Supplementary Material

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DMID 15-0052 PfSPZ-CVAC Study Team

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Statistical Methodology

As noted in the main text, vaccine efficacy against malaria infection by blood smear microscopy was assessed with multiple approaches:

- The hazard ratio approach: VE = 1 - HR, where HR is the unadjusted hazard ratio. This approach is based on comparing the time to first infection within 6 months of completing the vaccination regimen between treatment arms.
- The adjusted hazard ratio approach: VE = 1 - HR, where HR is the adjusted hazard ratio based on a Cox proportional hazards model including pre-specified covariates.
- The proportional approach: VE = 1 - RR, where RR is the risk ratio. This approach measures efficacy by comparing the probabilities of infection within 6 months of completing the vaccination regimen between treatment arms.
- The incidence rate ratio approach: VE = 1 - IRR, where IRR is the incidence rate ratio. This is the only one of these approaches that analyses multiple infections per individual.

As per the Statistical Analysis Plan (SAP), the following covariates were considered for inclusion in the Cox model to calculate the adjusted hazard ratio: age, sex, baseline anti-PfCSP antibody level, and baseline malaria infection. A log-transformation was chosen to ensure a linear relationship between anti-PfCSP antibody level and the log hazard. A model was fit including all covariates (transformed as appropriate). Covariates with significance levels greater than 0.10 in this model were dropped from the final model,

which included age and log-transformed anti-PfCSP antibody level. The same process was used to create the adjusted model for time to first PCR positive, which included age and sex.

For the incidence rate ratio approach, a negative binomial model was used. The outcome was the number of malaria infections per individual, and log-transformed time-at-risk was included as an offset. Because smears were collected on a monthly basis as well as during sick visits, two positive smears could represent a continuation of a single infection. Therefore, two positive smears were counted as distinct infections only when they were separated by a negative smear and/or the second positive smear occurred at least five days after a course of antimalarial treatment was completed. The same approach was used for PCR results. As specified in the protocol, the 24-week follow-up period began after the post-vaccination antimalarial treatment regimen was completed. Time on antimalarials during the follow-up period was subtracted from time at risk.

An error in executing the randomization process during the administration of Dose 1 caused several participants to receive a treatment different from what was assigned. These participants continued to receive the same treatment for subsequent doses and were analyzed according to the treatment received, in accordance with the SAP. Efficacy analyses were repeated on the Intention-to-Treat (ITT) population, which included all 62 participants because all participants received study product (Figure 1). The ITT analysis also analysed participants according to treatment received, in accordance with the SAP. The intention-to-treat principle is not violated by this approach as the randomization error is an artifact of the blinded trial environment and would not be reproduced in a natural public health setting.

Immune responses were summarized numerically and graphically. Numbers and percentages of participants experiencing seroconversion (defined as an additive change of at least 50 and multiplicative change of at least 3 in anti-PfCSP antibody from baseline levels) were summarized by treatment group and compared with Fisher's Exact Tests. Distributions of antibody levels were visualized by time point and treatment group via reverse cumulative distribution curves.[11] The additive change from baseline antibody level (net OD 1.0) and the multiplicative change (ratio OD 1.0) were compared between vaccinees and controls, and between infected and uninfected vaccinees with Brunner-Munzel Tests.

Cox proportional hazards modelling was used to estimate the relationship between anti-PfCSP antibody levels by ELISA and the time to first parasitemia. Separate models were fit for the time to parasitemia measured by TBS and by PCR. The model for time for parasitemia by TBS included treatment group and square root-transformed antibody level. A separate model included treatment group and the change from baseline antibody level, calculated as the post-vaccination antibody level minus the level measured on the day of Dose 1. The untransformed additive change was used because the untransformed variable satisfied the proportional hazards assumption while the transformed variable did not. A third model included treatment group and log-transformed multiplicative change from baseline antibody level, calculated as the ratio of post to pre-vaccination antibody level. Analyses were performed on the PP population and repeated on the ITT population. The same models were repeated for time to parasitemia measured by PCR except that a log transformation was used instead of a square root transformation for the model including antibody level.

Supplementary Table S1: Number and Proportions of Participants Infected by Thick Blood Smear Microscopy and PCR and Vaccine Efficacy Against Malaria Detected by Thick Blood Smear Microscopy at 12 Months after Last Vaccination, Per Protocol Population.

Number and Proportions of Participants Infected within 24 weeks after artesunate				
	PfSPZ-CVac (N=29)		**Control (N=30)**	
Infection definition	**n (%)**	**95% CI**	**n (%)**	**95% CI**
Thick Blood Smear	16 (55·2)	37·5, 71·6	22 (73·3)	55·6, 85·8
PCR	26 (89·7)	73·6, 96·4	29 (96.7)	83·3, 99·8
Vaccine Efficacy Against Malaria Detected by Thick Blood Smear Microscopy at 24 weeks after artesunate clearance				
Vaccine efficacy (VE) calculation Method	**Statistic**	**VE (%)**	**95% Confidence Interval**	**p-value**
Proportional method	VE=1-Kaplan-Meier estimated risk ratio	24·8	-4·8, 54·3	0·100
Hazard ratio method	VE=1-hazard ratio	33·6	-27·9, 65·5	0·221
Hazard ratio method, adjusted for covariates	VE=1-adjusted hazard ratio	17·6	-63·5, 58·5	0·579
Incidence rate ratio method	VE=1-incidence rate ratio	11·1	-55·0, 49·0	0·677
Number and Proportions of Participants Infected within 48 weeks after artesunate clearance				
	204,800 PfSPZ (n=29)		**Control (n=30)**	
Infection definition	**n (proportion)**	**95% CI**	**n (%)**	**95% CI**
Thick Blood Smear	17 (0·586)	40·7, 74·5	23 (76·7)	59·1, 88·2
PCR	27 (0·931)	78·0, 98·1	29 (96·7)	83·3, 99·8
Vaccine Efficacy Against Malaria Detected by Thick Blood Smear Microscopy at 48 weeks after artesunate clearance				
Vaccine efficacy (VE) calculation Method	**Statistic**	**VE (%)**	**95% Confidence Interval**	**p-value**
Proportional method	VE=1-Kaplan-Meier estimated risk ratio	23·2	-4·8, 51·1	0·104
Hazard ratio method	VE=1-hazard ratio	33·4	-26·0, 64·8	0·211
Hazard ratio method, adjusted for covariates	VE=1-adjusted hazard ratio	19·3	-58·4, 58·9	0·533
Incidence rate ratio method	VE=1-incidence rate ratio	01·0	-64·3, 40·3	0·969

Supplementary Table S2: Numeric Summaries of Anti-PfCSP Antibody Levels

Numeric Summaries of Anti-PfCSP ELISA OD 1.0 by Study Day and Treatment Group				
Treatment Group	**Statistic**	**Baseline**	**12 Days Post Dose 1**	**12 Days Post Dose 3**
PfSPZ-CVac	N	29	29	29
	Mean	587·2	714·6	1268·6
	Standard Deviation	505·5	593·2	1441·3
	Median	408	508	822
	(Minimum, Maximum)	(1, 2155)	(73, 2782)	(182, 7673)
	Geometric Mean	351·5	521·9	852·8
	95% CI for Geometric Mean	(204·1, 605·5)	(379·8, 717·2)	(611·8, 1188·9)
Control	N	30	29	30
	Mean	256·2	266·9	315·3
	Standard Deviation	174·1	202·3	350·8
	Median	199	225	216
	(Minimum, Maximum)	(67, 811)	(66, 868)	(58, 1915)
	Geometric Mean	210·2	208·1	218·3
	95% CI for Geometric Mean	(165·7, 266·6)	(158·8, 272·8)	(159·7, 298·2)

Number and Percentage of Seroresponders							
	PfSPZ-CVac			**Control**			
Study Day	**N**	**n (%)**	**95% CI[a]**	**N**	**n (%)**	**95% CI[a]**	**P-value[b]**
15	29	3 (10)	(4, 26)	29	0 (0)	(0, 12)	0.2368
71	29	8 (28)	(15, 46)	30	1 (3)	(1, 17)	0.0122

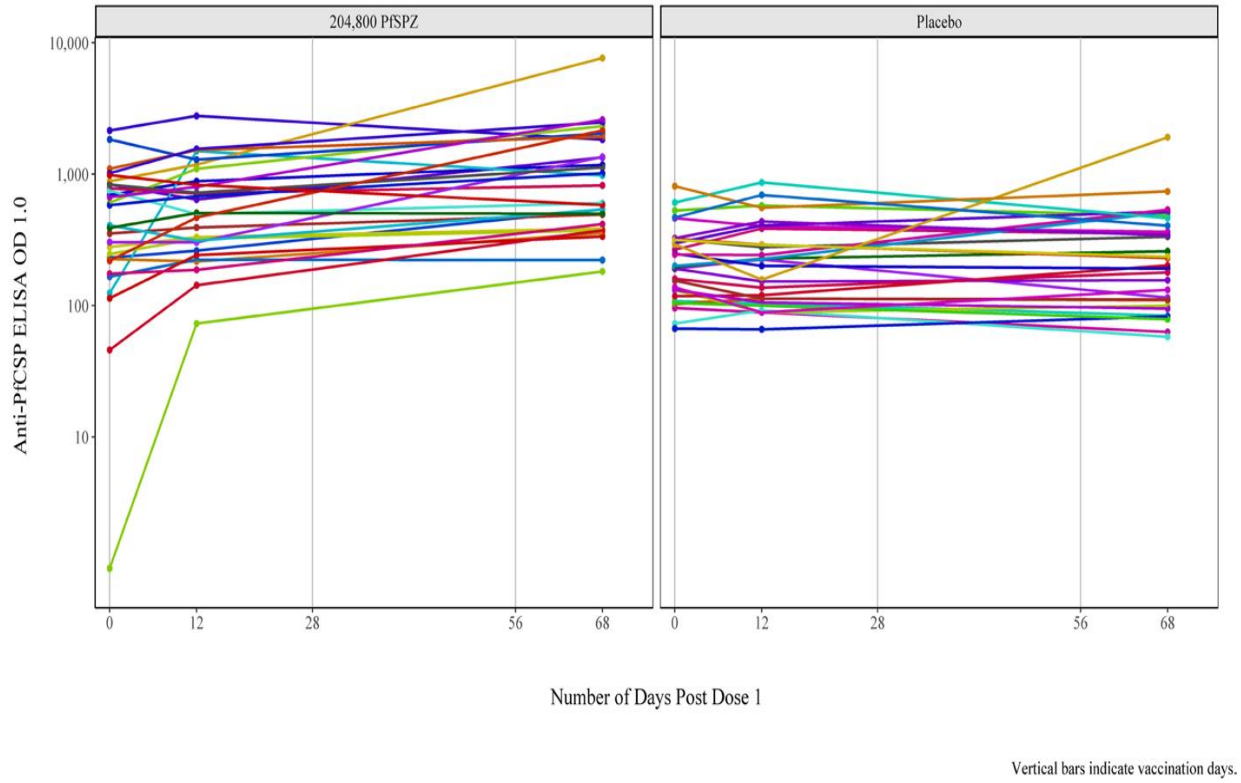
Note: N = number of participants in the PP population with an assessment at the study day.
n = Number of participants with seroresponse, i.e., net OD 1.0 ≥50 and ratio OD 1.0 ≥3.

[a] 95% CI - 95% confidence interval from Wilson score.

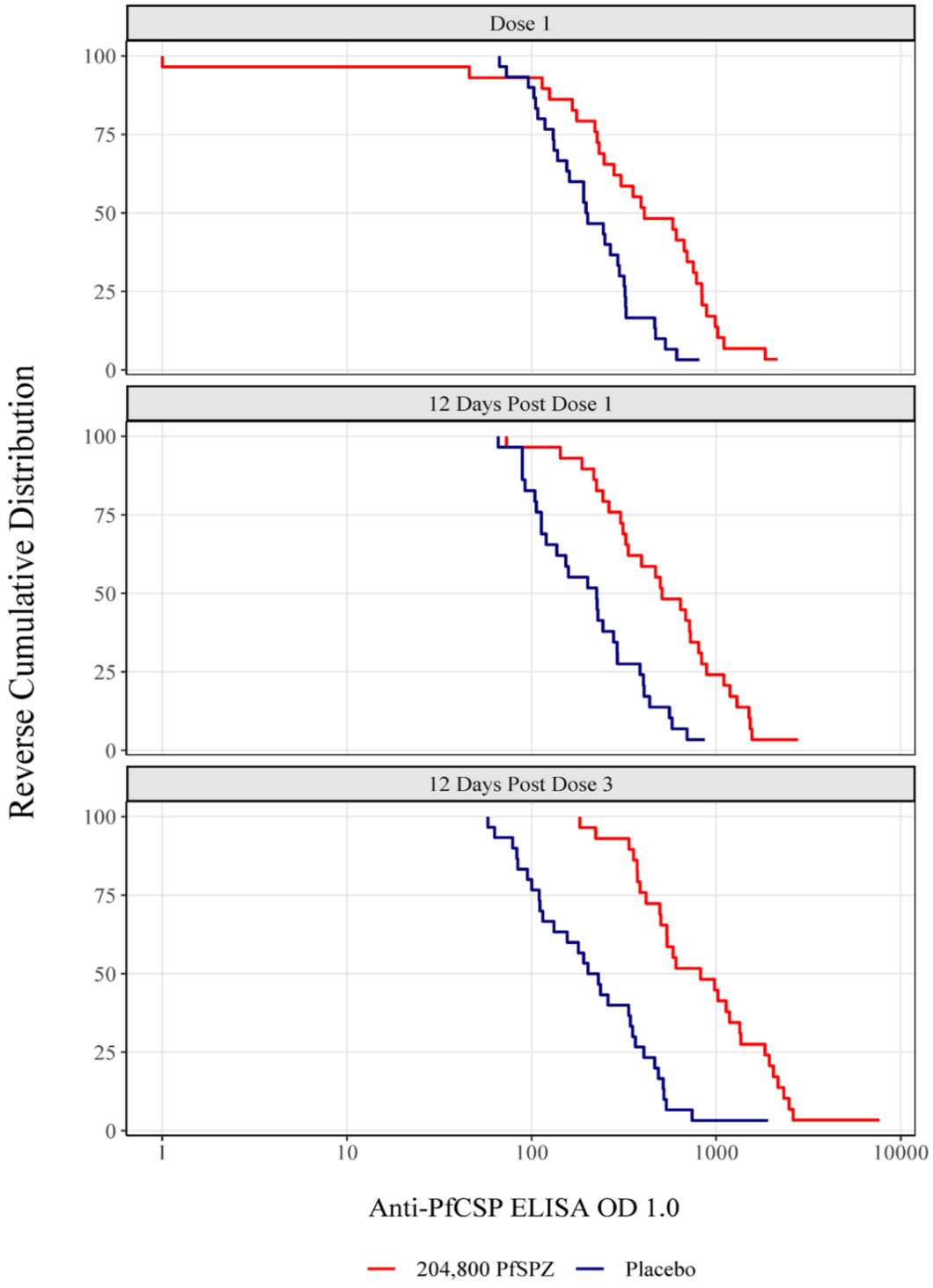
[b] p-values were calculated using Fisher's Exact Test.

Figure S1. Antibodies to PfCSP by ELISA. Serum dilution at which the optical density was 1.0 (OD 1.0). A. Individual Time Trend Plots of Anti-PfCSP ELISA OD 1.0 by Treatment Group - 204,800 PfSPZ PP Population. Each line represents one participant. Population tested: 29 in vaccine group and 30 in control group. Variability in antibody levels among participants, small increases in the vaccine group over time, and stability in the control group are documented. **B: Reverse Cumulative Distribution Plots of Anti-PfCSP ELISA OD 1.0 by Treatment Group and Time Point, PP Population.** The red lines represent the Reverse Cumulative distribution of Anti-PfCSP ELISA OD 1.0 for vaccine group and the blue line represent the control group. Population analysed: 29 in vaccine group and 30 in control group. To understand the RCD curve, observe that the x-axis displays the range of observed antibody values. For a given x-value, the height of the curve is the percentage of subjects whose antibody level was greater than or equal to that x-value. Letting xmin denote the smallest antibody value in the data set, the curve starts at the left with the point (x = xmin, y = 100), since 100% of the values are greater than or equal to the minimum value. The curve drops in a stepwise fashion at each value that was observed, with the height of the drop equal to the proportion of subjects with that value. The median is the x-value where the height of the curve is 50%, and the 95th percentile is the x-value where the height of the curve is 5%. When the RCD curve for one group is shifted to the right of that for another, the corresponding histogram is also shifted to the right. The RCD curves show that the baseline antibody levels are generally higher in the vaccine group than the control group (though the vaccine group has an outlier in the left tail) and that the vaccine group distribution shifts rightward over time.

A



B


Dose 1
12 Days Post Dose 1
12 Days Post Dose 3
Reverse Cumulative Distribution
100
75
50
25
0
1
10
100
1000
10000
Anti-PfCSP ELISA OD 1.0
204,800 PfSPZ
Placebo

SAFETY, TOLERABILITY, IMMUNOGENICITY AND PROTECTIVE EFFICACY AGAINST NATURALLY-TRANSMITTED MALARIA OF INFECTIOUS, CRYOPRESERVED *PLASMODIUM FALCIPARUM* SPOROZOITES (PFSPZ CHALLENGE) ADMINISTERED BY DIRECT VENOUS INOCULATION UNDER CHLOROQUINE CHEMOPROPHYLAXIS (PFSPZ-CVAC), IN MALIAN ADULTS: A RANDOMIZED, DOUBLE BLIND, PLACEBO-CONTROLLED TRIAL

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Draft or Version Number: 7.0

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This protocol format is adapted from the ICH guidance document E6 (Good Clinical Practices), Section 6.

STATEMENT OF COMPLIANCE

The study will be carried out in accordance with the US Code of Federal Regulations (CFR), local regulations, and Good Clinical Practice (GCP) as required by the following:

- 45 CFR 46; 21 CFR 50, 21 CFR 56, 21 CFR 11, 21 CFR 812, and 21 CFR 312
- International Conference on Harmonization (ICH E6); 62 Federal Register 25691 (1997)

All individuals responsible for the design and conduct of this study have completed Human Participants Protection Training and are qualified to be conducting this research before the enrollment of any participants. CVs for all investigators and sub-investigators participating in this trial are on file in a central facility (21 CFR 312.23 [a] [6] [iii] [b] edition).
The signature on the following page constitutes approval of this protocol and the attachments, and provides the required 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, applicable US federal regulations and (ICH E6) guidelines.

SIGNATURE PAGE

The signatures below constitute the approval of this protocol and the attachments, and provide 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, and the Declaration of Helsinki.

