and stored as indicated in the relevant SOP. Accurate records will be maintained regarding receipt of PfSPZ Vaccine, PfSPZ Challenge and normal saline, which include: date received, lot number, amount received and disposition. All products associated with PfSPZ Vaccine, PfSPZ Challenge, PBS, HSA and syringes, are also inventoried.

Sanaria will maintain records in parallel regarding disposition of product, which include:

- Participant identification number
- Date and time study drug dispensed
- Amount injected
- PfSPZ Vaccine or PfSPZ Challenge lot number
- Signature of the person preparing the syringe
- Signature of the person administering the injection

Any partly used PfSPZ Vaccine, PfSPZ Challenge, Diluent vials, or normal saline as well as the empty cryovials/vials will be returned or destroyed according to the applicable SOP and as determined by Sanaria. An inventory of the number of vials shipped versus used will be recorded. Unopened vials will be returned to Sanaria.

PfSPZ Vaccine and PfSPZ Challenge are not licensed products and must be distributed under an Investigational New Drug (IND) application in accordance with FDA regulations or under the appropriate regulatory approval in the EU. These products must be administered by or under the supervision of the site PI or his/her designee.

6.4 FORMULATION, PACKAGAING AND LABELING

6.4.1 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^{o}C to -196^{o}C, thawed, diluted in phosphate buffered saline (PBS) with human serum albumin (HSA) to achieve the correct dosage and administered by DVI.

Example of a label for PfSPZ Vaccine (not the lot to be used in the trial):

PfSPZ (NF54) VACCINE SANARIA®

Lot#092819 Manufactured: SEP 2019
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

6.4.2 PFSPZ CHALLENGE (7G8)

PfSPZ Challenge (7G8) for CHMI is identical to PfSPZ Vaccine except it is not radiation-attenuated (and therefore the PfSPZ are infectious and can cause malaria if injected into humans) and that it is a Brazilian strain (Pf7G8). Information on storage, stability, preparation and accountability is the same as for PfSPZ Vaccine. The dose used is 3.2 x10^3 PfSPZ for CHMI.

Example of a label for PfSPZ Challenge (not the lot to be used in the trial):

PfSPZ (7G8) CHALLENGE
SANARIA™ 20 µL/vial 15,000 SPZ
Manufactured: 10/14 Lot#100114-02
Store in liquid nitrogen vapor phase
Caution: Infectious Agent
Ltd by Federal Law to Investigational Use Only

6.4.3 NORMAL SALINE

The normal saline to be used in this trial will be an FDA licensed product and will be supplied to the clinical sites by Sanaria.

6.5 PRODUCT STORAGE AND STABILITY

PfSPZ Vaccine and PfSPZ Challenge are stored at Sanaria or Sanaria's designated storage site in LNVP at -150°C to -196°C until shipped to the study sites. Transfer from storage will follow the relevant Sanaria's SOPs. Throughout transportation and during temporary storage at the study site, the interior chamber temperature of the LNVP dry shipper will be continuously monitored and recorded. If the temperature rises above -150°C, the study staff will be alerted. Receipt and use of the vaccine material at the trial study site will be documented, respectively, in the "Shipping Log" and "Inventory Log" by study staff.

A chain of custody of all of the products to be administered to study participants will be maintained and documented. Unused PfSPZ Vaccine and PfSPZ Challenge cryovials retained in the dry shipper will be returned to Sanaria. Diluent for PfSPZ Vaccine and PfSPZ Challenge and normal saline used for placebo will be stored according to SOP. Details of storage and transfer of these products will be outlined in the protocol-specific SOP.

6.5.1 DOSE PREPARATION

Qualified and trained vaccine dilution and syringe preparation staff will prepare and fill syringes for each dose (vaccine or placebo) just prior to administration as described in the relevant SOPs according to randomized study number assignment. Cryopreserved vials will be thawed immediately before use per SOP. The preparation of all doses of vaccine material, from start to finish, will be monitored and inspected by Sanaria personnel or a designee, where necessary. Each form will have a requirement for sign-off by the study team preparing the injections. The pharmaceutical operations team will quarantine any vials that fail visual inspection.

The dose will be prepared using a 1 mL syringe with a 25-gauge needle and must be administered within 30 minutes of thawing. Further details regarding dilution and syringe preparation are included in Sanaria SOPs.

6.5.2 ADMINISTRATION OF VACCINE OR PLACEBO

Vaccine or placebo will be administered by blinded study staff given syringes labeled with the participant's study number, by the unblinded Pharmaceutical Operations team preparing injections. Each dose of PfSPZ Vaccine, PfSPZ Challenge and placebo will be administered in 0.5 mL by DVI into a vein in the participant's arm, wrist or hand using a needle and syringe according to SOP and the study schedule.

6.5.3 MODIFICATION OF STUDY INVESTIGATIONAL PRODUCT (IP) FOR A PARTICIPANT

Dosing of PfSPZ Vaccine or PfSPZ Challenge will be discontinued if found to be unacceptably reactogenic or unsafe for individual participants. If a participant experiences notable toxicity related to PfSPZ Vaccine, the investigators and the Sponsor, in consultation with the Local Safety Monitor and the SMC will determine if subsequent immunizations in that participant is acceptable or halted. If a participant experiences an SAE deemed to be related to PfSPZ Vaccine, the participant will not receive the relevant subsequent doses unless so recommended by the SMC, Sponsor, and PI.

6.5.4 ACCOUNTABILITY PROCEDURES FOR THE STUDY INVESTIGATIONAL PRODUCT

The site PI alone has the authority to distribute or dispose of the IP as instructed by Sanaria and is responsible for IP accountability. The site PI will normally delegate responsibility for IP accountability to the head of Pharmaceutical Operations so that he or she may remain blinded to treatment assignment. The site PI and designee will be responsible for maintaining complete records and documentation of IP, accountability, dispensation, temperature monitoring, storage conditions, and final disposition of the study product. All study IPs, whether administered or not, must be documented on the appropriate study IP accountability record or dispensing log.

Upon completion of each day of immunizations with PfSPZ Vaccine or injection with PfSPZ Challenge, any remaining unused study IP will be returned to the Sponsor according to the appropriate SOP(s).

6.6 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

6.6.1 RANDOMIZATION

On the day of first immunization (V1 = study day 1), each participant invited to enroll will be randomized to receive PfSPZ Vaccine or NS according to a 3:1 ratio with randomization blocked to assure an approximation of this ratio in each CHMI cohort.

In the situation where an eligible participant withdraws consent or is deemed to be ineligible after treatment assignment but prior to administration of the first dose (e.g. due to acute illness or a request to withdraw from participation), a back-up participant will be assigned the same randomization number in the place of the participant who has become ineligible or has withdrawn consent. The withdrawn participant will be considered a screening failure, and the replacement participant will assume the randomization number of the withdrawn participant in order to assure that the study group receives the correct number of vaccine and placebo assignments. Once an injection has been given, however, even if the study participant withdraws immediately thereafter, the randomization number will not be reused, and the individual will be considered a drop-out.

The randomization procedure will be conducted by the data management and statistics vendor for this trial, as described in the applicable SOPs. Access to the unblinded randomization list will be limited exclusively to the syringe preparation team and the independent statistician(s) at the data management vendor. These individuals will be unblinded and will not be involved in evaluation or care of the study participants. A copy of participant treatment assignments will be retained at the site in a secure file by the unblinded Pharmaceutical Operations staff.

6.6.2 UNBLINDING

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

Study participants, clinical investigators (including the PI) and all other staff involved in measuring study outcomes will remain blinded to treatment allocation until the data from (at least) 28 days after CHMI are cleaned and locked. The site PI will be responsible for strict maintenance of the blind. Only the Pharmaceutical Operations team responsible for syringe preparation and the responsible statistician from the data management vendor will be unblinded from the onset of the trial.

The regulatory team and select members of the clinical team of the Sponsor, Sanaria, may be unblinded at any time if there is an urgent regulatory or research need for unblinded safety, tolerability or efficacy data. This will be discussed with the site PI and the plan approved before putting it into effect.

Unblinding of individual participants: If knowledge of the treatment assignment is needed to provide appropriate medical care, and unblinding is recommended by the PI, the Local Safety Monitor, or the SMC, the treatment assignment of that participant will be unblinded and provided to the PI on a need-to-know basis. The PI must contact the Sponsor and provide documentation of the event and the reasons for unblinding. If unblinding is required, the treatment assignment from the on-site secure file will be provided by the unblinded staff.

If pausing criteria are met based on blinded data, the PI will provide the list of participants contributing to pausing criteria and inform the head of the Pharmaceutical Operations team how many of these participants have to be vaccine recipients (as opposed to normal saline recipients) in order to truly meet pausing criteria. The head of the Pharmaceutical Operations team or designee will privately identify the

treatment assignment of each participant on the list, and then inform the PI if the pausing criteria have been met but will not unblind the PI with respect to individual volunteers unless necessary.

Information or correspondence that could unblind individuals who interact with volunteers, including the Sanaria clinical team and any others involved with syringe preparation, will be held confidential until the official unblinding. When syringes are being prepared or treatment allocation lists are exposed, access to the syringe preparation room will be restricted and staff who interact with the volunteers will not be permitted to enter.

Similarly, only the clinical team will interact with the research participants. The team preparing injections will not interact with participants.

However unlikely, any case of inadvertent or unauthorized unblinding will be reported to the Sponsor within 7 days and prior to performing any scheduled study procedures with the affected study volunteer(s). Evaluation and treatment of acute medical problems and any procedures related to safety may proceed, even if the inadvertent blinding has not yet been reported to the Sponsor.

6.7 STUDY INTERVENTION COMPLIANCE

The investigational product will be administered via DVI by study staff. For participants who become parasitemic, anti-malarials will be administered by DOT.

6.8 CONCOMITANT MEDICATIONS/TREATMENTS

This protocol places no restrictions on the use of concomitant medications with the exception of the immunomodulators as detailed in the exclusion criteria, drugs with anti-malarial properties (e.g., doxycycline, clindamycin, azithromycin, trimethoprim/sulfamethoxazole) and drugs that might interfere with anti-malarials during the treatment phase post-CHMI in those participants who become parasitemic. Any pre-existing conditions that require routine or intermittent medications should be discussed at the time of screening and a study physician will determine if participation is safe and will not interfere with any data being collected. All concomitant medications received within 28 days before signing the consent should be recorded. Concomitant medications include prescription, over the counter and herbal medications and supplements.

Any new medications required during the course of participation in the study must be discussed with the study team to ensure both safety of the participant and integrity of the data being collected. Information regarding all over-the-counter and/or prescription medications taken will be collected and recorded at each scheduled study visit.

The investigator will recommend medication for symptomatic relief, if necessary. This will not affect the data collected from participants who take medication during the trial.

7. STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION / WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

The study intervention will be paused or discontinued if specified criteria in Sections 7.2 'Pausing Criteria, Unblinding and Safety Hold' or 7.3 'Temporary Hold of Immunization or CHMI of Individual Participants' are met (see below). Discontinuation from immunization does not mean discontinuation from the study, and remaining study procedures related to safety should be completed. Remaining study procedures related to research assays, such as collecting samples for immunology tests, may be continued if the participant agrees and the tests will provide meaningful data.