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Signed: Date:

Mahamadou A. Thera MD PhD
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LIST OF ABBREVIATIONS

AE	Adverse event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BSC	Biological Safety Cabinet
CBC	Complete blood count
CFP	Challenge Final Product
CFR	Code of Federal Regulations
CMF	Controlled Manufacturing Facility
CMI	Cell mediated immunity
CRF	Case Report Form
CSP	Circumsporozoite protein of *Plasmodium falciparum*
CTL	Cytotoxic T lymphocyte
CVD	Center for Vaccine Development, University of Maryland School of Medicine
DBS	Dried blood spot
DMID	Division of Microbiology & Infectious Diseases, NIAID, NIH
DNA	Deoxyribonucleic acid
DVI	Direct Venous Inoculation
ECG	Electrocardiogram
eCRF	electronic Case Report Form
ELISA	Enzyme linked immunosorbent assay
FDA	US Food and Drug Administration
FWA	Federal-Wide Assurance
GCP	Good clinical practice
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
HSA	Human Serum Albumin
ICH	International Conference on Harmonisation
ID	Intradermal
IDES	Internet Data Entry System (Advantage eClinical^{SM})
IEC	Institutional Ethical Committee
IFA	Immunofluorescence assay
IND	Investigational new drug
IRB	Institutional Review Board (ethical review committee)
IV	Intravenous
ISM	Independent Safety Monitor
LNVP	Liquid Nitrogen Vapor Phase
LSA1	Liver stage antigen 1 of *Plasmodium falciparum*

MRTC	Malaria Research and Training Center
NaCl	Sodium Chloride
NF54	A laboratory clone of *Plasmodium falciparum*
NHP	Non-human primates
NIAID	National Institute of Allergy and Infectious Diseases
NIH	US National Institutes of Health
NIST	National Institute of Standards and Technology
NMRC	Naval Medical Research Center
MRTC	Malaria Research and Training Center
OCRA	Office of Clinical Research Affairs, DMID, NIAID, NIH
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate Buffered Saline
PfSPZ	*Plasmodium falciparum* sporozoites
PI	Principal Investigator
QA	Quality assurance
QC	Quality control
RNA	Ribonucleic Acid
SAE	Serious adverse event
SC	Subcutaneous
SDW	Source Document Workbook
SMC	Safety Monitoring Committee
SNP	Single nucleotide polymorphism
SOP	Standard Operating Procedure
SPZ	Sporozoite
UMD	University of Maryland
USTTB	Université des Sciences, des Techniques et des Technologies de Bamako

PROTOCOL SUMMARY

Title	Safety, Tolerability, Immunogenicity and Protective Efficacy against Naturally-Transmitted Malaria of Infectious, Cryopreserved *Plasmodium falciparum* Sporozoites (PfSPZ Challenge) administered by Direct Venous Inoculation under Chloroquine Chemoprophylaxis (PfSPZ-CVac), in Malian Adults: A Randomized, Double Blind, Placebo-Controlled Trial
Short Title	Safety and Efficacy of Sanaria's PfSPZ-CVac in Malian Adults
Clinical Trial Phase	1
Population	Healthy, malaria-experienced adults aged 18-45 years
Number of Sites	1
Study Duration	24 months
Subject Participation Duration	Approximately 14-15 months
Description of Agent or Intervention	Three doses of Sanaria's non-irradiated, fully infectious *P. falciparum* sporozoites (PfSPZ Challenge) administered by direct venous inoculation to volunteers receiving chloroquine chemoprophylaxis, a vaccination strategy termed PfSPZ-CVac. Participants will receive 204,800 sporozoites or placebo (normal saline) per dose administration. All participants will receive a standard chemoprophylactic regimen of chloroquine (CQ) for 10 weeks.
Objectives	**Primary:** 1. To assess the safety and tolerability of PfSPZ Challenge compared to placebo among malaria-experienced adults taking chloroquine prophylaxis (PfSPZ-CVac) **Secondary:** 1. To assess the protective efficacy of PfSPZ-CVac against naturally transmitted *P. falciparum* malaria infection as diagnosed by thick blood smear microscopy 2. To assess protective efficacy of PfSPZ-CVac against naturally transmitted *P. falciparum* malaria infection as diagnosed by qPCR 3. To assess the expanded efficacy of PfSPZ-CVac compared to placebo

	4. To examine the immune response to *P. falciparum* malaria infection **Exploratory:** 1. To assess potential immune correlates of protection against infection by naturally transmitted *P. falciparum* malaria within 6 and 12 months after the last vaccination with PfSPZ-CVac 2. To measure strain-specific efficacy and vaccine selection for non-vaccine variant strains within 6 and 12 months after the last vaccination with PfSPZ-CVac 3. To determine if artesunate monotherapy selects for in vivo resistance to artesunate
Endpoints	**Primary**: 1. The number and severity of solicited local and systemic adverse events (AE) in the 12 days following PfSPZ challenge administration 2. The number and severity of unsolicited AEs related to study product in the 12 days following PfSPZ challenge administration 3. The number of serious adverse events (SAEs) **Secondary**: 1. Time to *P. falciparum* parasitemia, detected by thick blood smear microscopy, within six months after the last vaccination with PfSPZ-CVac 2. Time to *P. falciparum* parasitemia, detected by qPCR, within six months after the last vaccination with PfSPZ-CVac 3. Time to *P. falciparum* parasitemia, detected by thick blood film microscopy, within twelve months after the last vaccination with PfSPZ-CVac 4. Time to *P. falciparum* parasitemia, detected by qPCR, within twelve months after the last vaccination with PfSPZ-CVac 5. Antibody titers against *P. falciparum* circumsporozoite protein (CSP) and other *P. falciparum* proteins at serology time points; markers of cell-mediated immunity **Exploratory**: 6. Correlation of antibody titers against *P. falciparum* proteins to time to first parasitemia by qPCR using Cox proportional hazards modeling 7. Correlation of antibody titers against *P. falciparum* proteins to time to first parasitemia by microscopy using Cox proportional hazards modeling

	8. Genomic and genetic divergence between PfSPZ-CVac strain and post-vaccination *P. falciparum* infections 9. Malaria positivity by qPCR on the third day of artesunate administration 10. Post-vaccination PBMC Cytokine profiles 11. mRNA Gene expression profiles 12. Serologic antibody profiles against *P. falciparum* proteins measured by microarray
Description of Study Design	The proposed study is a single site, double-blinded, randomized, placebo-controlled clinical trial of PfSPZ-CVac safety, tolerability, immunogenicity and efficacy against naturally occurring malaria in malaria-exposed Malian adults. The overall goal of the study is to evaluate if a regimen of PfSPZ-CVac (PfSPZ Challenge under chemoprophylaxis) is safe, well-tolerated, and provides sterile protection against naturally-occurring malaria in malaria-experienced adults. Participants will receive three immunizing PfSPZ Challenge injections via direct venous inoculation (DVI) four weeks apart under chloroquine chemoprophylaxis. The PfSPZ Challenge dose will be 204,800 PfSPZ. This is based on results of studies in Europe and in Africa. In Tübingen, Germany, 100% of malaria-naïve adults who received three doses of 51,200 PfSPZ every four weeks under chloroquine chemoprophylaxis were protected against homologous controlled human malaria infection (CHMI). At the same time, studies of PfSPZ Vaccine in malaria-experienced adults in Mali and in Tanzania demonstrate that higher doses of PfSPZ are required to demonstrate immunogenicity and high grade protection in malaria-experienced adults that is comparable to that achieved in malaria-naïve adults studied in the USA. For this reason, the dose selected for this study is four-fold higher than the dose used for Tübingen, Germany. Controls will receive 0.9% sodium chloride (NaCl) as placebo. All participants will receive a standard chemoprophylactic regimen of chloroquine (CQ) for 10 weeks. Chloroquine will be given as a loading dose (600mg chloroquine base) two days before the first administration of PfSPZ Challenge, followed by weekly doses of chloroquine (300mg chloroquine base weekly). Participants will also be treated with a 7-day regimen of artesunate (200 mg per day) after the last PfSPZ Challenge dose of 204,800 sporozoites for malaria parasite clearance, one week after the last CQ dose is given.

	A total of 62 participants will be randomized in a 1:1 ratio to one of two groups and will be inoculated with PfSPZ Challenge or 0.9% NaCl by DVI so that a total of 62 adults will participate in the study. Participants will be recruited from the MRTC's Bougoula-Hameau site. All volunteers recruited will be healthy adults aged between 18 and 45 years. Safety and infectivity data will be collected. Volunteers, clinical and laboratory investigators will be blinded to group allocation. Participants will be followed every four weeks after the last vaccination as outpatients for active malaria diagnosis and treatment. Passive follow-up will be accomplished by continuous availability of study staff onsite to diagnose and treat malaria and other medical issues that arise. Study Arms: 1. Group 1 (n=31): 204,800 PfSPZ of PfSPZ Challenge every 4 weeks x 3 doses by DVI 2. Group 2 (n=31): NaCl placebo every 4 weeks x 3 doses by DVI
Estimated Time to Complete Enrollment	45 days

Figure 1 Study schema

Study Week	1	2	3	4	5	6	7	8	9	10	11	12 to 35	36 to 60
PfSPZ Challenge or Normal Saline	X				X				X				
Chloroquine dosing	C	C	C	C	C	C	C	C	C	C			
Artesunate dosing											A		
Primary Efficacy Follow-up												P	
Secondary Efficacy Follow-up													S
Safety Monitoring Committee review				R									

1 KEY ROLES

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background information

2.1.1 Malaria parasite life cycle

Among the five species of *Plasmodium* that cause human malaria, *Plasmodium falciparum* is responsible for the most disease and death. Its life cycle is complex (Figure 2). Anopheline mosquitoes inject worm-like sporozoites which travel to the liver and invade hepatocytes. About six to ten days later, each infected hepatocyte releases thousands of tiny merozoites into the bloodstream. Each merozoite can invade a different red blood cell. Within the red blood cells, *P. falciparum* merozoites multiply over the course of 48 hours to produce 15-30 new merozoites within each schizont (infected red blood cell). At 48 hours after merozoite invasion, each schizont bursts and releases this new generation of merozoites, each able to invade other red cells. The blood stages of the infection produce clinical symptoms and malaria disease.

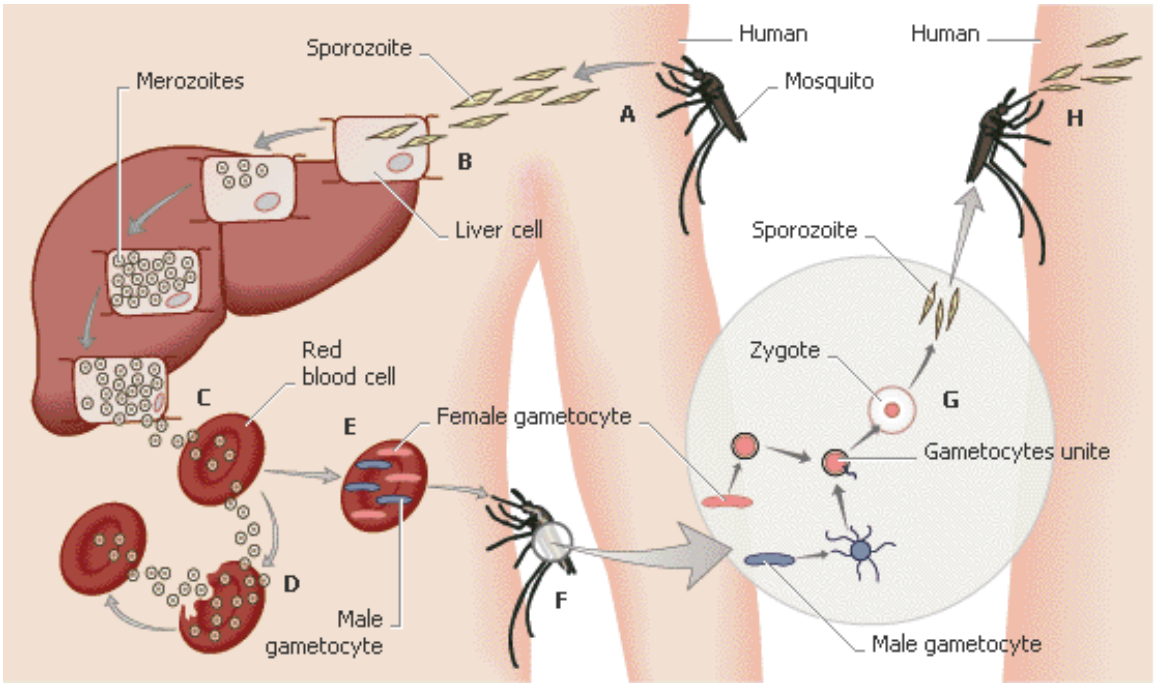

Figure 2 Malaria life cycle

Particular to *P. falciparum* is its ability to modify the surface of the red blood cell in a way that the infected cells can adhere to the vascular endothelium and other tissues, where they cause disease. Parasite sequestration in various organs (brain, heart, liver, kidney, and placenta) contributes to the pathogenesis of malarial disease. A small proportion of asexual parasites convert to sexual forms that transmit the infection to others through female Anopheline mosquitoes.

2.1.2 Disease burden

Africa bears the heaviest burden of malaria. In 2013, malaria caused 198 million clinical illnesses and 584,000 deaths, with 80% in children <5 years of age living in Sub-Saharan Africa [1]. The vast majority of these deaths are attributed to *P. falciparum*. In areas of stable malaria transmission, 25% of all-cause mortality in children aged four years or less has been directly attributed to malaria [2]. Studies done in West Africa indicate that malaria could account for as much as 60% of all-cause mortality in children aged less than five years old [3-5].

2.1.3 Malaria in Mali

In Mali, West Africa, where childhood mortality exceeds 20%, malaria is a leading cause of morbidity in the general population and of mortality in children aged less than ten years. Malaria is hyperendemic in Mali, characterized by a high intensity of transmission that is almost exclusively *P. falciparum*, and is estimated to have caused over 15,000 deaths in 2013 (http://www.who.int/countries). Malaria transmission in Bougoula Hameau, Mali peaks during the rainy season from May to November. The major vectors are *Anopheles gambiae ss*, *Anopheles arabiensis*, and *A. funestus*. The study site, Bougoula Hameau, Mali, is a suburban district with approximately 6,900 inhabitants and is located 370 kilometers southeast of Bamako in the Sikasso district. Bougoula Hameau is part of the health district of Sikasso (604,920 inhabitants living in 22 communes according to 2015 district health data). Bougoula is located in the Sudanian zone dominated by savanna woodland with tall grass dotted with trees. The climate is under the influence of the humid forest zone.

In Bougoula Hameau, the average number of clinical episodes of malaria per child and per transmission season is 2.7, with a few children experiencing up to 8-10 clinical episodes. Adults are all malaria-experienced and show an asymptomatic parasitemia prevalence of up to 40-50% during the dry and rainy seasons when measured by microscopy, and ~58% by PCR, but they rarely experience clinical malaria due to acquired immunity. The number of hospitalizations for severe malaria among children aged five years or less in Sikasso health district was 2608 from 2012-2013, based on regional and local health data sources.

2.1.4 Preclinical Investigations

PfSPZ Challenge contains fully infectious *P. falciparum* sporozoites (PfSPZ) purified from the salivary glands of *Anopheles stephensi* mosquitoes raised under aseptic conditions. The manufacturing process for PfSPZ Challenge is essentially identical to that of PfSPZ Vaccine, with the exception that sporozoites of PfSPZ Challenge are not irradiated, and have been cryopreserved for clinical trials at either 15,000 PfSPZ per vial, 50,000 PfSPZ per vial or 100,000 PfSPZ per vial, instead of 150,000 PfSPZ per vial for PfSPZ Vaccine trials conducted to date. Hence, the preclinical studies for PfSPZ Vaccine are referenced to support the profile of safety and tolerability for PfSPZ Challenge.

2.1.4.1 Toxicity Study of PfSPZ Vaccine in New Zealand White Rabbits

The safety and immunogenicity of PfSPZ Vaccine administered by SC, ID, and IV routes was demonstrated in New Zealand White (NZW) rabbits and mice. Three preclinical toxicology studies in rabbits, giving 5-7 doses of

135,000 PfSPZ each by the ID, SC and IV routes were performed with the radiation-attenuated PfSPZ Vaccine. In all 3 studies, PfSPZ Vaccine was safe and well tolerated.

2.1.4.2 Biodistribution studies

Formal biodistribution studies were performed, which examined the distribution and persistence in mouse tissues of *P. falciparum* DNA following SC, ID, or IV inoculation of a single 135,000 PfSPZ dose of PfSPZ Vaccine. The studies showed that PfSPZ Vaccine was safe and well tolerated in mice and that there was no persistence of PfSPZ in the host tissue beyond 144 hours. The dose of sporozoites in these studies was equivalent to the highest dose administered in the first two Phase 1 clinical trials of PfSPZ Vaccine, a dose that has now been shown to be safe and well tolerated in adult participants administered PfSPZ Vaccine by SC, ID, and IV routes.

2.1.5 Clinical Investigations

Based on the results of pre-clinical and clinical studies of PfSPZ Vaccine administered SC and ID, clinical trials of PfSPZ Challenge were initiated without additional pre-clinical toxicology studies (IND 14267). In the first PfSPZ Challenge Phase 1 clinical trial (TIP2), eighteen healthy Dutch participants received PfSPZ Challenge with Group 1 (n=6) receiving 2,500 PfSPZ, group 2 (n=6) receiving 10,000 PfSPZ and group 3 (n=6) receiving 25,000 PfSPZ in two ID injections [6]. There were no acute systemic allergic reactions or local adverse events (AE) following the injection. One serious adverse event (SAE) occurred in a volunteer who reported chest pain the day after initiation of Malarone treatment. The chest pain resolved spontaneously within 60 minutes without treatment. Since the cause of chest pain was not clear, the clinical data suggested that this SAE was not a serious cardiac event and this event was deemed “possibly related” to trial participation.

In the second clinical trial, PfSPZ Challenge was administered intramuscularly (IM) by needle injection at University of Oxford, UK in 2011 [7]. Here PfSPZ Challenge was administered ID at 2,500 PfSPZ and IM at 2,500 or 25,000 PfSPZ doses. The PfSPZ were infectious and PfSPZ Challenge was safe and well tolerated.

Subsequent trials in Tanzania (ID administration of up to 25,000 SPZ per dose) (IND 14267), University of Maryland (ID administration of up to 50,000 PfSPZ per dose) (IND 14954), and Germany (IV administration of up to 3200 PfSPZ per dose) (IND 15012) [8], also indicate that PfSPZ Challenge is safe and well-tolerated in humans. In addition, trials in Barcelona (IM administration of up to 75,000 PfSPZ per dose and IV administration of up 3,200 PfSPZ per dose) (IND 14267) and Kenya (IM administration of 25,000 PfSPZ per dose; 75,000 PfSPZ per dose and 125,000 PfSPZ per dose) (IND 14267) [9] also indicate that PfSPZ Challenge is safe and can be safely administered in human studies. A clinical study of PfSPZ Challenge administered by direct venous inoculation (DVI) in individuals with previous malaria exposure with or without the sickle cell trait was performed in Gabon (IND 15012); here, DVI of 3200 PfSPZ per dose was also well-tolerated.

PfSPZ Challenge has also been administered to participants in combination with the antimalarial drug, chloroquine, in an approach to vaccination called PfSPZ-CVac, or PfSPZ Chemoprophylaxis Vaccine. The

PfSPZ-CVac approach was assessed via the ID route in a Phase 1 trial in the Netherlands (IND 15191). Immunizations were well tolerated in all participants. However, there was one serious adverse event (SAE) that occurred 73 days after the last dose of PfSPZ-CVac in a volunteer who had controlled human malaria infection (CHMI) by mosquito bite to assess the efficacy of PfSPZ-CVac. This volunteer developed malaria and antimalarial treatment was initiated. The next day he developed elevation in troponin levels suggesting cardiac problem. Later that same day, he experienced chest pain and a heavy feeling in his left arm. After further evaluation, he was diagnosed with myocarditis. He had no other symptoms or signs. Troponin levels were normal within 16 days. He recovered without sequelae. The etiology of the myocarditis could not be determined; of note, the study subject received six different immunizations (diphtheria, poliomyelitis, tetanus, parenteral typhoid fever, hepatitis A and hepatitis B) between administration of the last dose of PfSPZ-CVac and CHMI.

A second trial of the PfSPZ-CVac approach via the IV route was performed in Germany (IND 15862). Immunizations were well tolerated in all participants and neither unexpected nor serious adverse reactions were observed during the seven days after injection. Significant protective efficacy was demonstrated in the high dose group (3 immunizations of 51,200 PfSPZ under chemoprophylaxis), where nine out of the nine immunized participants did not develop malaria and were therefore considered sterilely protected against the disease.

One volunteer was ostensibly non-compliant with administration of chloroquine despite the use of directly observed treatment (DOT), as he showed only trace levels in the blood on day 13 after PfSPZ Challenge, which was one day after receiving the second chloroquine dose. The trace levels of chloroquine were inconsistent with the known good bioavailability of this drug, and indicated that the volunteer most likely held the chloroquine tablets in his cheek during the initial loading dose and during the second dosing on day 13, and spit them out later. Consistent with subtherapeutic chloroquine levels, the volunteer developed rising parasitemias identified by blood smear on day 13 after PfSPZ Challenge, became symptomatic for clinical malaria, was given atovaquone/proguanil to eliminate the parasitemia and was withdrawn from the study.

In summary, the results from all pre-clinical studies of PfSPZ Vaccine, bolstered by the results of clinical trials of PfSPZ Vaccine (by SC, ID and IV routes), and of PfSPZ Challenge and the PfSPZ-CVac approach administered by the ID, IV and IM routes to participants, indicate that PfSPZ Challenge is safe and well tolerated in humans. The combination of PfSPZ Challenge and partner drug (the PfSPZ-CVac approach) is also safe and well tolerated as long as volunteers are compliant with chloroquine administration. Due to the importance of compliance, it is recommended that all chloroquine dosing be administered by DOT and that a post administration oral examination should be performed by the clinical investigator.

2.2 Rationale

A safe and effective malaria vaccine would represent an important tool for malaria prevention and control, and for malaria elimination and eradication.[10] Two critical steps of the malaria life cycle have been closely

examined as potential targets for vaccine-induced immunity to prevent malaria morbidity and mortality: the pre-erythrocytic sporozoite and liver stages and the asexual erythrocytic stages of the parasite life cycle. Vaccines directed against pre-erythrocytic stages, are intended to prevent disease and transmission by blocking infection, and those directed against blood stage parasites are intended to prevent disease by inhibiting parasite replication in the blood.

An ideal, single stage vaccine useful for elimination of *Plasmodium falciparum* would prevent infection at the pre-erythrocytic stage of the parasite life cycle, thereby preventing disease and transmission from humans to mosquitoes. The only approach to immunization shown to consistently induce greater than 90% protection against infection and protection sustained for at least 10-28 months has been immunization by mosquito bite with whole *P. falciparum* sporozoites (PfSPZ). Two types of whole PfSPZ immunization have been tested for efficacy in humans. The first type, radiation-attenuated PfSPZ, invades hepatocytes and expresses new proteins, but cannot replicate. The second type fully develops in hepatocytes, producing tens of thousands of merozoites that invade erythrocytes, but are unable to fully develop within erythrocytes because they are killed by an antimalarial drug. This latter approach, called chemoprophylaxis with sporozoites, harnesses the infectious agent's inherent replicative properties to amplify production of protective immunogens spanning multiple developmental stages, and then eliminates the infectious agent with an anti-infective drug before the onset of disease.

An early study of chemoprophylaxis with sporozoites was conducted in malaria-naïve adults in the Netherlands. Roestenberg et al. reported 100% efficacy against CHMI for 10 healthy volunteers to bites of *P. falciparum*-infected mosquitoes once a month for three months while receiving chloroquine chemoprophylaxis.[11] When six of these "protected" volunteers underwent a second CHMI 28 months after immunization, four (67%) were protected.[12] This novel approach to malaria vaccination was subsequently pursued as it demonstrated both short and long-term protection in this initial clinical trial.

Sanaria developed a product called PfSPZ Challenge that is comprised of non-irradiated, fully infectious PfSPZ. PfSPZ Challenge has been shown to infect 100% of malaria-naïve volunteers after administration by needle and syringe at adequate doses. It has been tested for optimization of administration by the ID, IM, and IV routes in the Netherlands, UK, Tanzania, U.S., Germany, Spain, and Kenya. The optimal dose and route of administration of PfSPZ Challenge in malaria-naïve individuals was recently established in studies in Europe.[8] This is to give 3,200 PfSPZ by direct venous inoculation (DVI).

To mimic chemoprophylaxis with sporozoites, Sanaria has developed a strategy, PfSPZ-CVac, comprised of PfSPZ Challenge administered to volunteers receiving chloroquine chemoprophylaxis. Chloroquine has been selected because of the durable high-grade protection induced in the Nijmegen study, and because the NF54 strain of malaria that comprises PfSPZ Challenge is extremely sensitive to this drug. When PfSPZ-CVac strategy was recently tested in malaria-naïve individuals in Tübingen using three doses of 51,200 PfSPZ of PfSPZ Challenge administered at four week intervals under chloroquine prophylaxis, 100% protection (9/9

volunteers) was demonstrated against controlled human malaria infection (CHMI) administered 9-10 weeks after the last immunization (8-9 weeks after chloroquine prophylaxis was discontinued) (NCT02115516). On a dose for dose basis, this approach is 10-20 times more potent than radiation attenuated sporozoites (PfSPZ Vaccine). In addition, the dose regimen providing high-grade protection can be completed in a shorter time frame – three doses every four weeks rather than every 8 weeks, the latter being the regimen of choice for PfSPZ Vaccine. This promising PfSPZ-CVac approach now needs to be optimized and tested in persons living in malaria-endemic areas with the overall objective to establish a regimen that can be used for malaria elimination campaigns.

2.2.1 Rationale for the Clinical Trial Vaccine Dosages and Schedule

The nine participants protected in the Tübingen study (NCT02115516) were dosed with 51,200 PfSPZ per dose. As described above, this provided 100% protection against CHMI conducted 9-10 weeks after the last immunization. Participants in the same clinical trial who received three doses of the 12,800 PfSPZ dose had 67% efficacy (6/9 volunteers protected), a marked reduction. Thus 51,200 PfSPZ appeared to be just above the threshold needed for high-grade protection.

Other variables besides dose also likely influence the outcome: (1) the Tübingen trial used a regimen characterized by 4 week intervals, allowing maturation of the immune response prior to re-dosing, whereas a more condensed regimen may have been less effective; (2) the 9-10 week interval between last immunization and CHMI was relatively short, minimizing any effects of waning immunity; (3) the parasites used for CHMI were the African Pf strain NF54, which is homologous to the vaccine, and thus did not assess heterologous (cross-strain) protection; and (4) the volunteers were malaria-naïve and therefore without the modulated immune responses that characterize individuals with prior malaria exposure. We hypothesize that greater stringency with regarding any of these variables could have pushed the efficacy induced by the Tübingen regimen below the protection threshold just as effectively as a reduction in dose of PfSPZ administered.

These concerns have been support by additional findings in the Tübingen and other trials. In the second stage of the Tübingen study, two condensed regimens were assessed: 6/9 volunteers (67%) were protected against CHMI when three doses of 51,200 PfSPZ were given every 14 days rather than every 28 days, and 5/8 volunteers (63%) were protected when three doses of 51,200 PfSPZ were given every 5 days. A new study will soon be initiated in the US to see if higher doses can restore the 100% efficacy seen in the first stage of the Tübingen trial while maintaining a condensed injection schedule.

A second variable is the interval between immunization and CHMI. As with other models of disease protection mediated by live, attenuated vaccines,[13;14] the antimalarial efficacy achieved by the PfSPZ Vaccine, which has been more extensively studied than PfSPZ-CVac, has waned during the initial several months after CHMI. It can therefore be reasoned that the vaccine-induced immunity required to convey long-term protection after PfSPZ-CVac would require higher doses of PfSPZ than used in the Tübingen trial. Interestingly, however, once efficacy is established following PfSPZ Vaccine administration, it appears to be relatively long lived. For

example, 5/5 (100%) volunteers protected against homologous CHMI at ~20 weeks after immunization with PfSPZ Vaccine remained sterilely protected against homologous CHMI at 59 weeks [Ishizuka 2016], and 5/6 (83%) volunteers protected against homologous CHMI at 18 weeks were still sterilely protected against heterologous (7G8) CHMI at 33 weeks (Lyke, submitted).

A third variable is the strain of Pf used in the CHMI. CHMI with a strain that is antigenically divergent from NF54 might diminish the protective efficacy of the vaccine. Studies with PfSPZ Vaccine indicate that this is indeed the case, although the loss of protection associated with a heterologous strain is relatively minor. In the Warfighter 1 trial, conducted by NMRC and WRAIR, sterile protection was 80% (4/5 volunteers) following CHMI with 7G8-infected mosquitoes 3 weeks after immunization, and only slightly higher, 92% (12/13 volunteers), in a parallel group receiving CHMI with 3D7-infected mosquitoes (Epstein, submitted). 3D7, which was cloned from NF54, differs from NF54 by only 15 single nucleotide polymorphisms, while 7G8, a clone from a Brazilian isolate, is strongly divergent. It thus appears that PfSPZ Vaccine does induce relatively strong cross-strain protection. A newly initiated study of PfSPZ Vaccine in the USA will exclusively assess long-term heterologous CHMI in order to impose the most stringent antigenic challenge possible.

Finally, increased doses of vaccine may also be needed to induce seroconversion in vaccinees with prior malaria exposure, due to pre-existing antimalarial immunity or to blunted immune responses due to immunosuppression induced by prior malaria infection.[15] The best evidence that malaria-exposed individuals may be disadvantaged is provided by the immunogenicity of identical PfSPZ Vaccine regimens in the USA (malaria-naïve volunteers), Tanzania (minimally malaria-exposed volunteers), and Mali (heavily malaria-exposed volunteers). Humoral responses, as measure by CSP ELISA or sporozoite IFA, diminished progressively with increasing history of malaria exposure. Concurrent with the reduced immunogenicity, the protection rate in Tanzania following CHMI was lower than that in the USA despite identical immunization regimens (Epstein, submitted; Shekalaghe, unpublished). Nevertheless, highly significant sustained protection against naturally transmitted malaria was demonstrated in the Mali trial, despite the marked heterogeneity of the parasites transmitted in the field and the heavy prior malaria exposure. Thus the hypothesized dose adjustment to overcome the deleterious effects of prior exposure may be relatively minor. Based on these several rationales, a four-fold increase in dose relative to the 51,200 PfSPZ used in the Tübingen trial has been selected for this trial. The dose of 204,800 PfSPZ was chosen in order to address three of the four variables described above: (1) to overcome preexisting antimalarial immunity in Malian participants, (2) to convey long-term protection over 24 weeks of follow-up, and (3) to overcome malaria parasite antigenic diversity seen in malaria-endemic areas. The fourth variable discussed above – the interval between immunizations – will be kept at four weeks, as in the Tübingen trial. The dose of 204,800 PfSPZ of PfSPZ Challenge has not yet been tested in humans. It is worth noting that doses of up to 2,700,000 PfSPZ of PfSPZ Vaccine have been administered to healthy volunteers via DVI in the USA and Africa, and have been safe and very well-tolerated with no safety signal detected.

2.2.2 Rationale for Placebo

Having a control or comparator arm is important in malaria vaccine trials conducted in malaria-endemic areas, because background immunity and natural exposure to malaria make it difficult to interpret immunogenicity data and establish correlates of protective efficacy. In this setting, rising titers of antibody or increased cellular immune responses to malaria antigens could be due to vaccination or to natural exposure or both. The use of a control group will permit comparison of immune responses and malaria infection rates, and allow clearer interpretation of immunogenicity and efficacy results. Moreover, in a population with high rates of infectious and other illnesses, a comparator arm is helpful in assessing rates of AEs experienced by vaccine recipients. Recognizing that a comparator vaccine (or vaccines) with direct benefit would favorably alter the benefit:risk ratio, no licensed vaccine exists that can be given via the IV route. As normal saline is a physiologic solution that can be injected intravenously in small volumes with virtually no expected side effects, control vaccinees will be given normal saline IV.

2.2.3 Rationale for Presumptive Malaria Treatment

Participants will initiate a 7-day course of presumptive malaria treatment with oral artesunate therapy beginning one week after the last chloroquine dose is given. This will eliminate blood-stage infections and allow assessment of infection rate and occurrence of infection, permitting evaluation of PfSPZ-CVac efficacy against malaria infection. As has been used in several clinical trials in Africa and other endemic areas for malaria treatment,[16-21] artesunate monotherapy will be administered for seven days as oral therapy (200mg/day). Artesunate monotherapy for presumptive treatment is proposed for several reasons. First, unlike primaquine, artesunate can be given to persons who are deficient in the enzyme glucose-6-phosphatase-dehydrogenase without significant risk of serious side effects. Second, a drug with a short half-life is needed before the onset of the malaria season so that vaccine efficacy can be tested without lingering prophylactic effects of a longer-acting antimalarial drug. Lastly, artesunate monotherapy has been used in clinical trials of children and adults in malaria-endemic areas without evidence of toxicity or other serious consequences.[16-21] To evaluate if artesunate monotherapy selects for *in vivo* resistance in this clinical trial setting, we will collect filter paper specimens on the first three days of artesunate administration and determine if these are positive by PCR testing. Chloroquine itself is not sufficient to clear existing parasitemias due to the presence of chloroquine resistant parasites in Mali (chloroquine is administered solely to kill the highly chloroquine sensitive PfNF54 parasites comprising PfSPZ Challenge).

2.3 Potential risks and benefits

2.3.1 Potential Risks

2.3.1.1 Potential Risks of Vaccination

As of June, 2016, 2181 doses of PfSPZ products (PfSPZ Vaccine, PfSPZ Challenge) have been administered by the intravenous or direct venous inoculation routes to 890 subjects, and all have been safe and well tolerated, with no adverse reactions linked to administration. In four randomized, double-blind, placebo-controlled studies conducted in Africa (3 studies) and Germany (1 study), after unblinding the data, no differences in side effect profiles were identified comparing PfSPZ recipients to normal saline placebo recipients. Potential side effects that were evaluated included local and systemic side effects (both solicited and unsolicited) and laboratory abnormalities. Nevertheless, there are a number of theoretical risks that should be considered.

Theoretical local risks (by analogy to other vaccines) associated with administration of PfSPZ Challenge include local inflammatory reactions, pruritus, and larger local reactions involving the whole forearm. Theoretical systemic risks associated with PfSPZ Challenge include systemic allergic reactions, fever, chills, malaise, fatigue, headache, myalgia, arthralgia, dizziness, and nausea. Regardless of the precautions taken, the risk of serious, or even life-threatening, allergic reactions is possible. Emergency equipment and supplies, including epinephrine, diphenhydramine, and prednisone, will be available to treat acute allergic symptoms as per standard medical procedures. Venipuncture and associated intravenous inoculation, or intramuscular or intradermal injection, always carry risks of minor discomfort and the possibility of bruising at the site of the needle puncture, or, rarely, infection. Participants may also have difficulty sleeping, anxiety, confusion, swelling, shaking chills, cough, and shortness of breath or heart racing related to PfSPZ Challenge. As with all research, there is the remote possibility of risks that are unknown or that cannot be foreseen based on what is currently known about the product.

In Equatorial Guinea two serious adverse events possibly related to PfSPZ Vaccine have been described. One volunteer who received PfSPZ Vaccine had a miscarriage at 10 weeks after getting pregnant while participating in a PfSPZ Vaccine trial. Miscarriages frequently occur without known causes and although it is unlikely the vaccine caused the miscarriage, the temporal relationship meant this was a possibility. All women of child-bearing age are required to take birth control measures as specified within the protocol and/or consent form to avoid getting pregnant while participating in trials of PfSPZ-based products. Another volunteer, a 15-year-old boy who received PfSPZ Vaccine, had a generalized seizure 3 ½ hours after receiving his third dose. The boy fully recovered from the seizure. The EEG showed that he was predisposed to having seizures. It is unlikely the vaccine caused the seizure, but like all vaccines, PfSPZ Vaccine causes an immune response in the body which may increase the chance that those individuals predisposed to seizures experience a seizure.

Risks from malaria infection

Because participants will be taking chloroquine (CQ) preventive therapy during PfSPZ Challenge administration, and the NF54 parasite used in this PfSPZ Challenge product is highly susceptible to CQ prophylaxis, clinically significant parasitemias due to PfSPZ Challenge are not likely to occur. However, due to the presence of circulating malaria at the study site, any individual with clinical signs of malaria will be

evaluated with a thick blood smear and, if positive, treated with the standard antimalarial therapy according to Mali Ministry of Health guidelines, currently artemisinin combination therapy. Most malaria infections in adults at the study site are asymptomatic due to preexisting immunity. Should infections become symptomatic, a volunteer may experience mild malaria symptoms that commonly include headache, myalgia, chest pain, fever, chills, sweats, nausea, vomiting, and diarrhea. In the absence of chloroquine prophylaxis, typical onset of symptoms and signs of malaria occur approximately 9 days post-inoculation.

Risks from antimalarials

Potential side effects of chloroquine include headache, malaise, dizziness, blurred vision, difficulty focusing, muscle weakness, tinnitus, sun sensitivity, mild gastrointestinal upset, and hearing loss in persons with preexisting auditory damage. Non-urticarial pruritus, without rash, is a problem that is more common among dark-skinned patients. The symptom usually begins within the first day after the initial dose and may last up to seven days. Severe adverse reactions, including seizures, are extremely rare.[22] CQ is well known to be safe in pregnancy and is routinely recommended for both malaria treatment and malaria prophylaxis in pregnant women.

Potential side effects of artesunate include dizziness, vomiting, stomach pain, nausea, rash, pruritus, urticaria and loose stools. Precautions taken to minimize risks associated with artesunate therapy will include administration of at least 20 mL of liquid beverage and a small snack under direct observation. Participants will be encouraged not to drive or operate machinery should they experience dizziness after taking artesunate. The following side effects are uncommon (between 1 in 1,000 and 1 in 100 patients treated): slow heart rate, mild gastrointestinal disturbances, abnormal liver function tests (high liver enzyme levels in blood), low counts of pre-stages of red blood cells (reticulocytopenia), and low white blood cell counts. Although artesunate is not usually recommended, reasons for this therapy to be used in the current protocol have been elaborated in Section 2.2.3. Artesunate has not been studied specifically in patients with special hereditary disorders of the blood called thalassaemia, sickle cell anemia and G6PD deficiency. Persons taking artesunate simultaneously with other drugs including amodiaquine and efavirenz are at risk for hepatotoxic are at risk for liver damage. Use of co-therapy with these medicines should be avoided.

Risks from blood drawing

Risks associated with blood drawing include small risks of bleeding, hematoma and infection. To minimize this risk, the skin is cleaned with alcohol before puncture; sterile, single-use needles and lancets will always be used; and pressure will be held at the puncture site after removal of the needle or lancet. Although the quantity of blood drawn would not lead to any ill effects on the participants' health, some adults feel faint with phlebotomy. This risk will be minimized by having trained technicians perform the procedure, and by placing participants in a recumbent position if they feel light headed or appear as if they are about to faint. Clinicians will be available for evaluation if there is any untoward effect.

Risks from PfSPZ Challenge injection at the injection site

Risks associated with PfSPZ Challenge injection include small risks of bleeding, hematoma and infection. To minimize this risk, the skin is cleaned with alcohol before injection; sterile, single-use needle and syringe will always be used; and pressure will be held at the puncture site after removal of the needle.

Free medical treatment will be provided to enrolled participants during the active vaccination phase and the surveillance period, at a level that meets the local country standards of medical diagnosis and treatment. Medical care for ailments not related to vaccination will not extend beyond the study follow-up period. Medical care for ailments related to vaccination will extend, at minimum, until the condition has resolved or stabilized.