If a clinically significant finding is identified in a study participant after enrollment, the investigator will determine if any change in participant management is needed. This may involve a temporary suspension of the study intervention for an individual participant (e.g. delaying immunization until an acute illness has resolved) or for all participants (the latter after consultation with the Sponsor). The reason for pausing or discontinuation will be recorded on the Case Report Form (CRF) and any additional supplemental clinical notes that are required for complete documentation.

7.2 PAUSING CRITERIA, UNBLINDING AND SAFETY HOLD

As described in the Background and Risk sections above, PfSPZ Vaccine has been safe and well tolerated in 11 randomized, double-blind, normal saline placebo-controlled trials including three trials in African children: BSPZV2 in Tanzania [40], KSPZV1 in Kenya [41, 42], 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. In Part 2 of the Kenya trial, approximately 80 infants received three doses of $1.8x10^6$ PfSPZ at 8-week intervals [42] – twice the dose planned in the USSPZV7 trial. Thus, it appears unlikely that safety issues will arise. Nevertheless, it will be important to continue careful recording of adverse events and to check safety labs.

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, the following two criteria have been established. If either criterion is met, the clinical team will contact the Sponsor, which will contact the SMC to review the events in question:

- Three or more participants experience the same severe (grade 3) AE during the periods of AE collection and the AEs are deemed possibly, probably or definitely related to study product administration (PfSPZ Vaccine or NS), or three or more participants experience the same grade 3 laboratory abnormality post vaccination. 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 these criteria are met, unblinded study staff (e.g., the head of Pharmaceutical Operations) will need to confirm that the total indeed constitutes 3 or more vaccinees.

- 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 6.6.2 'Unblinding'). To meet the criteria, the participants must actually have received PfSPZ Vaccine and not NS placebo.

After confirmation that a pausing criterion has been met, the Sponsor will contact the SMC to describe the circumstances of the event(s). After an email discussion or, if recommended by the SMC, an ad hoc teleconference, the SMC, which will include the Local Safety Monitor as a voting member, will make a written recommendation about whether or not the study should resume, 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 SMC recommendation, a decision will be made by the Sponsor and principal investigator to either resume 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 resume the study but will be required to reverse a safety hold.

Irrespective of the pausing criteria, the Sponsor, LSM, 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 3 vaccinee 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 at another study site or 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 IRBs of record.

If a safety hold or study termination is deemed necessary, the applicable ethical committees and regulatory agencies will be notified. For a safety hold to be lifted, there must be written concurrence by the entity or entities that initiated the hold that the study can resume.

7.3 TEMPORARY HOLD OF IMMUNIZATION OR CHMI OF INDIVIDUAL PARTICIPANTS

The administration of PfSPZ Vaccine or PfSPZ Challenge for CHMI to a study participant may be temporarily put on hold for the following reasons:

1. The participant is ill on the day of immunization or CHMI (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 that has been determined to be clinically significant.
3. The participant has taken medication for another acute illness that may affect response to PfSPZ Vaccine or CHMI.
4. The participant has received 3 or more non-live vaccines 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 vaccine.
5. At the request of the participant.
6. On the advice of the LSM, IRB, SMC, Sponsor, or FDA.
7. On the advice of the investigators.

Immunization or CHMI will be rescheduled at the earliest possible time within the acceptable window of the originally scheduled immunization or CHMI. Indicated intervals can be shortened or lengthened if circumstances dictate, there is no apparent risk to the participant, and the Sponsor agrees.

7.4 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request. If voluntary withdrawal occurs, the participant will be asked to return for safety follow-up as per determination of the PI. If the participant declines, the end of study form will be completed, and the participant excluded from further study procedures. If a participant withdraws from the study, blood samples previously collected for protocol-specified analyses will be retained. If at the time of consent, a participant gave permission for samples to be collected and retained for non-specified future research they can withdraw this permission at the time of withdrawal from the study and these samples will be destroyed. This withdrawal of consent for future research will be documented. Participants who have received PfSPZ Challenge who withdraw or are withdrawn will receive a complete, appropriate, curative course of antimalarial therapy starting no sooner than the seventh day after PfSPZ Challenge administration. The participant will also be asked to return to the research center for a final safety follow-up on CHMI+28.

The investigator may also permanently discontinue or withdraw a participant from the study procedures for the following reasons:

- The participant starts taking a concomitant medication for a chronic illness that could affect the study outcome variables.
- Pregnancy. The participant will be asked for permission to continue scheduled safety follow-up visits and to complete an end-of-study evaluation as scheduled. The participant will also be asked to allow access to all subsequent health and medical records that pertain to the pregnancy for the duration of the pregnancy and for access to the health and medical records of her infant through one year of age.
- Significant study intervention non-compliance.
- Any clinical adverse event, laboratory abnormality, or other medical condition or situation that occurs such that the site PI considers that continued participation in the study would not be in the best interest of the participant.

- The participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.
- The participant is unable to be immunized or undergo CHMI for a period of 30 days or more beyond the date scheduled (plus applicable window) for these procedures.
- Participant is lost to follow-up (see Section 7.5 'Lost to Follow-Up' below).
- On the advice of the LSM, site PI, Sponsor, SMC, IRB, or FDA.

The investigator must promptly notify the Sponsor of this decision and record the reason for participant discontinuation or withdrawal from the study on the CRF and any additional supplemental clinical notes that are required for complete documentation.

7.5 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for three or more scheduled visits and/or is unable to be contacted by the study site staff, as noted below. 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 participant and reschedule the missed visit within one week, counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant 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 participant (where possible, via at least 3 emails, texts, telephone calls and/or letters). These contact attempts should be documented in the participant's study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of "lost to follow-up".
- If loss to follow-up takes place after injection with PfSPZ Challenge, the participant has not been treated for malaria, and attempts to locate the participant have been unsuccessful, the police or other authorities may be requested to aid in the search, due to the potential grave danger posed by untreated malaria.

8. STUDY ASSESSMENTS AND PROCEDURES

8.1 STUDY VISIT SCHEDULE AND FOLLOW-UP PERIODS

The study recruitment, screening, immunizations, administration of PfSPZ Challenge, and follow-up visits will take place at the site clinical center. All participants will actively participate for 15 to 24 weeks (starting from immunization and including CHMI, follow-up visits and final visit but excluding the screening period) depending upon their CHMI cohort. Evaluation of the study vaccine will include collection of solicited and unsolicited AEs, laboratory studies, and physical assessment by clinicians following immunization. The complete schedule of study visits, permitted windows for completing the visits, and evaluations performed at each visit is provided in Section 1.3 'Schedule of Activities'. There will be a specifically defined window for each study visit (allowable days before or after), although participants will be encouraged to be present on the targeted study visit day. The blood volume drawn from each participant will not exceed 5 mL/kg (250 mL for a 50 kg participant) during a 24-hour period and 525 mL per 8-week period (per guidelines of the American Association of Blood Banks). The clinical center may

provide light snacks and refreshments to participants for nutritional support and safety during scheduled and unscheduled study visits, although this is not required if food and drink are readily available from nearby sources.

8.1.1 SCREENING

Screening procedures may occur over multiple visits. The screening visit(s) for participants (immunized and placebo) will be scheduled within 90 days of first immunization. Informed written consent must be obtained from each participant prior to conducting any study procedure. Once consent forms are signed, a unique identification number will be assigned to each study participant. Procedures will be in place to ensure anonymity and de-identification of data. The following activities and procedures will be carried out during the screening visit(s):

- Information about the study will be provided to include a description of clinical trial design, risks, and study schedule. This may be accomplished in person with a member of the study team, or by using IRB approved briefing slides in person or by means of a pre-recorded audio recording.
- Participants will have the opportunity to ask research staff question(s) to assist them in making informed decision about participating in the study.
- Informed consent process will be performed, and the informed consent document (ICD) and other research-related documents will be signed.
- Participants will take a quiz to assess understanding of the study (minimum passing score of 80% required for participation and one repeat testing is allowed if necessary).
- Temperature, pulse rate, blood pressure, height, weight, and BMI will be measured and recorded.
- Inclusion and exclusion criteria will be reviewed.
- Participants will be interviewed to obtain a medical history to include concomitant medications and known allergies.
- Analysis of 5-year cardiovascular risk: This will be performed based upon the method of Gaziano et al (2008) [69]. The risk factors will include sex, age, body mass index (BMI), blood pressure, history of diabetes mellitus, and history of smoking.
- Physical examination will be performed.
- A baseline 12 lead EKG will be performed and results will be reviewed by a cardiologist if deemed necessary.
- Blood samples, as indicated in Section 1.3 ‘Schedule of Activities’, will be collected for:
  - Hematology: (CBC with differential: white blood count, hemoglobin, platelet count, absolute neutrophil count and absolute lymphocyte count);
  - Serum chemistry: glucose, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST);
  - Serology: HBsAg, HCV antibody, HIV-1/2 antigen-antibody 4th generation screen and confirmation (consent will be obtained prior to evaluation of HIV serology);
  - Pregnancy test (serum or urine ß-HCG): pregnancy tests will be required of all female participants regardless of age unless reported sterilization, or the participant is menopausal defined as appropriate age with 1 year of amenorrhea. Urine pregnancy tests will be performed on site whereas sera will be sent out for analysis.

All screening laboratories, with the exception of hepatitis and HIV serologies, will be considered expired if performed more than 90 days prior to first immunization. In the event that the screening laboratories

are expired, CBC with differential, glucose, creatinine, ALT, AST and urine/serum pregnancy test will be repeated during the screening follow-up visit and results will be reviewed prior to immunization.

During screening, participants will have blood drawn to determine if any clinical laboratory abnormalities exist that would preclude study participation. In participants who meet all of the inclusion criteria but have an abnormality in one or more screening safety laboratory tests, the site PI may elect to repeat such screening laboratory test(s) to exclude spurious results due to laboratory processing error, etc. The site PI or designee will identify values as abnormal and clinically significant or not clinically significant. Participants that have only mild (Grade 1) abnormalities that are not clinically significant may be included if the study investigator determines that their participation will not present undue risk to the participant. Participants with abnormal labs that are clinically significant will only be enrolled after consultation with the LSM and/or appropriate expert. Participants with one or more clinical laboratory abnormalities greater than mild severity (Grade ≥ 2) will not be enrolled in this clinical trial. If the laboratory abnormality appears related to an intercurrent illness, the site PI can wait until the participant recovers and re-test.

The clinical and laboratory toxicity grading scales that will be used in this trial are based on the Guidance from the Center for Biologics Evaluation and Research, FDA [70] , as well as the site specific laboratory normal reference ranges. Laboratory testing may be repeated if the investigator wishes to confirm specific result(s) that may affect the participant's ability to participate.

Participants who meet all inclusion criteria and none of the exclusion criteria, who have signed the ICD, and have passed the Assessment of Understanding, may be scheduled for immunization and enrollment into the study. Participants excluded from this study because of significant abnormalities will be referred to outside care for evaluation as necessary. In the event of a positive HIV test, the participant will be counseled by a physician investigator and referred for appropriate follow-up care. Notification of state and federal authorities, as required by law, will be the responsibility of the site PI or their designee. For members of the military, notification of command will also be the responsibility of the site PI as required by military regulations.