Precautions taken to minimize risks associated with vaccination include a stock of medication and equipment to treat possible but unlikely allergic reactions and will be available in the vaccination area. A study doctor will be present to monitor the vaccination process and the participants for at least 30 minutes after vaccination.

2.3.1.2 Potential Risk of loss of confidentiality

There is a potential risk of loss of confidentiality to study participants. Private health information recorded on participant case report forms could theoretically become available to non-study personnel. To protect against this potential risk, the principal investigator will carefully monitor study procedures to protect the safety of participants and the quality of the data. Participant samples will be labeled with a participant ID number. The key to this number, and all private health information will be kept in a locked cabinet in a locked room that is accessible only to study personnel.

2.3.1.3 Risks to study personnel

The main risks to study personnel are from accidental exposure to blood and body fluid-borne infections. SOPs for staff safety are used in clinical and laboratory areas, including sharps management, hazardous waste management, etc. Universal precautions are used for handling all body fluids.

2.3.2 Known potential benefits

It is not known if this vaccine strategy will be effective. If it is effective, participants may directly benefit from development of protective immunity from malaria, which is present in their community. Participants will receive outpatient follow-up medical care at the Bougoula Hameau research clinic. Routine and emergency health care including hospitalization will be provided at the Sikasso District Hospital, in collaboration with District Hospital physicians.

If this research is successful and a safe and effective malaria vaccine is developed and licensed, the community of Bougoula Hameau, Sikasso District, Mali and the rest of the malaria-endemic world will benefit.

3 STUDY OBJECTIVES

3.1 Study Objectives

3.1.1 Primary objective

1. To assess the safety and tolerability of PfSPZ Challenge compared to placebo among malaria-experienced adults taking chloroquine prophylaxis (PfSPZ-CVac)

3.1.2 Secondary objectives

1. To assess the protective efficacy of PfSPZ-CVac against naturally transmitted *P. falciparum* malaria infection as diagnosed by thick blood smear microscopy
2. To assess protective efficacy of PfSPZ-CVac against naturally transmitted *P. falciparum* malaria infection as diagnosed by qPCR
3. To assess the expanded efficacy of PfSPZ-CVac compared to placebo
4. To examine the immune response to *P. falciparum* malaria infection

3.1.3 Exploratory objectives

1. To assess potential immune correlates of protection against infection by naturally transmitted *P. falciparum* malaria within 6 and 12 months after the last vaccination with PfSPZ-CVac
2. To measure strain-specific efficacy and vaccine selection for non-vaccine variant strains within 6 and 12 months after the last vaccination with PfSPZ-CVac
3. To determine if artesunate monotherapy selects for in vivo resistance to artesunate

3.2 Study Outcome Measures

3.2.1 Primary outcome measures

1. The number and severity of solicited local and systemic adverse events (AE) in the 12 days following PfSPZ challenge administration (day of vaccination and 11 subsequent days)
2. The number and severity of unsolicited AEs related to study product in the 12 days following PfSPZ challenge administration
3. The number of serious adverse events (SAEs) during the entire study period

3.2.2 Secondary outcome measures

1. Time to *P. falciparum* parasitemia, detected by thick blood smear microscopy, within six months after the last vaccination with PfSPZ-CVac
2. Time to *P. falciparum* parasitemia, detected by qPCR, within six months after the last vaccination with PfSPZ-CVac
3. Time to *P. falciparum* parasitemia, detected by thick blood film microscopy, within twelve months after the last vaccination with PfSPZ-CVac
4. Time to *P. falciparum* parasitemia, detected by qPCR, within twelve months after the last vaccination with PfSPZ-CVac
5. Antibody titers against *P. falciparum* circumsporozoite protein (CSP) and other *P. falciparum* proteins at serology time points; markers of cell-mediated immunity

3.2.3 Exploratory outcome measures

1. Correlation of antibody titers against *P. falciparum* proteins to time to first parasitemia by qPCR using Cox proportional hazards modeling
2. Correlation of antibody titers against *P. falciparum* proteins to time to first parasitemia by microscopy using Cox proportional hazards modeling
3. Genomic and genetic divergence between PfSPZ-CVac strain and post-vaccination *P. falciparum* infections
4. Malaria positivity by qPCR on the third day of artesunate administration
5. Post-vaccination PBMC Cytokine profiles
6. mRNA Gene expression profiles
7. Serologic antibody profiles against *P. falciparum* proteins measured by microarray

Exploratory outcomes 1, 2, 4, and 5 will be included in the clinical study report (CSR). Results of exploratory outcome measures 3, 6 and 7 are expected at a point in time later than CSR development and will be addended to the CSR if available.

4 STUDY DESIGN

4.1 Overview

- Single site, double blind, randomized, placebo controlled, Phase 1 study
- Study population includes 62 healthy, malaria-experienced adults aged 18-45 years, inclusive, residing in Bougoula Hameau and surrounding villages, Mali
- Participants will be recruited into a single cohort comprised of 62 participants
- Analysis of cardiovascular risk and safety evaluation including a screening electrocardiogram (ECG) will be performed at screening. Subjects with abnormal cardiovascular symptoms or findings will be referred to a cardiologist for further evaluation. A study investigator will review study ECGs.
- Participants will be given a single loading dose of chloroquine (600 mg chloroquine base) two days before the first vaccination followed by weekly prophylaxis (300 mg chloroquine base once weekly) so that chloroquine is given for a total of 10 weeks (10 doses), starting two days before the first vaccination. During vaccination weeks, chloroquine administration will continue to occur 2 days before injections.
- Participants will be randomized in a 1:1 ratio to receive either PfSPZ Challenge or 0.9% sodium chloride (NaCl) via direct intravenous inoculation (DVI) on a 0, 4 and 8 week schedule (study days 3, 31 and 59)
- The dose for the PfSPZ Challenge is 204,800 PfSPZ per dose
- One week after the last chloroquine administration, participants will be given a 7-day course of presumptive treatment with artesunate for malaria infection
- Safety oversight from an independent safety monitor and SMC
- Study monitoring delegated to DMID or DMID representative
- Screening will be done within 45 days before enrollment
- Vaccination schedule will be within a 5-day window of the assigned study vaccination days for second and third vaccinations

- Once the first vaccinations are complete and 12-day post-vaccination safety assessments are completed including safety laboratory results, the SMC will review safety data to determine if the study will proceed to administer dose 2 to participants.

- Data collection forms will serve as source documents. Only information that cannot be collected initially onto data collection forms (namely, clinical laboratory test results and AE medical records) will first be collected onto separate source documents before transcription into data collection forms. The information in the data collection form will then be entered directly into the Internet data system.

- Clinical laboratory evaluations will occur at the clinical laboratory onsite in Bougoula Hameau and/or at the MRTC's central laboratory in Bamako, Mali

- Immunogenicity evaluations will occur at the University of Maryland and/or at Sanaria Incorporated

- Exploratory analysis #2 will be conducted by the University of Maryland School of Medicine Institute for Global Health's Division of Malaria Research and other collaborating laboratories, and will include PCR analysis to determine the presence or absence of *P. falciparum* parasitemia, gene expression profiling of participants by microarray analysis, serological profiling against *P. falciparum* protein arrays, and genomic analysis of infecting strains of *P. falciparum*.

- Study duration will be up to 15 months per participant, and the entire clinical trial will last approximately 24 months

- Direct observation will be for 30 minutes following each PfSPZ Challenge injection and each artesunate and chloroquine administration

- 12-day surveillance (day of vaccination and 1, 3, 7 and 12 days after vaccination) for solicited AEs

- Surveillance for SAEs for the duration of the study follow-up period

- Active and passive case detection of AEs including clinical malaria and SAEs

- Active surveillance for malaria infection

- Follow-up of SAEs until resolution or stability; follow-up of AEs until resolution or stability or until the end of follow-up

- Participants will be followed every 28 days after presumptive treatment with artesunate for safety and for malaria surveillance

- For participants with malaria symptoms, thick blood smears will be read in real-time; those who test positive will be treated with standard antimalarial therapy (artesunate combination therapy as first line) and then continue to follow-up as scheduled. Participants without malaria symptoms will have blood smears read retrospectively for study endpoint determination and not for treatment decisions. As unplanned visits may occur, participants may have different numbers of unplanned visits but all have the same number of planned visits.

- The analysis of all primary outcome measures and for secondary outcome measures 1 and 2 will be conducted at a data-lock-point 24 weeks after the last artesunate dose is administered to participants, after which a Primary Analysis Report will be produced.

- Neither study staff responsible for participant follow-up and evaluation or study participants will be unblinded to the study treatment assignment prior to Study Day 245. However, it is acknowledged that the public release of the Primary Analysis Report may compromise the study blind because information in this report may reveal some treatment assignments.

- Data gathered during the second follow-up period (study weeks 36-60) will be reported in the clinical study report.

- At the end of the study (study week 60), participants will be informed regarding what intervention they received (PfSPZ challenge or saline placebo). This procedure is described in the study manual of procedures (MOP).

5 STUDY ENROLLMENT AND WITHDRAWAL

Site description

Participants for this study will be drawn from the population of healthy adults aged 18-45 years residing in Bougoula Hameau and environs in Mali (Figure 3). Bougoula is a suburban district of 6,900 inhabitants, located five kilometers from Sikasso town. It is located in the Sudanian zone dominated by savanna woodland with tall grass dotted with trees. The climate is under the influence of the humid forest zone with a rainy period up to six months or more. Here malaria transmission is seasonal with peak transmission occurring from May through November. The predominant ethnic groups are Senoufo, Samago, Mossi, Fulani and Bambara. The area is rural, with agriculture being the main economic activity. The Sikasso region receives more rain than any other Malian region and is known for fruits and vegetables.

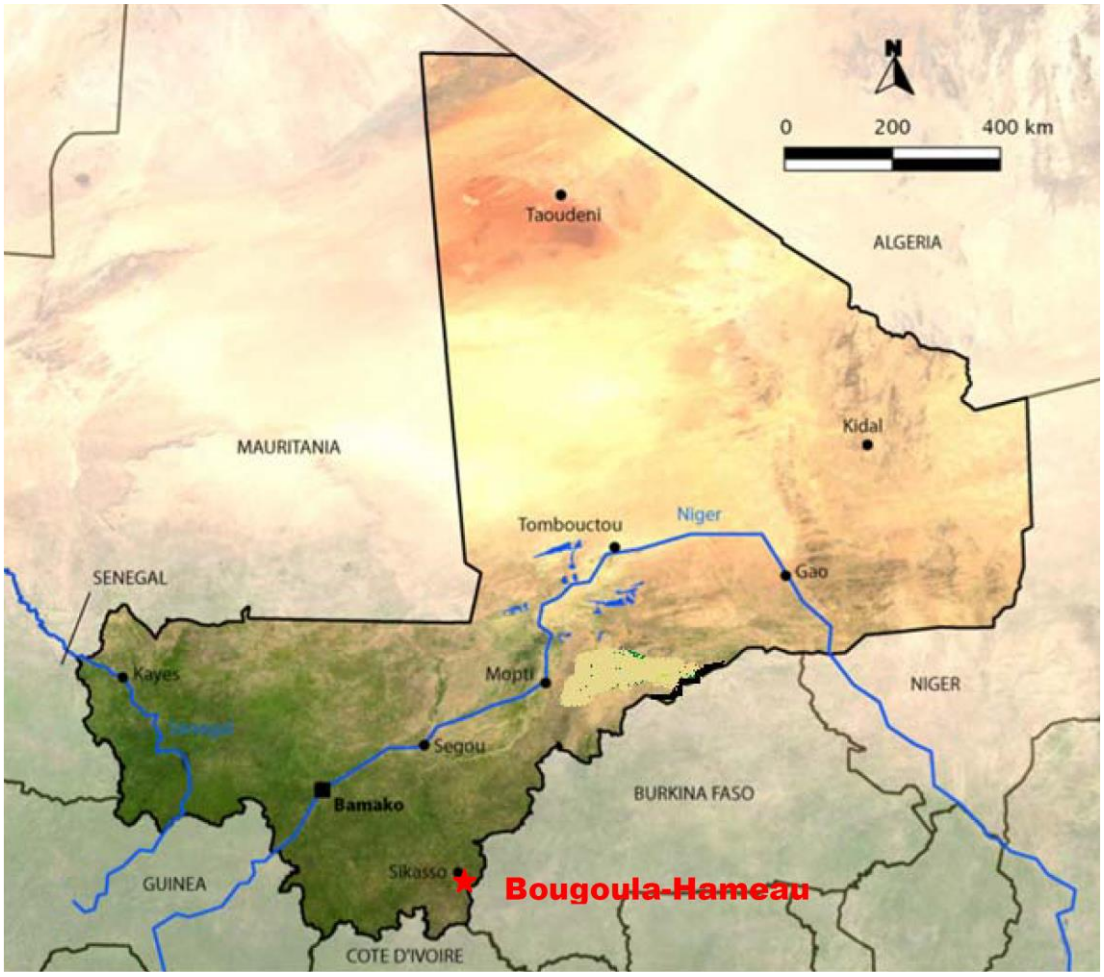

Figure 3 Map of Mali, West Africa

Availability of medical and preventive care

In the Sikasso district, a central district hospital and community clinics are staffed by nurses and midwives. Sikasso is a district located within a few hours' drive of the capital city Bamako where main reference referral hospitals are staffed by experienced physicians.

Insecticide treated nets

At the study site in Bougoula Hameau, Sikasso District, Mali, bed net coverage is estimated at 80%, and usage depends on the season. Treated bednets are distributed periodically by the government and other groups. The distribution of bednets is not consistent as it depends on private donors. Pregnant women are prioritized to

have one bednet each. They are also for sale in the private sector. As part of the informed consent process, study investigators will counsel potential participants to use bednets as a proven method to prevent malaria transmission.

5.1 Subject inclusion criteria

1. A male or non-pregnant female aged 18-45 years inclusive at the time of screening.
2. For women of childbearing potential, willingness not to become pregnant or breastfeed until one month after the last CQ dose[1]

   [1]Pre-menopausal female participants will be referred to the local family planning clinic, which offers several means of contraception that are approved and recommended by the Mali Ministry of Health. Contraception (male or female condoms, diaphragm or cervical cap with spermicide, intrauterine device, or hormone-based contraceptive) should be started 30 days before the first vaccination and continue until 30 days after last vaccination.
3. Written informed consent obtained from the participant before screening
4. Available and willing to participate in follow-up for the duration of study
5. Residing in Bougoula Hameau region and environs
6. In general good health based on clinical and laboratory investigation

5.2 Subject exclusion criteria

1. Previous vaccination with an investigational malaria vaccine
2. Use of an investigational or non-registered drug or vaccine other than the study vaccine(s) within 30 days before the first study vaccination, or planned use up to 30 days after last vaccination
3. Chronic administration (defined as more than 14 days) of immunosuppressants or other immune-modifying drugs within six months before the first vaccination[2]

   [2]This includes any dose level of oral steroids, but not inhaled steroids or topical steroids.
4. Planned administration/administration of a vaccine not foreseen by the study protocol within 30 days before the first study vaccination with the exception of tetanus toxoid
5. Confirmed or suspected immunosuppressive or immunodeficient condition

6. Confirmed or suspected autoimmune disease

7. History of allergic reactions or anaphylaxis to chloroquine, 4-aminoquinolone derivatives, artesunate and artemisinin derivatives, vaccinations or to any vaccine component

8. History of serious allergic reactions to any substance, requiring hospitalization or emergent medical care

9. History of allergy to any component of the PfSPZ Challenge product, including human serum albumin

10. Use or planned use of any drug with anti-malarial activity during the course of the study except for antimalarial medication administered by study clinicians

11. History of splenectomy

12. Confirmed pregnancy

13. Laboratory evidence of liver disease (ALT > upper limit of normal)

14. Laboratory evidence of renal disease (serum or plasma creatinine > upper limit of normal)

15. Laboratory evidence of hematologic disease (platelet count <114,000/mm^3 for males and <144,000/mm^3 for females, or hemoglobin <11.2 g/dL for males and <9.5 g/dL for females).

16. Seropositive for hepatitis B surface antigen or hepatitis C virus (hepatitis C antibody)

17. Seropositive for HIV

18. Sickle cell trait carriage or sickle cell disease

19. Administration of immunoglobulin and/or any blood products within the three months preceding the first study vaccination or planned administration during the study period.

20. Simultaneous participation in any other interventional clinical trial

21. Acute or chronic pulmonary, cardiovascular, hepatic, renal or neurological condition, severe malnutrition, or any other clinical findings that may increase the risk of participating in the study[3]

    [3] As determined by the PI

22. Has evidence of increased cardiovascular disease risk (defined as > 10%, 5 year risk) as determined by the method of Gaziano

[4] Risk factors include sex, age (years), systolic blood pressure (mm Hg), smoking status, body mass index (BMI, kg/mm^2), reported diabetes status, and blood pressure

23. Abnormal screening ECG[5]

[5] Pathologic Q wave and significant ST-T wave changes, left ventricular hypertrophy, non-sinus rhythm except isolated premature atrial or ventricular contractions, right or left bundle branch block, advanced A-V heart block (secondary or tertiary), QT/QTc interval >450 ms

24. Other condition that in the opinion of the PI would jeopardize the safety or rights of a participant in the trial or would render the participant unable to comply with the protocol

25. Documented history of non-febrile seizures or atypical (complex) febrile seizures

5.3 Treatment assignment procedures

5.3.1 Randomization procedures

Individual participants will be randomized to receive either PfSPZ Challenge or saline placebo without stratification. Participants will be randomized within a single cohort of 62 participants in a 1:1 ratio to receive the PfSPZ challenge or placebo via DVI, such that 31 participants will receive PfSPZ challenge and 31 participants will receive saline placebo on a 0, 4 and 8 week schedule (study days 3, 31 and 59). All participants will receive a standard chemoprophylactic regimen of CQ for ten weeks, followed a week later by a seven day regimen of artesunate. The vaccine (or placebo) as assigned during the first vaccination will be maintained for subsequent vaccinations. Randomization to either vaccine or placebo will be done online using the enrollment module of The Emmes Corporation's Advantage eClinical^{SM} electronic data capture system. The randomization codes will be included in the enrollment module for the trial. Each participant enrolled into the trial will be assigned a treatment code after demographic and eligibility data have been entered into the system. The study site will be provided with a treatment code list to be kept in a secure place with access permitted only to the unblinded pharmacist. Participants who receive the first vaccination will not be replaced. Participants who withdraw before the first vaccination may be replaced with newly randomized subjects. The independent safety monitor (ISM) will also keep one set of the randomization codes in a sealed envelope, for use in the event that emergency unblinding is required. The reason for any unblinding will be documented, as well as any steps taken to prevent further such unblinding. Participant ID numbers will be assigned to participants in the order in which they are enrolled in the trial. Backup randomization procedures and materials will be available and specified in the manual of procedures (MOP) in the event that the internet is unavailable.

5.3.2 Masking procedures

Measures will be taken to keep participants, clinical investigators and all other staff involved in measuring study outcomes blinded to treatment allocation. Masking procedures are described in the study MOP for randomization and vaccine preparation and administration. The PfSPZ Challenge product has a colorless appearance and will require injection using a needle and syringe. The saline placebo will appear as a colorless clear liquid of the same volume and administered in an identical-appearing syringe to the vaccine. The two products cannot be distinguished visually, by odor or consistency. Syringes will be prepared behind closed doors and labeled with the participant's identification number, then delivered to the vaccinator. This procedure will ensure that neither the participant nor the vaccinator will know if vaccine or placebo is given. The vaccine preparation and dilution staff will include the unblinded study site pharmacists with experience and training in PfSPZ Vaccine or PfSPZ Challenge preparation, and their role in the trial will be restricted to vaccine handling and preparation, with no role in post-vaccination assessments or follow-up of study participants.

Vaccinations will be carried out simultaneously in dedicated vaccination rooms near the vaccine preparation room. Vaccinators will be health care providers who are not involved with surveillance activities, such as in the assessment of AEs following vaccination. Each participant will be vaccinated in a closed room out of view of anyone other than the vaccinators.

The Statistician at The Emmes Corporation in the United States and the vaccine preparation staff will have the randomization list. Access to unsealed copies of the randomization list will be limited exclusively to the vaccine preparers and The Emmes Corporation statistician. These individuals will be unblinded and will not be involved in study participants' further evaluation.

5.3.3 Reasons for withdrawal

The following criteria will be checked before each vaccination. If any become applicable before completion of vaccinations, further vaccinations will not be administered but the participant will be followed for the duration of the study. If any become applicable during the study but after vaccinations are completed, the participant will not be required to discontinue the study, but a separate immunogenicity analysis may be done that excludes these individuals.

- Use of any investigational drug or vaccine other than the study vaccine(s) during the study period.
- Chronic administration (defined as more than 14 days) of any dose level of immunosuppressants or other immune-modifying drugs during the study period and chronic daily use of inhaled and topical steroids. Intermittent use of inhaled and topical steroids is allowed.
- Administration of a vaccine not foreseen by the study protocol during the period starting from 28 days before the first study vaccination and ending 28 days after the last vaccination.

- Administration of immunoglobulin and/or any blood products up to 28 days after the last scheduled study vaccination.

- Unresolved laboratory abnormalities that are deemed by the PI and/or the independent safety monitor (ISM) to be clinically significant. Transient laboratory abnormalities that have been documented to have returned to normal range before vaccination or which are not deemed clinically significant may not necessitate withdrawal, depending on the judgment of the PI in consultation with the ISM.

- Grade 3 hypersensitivity to chloroquine, artesunate or the PfSPZ Challenge study product

- Severe side effects following chloroquine or artesunate administration

The following criteria will be checked before each vaccination and are contraindications to further vaccination. However, the study participants will be encouraged to continue to participate in the surveillance schedule for safety and immunogenicity evaluation.

- Systemic hypersensitivity reaction (anaphylaxis) or other Grade 3 systemic adverse events considered related to study vaccination. Severe (i.e., Grade 3) local reactions will be evaluated to determine whether or not further study vaccinations should be administered. This will not be assessed before the first vaccination, but will be assessed before subsequent vaccination.

- Positive urine β-HCG. If a study participant becomes pregnant during the study, no further vaccinations or presumptive treatment for malaria (artesunate) will be given and the participant will be encouraged to continue to participate in the safety surveillance schedule. The pregnancy outcome will be followed by study clinicians and reported.

Subjects may also withdraw voluntarily from receiving the study intervention or from continuing study follow-up upon request for any reason.

The following AEs constitute contraindications to administration of vaccine at that point in time; if any one of these AEs occurs at the time scheduled for vaccination, the participant may be vaccinated at a later date, within the time window specified in the protocol, or withdrawn at the discretion of the investigator. The participant must be followed until resolution of the event, as with any AE.

- Acute disease at the time of vaccination. Acute disease is defined as the presence of a moderate or severe illness with or without fever. All vaccines can be administered, at the investigator's discretion, to persons with a minor illness such as mild diarrhea or mild upper respiratory infection with or without low grade fever, i.e., axillary temperature < 38.0°C

- Axillary temperature ≥ 38.0°C.

5.3.4 Handling of withdrawals

Every effort will be made to collect safety data on any participant discontinued from receipt of additional vaccinations because of an AE or SAE or any other reason by continuing the safety follow-up procedures. If administration of subsequent vaccinations occurs, the participant will be asked to continue scheduled evaluations and be given appropriate care under medical supervision until the symptoms of any AE resolve or the participant's condition becomes stable. If withdrawal occurs at a time when a participant would potentially develop malaria from the PfSPZ Challenge product (within 14 days after administration), terminal treatment with either a treatment course or a prophylactic course of antimalarial therapy will be given. If possible, subjects who leave the study area will be traced and visited by clinical investigators to collect safety follow-up data.

5.3.5 Termination of study

The trial may be suspended or terminated by DMID or by the PI due to development of serious laboratory toxicities or other major safety concern identified by the ISM. The trial may also be suspended by the IRBs if deemed necessary, and may be recommended for suspension by the SMC.

6 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

6.1 Study product description

PfSPZ Challenge

Sanaria Incorporated (Sanaria) has developed a method to produce *A. stephensi* mosquitoes infected with PfSPZ under aseptic conditions. In brief, this includes the production, under traditional environmental conditions, of eggs from a colony of *A. stephensi* mosquitoes housed in a controlled environmental chamber. Disinfection of eggs is initiated before the eggs are accepted into Sanaria's Clinical Manufacturing Facility (CMF). In the CMF, all procedures, materials and supplies are handled using aseptic methods to ensure that contaminating microorganisms are not introduced to and carried through the process. Surface disinfection of the eggs is continued by exposure to chemical agents in a Class II biological safety cabinet (BSC). Surface-disinfected eggs are inoculated into sterile, vented flasks containing aseptic growth medium. The eggs hatch and develop into pupae, which are transferred to an adult mosquito container where the adult mosquitoes emerge. These adult mosquitoes, which have been raised under aseptic conditions, are fed *P. falciparum* gametocyte-infected blood in a BSC in a High-Security Insectary. The *P. falciparum* gametocyte- infected blood has been produced from cultures of the Pf strain NF54 derived from a Working Cell Bank of the well-characterized *P. falciparum* strain NF54. Infected adult mosquitoes are reared until PfSPZ migrate to the salivary glands, and are maintained under aseptic conditions at all times. The investigational product, PfSPZ Challenge, is cryopreserved *P. falciparum* (Pf) (NF54) sporozoites (SPZ) (PfSPZ Challenge). PfSPZ Challenge contains fully infectious PfSPZ purified from the salivary glands of *Anopheles stephensi* mosquitoes raised under aseptic conditions.

Placebo

0.9% Sodium Chloride injection, USP (normal saline) will be used for the placebo.

Chloroquine (CQ)

Chloroquine phosphate is a 4-aminoquinolone, antimalarial agent for oral administration. It is active against the erythrocytic forms of *P. vivax, P. malariae,* and susceptible strains of *P. falciparum* (but not the sporozoite, liver or gametocyte stages of *P. falciparum*).

Artesunate

Artesunate is an artemisinin derivative with clinical efficacy against *Plasmodium falciparum.*

6.1.1 Acquisition

PfSPZ Challenge is developed, manufactured and provided by Sanaria. It is composed of *P. falciparum* NF54 strain sporozoites. All vaccine components (cryopreserved sporozoites, phosphate buffered saline, and human serum albumin) will be shipped through the DMID Clinical Agents Repository (DMID CAR, Fisher BioServices) under documented temperature-controlled conditions with temperature monitoring.

Sanaria, Inc, shipped samples of a similar product, PfSPZ Vaccine, cryopreserved in liquid vapor phase (LNVP) in dry shippers from its manufacturing facility in Rockville, Maryland to Navrongo, Ghana and then returned them to Rockville, Maryland. Potency assays performed in Navrongo and in Rockville upon the return found the product to be stable and retain potency. A similar shipping exercise was conducted in November 2009 from the Sanaria manufacturing facility in Rockville, Maryland to Ouagadougou, Burkina Faso, continuing out to the study site at Balonghin and then returning back to Rockville, Maryland. The investigational product remained below the accepted temperature threshold of -150°C, and assays performed upon return to Rockville have confirmed equivalent viability and potency of the shipped study product to non-shipped controls. Other experimental malaria vaccines and comparator vaccines have been successfully shipped by the University of Maryland to a remote area in Mali for four previous malaria vaccine trials.[23;24] Reliable supplies of liquid nitrogen are available in Bamako near the study site and liquid nitrogen storage and transport systems have been used to store and ship cryopreserved parasites and peripheral blood mononuclear cells.

The placebo (sterile normal saline-0.9% sodium chloride), artesunate and chloroquine will be supplied through the DMID CAR.

Artesunate 50mg tablets will be procured from Guilin Pharmaceutical Co. Ltd., Shanghai, China, a manufacturer prequalified by the World Health Organization.

The DMID CAR will oversee shipment of the all vaccine components, sterile normal saline, chloroquine, and artesunate to the research site upon request and approval by DMID.

6.1.2 Formulation, Packaging and Labeling

PfSPZ Challenge

The salivary glands from the PfSPZ-infected mosquitoes are removed by hand dissection. Salivary glands are then triturated to release the PfSPZ. The sporozoites are purified, counted, and, at a specified concentration, cryopreserved. PfSPZ are cryopreserved at different specified concentrations. Cryopreservation commences with the addition of cryoprotective additives to the purified PfSPZ Challenge Bulk Product to produce PfSPZ Challenge. PfSPZ Challenge is formulated into screw-cap vials containing either 15,000 PfSPZ in 20 μL, 50,000 PfSPZ in 20 μL, or 100,000 PfSPZ in 20 μL.

Phosphate buffered saline and human serum albumin diluent

The diluent for PfSPZ Challenge is composed of phosphate buffered saline (PBS) and 1% human serum albumin (HSA). PBS is manufactured by Sanaria in compliance with good manufacturing practice (GMP). Sanaria purchases HSA (25%) approved for parenteral, IV administration to humans. After thawing, PfSPZ Challenge will be diluted in a mixture of phosphate buffered saline (PBS) and human serum albumin (HSA) according to the protocol-specific MOP.

Sterile Normal Saline placebo

0.9% Sodium Chloride Injection, USP is a sterile, nonpyrogenic, isotonic solution of sodium chloride and water for injection (WFI) and will be used as the placebo. Each mL contains sodium chloride 9 mg. It contains no bacteriostatic, antimicrobial agent, or added buffer and may contain hydrochloric acid and/or sodium hydroxide for pH adjustment.

Chloroquine

Chloroquine phosphate is available as a 500 mg tablet for oral administration. Each 500 mg of chloroquine phosphate is the equivalent of 300 mg chloroquine base.

Artesunate

Artesunate is available as a 50mg tablet for oral administration. The tablets appear as white, round, scored and imprinted on both sides of the tablet; imprint AS and 50mg on opposite sides of the score.

6.1.3 Product storage and stability

PfSPZ Challenge will be stored at Sanaria Inc., Rockville, MD or SriSai Biopharmaceuticals, Frederick, MD in LNVP at -150°C to -196°C until it is shipped in conjunction with the DMID Clinical Agents Repository (DMID CAR, Fisher BioServices) for use in this study. Shipping will be performed in accordance with all FDA, U.S.

Department of Transportation, and United Nations transport guidelines. Upon arrival in Mali, the PfSPZ Challenge will be held in the transportation dry shipper and the shipper stored with appropriate security and monitoring. The storage temperature and refrigerant levels of the cryostorage tank will also be monitored. Specifics of study product receipt, monitoring, release and site notification will be included in the protocol-specific MOP.

Unused PfSPZ Challenge cryovials retained in the dry shipper will be returned to Sanaria.

Human serum albumin and phosphate-buffered saline used in PfSPZ Challenge dilution and normal saline used for placebo will be stored according to the manufacturer's instructions. Details of storage and transfer of these products will be outlined in the protocol-specific MOP.

Artesunate and chloroquine tablets must be stored at controlled room temperature (15-30°C) and should be kept away from light.

Any cold chain deviation for the vaccine will be recorded and reported as per DMID SOPs.

6.2 Dosage, Preparation and Administration of Study Investigational Product

Study vaccine will be prepared by the unblinded vaccine preparation and dilution staff, including the site pharmacist, on the day of vaccination. The PfSPZ Challenge will be thawed and diluted with the PBS containing HSA vaccine diluent for dose administration. The dose will be prepared using an appropriately sized syringe and must be administered within 30 minutes of thawing. The placebo volume will be drawn using the same volume as the vaccine dose. Further details regarding dilution and syringe preparation are included in the protocol-specific Manual of Procedures (MOP).

6.2.1 Administration of vaccine and placebo

Vaccine and placebo will be administered by blinded study staff not involved with post-vaccination participant evaluation. Each dose of PfSPZ Challenge and placebo will be administered by DVI into a vein in the participant's arm or hand using a needle and syringe according to the study schedule.

6.2.2 Dosing and Administration of Chloroquine

Loading Dose

Chloroquine 600 mg base will be orally administered as a single loading dose (as two- 500 mg tablets [equivalent to 300 mg base per tablet for a total of 600 mg base]) to the participant by the study staff via directly observed therapy on study day 1.

Weekly Dose

Subsequent doses of 300 mg chloroquine base will be given weekly as a single dose (one- 500 mg tablet [equivalent to 300 mg base per tablet]) for ten weeks. Doses will be given in a single calendar day either all at once or individually within one hour under direct observation with at least 20 mL of liquid beverage to ensure compliance. Tablets that make up a single dose may be consumed at once or in any combination such that tablets are consumed in a single calendar day. Participants will be observed for 30 minutes following the last chloroquine tablet administration to ensure that drug is not vomited. If vomiting occurs within 30 minutes of administration, dosing of the product consumed within 30 minutes before vomiting will be repeated up to two additional times.

6.2.3 Dosing and Administration of Artesunate

Artesunate tablets will be orally administered to the participant by the study staff via directly observed therapy according to the study schedule. Four tablets of 50 mg of artesunate each, totaling 200 mg, will be given in a single calendar day either all at once or individually within one hour under direct observation with at least 20 mL of liquid beverage to ensure compliance. Tablets may be consumed at once or in any combination such that four tablets are consumed in a single calendar day. Participants will be observed for 30 minutes following the last artesunate tablet administration to ensure that drug is not vomited. If vomiting occurs within 30 minutes of administration, dosing of the product consumed within 30 minutes before vomiting will be repeated up to two additional times.

6.3 Modification of Study Investigational Product for a Participant

Dosing of PfSPZ Challenge, chloroquine or artesunate will not be adjusted due to toxicity or other reasons. If participant experiences toxicity related to PfSPZ Challenge, chloroquine or artesunate, then the investigators and the sponsor, in consultation with the independent safety monitor, will determine if subsequent vaccinations, chloroquine or artesunate therapy should be given. If a participant experiences an SAE deemed to be related to PfSPZ Challenge, they will not receive subsequent injections.

6.4 Accountability Procedures for the Study Investigational Product

The Site Principal Investigator (PI) is responsible for the distribution and disposition of study product, and has ultimate responsibility for accountability. The Site PI may delegate to the Site Research Pharmacist responsibility for study product accountability. The Site Research Pharmacist will be responsible for maintaining complete records and documentation of study product receipt, accountability, dispensation, temperature monitoring, storage conditions, and final disposition of the study product. All study products, whether administered or not, must be documented on the appropriate study product accountability record or dispensing log. Unused study product will be retained as per DMID requirements.

Upon completion of the study and after the final monitoring visit, any remaining unused study product will either be returned or destroyed appropriately at the clinical site as per sponsor requirements and instructions, or in accordance with disposition plans. Further details regarding final accountability and disposition of used and unused study product are included in the protocol-specific MOP.

6.5 Concomitant Medications/Treatments

At each study visit/contact, investigators will question the participant about any medication taken, including traditional, herbal, supplements and over-the-counter medicines. Concomitant medication, including any vaccine other than the study vaccines, including any specifically contraindicated or administered during the period starting from 30 days before study start and ending at the end of the study follow-up period will be recorded with trade name and/or generic name of the medication, medical indication, start and end dates of treatment.

Medications that are not permitted during the follow-up period include those with any antimalarial activity, except as planned for presumptive treatment before the first dose of vaccine, after the last dose of vaccine, and unless otherwise prescribed by a clinician as needed for treatment of clinical malaria and/or other indications. These medications include, but are not limited to: chloroquine, sulfadoxine-pyrimethamine, trimethoprim-sulfamethoxazole, azithromycin, amodiaquine, artemether-lumefantrine and other artesunate combination therapies, halofantrine, quinine, doxycycline, mefloquine, primaquine and atovaquone/proguanil. These medications will be prescribed by study clinicians when indicated and adjustments will be made as needed for analyses of time period under risk for malaria infection in the statistical analysis plan.

7 STUDY SCHEDULE

7.1 Screening

Recruitment will be progressive until 62 adults of either gender who fulfill the inclusion criteria are enrolled. Participants will be recruited by non-coercive methods among adults aged 18-45 years residing near the study site. After community information is disseminated as described below, all interested, potentially eligible participants will be invited to visit the study clinic on a specific date. After the study has been explained to the potential participants they will be provided with a copy of the consent form and may leave and return later with their decision; this will allow time for them to discuss the study with their family and carefully consider their involvement in the study. The individual consent process will be conducted in private areas to ensure confidentiality, to reduce the likelihood of other participants influencing their decision, and to allow further time to make a final decision. A single informed consent document will be used for screening and other study procedures. Only participants who receive chloroquine are considered enrolled in the study.

All screening tests, medical history and examinations will be performed only after study consent is obtained. If an individual's medical chart or results diagnostic tests performed as part of an individual's medical care are going to be used for screening, written informed consent must be obtained before review of that information. Study clinicians will generally handle acute, simple conditions such as malaria or other acute infections. More complicated or chronic conditions, such as chronic renal or heart disease, will be referred to appropriate sources of medical care.

A 12-lead ECG will be done and analysis of five year cardiovascular event risk will be performed, based on the method published by Gaziano. The risk factors assessed will include sex, age, body mass index, blood pressure, history of diabetes mellitus, and history of smoking.

An eligibility checklist will be prepared for each participant and will later become part of the source document for participants enrolled in the vaccine trial. A unique identification number will be assigned to each study participant. A medical history will be taken with special attention to recurrent infections to suggest immune suppression, previous history of splenectomy and prior vaccine reactions. Concomitant medications will be documented. Physical examination and laboratory screening tests will include: complete blood count (CBC), creatinine, ALT, hepatitis B surface antigen testing, hepatitis C antibody testing, HIV testing, sickle cell testing, and urine pregnancy test for women of childbearing potential. Unused blood from screening tests of all potential and enrolled participants may be preserved for parasite genotyping. A participant who meets any of the exclusion criteria will be excluded. Participants excluded from this study because of significant abnormalities will be managed initially by study clinicians and referred to the local health center for evaluation as necessary. Screening tests will be completed within 45 days before the enrollment. Laboratory studies may be conducted at other times during the course of the trial if the investigators judge it necessary for the safety of the participant. Screening and follow-up diagnostic laboratory testing will be performed at the clinical laboratory at the study site and at a local reference laboratory if needed. Recruitment will continue until 62 eligible

participants have fulfilled all of the inclusion criteria and none of the exclusion criteria, and signed the study consent form. Should a participant change his/her mind and decline to participate before vaccination, additional participants may be screened until 62 participants are enrolled. A list of eligible participants will be generated and used to identify the participants eligible for vaccination. After the first vaccination doses are administered to the first 62 participants, no further participants will be added to the study even if a participant is unable to complete the planned vaccination schedule. If a participant does not proceed from enrollment to first vaccination, they may be replaced with another eligible participant after criteria for inclusion and exclusion have been met.

7.2 Enrollment/Baseline and Subsequent Vaccination Visits

Participants will be enrolled into the study on study day 1, at which time a loading dose of chloroquine will be given. At enrollment, each study participant will receive an ID card.

On study day 3, participants will be randomized to a study treatment assignment to receive either PfSPZ Challenge or saline placebo. The study treatment assignment that is given during the first vaccination will be maintained for subsequent vaccinations.