8.1.2 PRE-IMMUNIZATION VISIT

Participants meeting the entry criteria will return within 7 (±7)) days prior to immunization. The following will occur:

- Study education provided: review of study schedule, contact card (who to contact and how to contact the research site)
- Eligibility criteria will be reviewed
- Vital signs (temperature, blood pressure and pulse)
- Medical history update, physical exam (if indicated), and review of concomitant medications
- The following laboratory tests will be collected:
  - qRT-PCR for malaria (this can be run retrospectively)
  - CBC
  - Immunological Assays: Refer to Section 8.2.3 'Immunology Laboratory Evaluations'.
  - COVID-19 PCR – optional, if clinically indicated

If a participant decides not to continue after completing the Day -7 pre-immunization visit he/she will be a screening failure and will be replaced by an alternate participant on the day of first immunization. The

replacement participant (alternate) will have PCR and immunoassay samples drawn on the day of first immunization (if not completed on Day -7).

The participant may also be withdrawn or decide to withdraw after randomization and prior to first DVI. Up until the time of DVI, this will be considered a screening failure and the individual may be replaced using the same randomization number.

8.1.3 IMMUNIZATION VISITS

The following will occur prior to each immunization:

- Vital signs will be taken (temperature, blood pressure, pulse)
- Medical history, AEs (from V2), SAEs, Concomitant Medication update and targeted physical examination (if indicated) will be performed.
- For women of childbearing potential: results of pregnancy test (urine or blood ß-HCG) will be confirmed. Immunization will not proceed unless a negative pregnancy test has been obtained on each day of immunization.
- Eligibility criteria will be reviewed
- Randomization to vaccine and placebo group will occur prior to first immunization only
- The following laboratory tests will be collected (prior to V1 only):
  - Safety: CBC with differential, creatinine and ALT

Participants will be observed for at least 30 minutes following each immunization, have vital signs collected and given memory aids to record local and systemic reactions. After the 30-minute observation period, a post-immunization assessment will be completed before the participants leave including evaluation of vital signs, examination of the injections site, and the recording of solicited and unsolicited AEs. Although breakthrough infections are not expected, participants will be counseled about symptoms and signs of malaria and reminded how to contact study staff if needed at any time.

Alternates

Up to 5 Alternates may be recruited per cohort (not required). Alternates will undergo the pre-immunization visit (see Section 8.1.2 'Pre-Immunization Visit'). These alternates will be asked to be present on the day of first immunization. If an assigned participant does not present on the first day of immunization, elects to withdraw, or is found to have met an exclusion criterion, an alternate will be enrolled into the study, using the same randomization number as the participant who is replaced. An alternate may also replace a participant who withdraws or is withdrawn from the study by the site PI within a 7-day window post- first immunization only. This person will be issued a new randomization number.

Memory Aid

Participants will be provided with a daily memory aid on the day of each immunization. They will be asked to fill out the memory aid once a day for 7 days following each immunization. Participants will be given a thermometer and asked to record temperature, local (x 3d) and systemic symptoms daily. The study staff will review the memory aid with the participant prior to second immunization and 7 days after second and third immunizations. The memory aids will be collected by the study staff but not considered source documents. Failure by the participant to return a memory aid will not constitute a deviation. The memory

aid will be used to help identify solicited AEs during the post-immunization period. These results will be annotated in the participant's study file and/or CRFs.

8.1.4 POST-IMMUNIZATION FOLLOW-UP VISITS

Post-immunization follow-up visits will be scheduled for +2 and +7 days after immunization #2 and , +2, and +14 days after immunization #3. Additionally, other clinical concerns may prompt a study visit based on the judgment of a study clinician.

The following will occur at these visits:

- Vital signs collection (pulse, blood pressure, and temperature)
- Medical history, AEs, SAEs and concomitant medications update
- Targeted physical examination (if indicated)
- Solicited adverse events will be recorded by the study team through +7 days after each immunization
- Unsolicited adverse events will be collected through 21 days post 3rd vaccination for 3 week CHMI participants and through 28 days post 3rd immunization for 12 week CHMI participants.
- Blood collection for safety laboratories and research laboratories as indicated: (see Section 1.3 'Schedule of Activities', Section 8.2.2 'Clinical Laboratory Evaluations' and Section 8.2.3 'Immunology Laboratory Evaluations')
- On each of the days when samples are drawn for "Immune Regulation" studies (see Section 8.2.3.3 'Immune Regulation'), a CBC with differential will be obtained.

There will be a phone call follow-up for all participants on +7 days post immunization 2. The local AEs that will be solicited (Days 0, 1 and 2) are pain, tenderness, erythema, swelling, bruising, pruritus and induration. The systemic AEs that will be solicited are fever (oral temperature $\geq$ 100.4 F), subjective fever, chills, headache, fatigue/malaise, myalgia, arthralgia, rigors, rash, urticaria, pruritus, edema.

8.1.5 CONTROLLED HUMAN MALARIA INFECTION

CHMI with cryopreserved, aseptically prepared vialed infectious Pf sporozoites from the 7G8 clone (PfSPZ Challenge (7G8)) will occur by direct venous inoculation.

Prior to CHMI, the following activities and procedures will be performed:

- Inclusion and exclusion criteria will be reviewed
- Medical history, AEs and SAEs will be updated to include concomitant medications, a directed physical examination will be performed (if indicated), and vital signs will be recorded
- Results of pregnancy test (urine or blood ß-HCG) will be confirmed. CHMI will not proceed unless a negative pregnancy test has been obtained within 24 hours of CHMI.
- Blood samples will be collected for:
  - Safety: CBC with differential, creatinine and ALT
  - Immunological assays: refer to Section 8.2.3 'Immunology Laboratory Evaluations'

- Participants will be asked to verify and confirm their telephone contact information at this time, and the importance of maintaining contact and following study procedures will be re-emphasized.

Following CHMI, participants will remain at the clinical center for an observation period of at least 30 minutes. This will allow the study team to confirm that there are no immediate reactions in response to injection of PfSPZ Challenge (7G8) and to provide immediate care if any reactions do occur. After the 30-minute observation period, an assessment will be performed including the measurement of temperature, pulse rate, and blood pressure, local and systemic reactions from PfSPZ Challenge. Participants will be instructed to contact the clinical investigator if they experience any medical problems.

Participants will be instructed on the symptoms of malaria and complications of malaria to include, but not limited to, fever (oral temperature of $\geq$ 38°C [$\geq$100.4°F]), subjective fever, chills, rigors (shaking chills), sweats, cough, headache, dizziness, malaise/fatigue, arthralgia, myalgia, nausea, vomiting, abdominal pain, diarrhea, chest pain, shortness of breath, palpitations. Participants will be assessed for signs and symptoms of malaria from +7 days post CHMI through +28-day post CHMI or until the participant becomes parasitemic, is treated and is asymptomatic.

Memory Aid

Participants will be provided with a daily memory aid on the day of PfSPZ Challenge. They will be asked to fill out the memory aid once a day starting on the day of CHMI and continuing for 6 days following PfSPZ Challenge. Participants will be asked to record temperature, local and systemic symptoms daily. The study staff will review the memory aid on the visit at 7 days post PfSPZ Challenge. The memory aids will be collected by the study staff but not considered as source documents. They will be used to help identify solicited AEs during the post-PfSPZ Challenge period. Failure by the participant to return a memory aid will not constitute a deviation. These results will be annotated in the participant's study file and/or CRFs.

8.1.6 POST-CHMI FOLLOW-UP VISITS

8.1.6.1 +7 THROUGH +18 DAYS POST-CHMI

To facilitate daily close monitoring of clinical status and malaria diagnostics, all participants who underwent CHMI will be seen daily at the CVD starting + 7 days after CHMI until + 18 days after CHMI or until the participant finishes his/her anti-malarial treatment. Participants will be required to remain vigilant about maintaining lines open for rapid communication with the study team. A study physician will be available for consultation 24 hours per day.

The following will occur at each visit:

- Vital signs will be recorded.
- Medical history, AEs, SAEs and concomitant medication update.
- Targeted physical exam (if indicated).
- Participants will be assessed for signs and symptoms of malaria as mentioned in Section 8.3.1.2 "Malaria Events". Signs and symptoms of malaria infection will be collected on the visit CRF specific to CHMI follow-up and will not be considered as adverse events.
- Unsolicited AEs will be recorded through 28 days post-CHMI.
- Collection of blood samples for qRT-PCR.

- Symptomatic study participants may have blood samples for qRT-PCR collected as frequently as every 6-8 hours or at any time post-CHMI based upon the clinical judgment of the investigator
- Blood collection for qRT-PCR will be discontinued upon malaria diagnosis and treatment.
- The clinical center will have the capability to prepare and read TBS. (See Section 8.2.3.5 'Thick Blood Smear (TBS)').

8.1.6.2 MANAGEMENT AND TREATMENT OF MALARIA PARASITEMIC PARTICIPANTS

Malaria infection will be treated when the criterion for a case is met. The case definition in this protocol identifies cases of malaria before gametocytes can develop. Detection of 2 positive qRT-PCR results at any level of parasitemia from samples collected $\geq$ 12 hours apart will constitute a diagnosis of malaria. In the case of an acutely ill participant for whom the site PI (or designee) determines rapid diagnosis should be implemented, TBS may be utilized for diagnosis. If diagnosed, the study participant will be called to return to the clinical center to receive treatment. After completion of treatment participants will be asked to return to the clinical center +28 days post-CHMI.

- Safety laboratories will be drawn at time of diagnosis and at any other time point post-CHMI when deemed clinically indicated by the investigator.
- Just prior to the first dose of antimalarial medication, samples for preparation of TBS (to be read retrospectively) and confirmatory PCR will be collected.

All participants who develop blood-stage malaria infection will be treated with a standard dose of Malarone® (250 mg atovaquone/100 mg proguanil tablets) 4 tablets once per day for 3 days, as first line therapy. This will be done by directly observed treatment (DOT); that is, a study team physician or nurse will witness the swallowing of the Malarone® dose by the participant. In the event that a participant is allergic to, or unable to tolerate Malarone®, he or she will be treated with Coartem® (artemether/lumefantrine 20/120 tablets), an alternative, but equally effective, antimalarial agent; the adult dose for Coartem is 4 tablets orally twice daily for 3 days, with the first two doses on the day of parasitemia to be administered approximately 8 hours apart. The first dose each day will be administered by DOT whereas the second dose each day can be self-administered by the participant. Participants will be reminded of the potential side effects of the antimalarial treatment. All participants exposed to malaria will be unable to donate blood for 3 years.

If symptoms develop, provided there are no contraindications, participants may be given acetaminophen or ibuprofen. In case of vomiting, particularly if it interferes with the administration of antimalarial medication, participants can be treated with prochlorperazine or ondansetron. Antimalarial medication administration will be repeated once if vomiting occurs within 30 minutes of initial administration. If vomiting occurs twice, an alternative antimalarial treatment will be selected (see above).

8.1.6.3 +20, +22 AND +25 DAYS POST-CHMI

Participants who remain negative for patency +18 days post-CHMI will be followed and evaluated during visits that occur +20, +22, and +25 days after CHMI. The evaluation of participants will include:

- Vital signs, medical history, concomitant medication, AEs, SAEs update, collection of unsolicited AEs and directed physical exam (if indicated)
- qRT-PCR

- Solicited signs and symptoms of malaria
- Safety labs may be drawn if clinically indicated.

Any participants who develop blood-stage malaria infection as measured by qRT-PCR will be treated as outlined above.