The day before, or the day of each vaccination, criteria for continued eligibility will be reviewed and verified using an eligibility checklist and targeted physical exam with documentation of vital signs (axillary temperature, blood pressure, pulse, respiratory rate). Venous blood will be collected for laboratory analysis and for baseline safety before vaccination, but results are not necessarily resulted and reviewed before vaccination. Also on the day before, or the day of each vaccination, female participants of childbearing potential (defined as women with no history of surgical sterilization and who have had menses within the previous 2 years) will undergo urine pregnancy testing and must have documentation of a negative urine pregnancy test before being vaccinated.

The following criteria will be reviewed using a checklist before each vaccination. If any become applicable before completion of vaccinations, further vaccinations will not be administered but the participant will be followed for the duration of the study.

- Use of any investigational drug or vaccine other than the study vaccine(s) during the study period.
- Chronic administration (defined as more than 14 days) of any dose level of immunosuppressants or other immune-modifying drugs during the study period and chronic daily use of inhaled steroids. Intermittent use of inhaled and topical steroids is allowed.
- Administration of a vaccine not foreseen by the study protocol during the period starting from 28 days before the first study vaccination and ending 28 days after the last vaccination.

- Administration of immunoglobulin and/or any blood products up to 28 days after the last study vaccination.

- Unresolved laboratory abnormalities that are deemed by the PI and/or the ISM to be clinically significant. Transient laboratory abnormalities that have been documented to have returned to the site normal range before vaccination may not necessitate withdrawal, depending on the judgment of the PI in consultation with the ISM.

The following criteria will be checked using a checklist before each vaccination and are contraindications to further vaccination. However, the study participants will be encouraged to continue to participate in the surveillance schedule for safety and immunogenicity evaluation.

- Systemic hypersensitivity reaction (anaphylaxis) or other Grade 3 systemic adverse events considered related to study vaccination. Severe (i.e., Grade 3) local reactions will be evaluated to determine whether or not further study vaccinations should be administered. This will not be assessed before the first vaccination, but will be assessed before subsequent vaccination.

- Positive urine β-HCG. If a female participant becomes pregnant during the study, no further vaccinations or presumptive malaria treatment (artesunate) will be given and the participant will be followed in the safety surveillance schedule. The pregnancy outcome will be followed by study clinicians and reported.

The following AEs constitute contraindications to administration of vaccine at that point in time; if any one of these AEs occurs at the time scheduled for vaccination, the participant may be vaccinated at a later date, within the time window specified in the protocol, or withdrawn at the discretion of the investigator. The participant must be followed until resolution of the event, as with any AE.

- Acute disease at the time of vaccination. (Acute disease is defined as the presence of a moderate or severe illness with or without fever). All vaccines can be administered, at the investigator's discretion, to persons with a minor illness such as diarrhea, mild upper respiratory infection with or without low grade fever, i.e., axillary temperature < 38.0°C

- Axillary temperature ≥ 38.0°C.

After the above procedures are completed and the participant's identity is checked by comparing his/her name with the list of eligible participants, he/she will be vaccinated according to the vaccine administration SOP. Vaccination will be done on study days 3, 31 and 59.

After each vaccination, participants will be observed for local and systemic reactions for a minimum of 30 minutes. Physicians trained in cardiopulmonary resuscitation will be present on site on vaccination days. Signs and symptoms will be solicited from participants and recorded by the investigators, and vital signs including

pulse, respiratory rate, blood pressure, and axillary temperature will be noted. Participants will then be followed for a 12-day surveillance period after each vaccination to record solicited and unsolicited AEs during planned visits at 1, 3, 7 and 12 days after vaccination and unplanned visits occurring on any day after vaccination. All AEs occurring during the study follow-up period will be followed as indicated until resolution of the AE or until the end of the study follow-up period.

Details of the schedule of events, including post-vaccination safety and immunogenicity laboratory analyses, are included in the Study Schedule (Table 1).

7.3 Follow-up

After the assigned date of the last artesunate dose (day 77), participants will be followed every four weeks for follow-up. A targeted clinical examination will be performed as needed and information on any symptoms since the last visit will be collected. A blood sample will be collected during the course of these follow-up visits. AEs will be recorded for the entire length of follow-up for study participants. Every effort will be made to ensure compliance with visits. If a participant does not appear for a scheduled clinic visit, a study site staff member will visit him/her and accompany the participant to the clinic center if desired. If an SAE has occurred, appropriate measures will be taken to notify the PI, independent safety monitor, DMID, and all IRBs per protocol.

Details of the schedule of events, including laboratory analyses, are included in the summary of study procedures.

7.4 Final Study Visit

The final study visit should occur 48 weeks after presumptive treatment with artesunate (study day 413). Any AEs or SAEs that are unresolved at that time will be continue to be followed until resolution, or, if a chronic condition lasting >8 weeks has developed, until the end of the study follow-up period.

Evaluations to be done during the final study visit are listed in the daily study procedures and in the summary of study procedures. Procedures include the following:

- Brief medical history
- Concomitant medication documentation
- Record blood pressure, pulse and axillary temperature
- Targeted physical examination if needed
- Record any unsolicited AEs occurring since the last visit
- Collect blood for malaria smear, parasite genotyping, DBS, antibody assays, and CMI and gene expression analyses

7.5 Early Termination Visit

If a participant wishes to end their participation early and is willing to have evaluations performed, a physical examination should be done and 7-20 mL venous blood may be drawn for CBC, creatinine, and ALT determination, malaria blood smear, dried blood spot and/or for immunology assays. Presumptive malaria treatment (artesunate) will not be given to participants who end their participation early.

Investigators will make every effort to continue follow-up visits for any participant who has received one or more vaccinations for the duration of the study even if it is determined that subsequent vaccination should not be administered, including participants who become pregnant. Participants who do not receive subsequent vaccinations will not be replaced by new participants.

7.6 Unscheduled Visit

Unscheduled visits will prompt a history and targeted physical examination when indicated, clinical laboratory tests including malaria smear if indicated, documentation of any AEs, and any other medically indicated diagnostic or therapeutic procedures. These visits will be recorded as observations in the participant's study record.

7.6.1 Active surveillance

Active surveillance to detect AEs will consist of the scheduled visits during the vaccination phase of the trial with additional scheduled visits every four weeks (28 days) over the entire follow-up period for clinical assessment, parasite genotyping and malaria smear collection.

Consistent with standard clinical practice in settings with high prevalence of asymptomatic malaria infection, routine scheduled malaria smears will not be read immediately unless symptoms are present. Asymptomatic infections will therefore only be detected retrospectively. Should symptoms suggestive of malaria be identified during scheduled visits, participants will undergo the same full assessment given to self-referred participants who present to the clinic due to illness. Whenever a malaria smear is performed filter paper and venous blood samples will be collected for PCR diagnosis of malaria, which will be performed retrospectively at the University of Maryland.

Participants who present with fever will be evaluated for malaria and other potential etiologies based on their symptoms and clinical judgment of the investigators and clinical staff. Guidelines for evaluation and management of fever in adults have been established by the Mali Ministry of Health and will be followed.

7.6.2 Passive surveillance

For the efficacy endpoint, passive surveillance will consist of continuous availability of free, expeditious, high quality medical care at the research clinic, including rapid microscopic diagnosis of malaria. All study participants will reside within close distance to the study clinic. The outpatient research clinic will be staffed by study staff 7 days a week, and the study physicians will be on call 24 hours a day, seven days a week to assess cases of severe malaria or other medical emergencies. When a study participant presents at any time when the outpatient research clinic is closed, a guard will promptly contact a research physician. Whenever a malaria smear is performed a DBS sample will be collected for PCR diagnosis of malaria and studies of parasite genotyping, which will be performed retrospectively at the University of Maryland.

8 STUDY PROCEDURES/EVALUATIONS

8.1 Daily study procedures

Day -45 to –1 Screening /inclusion of participants

Before screening, meetings will be held with Sikasso District administrative and medical authorities to explain the purpose of the study. These meetings will be followed by meetings with the traditional authorities and community leaders to request community-level “permission to enter.” The target population will be invited for screening as described in participant recruitment SOPs. Screening will be performed until 62 eligible participants are identified.

Screening (may take place over more than one visit) up to 45 days before enrollment

- Written informed consent
- Participant identification number assigned
- Medical history of participant
- Concomitant medication documentation
- Complete physical examination and vital signs (pulse, respiratory rate, blood pressure, axillary temperature)
- A 12-lead ECG will be done and analysis of five year cardiovascular event risk will be performed, based on the method published by Gaziano. The risk factors assessed will include sex, age, body mass index, blood pressure, history of diabetes mellitus, and history of smoking.
- Collect venous blood sample for:
    - Hematology: CBC (hemoglobin, WBC, platelets)
    - Biochemistry: serum creatinine, and ALT
    - Serology: hepatitis B, hepatitis C, HIV
    - Sickle cell testing: hemoglobin electrophoresis or high performance liquid chromatography (HPLC)
- Collect urine for females of childbearing potential: pregnancy test
- Check of inclusion and exclusion criteria

Day 1 Enrollment and chloroquine administration

- Review inclusion/exclusion criteria
- Concomitant medication documentation
- Enroll persons meeting inclusion and exclusion criteria
    - Administer chloroquine oral therapy (600-620mg chloroquine base); observe for 30 minutes after administration
- Prepare a participant ID card

- Record any SAEs during the entire study period

Day 3: Vaccination 1

Before vaccination:

- Urine pregnancy test for females of childbearing potential
- Review criteria checklist for immunizations and check of contraindications/precautions
- Concomitant medication documentation
- Record any complaints or new medical history, targeted physical examination
- Record vital signs: axillary temperature, blood pressure, pulse, respiratory rate
- Record baseline data for solicited general symptoms
- Collect venous blood sample for:
    - CBC, creatinine, and ALT
    - Antibody assays
    - Cell-mediated immune responses (CMI), including PBMC
    - Gene expression analyses
    - Malaria smear, parasite genotyping and DBS
- Enrolled subjects will be randomly assigned to receive either PfSPZ Challenge or saline placebo
- Administer study vaccination 1 and ask participants immediately to subjectively assess pain after immunization (no pain, mild pain, moderate pain, or severe pain)

After vaccination:

- Observe for a minimum of 30 minutes
- Examination of the vaccination site(s) for any abnormalities.
- Record blood pressure, pulse, respiratory rate, axillary temperature
- Record solicited and unsolicited AEs
- Instruct participants to return to the research clinic immediately should they manifest any signs or symptoms they perceive as concerning or serious
- Record any SAEs during the entire study period

Days 4 and 6: Post-vaccination surveillance visits (also study days 32, 34, 60 and 62)

- Brief medical history
- Concomitant medication documentation
- Record blood pressure, pulse respiratory rate and axillary temperature
- Record solicited and unsolicited AEs
- Targeted physical examination including vaccination site
- Record any SAEs during the entire study period

Day 8: 5 days post-vaccination surveillance visit (also study days 15, 22, 29, 36, 43, 50, 57, and 64)

- Brief medical history
- Concomitant medication history
- Administer chloroquine oral therapy (300-310 mg chloroquine base); observe for 30 minutes after administration
- Record unsolicited AEs
- Record solicited AEs (only for 12 days after injection)
- Record any SAEs during the entire study period

Day 10: 7 days post-vaccination surveillance visit (also study days 38 and 66)

- Brief medical history
- Concomitant medication documentation
- Record blood pressure, pulse, respiratory rate and axillary temperature
- Targeted physical examination including vaccination site
- Record any solicited and unsolicited AEs occurring since the last visit
- Record any SAEs during the entire study period

Day 15: 12 days post-vaccination surveillance visit (also study days 43 and 71)

- Brief medical history
- Concomitant medication documentation
- Record blood pressure, pulse, respiratory rate and axillary temperature
- Targeted physical examination including vaccination site
- Record any solicited and unsolicited AEs occurring since the last visit
- Collect venous blood for CBC, creatinine, ALT, antibody assays, CMI and gene expression (day 15 only)
- Administer chloroquine oral therapy (300-310 mg chloroquine base); observe for 30 minutes after administration (study days 15 and 43 only)
- Record any SAEs during the entire study period

Subsequent vaccination visits (Study days 31 and 59)

Before vaccination:

- Check participant's ID to confirm identity
- Brief medical history
- Concomitant medication documentation
- Targeted physical examination as indicated
- Check of contraindications/precautions
- Record vital signs: axillary temperature, blood pressure, pulse, respiratory rate
- Record unsolicited AEs occurring since last visit

- Record baseline data for solicited general symptoms
- Perform urine pregnancy testing for female participants of childbearing potential
- Collect venous blood sample for antibody assays, malaria smear, parasite genotyping, DBS, CBC, creatinine, ALT, CMI and gene expression
- Administer study vaccination and ask participants immediately to subjectively assess pain after immunization (no pain, mild pain, moderate pain, or severe pain)
- Record any SAEs during the entire study period

Administer study vaccination

After vaccination:

- Observe for at least 30 minutes
- Examination of the vaccination site for any abnormalities
- Record blood pressure, pulse, respiratory rate, axillary temperature
- Record solicited and unsolicited AEs
- Instruct participants to return to the research clinic immediately should they manifest any signs or symptoms they perceive as serious or concerning
- Record any SAEs during the entire study period

Days 71-77: Artesunate therapy administration

- Brief medical history
- Concomitant medication documentation
- Record any unsolicited AEs occurring since the last visit
- Targeted physical examination as indicated
- Presumptive malaria treatment: Administer artesunate oral therapy (200mg/day) for seven days; observe for 30 minutes after each administration
- Concomitant medication documentation
- Collect blood for malaria smear, parasite genotyping and DBS on days 71, 72 and 73 only
- Record any SAEs during the entire study period

Day 87: Immunogenicity assessment

- Brief medical history
- Concomitant medication documentation
- Record blood pressure, pulse, respiratory rate and axillary temperature
- Targeted physical examination as indicated

- Record any unsolicited AEs occurring since the last visit
- Collect blood for malaria smear, parasite genotyping and DBS
- Collect venous blood sample for antibody assays, CMI and gene expression
- Record any SAEs during the entire study period

Days 105, 133, 161, 189, 217, 245, 273, 301, 329, 357, 385, 413: Post-last vaccination visits (approximately every 28 days)

- Brief medical history
- Concomitant medication documentation
- Record blood pressure, pulse, respiratory rate and axillary temperature
- Targeted physical examination as indicated
- Record any unsolicited AEs occurring since the last visit
- Collect blood for malaria smear, parasite genotyping and DBS
- Record any SAEs during the entire study period
- Additional testing on days 245 and 413:
    - Collect venous blood sample for antibody assays, CMI and gene expression

Table 1. Summary of Study Procedures

Study Days	**-45 to –1 Screen**	**1**	**3**	**4,6**	**8**	**10**	**15**	**22**	**29**	**31**	**32, 34**	**36**	**38**	**43**	**50**	**57**	**59**	**60, 62**	**64**	**66**	**71-77**	**87**	**105,133, 161,189, 217**	**245**	**273,301, 329,357, 385**	**413**
Visit Window (± number of days)		**2**	**2**	**1**	**2**	**3**	**2**	**2**	**2**	**5**	**1**	**2**	**3**	**2**	**2**	**2**	**5**	**1**	**2**	**3**	**1**	**3**	**7**	**7**	**7**	**7**
Visit Number	**00**	**1**	**02**	**03, 04**	**05**	**06**	**07**	**08**	**09**	**10**	**11, 12**	**13**	**14**	**15**	**16**	**17**	**18**	**19, 20**	**21**	**22**	**23-29**	**30**	**31-35**	**36**	**37-41**	**42**
Village & family information & discussion	x																									
Written individual Study Consent	x																									
Check of inclusion/exclusion criteria	x	x	x																							
Enrollment		x																								
Administration of oral chloroquine		x			x		x	x	x			x		x	x	x			x							
Administration of oral artesunate																					x					
Check of contraindications to vaccination			x							x							x									
Medical history	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	x		x	x		x	x			x	x		x	x			x	x		x		x	x	x	x	x
Physical examination (complete), ECG	x																									
Physical examination (targeted)			x	x		x	x			x	x		x	x			x	x		x	x	x	x	x	x	x
Vaccination			x							x							x									
Post-vaccination recording of solicited AE			x	x	x	x	x			x	x	x	x	x			x	x	x	x	x*					
Recording of unsolicited AE			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Recording of concomitant medication	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
Recording of SAEs during the study period		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
Urine pregnancy test for female participants	x		x							x							x									
Complete blood count 3ml	x		x				x			x				x			x				x*					
Serum chemistry (Creatinine and ALT) 3ml	x		x				x			x				x			x				x*					
Hepatitis B & C, HIV, sickle cell testing 9ml	x																									
Serum for antibody assays 5ml			x				x			x				x			x				x*	x		x		x
CMI and gene expression 25ml			x				x			x							x					x		x		x
Malaria smear, genotyping, and DBS 2ml			x							x							x				x†	x	x	x	x	x
Scheduled blood volume (mL)	15		38				36			38				11			38				17	32	10	32	10	32
Cumulative Blood Volume (mL)	15		53				89			127				138			176				193	225	235	267	277	309

*Study Day 71 only; †Study Days 71, 72 and 73 only

8.2 Clinical Evaluations

8.2.1 Medical History

A medical history from childhood onward will be taken with special attention to 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 hematology.

8.2.2 Medications History

As part of the medical history, a medications history for the past 30 days will be taken with special attention to immunosuppressive medications including corticosteroids. Participants will be queried about medications prescribed by a clinician, over-the-counter medications, and any homeopathic or traditional medications. These medications will be reviewed before enrollment and throughout the study with special attention for prohibited medications.

8.2.3 Electrocardiogram (ECG)

As part of the screening process, an ECG will be performed for potential participants and read by study physicians. Participants with the following ECG findings will be excluded: pathologic Q wave and significant ST-T wave changes, left ventricular hypertrophy, non-sinus rhythm except isolated premature atrial or ventricular contractions, right or left bundle branch block, advanced A-V heart block (secondary or tertiary), QT/QTc interval >450 ms.

8.2.4 Physical Examination

As part of the screening process, a physical examination will be performed by study clinicians. Vital signs, including axillary temperature, blood pressure, respiratory rate and pulse, will be assessed with a complete physical examination. Organ systems assessed as part of the physical examination include General Appearance, Eyes, Ears/Nose and Throat, Respiratory, Cardiovascular, Gastrointestinal, Genitourinary, Skin, Lymphatic, Extremities, Musculoskeletal, and Neurological. Subsequent physical examinations will be 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.

8.2.5 Reactogenicity Assessments

Study participants will be evaluated for solicited and unsolicited systemic and local reactogenicity to PfSPZ Challenge at 1, 3, 7 and 12 days after vaccination and during unscheduled visits up to 12 days after vaccination. Solicited local reactogenicity assessments include injection site pain, tenderness, erythema/redness, bruising and swelling. Solicited systemic reactogenicity assessments are, for vital signs: fever, tachycardia, bradycardia, systolic hypertension, systolic hypotension, and tachypnea; and for systemic symptoms: malaise, nausea, arthralgia (joint pain), myalgia (muscle pain), chills, and headache; solicited laboratory reactogenicity assessments include hemoglobin, white blood cells, platelets, alanine aminotransferase and creatinine. The rating scale used for solicited reactogenicity assessments is as noted in Section 9 Assessment of Safety.

Unsolicited AEs will be documented as related, probably related, possibly related, unlikely related, or not related to vaccination. Grading of these AEs will be according to the grading system for AEs in Section 9 Assessment of Safety.

8.2.6 Health care provision

Routine and emergency health care will be provided by clinicians at the research clinic and Sikasso District Hospital. The clinical research facility includes private consultation rooms, a procedure room, a resuscitation suite with oxygen, suction and resuscitation kits, and a post-vaccination observation room. The pharmacy at the research clinic will have sufficient stocks to provide participants with oral and parenteral drugs for the treatment of common illnesses (including uncomplicated and severe malaria) free of charge, using essential medicines and treatment regimens that meet or exceed standards recommended by the Mali Ministry of Health. Blood transfusion will be available, and twenty-four hour hospitalization and basic emergency surgery services are available at the Regional Hospital located in Sikasso. Twenty-four hour nursing staff and on call physicians will be available.

If the investigators or the independent safety monitor judge that a participant requires hospitalization at the National tertiary referral hospital in Bamako (approximately 3 hours by car from the study site), referral and transportation will be arranged and the medical management of the participants will be monitored by senior physician investigators and/or the independent safety monitor. The regional hospital in Sikasso, a 20-minute drive from the study site, has laboratory, surgical capabilities, radiography, medical subspecialty care, an intensive care unit with mechanical ventilation, and a computerized axial tomography scanning facility. The National tertiary referral hospital in Bamako has these capacities and in addition has other advanced medical and surgical care.

Cases of clinical malaria will be managed according to Mali National Malaria Control guidelines and the study SOPs.

8.3 Laboratory Evaluations

The Investigators will maintain detailed SOPs for all laboratory assays at the study site and central laboratories at study center headquarters in Bamako. These SOPs will include sample collection, handling (e.g. serum separation), labeling, preservation (e.g. PBMC cryopreservation), storage, transport, and shipping. All staff and investigators will be trained in the SOPs relevant to their duties and sign copies of the SOPs to document this training. Copies of SOPs will be available for inspection and review by study monitors. The general methods that will be used are summarized in the following section.

8.3.1 Clinical Laboratory Evaluations

Routine safety laboratory testing for CBC, creatinine, and ALT will be performed at the clinical laboratory at the Bougoula Hameau site, with back-up testing available at the Malaria Research and Training Center clinical laboratory in Bamako if needed. Results will be entered into the study database. Screening laboratory values will serve as the basis for initial and ongoing eligibility for vaccinations. If baseline clinical labs (Visit 02—Pre-injection) fall within Grade 1 parameters, then an AE is reported only if there is a change (increase) to a severity of Grade 2 or higher parameter for the clinical lab.

8.3.1.1 Hematology and biochemistry

A complete blood count (CBC) with automated differential cell count including lymphocyte and granulocyte enumeration, serum creatinine, and ALT will be measured at defined time points throughout the study period. The Principal Investigator will maintain laboratory reference intervals in the study file, and copies will be made available upon request to study monitors and sponsors. Hematology and serum biochemistry assays will be performed at the clinical laboratory at the study site. In the rare event that a participant has traveled to Bamako, hematology and biochemistry tests may also be done in the Malaria Research and Training Center clinical laboratory.

8.3.1.2 Hepatitis B and C, and HIV testing

Rapid test kits will be used to test for hepatitis B and C at screening. HIV testing will also be performed at screening in accordance with the study manual of procedures. If an individual tests positive, they will be referred to the local clinic for appropriate counseling and follow-up treatment according to Mali national guidelines.

8.3.1.3 Sickle Cell testing

Hemoglobin electrophoresis or high-performance liquid chromatography (HPLC) testing for sickle cell trait and sickle cell disease will be done according to the study manual of procedures.

8.3.1.4 Urine pregnancy testing

For female participants of childbearing potential, urine will be tested for pregnancy using urine pregnancy test kits as specified in the study MOP at screening and before each vaccination.

8.3.1.5 Malaria smears

Both thick and thin smears will be obtained during the study. Parasite density will be quantified each time malaria smears are obtained, either at the time of the visit if the participant is ill or at a later time. Standard operating procedures are followed to assure uniform and high quality malaria smear preparation. Accurate speciation and quantification will be assured based on SOPs. Malaria microscopists have been trained in malaria diagnostic methods and will be evaluated regularly to assess their competency.

8.3.1.6 PCR for malaria infection

DBS samples will be tested for the presence of *P. falciparum* infection at the University of Maryland using extraction and PCR protocols as described at http://medschool.umaryland.edu/malaria/protocols.asp.

8.3.2 Special Assays or Procedures

8.3.2.1 Serology Assays

Assays for antibodies against *P. falciparum* circumsporozoite protein (CSP) and potentially for other *P. falciparum* proteins will be performed at laboratories at the University of Maryland, Sanaria Inc and/or the collaborating institutions, following SOPs for methods that have been used for the previous trials of this vaccine in North American and African volunteers.

Immunogenicity will be determined by evaluating antibody (IgG) responses as measured using standard enzyme linked immunosorbent assays (ELISA) using CSP antigen as the primary immunogenicity outcome. Immunofluorescent assays (IFA) with appropriate capture antigens, and/or the inhibition of sporozoite invasion assay may also be used to measure immunogenicity. Protein microarrays to measure seroreactivity to large numbers of *P. falciparum* antigens will also be performed as exploratory analyses to evaluate cross-reactive antibody responses to

diverse variants of *P. falciparum* antigens, using methods established at the University of Maryland [25]. Raw protein microarray intensity data will be shared with Emmes for data analysis.

8.3.2.2 Cell-Mediated Immunity assays

Peripheral blood mononuclear cells (PBMC) will be cryopreserved in a linear rate fashion and transported to the MRTC in Bamako for CMI assays. Cytokine production will be measured by cryopreserved PBMC samples following in-vitro stimulation with specific antigens, i.e., PfSPZ and circumsporozoite protein (CSP). Multiplexed cytometric bead array assays or an equivalent multiplexed technology to concomitantly measure multiple analytes will be used to quantify production of cytokines, including interferon gamma and IL-2. At minimum, these studies will be done on samples collected at baseline, two weeks (14 days) after the first vaccination, and four weeks (28 days) after the first and last vaccinations. If a significant rise in interferon gamma and/or IL-2 is documented in this primary analysis, additional time points may be added. If cells remain, T and B memory cells (e.g. intracellular cytokines, proliferation) may be performed.

8.3.2.3 Genome sequencing and genotyping

In preliminary studies aimed at identifying antigen(s) responsible for protective immunity elicited by the PfSPZ-CVac we will conduct parasite genome-wide association studies to identify *P. falciparum* genetic loci selected by vaccination with PfSPZ challenge and associated with vaccine escape. Parasite DNA extracted from leukocyte-depleted blood collected during clinical episodes and follow-up visits from vaccinees and controls will be subjected to genome sequencing and analyses performed to measure vaccine selection and allele-specific efficacy.

Whenever venous blood is obtained for research purposes, blood cell pellets will be frozen for subsequent DNA extraction and genotyping. When sufficient volumes are available after blood is allocated for other uses, blood pellets may be cryopreserved for later expansion in culture for further parasite characterization by molecular and in vitro methods such as growth inhibition assays. Whenever malaria smears are obtained, a few drops (less than 0.5 mL) of blood will be blotted onto filter paper and preserved for parasite DNA analysis, and an additional 1-2 mL of venous blood will be collected for genome-wide parasite genotyping. These samples will be subject to sequencing at the University of Maryland to identify polymorphic amino acid residues that may be selected by the PfSPZ-CVac and other parasite genotyping studies including microsatellite and genome-wide analyses to aid with characterization of vaccine-resistant strains.

Rationale. Vaccine-induced protection will be based on the immune system's recognition of the Pf antigens presented by the vaccine strain sporozoites. At these target loci, alleles that are antigenically similar to those encoded by the Pf strain in the vaccine, NF54, may be present in

infections in controls but will be absent in infections in vaccinees. This selective elimination of NF54-like alleles at the loci targeted by the immune system forms the basis for the identification of these loci. When compared with the Pf isolates in controls, the set of Pf isolates in vaccinees should exhibit lower polymorphism, lower haplotype diversity and reduced frequency of the vaccine allele at the targeted antigenic loci. In addition, at these loci, F_{ST} (a measure of genetic divergence) between vaccinees and controls should be higher than expected.

Locus selection. PfSPZ-CVac should prime the immune system against antigens expressed during the sporozoite, liver and early blood stages. We will investigate several hundred loci, including known pre-erythrocytic antigens, as well as all other loci known to be expressed before and up to early in the blood stage and for which there is evidence of immunogenicity from seroreactivity surveys on peptide or protein arrays.

8.3.2.4 Microarray analysis

Microarray analysis will be utilized to determine whether up- or down-regulation of any specific genes is correlated with protection against *P. falciparum* infection. Preparation of cellular RNA for GENE Chip®microarray analysis, cDNA preparation, in vitro transcription, staining and scanning of Affymetrix® U133A Gene Chips (Affymetrix, Santa Clara, CA) microarrays will be carried out at the University of Maryland, Institute for Genome Sciences.

Rationale. Host factors may affect the incidence of malaria infection during a vaccination trial. We will test two hypotheses, in particular (1) that there are differences among subjects in susceptibility to malaria and (2) that there are differences among subjects in response to immunization. Hypothesis (1) will be addressed with samples from the control study arm. Gene expression will be compared between subjects who did not acquired malaria within the time span of the study with those who did. Gene expression will be evaluated at 3 time points: start of the study; peak of malaria season; at the time of first infection (first positive parasitemia) or at the end of the study, whichever comes first. Hypothesis (2) will be addressed with samples from the vaccinee study arm. Gene expression will be compared between subjects who did not acquired malaria within the time span of the study with those who did.
Gene expression will be evaluated at three time points: start of the study; four weeks after last immunization; at the time of first infection (first positive parasitemia) or at the end of the study, whichever comes first.

8.3.3 Specimen Preparation, Handling and Shipping

Detailed SOPs are maintained for these activities. Briefly, blood samples are obtained at the research clinic and processed in the sample processing laboratory according to SOPs. Filter paper blood samples are stored at room temperature in sealed desiccant pouches. Sera, plasma, cells and parasites are frozen in freezers or in liquid nitrogen containers according to SOPs. Frozen samples will be transported to the central immunology laboratory in Bamako, and either stored there in temperature-monitored freezers, a liquid nitrogen storage system or shipped to the University of Maryland or to the DMID clinical repository. International Air Transport Association (IATA) guidelines will be followed for specimen handling, transport and shipping.

8.3.4 Standard operating procedures (SOPs)

The Investigators will maintain detailed SOPs for vaccine transport, storage, preparation, reconstitution and administration. All staff and investigators will be trained in the SOPs relevant to their duties and will sign copies of the SOPs to document this training. Copies of SOPs will be available for inspection and review by the DMID, Sanaria and study monitors. During the study SOPs may be modified to improve them and new SOPs may be developed as needed to improve operations and ensure adherence with the protocol.

9 ASSESSMENT OF SAFETY

9.1 Specification of safety parameters

The primary safety outcome measures for this trial are:

- Occurrence of solicited local and systemic AEs within 12 days following vaccination (day of vaccination and 11 subsequent days)
- Occurrence of unsolicited AEs considered related to vaccination within 12 days following vaccination (day of vaccination and 11 subsequent days)
- Occurrence of SAEs at any point during the study period

9.2 Methods and timing for assessing, recording, and analyzing safety parameters

9.2.1 Adverse Events

An adverse event includes any noxious, pathological or unintended change in anatomical, physiological or metabolic functions as indicated by physical signs, symptoms and/or laboratory-detected changes occurring in any phase of the clinical study whether associated with the study product and whether or not considered related to the intervention. This definition includes an exacerbation of pre-existing conditions or events, intercurrent illnesses, or drug interaction. Anticipated day-to-day fluctuations of pre-existing conditions that do not represent a clinically significant exacerbation need not be considered adverse events. Discrete exacerbations of chronic conditions that are deemed to be different than regularly sustained day-to-day fluctuations, occurring during a study period will be reported as adverse events in order to assess changes in frequency or severity.

Adverse events will be documented in terms of a medical diagnosis. When this is not possible, the adverse event will be documented in terms of signs and/or symptoms observed by the investigator or reported by the subject at each study visit. All AEs occurring while on study will be documented appropriately regardless of relationship. Pre-existing conditions or signs and/or symptoms (including any which are not recognized at study entry but are recognized during the study period) present in a participant before the start of the study will be recorded on the participant's CRF and will be recorded as an AE if deterioration or exacerbation in the condition occurs during the study. Any hospitalization other than the planned inpatient evaluation for the malaria event will be considered a serious adverse event. Information to be collected include event description, date of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and date of

resolution/stabilization of the event. All AEs will be followed to adequate resolution or stabilization.

All AEs must be graded for severity and relationship to study product.

FDA defines AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Severity of Event: All AEs will be assessed by the Principal Investigator or appropriate sub-investigator using a protocol defined grading system in this protocol and the adult DMID Toxicity Tables (Tables 3-4). For unsolicited events not included in the protocol-defined grading system, the following guidelines will be used to quantify intensity:

- **Mild** (Grade 1)- events require minimal or no treatment and do not interfere with the participant's daily activities.

- **Moderate** (Grade 2)- events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning and daily activities.

- **Severe** (Grade 3)– events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

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

Relationship to study products: The clinician's assessment of an AE's relationship to test article (vaccine or study drug) is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship to study product assessed using the following terms: related or not related. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used:

- Related – there is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.

- Not related – there is not a reasonable possibility that the administration of study product caused the event.

In order to accommodate the industry partner, all AEs will also be graded for relatedness according to a five-grade scale. Details on the scale will be found in the study MOP. The five-grade scale will not be used for any decision making in this trial, nor affect the conduct of the trial in any way. Before database lock and after all data has been collected and monitored, the data center will conduct a one-time reconciliation between the two grading system.

9.2.2 Reactogenicity

Reactogenicity events are AEs that are common and known to occur for the intervention/investigational product being studied and should be collected in a standard, systematic format using a grading scale based on functional assessment or magnitude of reaction.

The following are expected AEs related to using a syringe and needle to deliver vaccine into a vein: pain, bruising, and minor swelling or bleeding at the insertion site. Rarely, use of a syringe and needle to inject into a vein may lead to an infection, an irritation of the vein or a blood clot.

The following are AEs considered at least potentially related to vaccination with PfSPZ-challenge, in the absence of an alternative clear etiology: injection site pain, tenderness, erythema, bruising and swelling/induration; systemic events include, for vital signs: fever, tachycardia, bradycardia, systolic hypertension, systolic hypotension, and tachypnea; and for systemic symptoms: malaise, nausea, arthralgia (joint pain), myalgia (muscle pain), chills, and headache. These reactogenicity events will be documented by research team staff on a reactogenicity CRF on the days of vaccination and 1, 3, 7 and 12 days following vaccination and on other days that participants present to the clinic for unscheduled follow-up. Laboratory safety testing may reveal a temporary increase in alanine aminotransferase (ALT) levels and will be conducted 14 days after vaccination to measure ALT, creatinine and hemoglobin as part of the primary outcome reactogenicity assessment.

Pulse, blood pressure, and respiration assessed at Visit 02 (Day 3—Pre-injection) will be considered baseline. In the case of baseline values that fall within Grade 1 parameters, an AE at subsequent assessments will be reported only if there is a change from baseline to a severity of Grade 2 or higher. For example, if baseline systolic blood pressure (Visit 02—Pre-injection) is 141-150 mmHg, then an AE is reported only if on subsequent visits there is a change (increase) to Grade 2 or higher parameters.

The following are expected AEs related to placebo administration: none in addition to those expected related to using a syringe and needle to deliver vaccine into a vein.
Solicited reactogenicity to be recorded as endpoints will include evaluation of local and systemic reactogenicity and corresponding grading listed in Tables 2-4.

Table 2: Assessment of solicited adverse event intensity

Adverse Event	Grade	Intensity Definition
Pain at injection site	0	Absent
	1	Does not interfere with activity
	2	Repeated use of non-narcotic pain reliever >24 hours or interferes with activity
	3	Any use of narcotic pain reliever or prevents daily activity
Tenderness at injection site	0	Absent
	1	Discomfort only to touch
	2	Discomfort with movement
	3	Significant discomfort at rest
Erythema/redness at injection site (greatest single diameter)	0	None or <2.5 cm
	1	2.5 cm to 5 cm
	2	5.1 cm to 10 cm
	3	>10 cm
Bruising at injection site (greatest single diameter)	0	None or <2.5 cm
	1	2.5 cm to 5 cm
	2	5.1 cm to 10 cm
	3	>10 cm
Induration/swelling at injection site	0	None or <2.5 cm and does not interfere with activity
	1	2.5 cm to 5 cm and does not interfere with activity
	2	5.1 cm to 10 cm or interferes with activity
	3	>10 cm or prevents daily activity
Fever (axillary temperature*)	0	< 38.0°C
	1	38.0-38.4°C
	2	38.5-38.9°C
	3	≥39.0°C
Malaise	0	None
	1	No interference with activity
	2	Some interference with activity
	3	Prevents daily activity
Chills	0	None
	1	No interference with activity
	2	Some interference with activity
	3	Prevents daily activity
Nausea	0	None
	1	No interference with activity
	2	Some interference with activity
	3	Prevents daily activity
Headache	0	None
	1	No interference with activity
	2	Repeated use of non-narcotic pain reliever >24 hours or some interference with activity
	3	Significant; any use of narcotic pain reliever or prevents daily activity
Arthralgia	0	None
	1	No interference with activity

	2	Some interference with activity
	3	Significant; prevents daily activity
Myalgia	0	None
	1	No interference with activity
	2	Some interference with activity
	3	Significant; prevents daily activity
Fever, subjective	0	None
	1	No interference with activity
	2	Some interference with activity
	3	Significant; prevents daily activity

*Axillary temperature will be recorded at the time of the clinic visit. If additional temperature measurements are recorded at another time of the day, the highest temperature will be recorded.

Table 3. Vital signs toxicity grading

Vital Signs*	Grade 1	Grade 2	Grade 3
Tachycardia- beats per minute	101-115	116-130	>130
Bradycardia- beats per minute	50 – 54 or 45-49 bpm if baseline <60 bpm	45 – 49 or 40-44 if baseline <60bpm	< 45 or <40bpm if baseline <60bpm
Hypertension (systolic) mm Hg	141-150	151-160	> 160
Hypotension (systolic) mm Hg	85-89	80-84	< 80
Tachypnea- breaths per minute	21-25	26-30	>30

* Pulse, blood pressure, and respiration assessed at Visit 02 (Day 3—Pre-injection) will be considered baseline. In the case of baseline values that fall within Grade 1 parameters, an AE at subsequent assessments will be reported only if there is a change from baseline to a severity of Grade 2 or higher. For example, if baseline systolic blood pressure (Visit 02—Pre-injection) is 141-150 mmHg, then an AE is reported only if the value changes such that it falls into Grade 2 or higher criteria at subsequent visits.

Table 4. Laboratory toxicity grading

HEMATOLOGY*			
	Grade 1	Grade 2	Grade 3
Hemoglobin Female	8.5 - 9.4 gm/dL	8.4 – 7.5 gm/dL	≤7.4 gm/dL
Hemoglobin Male	11.0 – 12.1 gm/dL	9.5 – 10.9 gm/dL	≤9.4 gm/dL
Platelets	75,000 -125,999/mm^3	50,000 -74,999/mm^3	≤49,999/mm^3 or > 1,000,000/mm^3
WBCs	11,000-13,000/ mm^3 or 2001-2499/mm^3	13,001-15,000 /mm^3 or 1501-2000/mm^3	>15,000/mm^3 or ≤1500/mm^3
CHEMISTRIES*			
	Grade 1	Grade 2	Grade 3
Creatinine	123.79 – 176.84 µmol/L	176.85 - 344.84 µmol/L	>344.84 µmol/L
ALT	61.1 – 121.9 IU/L	122 – 182.9 IU/L	>182.9 IU/L

* If baseline clinical labs (Visit 02—Pre-injection) fall within Grade 1 parameters, then an AE is reported only if the value changes such that it falls into Grade 2 or higher criteria at subsequent visits. For laboratory results that are abnormal according to the local reference range found in Appendix A, but not considered a Grade 1 abnormality according to Table 4 above, these will not be considered adverse events and will thus not be graded, but will be followed-up clinically at the discretion of the study team onsite.