8.1.6.4 +28 DAYS POST-CHMI

This follow-up visit shall include:

- Vital signs, medical history, concomitant medication, AEs, SAEs update, collection of unsolicited AEs and directed physical exam (if indicated)
- qRT-PCR
- Solicited signs and symptoms of malaria
- CBC with differential, ALT and creatinine
- Immunology Assays: Refer to Section 8.2.3 'Immunology Laboratory Evaluations'

Any participants who develop blood-stage malaria infection as measured by qRT-PCR at this visit will be treated as outlined above.

8.1.6.5 FINAL VISIT: +56 DAYS POST-CHMI

Participants will have medical history and concomitant medication update, vital signs taken and directed physical exam performed (if indicated). Any AEs or SAEs that are unresolved at that time will be continued to be followed until resolution or stabilization, or, if a serious or chronic condition has developed, until it has stabilized.

8.1.7 UNSCHEDULED VISITS

Unscheduled visits, requested by the participant or the study physician, may prompt a medical history update, vital signs, clinically indicated laboratory tests, documentation of any AEs, and/or any other medically indicated diagnostic or therapeutic procedures. A targeted physical examination (if indicated) may be conducted.

8.1.8 EARLY TERMINATION VISIT

If a participant wishes to end their participation early, and is willing to have evaluations performed, a physical examination may be performed, and blood may be collected for safety laboratory analysis, qRT-PCR, and/or immunology assays.

Investigators will make every effort to continue follow-up visits for any participant who has received one or more immunizations for the duration of the study even if it is determined that subsequent immunizations should not be administered.

8.1.9 MEDICAL CARE FOR RESEARCH RELATED INJURY

All study-related medical care will be provided without cost. Should a participant be injured as a direct result of participating in this research project, he or she will be provided medical care by the clinical center at no cost to the participant, for that injury. The participant will not receive any injury compensation, only

medical care. The participants will not be compensated for care if he/she chooses to seek care from his/her own physician.

If a participant is injured because of participation in this research the participant is entitled to free medical care for that injury at a hospital or clinic designated by the clinical center. If the participant receives care for research-related injuries outside of the designated hospital or clinic, the participant or the participant's insurance will be responsible for medical expenses.

For all participants: Transportation will not be provided from domicile to study location, hospitals, clinics, or the clinical center. No reimbursement is available if the participant incurs medical expenses to treat research-related injuries. No compensation is available for research-related injuries or lost time from work. The participant is not waiving any legal rights. The participant should contact the site PI if the participant believes he or she has sustained a research-related injury. The participant should contact the site PI for any questions.

Employees retain the right to pursue relief through established workers compensation processes, but these benefits are not guaranteed. This issue is addressed in the informed consent and will be discussed with the participant by the investigator or designee before the participant signs the informed consent to participate in the study. Requests for other benefits, such as compensation for lost time from work, are processed independently of this protocol.

8.2 EVALUATIONS: CLINICAL, IMMUNOASSAYS AND DIAGNOSTIC

8.2.1 CLINICAL EVALUATIONS

8.2.1.1 MEDICAL HISTORY

A medical history from childhood onward will be taken with special attention to neurologic disorders including seizures and migraine, recurrent infections that suggest immune suppression, previous history of splenectomy and prior vaccine reactions. Systems to be reviewed include head/eyes/ears/nose/throat, pulmonary, cardiovascular, gastrointestinal, genitourinary, skin, musculoskeletal, neurological, allergy/immunology, endocrine, and hematological.

8.2.1.2 ELECTROCARDIOGRAM

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

8.2.1.3 PHYSICAL EXAMINATION

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

8.2.1.4 ADVERSE EVENTS (AES) ASSESSMENTS

Solicited AEs:
PfSPZ Vaccine: Surveillance for solicited local AEs will be done for 2 days after each dose of PfSPZ Vaccine. Surveillance for solicited systemic AEs will be done for 7 days after each dose of PfSPZ Vaccine. The list of specific events is detailed in section 8.3.1.1.1 'Solicited Adverse Events'.
PfSPZ Challenge: Surveillance for solicited local AEs will be done for 2 days after the administration of PfSPZ Challenge using the same solicited AE list as is used for PfSPZ Vaccine. Surveillance for solicited systemic AEs will be done for 6 days after PfSPZ Challenge using the same solicited AE list as is used for PfSPZ Vaccine. Surveillance for malaria events will be done from 7 to 28 (CHMI+7 to CHMI +28) days after the administration of PfSPZ Challenge using an expanded list that include the expected signs and symptoms of malaria (See section 8.3.1.2 Malaria Events'). These events are expected as a consequence of Pf parasitemia and will not be considered adverse events.

Unsolicited AEs:
Unsolicited AEs will be collected within 28 days after immunization or CHMI. Collection of solicited and unsolicited AEs will be done before participants leave on the day of immunization and at scheduled visits post immunization. SAEs will be recorded throughout the study.

8.2.1.5 HEALTH CARE PROVISION

Upon enrollment, study participants will have access to routine and acute care by the research team 24 hours a day, 7 days a week until the end of study participation. This is fully described in Section 2.2 'Risk/Benefit Assessment' above and Section 8.2.1.5 'Health Care Provision'.

8.2.2 CLINICAL LABORATORY EVALUATIONS

Routine safety laboratory testing will be performed by clinical laboratory associated with the clinical center. Results may be entered directly in to the database from the laboratory results provided by the performing laboratory. The designation of clinical significance by the site PI or designee will also be taken into consideration. Laboratory abnormalities will be followed up as clinically indicated, in most cases by repeating the test. The follow-up plan will be tailored to each individual case.

8.2.2.1 HEMATOLOGY AND BIOCHEMISTRY

Screening and safety laboratories will be collected on specified time points as listed in Section 1.3 'Schedule of Activities'. Clinical laboratories may be drawn at any other time points when deemed clinically indicated by the investigators. The Principal Investigator will maintain laboratory reference intervals in the study file, and copies will be made available upon request to the Local Safety Monitor, clinical monitors and Sponsor.

8.2.2.2 PREGNANCY TEST: FEMALE (URINE β-HCG)

Urine pregnancy testing will be performed at the study site on screening, each day of immunization and prior to CHMI. Serum testing may be substituted at the discretion of the study investigators – this can be run on the serum collected at the first and second immunization visits but will require an extra blood draw if needed for the 3rd immunization visit. Immunization and CHMI will not proceed unless a negative pregnancy test (urine or blood) has been obtained.

8.2.3 IMMUNOLOGY LABORATORY EVALUATIONS

8.2.3.1 ANTIBODY

Time points: pre-immunization, 2 weeks after V2, 2 weeks after V3, CHMI, 28 days post CHMI.

Table 9. Antibody Assays

anti-CSP ELISA (all), IFA and ISI (exploratory); ELISA for other antigens (exploratory), systems serology	Serum 25 mL	3-week CHMI: pre-V1, D15, D43, D50, D78 12-week CHMI: pre-V1, D15, D43, D113, D141

Sanaria will conduct its standard ELISA to determine levels of antibodies to Pf circumsporozoite protein (CSP) and may also conduct whole PfSPZ immunofluorescence assay (IFA), inhibition of sporozoite invasion assay (ISI) and ELISA's using other Pf antigens. The laboratory of Dr. Galit Alter, Professor of Medicine at Harvard Medical School and a Group Leader at the Ragon Institute of MGH, MIT and Harvard, has developed a number of antibody profiling approaches that allow deep interrogation of the role of antibodies in the response to immunization and pathogens. The lab has a particular interest in malaria and malaria vaccines with a goal to understand the role of antibodies in protection against infection following immunization with PfSPZ Vaccine. As a contribution to the USSPZV7 trial, the lab will conduct relevant systems immunology assays including biophysical measurements (isotype, subclass, Fc-receptor binding levels), antibody effector function assays, as well as potential malaria restriction assays in primary human livers.

8.2.3.2 CELLULAR

Time points: pre-immunization, 2 weeks after V2, 2 days after V3, and 2 weeks after V3 and day of CHMI

Table 10. Cellular Assays

Flow Cytometry	PBMC 30 mL	3-week CHMI: pre-V1, D15, D31, D43, D50, D57 12-week CHMI: pre-V1, D15, D31, D43, D113, D120

This work will be done by the Cellular and Molecular assay team (led by Dr. Irfan Zaidi) within the laboratory of Dr. Patrick Duffy, Chief, Laboratory for Malaria Immunology and Vaccinology, NIAID. For assessment of vaccine-specific T cell responses, peripheral blood mononuclear cells (PBMCs) will be separated and cryopreserved as per the standard NIH operating procedure. Multi-parameter flow cytometry assays will be performed on PBMCs to assess the phenotype and cytokine-producing capacity of T cells. In addition, T cell responses may be assessed using specific antigenic stimulants of proteins such

as PfCSP, PfCeltos and PfLSA1 amongst others. Expression profiling of RNA from PBMCs following vaccination and infection and additional assays (Fluidigm, Proteomics, and mapping for "antigen discovery") may also be conducted.

8.2.3.3 IMMUNE REGULATION

This analysis is being conducted to provide a systems-level, global understanding of participant immune responses pre-immunization, post-immunization and pre- and post-CHMI. Up to three different samples (serum, PBMCs, PAXgene) will be collected at each relevant timepoint to be analyzed for multiple parameters.

PAXgene tube (1.0 mL)

- Whole blood transcriptome as analyzed by RNA-sequencing from stabilized RNA
- Measurement of relative abundance of serum proteins with SomaLogic's SOMAscan assay or other assays
- Antibody analysis, e.g. titers against pathogens, glycosylation levels

Whole blood (EDTA) tube (20 mL, except 30 mL pre-immunization):

- Flow cytometry on whole blood and/or immune cell subsets
- Cellular transcriptional activity in immune cell subsets as analyzed by RNA-sequencing and/or microarrays
- Characterization of chromatin accessibility and promoter/enhancer landscapes in immune subsets with ATAC-sequencing and/or other techniques
- Quantification of gene expression (transcriptome) of PBMCs with RNA-sequencing and/or microarray
- Additional "omics" assays for immune receptor repertoires (T-cell and B-cell receptor profiling)

Immune Regulation	Samples and volumes as above	3-week CHMI: pre-V1, D15, D 31, D43, D50, D57 12-week CHMI: pre-V1, D15, D31, D43, D113, D120

- At each of these timepoints, a CBC with differential will be collected. The WBC results will be critical for interpreting the results of these immunological assays.

The work will be done by the laboratory of Dr. John Tsang at Yale University School of Medicine. The lab will use systems and quantitative immunology approaches including high parameter flow cytometry phenotyping, single cell assays including CITE-seq and scATAC-seq, and potentially Somalogic proteomics to measure close to 5000 circulating proteins to examine inflammatory and tissue statuses. These multi-dimensional phenotypes will be integrated with other participant data from the collaboration, particularly participant level clinical protection and immunogenicity information, for computational modeling to tease apart the baseline differences in immune state and features of responses that associate with PfSPZ vaccination efficacy in humans. These systems immunology efforts and particularly single cell multi-omics and proteomic generation will allow multimodal data integration using machine learning and advanced computational and statistical modeling approaches. This work will build upon the laboratory's expertise in human immunology and translate surrogate predictive markers identified in blood that capture immune

statuses at baseline, such as due to age, sex, and ethnic group to develop novel predictive signatures in humans.

8.2.3.4 DIAGNOSTIC PCR

In this trial, quantitative reverse transcriptase PCR (qRT-PCR) assay for Pf 18S rRNA (qRT-PCR or qPCT) will be the primary method used to detect malaria parasitemia after vaccination and after CHMI. Nucleic acid-based methods offer significant advantages over traditional methods such as TBS evaluation for detection of malaria parasitemia. Nucleic acid-based methods have significantly increased sensitivity for detection of Pf blood-stage infection approaching 20 parasites/mL, often two to four days earlier than by paired TBS [71-74]. Quantification of parasite density also allows for calculation of the liver-to-blood inoculum. The assay to be utilized will be specific to each clinical center and detailed in relevant SOPS. As the assays in each center differ in sensitivity, the data in each center may not be directly comparable, but in all cases the sensitivity is much higher than that of TBS and will result in earlier detection and treatment than a TBS-based follow-up approach.

In order to provide standardization of results, all positive samples will be confirmed retrospectively by a qRT-PCR assay for Pf 18S rRNA developed by Dr. Sean Murphy at the University of Washington Department of Laboratory Medicine [75]. This assay has been established as a qualified biomarker by the US FDA (**see** FDA's website *https://www.fda.gov/drugs/biomarker-qualification-program/fda-reviews-qualified-biomarker-plasmodium-18s-rrnardna*).

Samples for qRT-PCR will be collected pre-CHMI (baseline), and daily from days 7 until 18 post-CHMI, and then again, for those participants who remain negative, on days 20, 22, 25 and 28 post-CHMI (with allowable windows). Samples may also be collected as often as every 6 to 8 hours as required if a participant has signs and/or symptoms of malaria. qPCR detection of malaria parasitemia is usually positive 1-4 days before a thick blood smear [1], resulting in earlier treatment (almost invariably prior to symptom onset) than would occur if a positive thick blood smear were the basis for designating a malaria endpoint. The same sample used for qPCR can also be used to make thick blood smears, keeping this as a backup diagnostic modality should circumstances require. A case of malaria parasitemia will be defined as two positive qRT-PCR results from blood samples drawn $\geq$ 12 hours apart. Blood draw for qRT-PCR will be discontinued once treatment has been begun. A final qPCR sample will be drawn from all participants at the end of CHMI surveillance (day +28).

8.2.3.5 THICK BLOOD SMEAR (TBS)

Blood smear diagnosis will not be conducted as a routine part of this trial. If an investigator determines that a participant is acutely ill (for example, a participant who is non-compliant and misses several daily blood draws for PCR) and the investigator would like to have a diagnosis on an urgent basis, the clinical study site will have the capacity to have a TBS prepared and read. Treatment may be initiated based on a positive TBS. Prepared smears will be held to allow for later reading at another laboratory if needed.

Additionally, at the time of first anti-malarial dosing, a sample will be drawn for preparation of a TBS to be read retrospectively (Section 1.3 'Schedule of Activities', Footnote 5 to Schedules A, B and C). This TBS, which may not be positive, will not be used for diagnosis but will provide important data.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS

The ICH guideline for GCP describes an adverse event as follows: "Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related."

An AE is considered any adverse change or exacerbation from a baseline condition which occurs following the initial administration of an investigational product whether or not the event is considered to be related to the investigational product.

8.3.1.1 SOLICITED AND UNSOLICITED ADVERSE EVENTS

8.3.1.1.1 SOLICITED ADVERSE EVENTS

A solicited AE is a pre-specified sign or symptom which reflects safety concerns related to the investigational product or procedures. Solicited and unsolicited AEs will be systematically collected in this protocol to measure the safety and tolerability of PfSPZ Vaccine, normal saline and PfSPZ Challenge. Solicitation of local and systemic AEs with respect to each injection will begin after injection of study product. To collect solicited AEs, a specific list of symptoms will be reviewed with the participant face-to-face or via telephone, or specific signs will be identified by physical examination. This approach assures systematic and comprehensive collection of AE data. Trial participants may use a memory aid to help with remembering the AEs they have experienced.

PfSPZ Vaccine or Normal Saline Placebo
Solicited Local AEs (collected for 2 days after each immunization): pain, tenderness, erythema, swelling, bruising, pruritus and induration.

Solicited Systemic AEs (collected for 7 days after each immunization): Fever (oral temperature $\geq$ 100.4 F), subjective fever, chills, headache, fatigue, malaise, myalgia, arthralgia, rigors, systemic allergic type reactions (rash, urticaria, pruritus, edema).

PfSPZ Challenge (7G8)
Solicited Local AEs (collected for 2 days after PfSPZ Challenge – CH to CH+2): pain, tenderness, erythema, swelling, bruising, pruritus and induration.
Solicited Systemic AEs (collected for 6 days after PfSPZ Challenge – CH to CH+6): Fever (oral temperature $\geq$100.4°F), subjective fever, chills, headache, fatigue, malaise, myalgia, arthralgia, rigors, systemic allergic type reactions (rash, urticaria, pruritus, edema).

8.3.1.1.1 UNSOLICITED ADVERSE EVENTS

Unsolicited adverse events will be identified through open-ended questions such as "do you have any other symptoms?" This will be asked to prompt the identification of unsolicited adverse events after

reviewing the solicited adverse event list, or as the sole means of collecting adverse event data during visits when solicited adverse events are not being collected.

A syndromic classification will be used for unsolicited adverse events; e.g., cough, nasal congestion, sore throat will be combined and recorded as "upper respiratory tract infection." Thus, whenever applicable, the term for the unifying diagnosis will be recorded as the adverse event, not each individual sign or symptom.

Any event not included under the specific list for solicited local or systemic adverse events (see Section 8.3.1.1.1 'Solicited Adverse Events') or malaria events (see Section 8.3.1.2 'Malaria Events' below) will be captured as an unsolicited AE if the AE occurs within 28 days after immunization or CHMI. Events outside of this period will be collected as clinical events.

8.3.1.2 MALARIA EVENTS

Starting at 7 days post-CHMI through either 28 days post-CHMI (CH+7 to CH+28) or 3 days after treatment commences for parasitemia, participants will be monitored for signs and symptoms consistent with malaria infection. These "Malaria Events" will be documented but will not be categorized as adverse events since they are expected outcomes of malaria infection.

Malaria Events (collected day CH+7 and onward during CHMI): Fever (oral temperature $\geq$100.4°F), subjective fever, chills, headache, fatigue, malaise, myalgia, arthralgia, rigors, sweats, cough, nausea, vomiting, dizziness, abdominal pain, diarrhea, chest pain, palpitations, shortness of breath, and tachycardia.

8.3.1.3 LABORATORY ABNORMALITIES

Laboratory abnormalities will be collected in separate listings and tables from AEs. They will be graded as Grade 1 (mild), Grade 2 (moderate), or Grade 3 (severe) using pre-defined ranges (Appendix B). In addition, they will be graded as "clinically significant" or "not clinical significant," with "clinically significant" depending on the clinician's evaluation.

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS

An adverse event 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
- Life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Congenital anomaly/birth defect (abortion, stillbirth and any malformations/disease must be reported as an SAE).

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

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

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1 SEVERITY OF EVENT

All AEs will be assessed for severity by the investigator. Inherent in this assessment is the medical and clinical consideration of all information surrounding the event including any medical intervention required. Each event will be assigned one of the following categories: mild (Grade 1), moderate (Grade 2), or severe (Grade 3). Grade 4 AEs will be automatically classified as SAEs and reported as such. All Grade 1 to 3 AEs will additionally be assessed to determine if they meet the criteria for characterization as a serious adverse event (SAE) or suspected unexpected serious adverse reaction (SUSAR). Refer to the grading scale in Appendix A for further guidance in the assignment of severity. The criteria below may be used for any symptom not included in the grading scale.

The CRF for AEs will reflect only the highest severity for continuous days an event occurred.

Mild	Grade 1 -	Does not interfere with routine activities Minimal level of discomfort
Moderate	Grade 2 -	Interferes with routine activities Moderate level of discomfort
Severe	Grade 3 -	Unable to perform routine activities Significant level of discomfort

FDA guidelines for toxicity will be followed; however, if a participant is evaluated in an emergency room for non-life-threatening illness or symptoms (i.e., visits emergency department on weekend for mild problems because the physician's office is closed), the information from that visit will be reviewed and severity of the adverse event will be assessed according to the participant's clinical signs and symptoms.

As defined by the ICH guideline for GCP, the term "severe" is often used to describe intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious", which is based on participant/event outcome or action criteria usually associated with events that pose a threat

to a participant's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

Laboratory abnormalities will be graded on a Grades 1 to 4 scale following FDA toxicity grades (Guidance from the Center for Biologics Evaluation and Research, FDA [70]).

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

The site PI or investigator must assign a relationship of each AE to the receipt of the investigational product. The investigator will use clinical judgment in conjunction with the assessment of a plausible biologic mechanism, a temporal relationship between the onset of the event in relation to receipt of the investigational product, and identification of possible alternate etiologies including underlying disease, concurrent illness or concomitant medications. The following guidelines should be used by investigators to assess the relationship of an AE to study product administration.

Not related: No relationship to investigational product. Applies to those events for which evidence exists that there is an alternate etiology.

Unlikely: Likely unrelated to the investigational product. Likely to be related to factors other than investigational product but cannot be ruled out with certainty.

Possible: An association between the event and the administration of investigational product cannot be ruled out. There is a reasonable temporal association, but there is an alternative etiology that is more likely.

Probable: There is a high likelihood that a relationship to the investigational product exists. There is a reasonable temporal association, and the event cannot be explained by known characteristics of the participant's clinical state or factors including other therapy. However, there are alternative explanations, even if less likely.

Definite: An association exists between the receipt of investigational product and the event. An association to other factors has been ruled out.

To convert these designations to a binary system, "not related" and "unlikely related" will be considered "unrelated" while "possibly related", "probably related" and "definitely related" will be considered "related."

The Sponsor will determine the final classification of relatedness and expectedness for an AE.

8.3.3.3 EXPECTEDNESS

An adverse event 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 and listed in the IB.

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

Changes in a participant's medical condition will be assessed at all study visits, documented in the source records, and where applicable, will be recorded on the CRF using accepted medical terms and/or the diagnoses that accurately characterize the event. Unsolicited AEs will be collected within 28 days after

immunization or CHMI; SAEs will be collected for the duration of the study. When an event has not fully resolved by study closure, the event will be followed until it has been determined to be medically stable or fully recovered.

8.3.5 ADVERSE EVENT REPORTING

All adverse events (solicited and unsolicited) will be accurately documented in the case report form (CRF) by the investigator. For each event the following details will be recorded:

1. Description of the event
2. Date and time of occurrence
3. Severity
4. Relationship to PfSPZ Vaccine or PfSPZ Challenge (not related, unlikely to be related, or possibly, probably, or definitely related)
5. Alternate etiology: if an adverse event is deemed unlikely related or unrelated, an alternative etiology should be provided.
6. Action taken, including treatment
7. Duration
8. Outcome

When an adverse event occurs, it is the responsibility of the site PI (or designee) to review all documentation (e.g., progress notes, laboratory, and diagnostics reports) relative to the event. The investigator will then record all relevant information regarding the adverse event on the CRFs. If the adverse event is unsolicited, the site PI (or designee) will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the adverse event and not the individual signs/symptoms. In case no diagnosis is possible, symptoms, signs or laboratory abnormalities may be documented as individual adverse events/SAEs. Adverse events that are not serious do not require special reporting unless they are determined to be an unanticipated problem (see Section 8.4 'Unanticipated Problems').

8.3.6 SERIOUS ADVERSE EVENT REPORTING

Clinical Center Reporting to the Sponsor:

Per Sanaria's SOP, the respective site PI or medical sub-investigator will notify the Sponsor within 1 calendar day/24 hours via e-mail or phone or fax from the time he/she learns about the event. Any SAE reports (including SUSARs) will be sent via fax (SAE fax line: 240-306-0596) to the Sponsor. The Sponsor representative may request additional information for purposes of the study.

Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSOs) will be reported to the Sponsor within 48 hours and a written report will follow within 10 days. This is discussed further below under Section 8.4 'Unanticipated Problems'.

Table 11. Study Contacts for Reporting Serious Adverse Events and Unanticipated Problems Involving Risk to Patients or Others

Sponsor's Representatives	Sanaria Inc. 9800 Medical Center Drive Rockville, MD 20850
Institutional Review Board	wcg IRB 1019 39th Ave SE Suite 120 Puyallup, WA 98374 855.818.2289
Local Safety Monitor	Office: Cell: Email:

Table 12. SAE Information to be Reported to the Sponsor

Notification Type	Notification Method	Information to be Provided
Initial	Email or Telephone or fax (within 24 hours)	IND number, Sponsor study number, name of the investigational product, and investigator name and contact number Participant identification number SAE, onset date, date of investigational product administration, severity, relationship, and participant's current status
SAE Report	Fax or email all Sponsor's Representatives listed in **Table 11**. In addition, the database, using direct data entry, is designed to automatically notify Sponsor when an SAE CRF is completed.	Cover sheet or letter; Adverse event report form; Supporting documentation of the event (i.e. Concomitant medication case report form or a list of concomitant medications; Medical record progress notes including pertinent laboratory/diagnostic test results)

In order to comply with regulations mandating Sponsor notification of specified SAEs to the FDA according reporting guidelines, investigators must submit additional information as soon as it is available. The Sponsor will report unexpected SAEs associated with the use of the drug to the FDA as specified by relevant guidance.

The Sponsor will be responsible for notifying the FDA of any unexpected fatal or life-threatening suspected adverse reaction (SUSAR) 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. All other adverse events and SAEs will be reported by the Sponsor to FDA in the annual report to the IND.

Clinical Center Reporting to the Local Safety Monitor and IRB:

The site PI or his/her designee should promptly (within 48 hours) report to the IRBs and regulatory bodies as needed by telephone or email SAEs, Suspected Unexpected Serious Adverse Reaction [SUSARs] or Unanticipated Problems Involving Risks to Subjects or Others (UPIRTSOs) when s/he becomes aware of the event and then must follow up in writing within 10 working days from knowledge of the event.

8.3.7 REPORTING EVENTS TO PARTICIPANTS

All SAEs judged to be possibly, probably, or definitely related to the investigational product(s) will be reported to study participants via group meetings and/or one-on-one sessions with a study clinician and/or modifications or supplements to the informed consent form. Every effort will be made to maintain the confidentiality of the affected participant(s) while still providing enough information to other participants to allow them to fully assess the risks of continued involvement in the study. Informed consent forms will be updated with information concerning all SAEs judged to be possibly, probably, or definitely related to the investigational products, and participants.

8.3.8 EVENTS OF SPECIAL INTEREST

Not applicable.

8.3.9 REPORTING OF PREGNANCY

Each pregnancy must be reported **within 72 hours of identification** by email or fax to Sanaria and to the clinical center IRB in accordance with IRB policy.

Participants who become pregnant after Day 1 will be followed to term, and the following information will be gathered for outcome: date of delivery, health status of the mother and child including the child's gender, height and weight. Complications and or abnormalities should be reported including any premature terminations. A pregnancy is reported as an AE or SAE only when there is suspicion that the investigational product may have interfered with the effectiveness of contraception or there was a serious complication in the pregnancy including a spontaneous abortion or an elective termination for medical rationale.

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEM INVOLVING RISKS TO PARTICIPANTS OR OTHERS (UPIRTSO)

An Unanticipated Problem Involving Risks to Subjects or Others (UPIRTSO) is defined as involving risks to participants or others to include, in general, 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 research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- At least possibly related to participation in the research; and
- Suggesting that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously recognized.

8.4.2 UNANTICIPATED PROBLEM REPORTING

The investigator will report unanticipated problems (UPs) to the reviewing IRBs, HRPO and to the Sponsor. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents a UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported as follows:

- UPs that are SAEs will be reported as SAEs.
- Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSOs) will be reported to the Sponsor within 48 hours and a written report will follow within 10 days.
- Any other UP will be reported to the IRB (excepting IRBs providing administrative review) and to the Sponsor within 7 days of the investigator becoming aware of the problem.
- All UPs should be reported to regulatory institutions as required by an institution's written reporting procedures.

The Sponsor may also be the source of the UP report (e.g., failure of sterility testing in the vaccine lot). The Sponsor will report UPs relevant to participant safety to the SMC, and may schedule a SMC meeting to review, if appropriate. In addition, the Sponsor will notify the FDA and all participating investigators of potential significant risks represented by a UP, and this will be done no later than 15 calendar days after the Sponsor determines that the information qualifies for reporting. Other UPs (not posing a significant risk) will be reported by the Sponsor to the SMC in periodic reports and to FDA in the annual report to the IND.

8.4.3 REPORTING OF UNANTICIPATED PROBLEMS TO PARTICIPANTS

All UPs, if they directly affect the safety risk of study participants, will be reported to study participants via group meetings and/or one-on-one sessions with a study clinician and/or modifications or supplements to the informed consent forms. Every effort will be made to maintain the confidentiality of the affected participant(s) while still providing enough information to other participants to allow them to fully assess the risks of continued involvement in the study. Informed consent forms will be updated with information concerning all such UPs, and participants will be asked to sign the updated ICF if they agree to continued involvement in the study.

9. STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES FOR PRIMARY ENDPOINTS

9.1.1 PRIMARY ENDPOINTS

VE computed as one minus the estimated risk ratio for Pf infection (parasitemia) detected by PCR beginning 7 days after CHMI and censoring participants at 28 days in the "modified intention to treat" (mITT) population. The mITT population includes any participant completing all 3 doses and CHMI and receiving at least 20% of the injectate IV with each dose (see below).

Null hypothesis: there will be no significant difference between the vaccine group and the control group with regard to proportion protected (no parasitemia) and any observed difference is due to sampling or experimental error.

Alternative hypothesis: a statistically significant difference exists between the vaccine group and the control group with regard to proportion protected (no parasitemia).

The sample size for this study was not designed to test a formal hypothesis concerning vaccine safety

9.2 SAMPLE SIZE DETERMINATION

The primary outcome for these studies will be VE measured as [1 – risk ratio] x100. The outcomes of both CHMI's will be combined. Sample sizes of 45 and 15 for the vaccine and placebo groups were selected to be able to show with 83% power, if there are six drop-outs from the vaccine group and two from the control group prior to CHMI, that an infection rate of 92.3% in the controls (12/13 developing parasitemia) and 51% in the vaccinees (20/39 developing parasitemia) is statistically significantly different (α=0.05, 2-tailed) (VE=44.4%). As we expect VE to be higher than this, the sample size should be adequate.

Regarding safety, the study provides the following power to identify adverse outcomes:

1. If no PfSPZ Vaccine-related SAEs are observed, the sample size of 45 provides 95% confidence that the true rate of SAEs caused by immunization is less than 6.7%, 90% confidence that the true rate of SAE's is less than 5.0%, 85% confidence that the true rate of SAEs is less than 4.2%, and 80% confidence that the true rate of SAEs is less than 3.5%.

2. With regard to the ability to make comparisons between vaccinees and placebo recipients in the rates of grade 2 and grade 3 adverse events, where significant differences might indicate poor tolerability, a sample size of 45 vaccinees and 15 placebo recipients will show a statistically significant difference ($p<0.05$, two-tailed) between 10/45 vaccinees experiencing poor tolerability on the one hand, and 0/15 controls on the other hand.

Immunogenicity:
Antibody responses (group means, standard deviations, medians and ranges) will be used to compare participants with controls, and to compare infected and non-infected participants, the latter to look for trends suggesting a threshold response above which VE is seen, using appropriate nonparametric tests such as Wilcoxon and Spearman correlation. The data management vendor is providing biostatistical consultation and will generate the Statistical Analysis Plan, which will be finalized in advance of the database lock. The same vendor will conduct the statistical analyses.

9.3 POPULATIONS FOR ANALYSIS

9.3.1 TOTAL COHORT

The total cohort, or screened population, includes all participants who provide informed consent and are screened, regardless of whether the participant is randomized or vaccinated. This population will be used to fully account for participant disposition.

9.3.2 SAFETY COHORT

The safety cohort is a subset of the total cohort and will include all participants receiving at least one immunization. Participants will be analyzed according to immunization received. Participants who have received at least one immunization will not be excluded from the safety population for ineligibility, missing follow-up immunization, or protocol deviations.

9.3.3 IMMUNOGENICITY COHORT

The Immunogenicity Population will include all participants completing the immunization series (no missed immunizations) and for whom immunogenicity endpoint data are available. Participants who missed any follow-up immunizations will be excluded in immunogenicity analyses thereafter. Participants will be analyzed according to immunization received.

9.3.4 CHMI (VACCINE EFFICACY) COHORTS

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, and receives 1, 2 or 3 vaccinations. Because individuals will not be permitted to proceed to CHMI if they have not received three vaccinations, the results of CHMI will be imputed in those individuals receiving only one or two immunizations (see sensitivity analysis below).

The Modified ITT Population (mITT) is a subset of the ITT Population that receives all three immunizations and undergoes CHMI. It includes participants to whom V2 and/or V3 are administered out of the pre-specified time window, and also participants receiving partial immunizations, as long as at least 20% of the injectate is administered for each immunization. The mITT Population will be used for the primary analysis of VE.

The According to Protocol (ATP) Population is a subset of the mITT Population where three complete and correct immunizations (all 3 immunizations within the protocol specified time windows and each injection delivering at least 80% of the IP) as well as CHMI were administered within the allowed time windows. The ATP Population will be used for an exploratory analysis of VE.

If a volunteer drops out part way through CHMI, a judgment will be made after CHMI is completed regarding whether the efficacy data can be included, depending on the timing and result of the last PCR sample. If the result of the last (or an earlier) PCR is positive, the volunteer will be included in the efficacy analysis as not protected, using the measured prepatent period. If the result is negative, the volunteer will be included in the efficacy analysis as protected only if this negative result is recorded on the same day (or later) as the day of the most delayed positive result in any of the other volunteer undergoing CHMI.

Volunteers significantly straying from protocol procedures (e.g., use of antimalarial drugs during CHMI as discussed in the section on estimands) will be excluded from primary efficacy analysis but included in the exploratory analysis.

9.4 STATISTICAL ANALYSIS

9.4.1 GENERAL APPROACH

Data collection will be performed on site at the clinical center by direct data entry into eCRFs. Paper versions of the CRFs will be available, if needed, in the event the direct data entry system is unavailable. Paper CRFs, if used, will be retained as source documents and transcribed into the database. Data analysis will be supported by the data management company and will be performed in a coordinated way by study investigators and collaborators from Sanaria.
A Statistical Analysis Plan (SAP) will be prepared and will describe the statistical analyses for the study. The SAP may include minor differences from what is stated in the protocol.

9.4.2 ANALYSIS OF DEMOGRAPHICS

An analysis will be conducted on data and samples collected through 8 weeks post CHMI. Baseline demographics will be assessed using descriptive statistics and presented as tables, figures and listings. Continuous variables will be summarized with mean, standard deviation, median, and range, while categorical variables will be presented as a number and percentage.

9.4.3 ANALYSIS OF EFFICACY

The geometric mean, median, minimum, and maximum time to parasitemia (prepatent period) by qPCR after each CHMI will be presented for the 3-week and 12-week cohorts and for the combined controls. Prepatent periods will be measured from the hour/minute of administration of PfSPZ Challenge (7G8) to the hour/minute when the first positive sample was taken, with the result for the prepatent period calculated as the difference.

Primary efficacy analysis: the primary efficacy endpoint of the study for each research participant will be the presence or absence of Pf7G8 parasitemia following CHMI in the mITT population, defined by positive qPCR testing during the 28 days following CHMI, comparing vaccinees in each group to the combined controls from all the groups. VE will be defined as (1- relative risk) of Pf parasitemia by PCR after PfSPZ Challenge)*100.

Secondary efficacy analysis: in the secondary analysis, each cohort will be examined individually for protection. A 2-tailed Fishers exact test will be used to compare vaccinees with the controls. A p value <0.05 will be considered significant. Fishers exact test is appropriate because it is expected that all or nearly all controls will become positive, a non-random outcome.

Exploratory efficacy analysis: The trial is not powered for comparisons between cohorts, although this will be examined as an exploratory outcome. A 2-tailed Barnard's test will be used to compare the proportion of participants in each group who become parasitemic after CHMI. A p value <0.05 will be considered

significant. Barnard's test is appropriate because we have no expectations regarding differences in the proportion of protected study participants undergoing CHMI at 3 or 12 weeks.

A sensitivity analysis will be done by computing VE in the entire group using the ATP population and using the ITT population. In the latter case, CHMI outcome will be imputed first as not protected for all individuals not undergoing CHMI, and then as protected. This will provide an indication of the robustness of the primary VE outcome.

Finally, the VE for each group will be determined by time-to-event analysis (1 minus the hazard ratio). The time at risk will end whenever one of the following conditions happens: diagnosis of malaria infection, withdrawal from the study after receiving CHMI, loss to follow-up, or end of 28 day follow-up period. A Kaplan-Meier analysis with a log-rank test will be used to compare distributions of malaria-free time following CHMI.

9.4.4 ANALYSIS OF SAFETY

The inclusion of randomized, blinded normal saline controls allows unbiased collection of AE data. Because normal saline is physiologically inert, the rate of AEs recorded in normal saline recipients will represent background rates in the population. The use of any vaccine comparator other than normal saline would make it impossible to measure AEs due to PfSPZ Vaccine because these would be obscured by AEs caused by the comparator vaccine.

AEs and laboratory abnormalities will be analyzed based on the safety cohort dataset. Solicited local and systemic adverse events will be assessed individually and as total local or systemic adverse events. Unsolicited adverse events will be classified by the Medical Dictionary for Regulatory Activities (MedDRA) at the preferred term level and also will be assessed individually and as total unsolicited adverse events. The overall percentage of participants with a solicited local adverse event, a solicited systemic adverse event, an unsolicited adverse event, a laboratory abnormality or an SAE during the appropriate surveillance periods after immunization will be tabulated. The severity and relationship to study product will be tabulated as well. Rates of adverse events will be compared between vaccine and control groups and compared statistically using Fisher's exact test. In addition, laboratory abnormalities deemed clinically relevant will be tabulated by study group and rates of occurrence compared between vaccinees and controls.

9.4.5 ANALYSIS OF ANTIBODY RESPONSES, CORRELATES OF PROTECTION

Antibody levels to PfCSP by ELISA as the initial measure of immunogenicity assay will be measured at specified time points. Immunofluorescence assay (IFA) and inhibition of sporozoite invasion (ISI) may also be measured as exploratory analyses. Pre- immunization and post-immunization responses will be compared for vaccinees vs. placebo recipients. Tables and figures will be prepared showing these comparisons using:

- for antibodies to PfCSP by ELISA, reciprocal titers at optical density 1.0 (OD1.0) with background (medium control) subtracted, and ratios of values post-immunization compared to pre-immunization.
- for antibodies to sporozoites by automated immunofluorescence assay, reciprocal titers providing arbitrary fluorescence units of 2×10^5 with background (medium control) subtracted.

- for inhibition of sporozoite invasion, percent reduction of the numbers of PfSPZ that invaded a human hepatocyte line in the presence of post-immunization serum as compared with pre-immunization serum.

Correlations with protection will be done using non-parametric tests based on rank, such as the Spearman rank correlation coefficient. Association between categorical measures will be assessed using chi-square or exact tests. Effects of any covariates (e.g. age) will be assessed using regression models.

9.4.6 ANALYSIS OF CELLULAR RESPONSES, CORRELATES OF PROTECTION

The frequency of T-cells specific to Pf sporozoite and selected pre-erythrocytic antigens will be identified as CD4/CD8 + T-cells expressing cytokines (e.g., IL-2, TNF-α and IFN-γ) upon short term in vitro stimulation with PfSPZ or specific pre-erythrocytic antigens. Results will be expressed as antigen-specific CD4/CD8 T-cells per million respectively of CD4 or CD8+ T-cells. Descriptive statistics (e.g., N, minimum, Q1, median, Q3, maximum, mean, standard deviation) will be tabulated by group, for pre and post vaccination time-points. These results may be presented graphically by box plots.

Correlations with protection will be done using non-parametric tests based on rank, such as the Spearman rank correlation coefficient. Log10 transformed data will be used when a logarithmic transformation results in a distribution more nearly normal than the distribution of untransformed values. Association between categorical measures will be assessed using chi-square or exact tests. Effects of covariates (e.g. age and ethnicity) will be assessed using regression models.

9.4.7 PLANNED INTERIM ANALYSIS

No formal interim analyses are planned.

9.4.8 TABULATION OF INDIVIDUAL PARTICIPANT DATA

Individual safety, immunogenicity, and parasitology data will be listed for each study participant.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL AND STUDY OVERSIGHT CONSIDERATIONS

This study will be performed under the regulatory oversight of the US FDA. It will be performed under an active IND held by the IND Sponsor, Sanaria Inc. Original protocol approval will be performed by the WCG IRB. This will later be supplemented by oversight provided by NIH Ethics Committee (to oversee the work done at the NIH Clinical Center), the Clinical Ethics Committee at the University of Maryland Medical Center (to oversee the work done at the University of Maryland) or the appropriate IRB for another center if the trial cannot be done as planned at NIH and UMB. Details will depend in part on whether or not there are reliance agreements between WCG IRB and the institutional IRBs.

10.1.1 RECRUITMENT OF PARTICIPANTS

Healthy adult men and women will be recruited from the clinical center area by use of advertisements in multiple media formats, to include, but not limited to: informational flyers, newspaper advertisements, websites, word of mouth, and e-mail. All recruitment materials will be prepared and submitted for review and approval by the IRB prior to use. When a participant calls the study site and discloses an interest in the study, the recruitment staff will discuss the trial from an IRB-approved script. If the participant is still interested, contact information will be obtained and an appointment for briefing and/or screening will be arranged.

10.1.2 INFORMED CONSENT PROCESS

10.1.2.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

The site-specific informed consent document will be reviewed and approved by the appropriate IRB(s) and regulatory bodies (FDA) prior to initiation of the study. The consent document shall, at a minimum, meet the requirement of 32 CFR 219.116(a)(6) (General Requirements for Informed Consent). The consent document will contain a full explanation of the possible risks, advantages, and alternate treatment options, and availability of treatment in the case of injury, in accordance with 21 CFR 50. The consent document indicates that by signature, the participant permits access to relevant medical records by the Sponsor and by representatives of the IRBs and FDA. The Sponsor will submit a copy of the initial IRB-approved consent form to the FDA and will maintain copies of revised consent documents that have been reviewed and approved by the IRBs.

Informed consent includes the principle that it is critical the participant be informed about the principal potential risks and benefits. This information will allow the participant to make a personal risk versus benefit decision and understand the following:

- Participation is entirely voluntary.
- Participants may withdraw from participation at any time.
- Refusal to participate involves no penalty.
- The individual is free to ask any questions that will allow him/her to understand the nature of the protocol.
- A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by US law.

10.1.2.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent will be obtained from each participant prior to any procedures being performed. The study briefing and informed consent process will be done at the clinical center, which may begin in a group setting, but then proceeds to individual discussion between an investigator and study participant prior to any procedures. Study individuals who are interested in learning about the trial will meet with a member of the study team and will undergo an informed consent process consisting of a detailed informational presentation of the study given by a study investigator or by using IRB approved briefing slides in person or by means of a pre-recorded audio recording. Following the briefing, the coordinator or designee will provide the participant ample time to read the informed consent document. A study investigator and/or designee will answer all questions raised during the session. Any question that cannot be answered will

be referred to the site PI. The participant should understand that the study product is an investigational product and is not licensed by the FDA for commercial use, but is permitted to be used in this clinical research. The participant will be asked to sign the consent. The participant will be allowed to take the consent document home to consider and discuss it with others and return to the clinical center at a later time to sign it. After signing the consent, the participant will take an Assessment of Understanding. The assessment is administered to aid the study personnel in identifying gaps in understanding. Participants must score at least 80% correct on the 20-question multiple-choice questionnaire. Any questions missed will be explained to the participant, and the participant's questions will be answered. The participant will be given one additional opportunity to take the comprehension test. Any participant who, in the opinion of the study investigator, does not understand the study well enough to consider their consent truly informed will be excluded. As the Test of Understanding is in English for the two US sites, passing the test will be considered an adequate assessment of English language comprehension.

Should the protocol be modified, the participant consent document must be revised to reflect the changes to the protocol. If a previously enrolled participant is directly affected by the change, the participant will receive a copy of the revised informed consent document. The approved revision will be read, signed, and dated by the participant.

10.1.3 COMPENSATION FOR PARTICIPATION

Compensation will occur at the time of each designated visit. Compensation will be provided only for completed study procedures designated for compensatory payment. The likely compensation for study participants is shown in **Table 14**

For the post-CHMI malaria phase monitoring follow-up, each participant who is compliant with all of the required daily visits, including all follow-up visits after initiation of treatment for malaria, will receive $100 per day while they remain PCR negative or during Directly Observed Therapy (x 3 days). Each participant will receive a minimum of $300 and a maximum of $1200 depending upon when or if they develop malaria during the post-CHMI period. Participants who remain negative for malaria after the initial post-CHMI malaria monitoring phase will return to the CVD on Days 20, 22, 25, and 28 post CHMI to collect blood for qPCR. Participants who developed and were treated for malaria will return in follow-up on Day 28. Completion of post-CHMI follow-up visits will occur 28 days post-CHMI.

Participants who serve as alternates but who do not undergo immunization will be compensated for the screening visit, visit prior to first immunization and the day when they serve as an alternate $100.

Compensation $100 will also be provided for necessary unscheduled and/or supplemental visits.

Table 14. Compensation Plan

Activities	No. of Visits	UMB CVD	
		Compensation per Visit ($)	Total Compensation ($)
Screening	1	100	100
Pre-Immunization	1	100	100
Immunization	3	200	600
Memory Aid	4	70	280
Telephone Visit	1	15	15
Post-Immunization Follow-Up[a]	4	100	400
CHMI	1	400	400
Post-CHMI Initial Malaria Monitoring Phase[b]	3-12	100	300-1200
Additional CHMI Monitoring[c]	1-3	100	100-300
Post-CHMI Follow-Up	2	100	200
Bonus for Completion	1	200	250
Total [d]	28		2,645-3,845

a Number of visits for post-immunization follow-up (prior to CHMI) will vary depending upon the participant's cohort. Cohort A and B may have 7 follow-up visits; Cohort C may have 6 follow-up visits.
b Post CHMI Initial Monitoring Phase will start on Day 7 post-CHMI until Day 18 post-CHMI.
c qPCR will be collected on Days 20, 22, 25 and 28 post CHMI for participants who remain malaria negative. Those participants who develop malaria will return for directly observed therapy for three days and will return on D28.
d Total compensation is approximate as the total number and type of visits will be unique for each participant. Amounts of compensation may exceed the planned maximum amounts if compensated unplanned visits are required (e. g., to evaluate AEs).

10.1.4 STUDY DISCONTINUATION AND CLOSURE

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

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

The study may resume once concerns about safety, protocol compliance, and data quality are addressed.

10.1.5 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy are strictly held in trust by the participating investigators, their staff, and the Sponsor. All study procedures will be conducted per GCP guidelines, and every effort will be made to protect participant privacy and confidentiality to the extent possible.

In this research, the participant's health information will be collected and used to conduct the study; to monitor the participant's health status; to measure effects of the study product; to determine research results, and possibly to develop new tests, procedures, and commercial products. Health information is used to report results of research to the Sponsor and regulators and may be reviewed during study audits for compliance with study plans, regulations, and research policies. After the study ends, each participant has the right to see and receive a copy of his/her information.

Representatives of Sanaria Inc. as the IND Sponsor and the FDA are eligible to photocopy and review records related to this protocol as a part of their responsibility to protect the participants of this clinical trial. Data collection forms, laboratory specimens, and other reports will be identified by a coded number to maintain participant confidentiality. No personal identifier will be used in any publication or communication used to support this research study. The participant's identification number will be used in the event it becomes necessary to identify data specific to a single participant.

During the informed consent process, participants may be asked to provide consent to have their contact data stored in a registry. Participant information, including name, address, social security number, study identity, and dates of participation may be entered into the registry. This is to ensure that all study participants can be contacted if and when new information becomes available and participants need to be contacted at a later date. This information is stored for a minimum of 75 years and kept confidential.

All study-related information will be stored securely at the study site. While not in use and under immediate control of study staff, all participant information will be stored in locked areas with access provided to the appropriate study staff. All local databases will be secured with a password-protected access system. Participants study information will not be released without their written permission, except as necessary for monitoring compliance with legal or regulatory requirements. Medical records containing identifying information will be available for review when the Sponsor or an authorized regulatory agency monitors the study. Direct access may include examining, analyzing, verifying and reproducing records and reports that are important to the evaluation of the study.

10.1.6 FUTURE USE OF STORED SPECIMENS AND DATA

Handling and storage of biological samples, data and document-handling, and study record retention will be managed in accordance with ICH-GCP. During the conduct of the study, participants will be asked to provide permission for the future use of their specimens. A participant can choose to withdraw consent to have biological specimens stored for future research. Refusal to allow samples to be stored for future use will not exclude participants from participation. However, withdrawal of consent with regard to biological sample storage and future use may not be possible after the study is completed if samples have been anonymized.

10.1.7 KEY ROLES AND STUDY GOVERNANCE

Principal Investigator	Local Safety Monitor
Kirsten E. Lyke	TBD
Professor of Medicine, University of Maryland's School of Medicine	
Phone: 410-706-0462 Cell: 202-236-2948	Phone: Cell:
Email: klyke@som.umaryland.edu	Email:

Representatives of Sanaria Inc. and the study site may meet periodically to be updated on progress of the clinical trial and to offer guidance as to its successful execution.

10.1.8 SAFETY OVERSIGHT

Safety monitoring will be conducted throughout the study allowing safety concerns to be identified by continuous review of the data by the site PIs and clinic staff, with assistance as needed from the Local Safety Monitor assigned at each study site. In addition, there will be a Safety Monitoring Committee (SMC) chartered by Sanaria that will provide safety oversight and will be available to meet any time on an ad hoc basis. This will include as voting members a chair, at least one other physician with experience in the conduct of clinical trials, the Local Safety Monitor from each study site, and a biostatistician.

There will be two scheduled meetings of the SMC: (1) prior to the start of the trial to review the SMC Charter and the design of the clinical trial; (2) after completion of the final safety report to assess the trial and safety data. In addition, there will be ad hoc meetings if any pausing criteria are met, or if requested by the investigators, the Sponsor or the Local Safety Monitor. The Sponsor will be responsible for constituting the SMC and for all communications with the SMC. The Sponsor will notify the SMC of all SAE's potentially related to PfSPZ Vaccine occurring at the clinical trial sites and also will report other events of interest, such as unanticipated problems involving risks to participants or others (UPIRTSOs).

If at any point during the trial, significant safety or tolerability concerns arise at another site conducting a clinical trial with PfSPZ Vaccine that are not described in the IB, Sanaria is obligated to provide this information to relevant regulatory agencies, IRBs, SMCs and safety monitoring committees (SMCs). In particular, the Sponsor will inform the SMC for this trial and also the site PIs. Sanaria has adhered to this policy in the past, informing FDA, collaborators, and non-collaborating malaria vaccine development enterprises across the world when a concerning safety signal was seen in a trial of a different product.

10.1.9 CLINICAL MONITORING

Clinical monitoring will be conducted by the Sponsor's designated monitor to ensure adherence to ICH-GCP standards and regulatory guidelines. Pre-trial monitoring visits will be made to the site. The site PI will provide direct access and allow the study monitor and regulatory authorities to access all study-related documents.

All site records will be made available to monitors, including regulatory files, CRFs and other source documents, QA/QC documentation, SOPs, etc. Additional site visits (in person or virtual) will be made during the course of the trial as delineated in a Monitoring Plan approved by the Sponsor and site PI. The

Monitoring Plan will include the percentage of participant charts to be reviewed, which and what proportion of data fields will be monitored, who will be responsible for conducting the monitoring visits. The external monitor will also inspect the clinical site's regulatory files to ensure that applicable regulatory requirements (FDA) and ICH guidelines are being obeyed. During the monitoring visits, the site PI and/or designated study staff will be available to discuss the study. Additionally, Sanaria will perform a site qualification visit for each site (in person or virtual) to assess the suitability of the site for conducting the trial. The trial cannot begin without written approval from Sanaria.

10.1.10 QUALITY ASSURANCE AND QUALITY CONTROLS

To ensure compliance with GCP and all applicable regulatory requirements, the Sponsor (or Sponsor representative) may conduct quality assurance audits. Sanaria and/or its representative will have the right to audit the site(s). During the audit the site PIs and/or designated study staff will be available to discuss the study. The site PI will provide direct access and allow the Sanaria representative access to all study-related documents. Auditing of the clinical trial may be conducted at any time during the study to ensure continued compliance with regulations, policies and procedures. Audit findings will be documented in a formal audit report that will detail the conduct of the audit and summarize the observations noted.

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.

10.1.11 DATA HANDLING AND RECORD KEEPING

10.1.11.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

The handling and storage of data and document handling, record keeping, reporting, study record retention and protocol deviations will be managed in accordance with the applicable US CFR, ICH-GCP and Sponsor guidelines, and are described in SOPs. All primary source documents for this study will be stored at each clinical site. The long-term archiving of study data will be the responsibility of the site PI, who will notify the Sponsor of any change of archiving location.

Data monitoring and management will be performed by a qualified vendor contracted by Sanaria.

10.1.11.2 STUDY RECORDS AND RETENTION

The site PI must maintain all documentation relating to the study for at least 15 years after completion or 2 years after the last marketing application approval, or if not approved for 2 years following the discontinuance of the investigational product for investigation. If it becomes necessary for the Sponsor or designee or the FDA to review any documentation relating to the study, the investigator must permit access to such records.

The study site will store the completed, monitored source documents. The completed, monitored study dataset will be stored in a secure location by the Sponsor or designee. A copy of the dataset will be retained by the investigator.

The site PI will be responsible for retaining sufficient information about each participant, i.e., name, address, telephone number, social security number, and participant identifier in the study, so that the Sponsor, the local IRB, or the FDA may have access to this information should the need arise.

10.1.12 PROTOCOL DEVIATIONS

All participant-specific deviations from the protocol (i.e., failure to return for follow-up visits or blood collection within the time indicated in the protocol) are to be documented. The site PI or designee will be responsible for identifying and reporting all deviations, which are defined as isolated occurrences involving a procedure that did not follow the study protocol or study-specific procedure. Deviations will be reported to the IRB follow the regulation of each IRB. The site PI will assess action taken in response to the deviation and the impact of the deviation. Any protocol deviation that adversely affects the safety or rights of a participant or scientific integrity of the study will be immediately reported to Sanaria and the appropriate IRB.

10.1.13 PUBLICATION AND DATA SHARING POLICY

All data collected during this study will be used to support this IND. All data may be published in the open medical or military literature with the identity of the participants protected. Anyone desiring to publish or present data obtained during the conduct of the study will conform to study site policies, including any publication review clearance procedure.

10.1.14 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence is critical. Therefore, any conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate for their participation in the design and conduct of this trial. The study leadership in conjunction with the Sponsor has established policies and procedures for all parties involved in conducting the study to disclose conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

11. ABBREVIATIONS

AE	Adverse Event
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data and Safety Monitoring Board
DRE	Disease-Related Event
EC	Ethics Committee
eCRF	Electronic Case Report Forms
EMA	European Medicines Agency
EU	European Union
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
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
IND	Investigational New Drug
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
ITT	Intention-To-Treat
LSMEANS	Least-squares Means
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
NCT	National Clinical Trial
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
OHRP	Office for Human Research Protections
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOC	System Organ Class

SOP	Standard Operating Procedure
UP	Unanticipated Problem
US	United States

12. PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Changes	Brief Rationale
1.0	18Feb2022	Original approved version	
1.2	18Jul2022	1. Rationale for USSPZV7 trial was changed on basis of trials in Mali and Indonesia. The new rationale indicates (a) a reduction in the number of CHMI's from 3 CHMIs at 2 wks, 6 wks, 10 wks to 2 CHMIs at 3 wks and 12 wks, (b) a reduced sample size (n=60 rather than n=80), and (c) a need for at least 50% of the study participants to be women. 2. Adding presumptive treatment on day 28 post CHMI for those participants remaining qPCR negative, as a safety measure. 3. The background section has been extensively updated with new data and references. 4. The term "subjects" has been changed to "participants."	The study was originally designed to be a confirmatory study following Warfighter 3, and thus had the same design (CHMIs at 2, 6 and 10 weeks) but larger study numbers, in order to estimate vaccine efficacy (VE) with greater precision. Due to new developments (replacement of PfSPZ Vaccine with PfSPZ-LARC2 vaccine for a traveler's indication), the study is now serving a different purpose: forming a bridge between the VE seen following heterologous CHMI in malaria-naive adults and (a) field VE data in malaria-naive adults and (b) field VE data in malaria-exposed adults.

13. REFERENCES

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