9.2.3 Serious Adverse Events

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

- Death,
- A life-threatening AE*,
- Inpatient hospitalization or prolongation of existing hospitalization,

- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment they may jeopardize the patient or subject 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.

* Life-threatening AE. An AE is considered “life-threatening” if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an AE, had it occurred in a more severe form, might have caused death.

All SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- recorded on the appropriate SAE form and eCRF
- followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator
- reviewed and evaluated by an Independent Safety Monitor (ISM), the SMC (periodic review), DMID, and the IRB, if indicated

9.2.4 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

Abnormalities in clinical findings and in clinical laboratory testing will be followed according to national guidelines in Mali. The toxicity tables define what laboratory values or findings are considered abnormal.

9.3 Reporting Procedures

AEs and SAEs will be documented from the first study intervention, through the end of the follow-up period.

All AEs and SAEs will be captured on the appropriate data collection form. Information to be collected includes event description, date of onset, investigator assessment of severity, relationship to study product, date of resolution of the event, and outcome.

Investigators will report promptly to the sponsor any adverse effect that may reasonably be regarded as caused by, or probably caused by the PfSPZ Vaccine. If the adverse effect is “alarming”, the investigators will report the adverse effect immediately.

Likewise, the sponsor will keep each participating investigator informed of new observations discovered by or reported to the sponsor, particularly with respect to adverse effects and safe use. The sponsor will notify participating investigators in a written investigational new drug (IND) safety report, of any adverse experience associated with the use of the PfSPZ Vaccine that is both serious and unexpected, and any finding from tests in laboratory animals that suggests a significant risk for human subjects.

9.3.1 Serious Adverse Events

AEs will be followed to adequate resolution or stabilization for participants enrolled in the study. After termination of the study, participants will be referred to appropriate care for follow-up of ongoing AEs. AEs that are associated with the experimental vaccine will be followed to resolution or stabilization with the expectation that it will remain chronic.

Any AE that meets a protocol-defined serious criterion must be submitted immediately (within 24 hours of site awareness) on an SAE form to the DMID Pharmacovigilance Group, at the following address:

DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20814, USA
SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)
SAE FAX Phone Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

Other supporting documentation of the event may be requested by the DMID Pharmacovigilance Group and should be provided as soon as possible.

The DMID medical monitor and clinical protocol manager will be notified of the SAE by the DMID Pharmacovigilance Group. The DMID medical monitor will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct.

At any time after completion of the study, if the investigator becomes aware of an SAE that is suspected to be related to study product, the investigator will report the event to the DMID Pharmacovigilance Group.
In addition to the reporting to DMID, the investigator must also report to the ethics committee and IRB. Any AE that meets a protocol-defined serious criterion and is considered **related** to the investigational product must be submitted, unless otherwise requested by the ISM, sponsor or the investigators:

- Within 48 hours to the Mali IRB
- Within 5 days to the University of Maryland IRB

For other AEs that meet a protocol-defined serious criterion an are considered **not related** to the investigational product, this information can be submitted, unless otherwise requested by the ISM, sponsor or the investigators:

- In the annual summary to the Mali IRB
- In the annual summary to the University of Maryland IRB

Table 5. Summary of Serious Adverse Event Reporting Requirements

	DMID Pharmacovigilance Group	**Mali Institutional Review Board**	**University of Maryland Institutional Review Board**
Contact information	SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US) SAE FAX Phone Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US) SAE Email Address: PVG@dmidcroms.com	Hand delivery or e-mail to Research and Ethics Committee	Via CICERO (Collaborative Institutional Comprehensive Evaluation of Research Online) system
Related SAEs	Within 24 hours	Within 48 hours	Within 5 days
Unrelated SAEs	Within 24 hours	Annual summary	Annual summary

Participants will be followed for 48 weeks after presumptive artesunate therapy (study day 413) to document any SAEs.

9.3.2 Regulatory Reporting for Studies Conducted Under IND

Following notification from the investigator, the IND sponsor will report any suspected adverse reaction that is both serious and unexpected. DMID will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the AE. DMID will notify FDA and all participating investigators (i.e., all investigators to whom DMID is providing drug under its INDs or under any investigator's IND) in an 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 as specified in 21 CFR Part 312.32. DMID will also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7

calendar days after the sponsor's initial receipt of the information. Relevant follow up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, DMID will submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

All serious events designated as "not related" to study product(s), will be reported to the FDA at least annually in a summary format.

9.3.3 Reporting of Pregnancy

If a study participant becomes pregnant during the study, no further vaccinations or scheduled antimalarials (CQ or artesunate) will be given and the participant will be encouraged to continue to participate in the safety surveillance schedule. The pregnancy outcome will be followed by study clinicians and reported on the pregnancy report form as provided by the statistical and data coordinating center.

9.4 Type and Duration of Follow-up of Subjects after Adverse Events

Investigators will follow up subjects with AEs and SAEs until the event has resolved, or, if a chronic condition has developed, until the end of the study follow-up period. Outcome will be assessed as Recovered/Resolved, Recovered/Resolved with Sequelae, Recovering/Resolving, Not recovered/Not resolved, or Fatal.

9.5 Halting rules

Decisions to halt or pause the study will be made by DMID based on the recommendations of the ISM and/or the investigators and/or the SMC. The following criteria will be used as rules to put the study on hold until reviewed by the ISM and DMID. If any of the following halting criteria are met after vaccination, then vaccinations will be stopped and data will be reviewed by the SMC:

1. One or more participants experience an SAE that is determined to be related to the study vaccine.

2. One or more participant(s) experiences systemic allergic reaction (i.e., bronchospasm, allergy-related edema/angioedema, hypotension or anaphylaxis) in the 2 days following vaccination that is related to the study vaccine.

3. Three or more participant(s) experience the same Grade 3 solicited local or systemic adverse event, including solicited laboratory safety values, in the 12-day post-vaccination follow-up period deemed related to the study vaccine.
4. Three or more participant(s) experience the same Grade 3 unsolicited adverse event in the 12-day post-vaccination follow-up period deemed related to the study vaccine.

Subsequent review of serious, unexpected, and related AEs by the Medical Monitor, SMC, ethics review committee, the sponsor(s), or the FDA or relevant local regulatory authorities may also result in suspension of further trial interventions/administration of study product at a site. The FDA and study sponsor(s) retain the authority to suspend additional enrollment and study interventions/administration of study product for the entire study, as applicable.

DMID retains the authority to halt the study enrollment or study product administration.

9.6 Safety oversight

9.6.1 Safety Monitoring Committee (SMC)

This clinical trial will utilize an SMC, which is an independent group of experts that advises DMID and the study investigators. The primary responsibilities of the SMC will be to 1) periodically review and evaluate the accumulated study data for subject safety, study conduct and progress and 2) make recommendations to DMID concerning the continuation, modification or termination of the trial. The SMC will be composed of at least three voting members. Procedures for SMC data reviews will be defined in a SMC charter that will include membership, responsibilities, and the scope and frequency of data reviews. The SMC will operate on a conflict-free basis independently of the study team. DMID or the SMC may convene ad hoc meetings according to protocol criteria or if there are concerns that arise during the study. The SMC will have access to unblinded data during its closed session. After its assessment, the SMC will recommend continuation, modification, or termination of the clinical trial.

The SMC will

- Review post-1st dose 12-day safety and reactogenicity data and provide a recommendation regarding proceeding to second vaccinations based on safety and reactogenicity. In addition, the SMC will review aggregate safety data (primary endpoint) for increased rate of occurrence of serious adverse reactions.
- Review available safety data if a halting rule is met.
- Convene an *ad hoc* meeting to discuss any issue of safety raised by an investigator, ISM, DMID medical monitor, the sponsor, or a member of the SMC.

- Conduct a final review meeting 6 to 8 months after final clinical database lock to review the cumulative unblinded safety and efficacy data for the study. The data will be provided in a standard summary format. The SMC may be asked to provide recommendations in response to questions posed by DMID.

Additional details of study safety oversight, including scheduled meetings and roles and responsibilities of SMC are described in the SMC charter. Safety results of a separate, related clinical trial sponsored by DMID will be available for review by the SMC for the current trial before study start.

9.6.2 Independent Safety Monitor

An independent safety monitor (ISM) is a physician with relevant expertise whose primary responsibility is to provide to DMID an independent safety assessment in a timely fashion. Participation is for the duration of the DMID study and is a voluntary position that does not receive payment.

The ISM:
- Is in close proximity to the study site and has the authority and ability to readily access study participant records in real time.
- May be a member of the participating institution's staff but preferably be from a different organizational group within the institution.
- Should not be in a direct supervisory relationship with the investigator.
- Should have no direct involvement in the conduct of the study.

The ISM will:
- Sign a COI certification at the time they are asked to participate and provide updates to this information as needed.
- Receive reports of Serious Adverse Events (SAEs) from the site investigator and will be notified by email when DMID is notified of the SAE.
- Evaluate the SAE and report their clinical assessment to DMID, through DMID-CROMS SOCS in a timely manner and email the report to DMID-CROMS SOCS.
- Communicate with the investigator at the participating site as needed.
- Review additional safety related events at the request of DMID.
- Provide additional information to DMID and/or the SMC by teleconference as requested.

10 CLINICAL MONITORING

10.1 Site monitoring plan

Study site monitoring will be conducted by the DMID clinical monitoring contractor to ensure that good clinical practice standards and regulatory guidelines are being followed. A pre-trial monitoring visit will be made to the study site, including the clinical laboratory. All records will be made available to monitors, including regulatory files, CRFs and source documents, QA/QC documentation, SOPs, etc. Additional study site visits may be made during the course of the trial and at the end of the surveillance period.

In addition, monitors from the University of Maryland may make study site visits, in coordination with the primary monitoring group designated by DMID.

In conjunction with the clinical monitoring contractor designated by the sponsor, a detailed monitoring plan will be developed. This document describes who will conduct the monitoring, at what frequency monitoring will be done, and what level of detail monitoring will be conducted. The clinical monitoring plan will be written by DMID and the DMID clinical monitoring contractor. This separate monitoring plan will be agreed upon with the Office of Clinical Research Affairs (OCRA), and will describe protocol-specific items to be monitored. The monitoring plan will include the number of participant charts to be reviewed, which/what proportion of data fields and what will be monitored, who will be responsible for conducting the monitoring visits, and who will be responsible for ensuring that monitoring findings are addressed.

11 STATISTICAL CONSIDERATIONS

Data entry will be performed onsite and in the data management unit in Bamako if needed. The Emmes Corporation will perform data analysis and report primary and secondary endpoint results in collaboration with the Biostatistician consultant and the MRTC Data Management Unit. In collaboration with the University of Maryland, Emmes will develop a Statistical Analysis Plan for protein microarray and gene expression analyses.

11.1 Study Hypotheses

1. The percent infected within 6 months of last artesunate dose does not differ between participants vaccinated with PfSPZ challenge and controls.

2. The distribution of time to first infection does not differ between participants vaccinated with PfSPZ challenge and controls.

11.2 Sample Size Considerations

This trial will compare the proportion of volunteers infected by *P. falciparum* malaria during follow-up between the vaccine and placebo groups. Sample size calculations are based on a type 1 error of 0.05 (alpha) and a requirement for 90% power.

Under these assumptions, if PfSPZ-CVac provides 60% efficacy and the incidence rate in the placebo group at the Bougoula-Hameau site is 75%, a sample size of 28 is required in each group. Anticipating a maximum 10% drop out rate, 31 volunteers will be enrolled into each group.

This trial is intended as a proof-of-concept and is not powered to detect small differences between groups. As designed, the study has ~90% power to detect a difference between groups of 30% infected (e.g., PfSPZ-CVac participants) vs. 75% infected (e.g., normal saline controls) (http://statpages.org/proppowr.html).

11.3 Final Analysis Plan

A final research analysis plan will be agreed upon by the investigators, DMID, and Sanaria before locking of the database for the final analysis. The analysis) of the following outcomes will be conducted on data and samples collected until 24 weeks after presumptive treatment with artesunate (study day 245:

- All primary outcome measures

- Secondary outcome measure 1 (time to *P. falciparum* parasitemia by thick blood smear), and 2 (time to *P. falciparum* parasitemia by qPCR)

The above outcomes will be analyzed in a 'Primary Analysis Report' which will be used for decision-making related to the product clinical development plan and will be available for public presentation and publication after the data for these outcomes is locked, and before the clinical study report is generated. The study will continue for additional surveillance and immunogenicity assessment until 48 weeks after the presumptive treatment with artesunate (study day 413). This additional information will be combined with the Primary Analysis Report in the Clinical Study Report. It is anticipated that the results of this study, including the primary analysis report, will be presented to the scientific community via oral presentations at meetings and written publications in scientific journals. The data to be presented and the authorship will be discussed between partners before any official communication.

The official report of the primary analysis will be written by the study investigators and the statistical consultants, reviewed by all partners, and submitted through appropriate channels for approval by DMID. This report will contain detailed information about the participants, their tolerance of the vaccines, their side effects and laboratory abnormalities, as well as their overall immune responses to vaccination.

11.3.1 Analysis of demographics

Demographic characteristics (age, gender, and neighborhood of residence) of each study group will be tabulated. The mean age (plus range and standard deviation) by sex of the enrolled participants, as a whole and per group will be tabulated.

11.3.2 Analysis of safety

The overall proportion of participants with at least one solicited local reactogenicity event and the proportion with at least one solicited systemic reactogenicity event during the 12-day surveillance period after vaccination will be tabulated.

The incidence, intensity and relationship of individual solicited AEs to the vaccine over the 12-day surveillance period will be calculated per group and vaccine administration. The incidence, intensity and relationship of individual unsolicited AE, classified using MedDRA System Organ Classes and Preferred Terms, reported up to 12 days after vaccination will be tabulated per group and vaccine administration.

SAEs will be described. Comparisons between study groups of incidence of events, including solicited local and systemic reactogenicity events will be made based on two-sided Fisher exact tests. Analysis of safety during the initial surveillance period will consist of comparison of incidence of SAEs, as well as hemoglobin, white blood cell, platelet, creatinine, and ALT levels.

11.3.3 Clinical laboratory parameters

Hematological (CBC) and biochemical (ALT, creatinine) laboratory parameters will be measured at specific time points, the days of vaccination and 12 days after each vaccination. Clinically relevant abnormal values based on reference intervals determined in a similar population will be tabulated and a trend analysis performed if deemed necessary. Laboratory abnormalities will be graded on a scale of 1-3 as defined in Table 4: Laboratory Toxicity Grading.

11.3.4 Analysis of humoral immunogenicity

The immunogenicity endpoints-- antibodies against *P. falciparum* CSP and other *P. falciparum* proteins will be assessed in several ways. A series of graphs will display immunologic responses.

There will be one primary method used for assessing antibody response: ELISA using a *P. falciparum* CSP recombinant protein as antigen. At a minimum the *P. falciparum* CSP ELISA will be used to determine antibodies in sera taken before vaccination and 12 days after the last dose in all participants. Titers will be examined to look for an association with protection. IFA using PfSPZ and potentially other parasite stages and ELISA using other *P. falciparum* proteins and inhibition of sporozoite invasion (ISI) assay may be done based on the results of the first set of assays. Corresponding summary statistics will show medians or means and standard deviations, and an effort will be made to determine a threshold for protection. Cox proportional hazards modeling will be used to estimate the relationship between antibody titers and the time to first parasitemia. Separate models will be fit for the time to parasitemia measured by qPCR and time to parasitemia measured by microscopy.

11.3.5 Analysis of CMI responses

For other trials of PfSPZ Vaccine cellular immune responses against PfSPZ are primarily assessed by flow cytometry using intracellular cytokine staining (ICS), and by the FluoroSpot assay to assess cells producing interferon gamma and/or IL-2. For this study, we will measure cytokine production by cryopreserved PBMC samples following in-vitro stimulation with specific antigens, i.e., PfSPZ and circumsporozoite protein (CSP). Multiplexed cytometric bead array or other multiplexed assays will be used to quantify production of cytokines, including interferon gamma and IL-2. At a minimum the studies will be done at baseline, 28 days after each

vaccination, and 24 weeks after presumptive treatment with artesunate. If a significant rise in interferon gamma and/or IL-2 is seen post-vaccination compared to baseline in this primary analysis, additional timepoints may be assessed to determine the kinetics of development of the maximal response and the effect of sporozoite infection on the responses. T and B memory assays, including intracellular cytokines by flow cytometry may also be conducted and will be dependent upon PBMC availability and funding support. Correlations between continuous measures of immune response will be assessed using the standard Pearson as well as Spearman rank correlation coefficients, on $\log_{10}$ transformed data when a logarithmic transformation results in a distribution more nearly normal than the distribution of untransformed values. Association between a continuous and a categorical measure will be assessed using t-tests or analysis of variance, or the analogous tests on ranks. Association between categorical measures will be assessed using chi-square or exact tests. Appropriate summary descriptive statistics (e.g., means and standard deviations or medians and ranges) will be presented. Effects of covariates (e.g., gender, ethnicity) will be assessed using regression models.

11.3.6 Analysis of efficacy against infection

Time-to-event analysis will be used to compare time until first parasitemia between the control and treatment groups. The Kaplan Meier method will be used to display survival curves for the two groups, and the log rank test will be performed to test whether the survival curves differ between treatment and control groups. The unadjusted hazard ratio will be presented along with its 95% confidence interval, calculated using the likelihood score method. As a secondary analysis, the hazard ratio will be computed adjusting for baseline characteristics using Cox proportional hazards modeling. Vaccine efficacy will be estimated as $1 – p_v/p_c$, where p_v and p_c are risks of at least one malaria infection in the 24-week period following the post-vaccination antimalarial treatment in recipients of vaccine and placebo, respectively. If all participants are followed for the entire 24 weeks, p_v and p_c will be estimated as simple proportions. However, if there is any loss to follow-up after the post-vaccination antimalarial treatment, p_v and/or p_c will be estimated by the Kaplan-Meier method. Two-sided 95% confidence intervals around the point estimates of efficacy will be calculated, using a likelihood score or Taylor series method. Vaccine and placebo recipients will also be compared with respect to the proportion of individuals experiencing at least one malaria episode after randomization (ITT analysis), the number of malaria infections occurring in each individual, and number of clinical malaria events, will also be done. Asexual *P. falciparum* parasite density, measured as area under the curve, will also be reported for vaccine and for placebo recipients.

11.3.7 Analysis of strain-specific efficacy and selection

Rationale

The PfSPZ-CVac provided 100% efficacy against CHMI by a strain of *P. falciparum* identical to the vaccine strain in malaria-naïve German participants. It is hoped that this vaccine will provide similar high efficacy in malaria-experienced African adults who are exposed to genetically diverse natural *P. falciparum* infection.[26;27] This initial test of PfSPZ-CVac against natural challenge by genetically diverse parasites will afford a valuable first opportunity to develop and use novel genomic epidemiology methods and analytical strategies for assessing the molecular basis of whole-parasite vaccine protection.[15] These exploratory analyses may lead to fundamental advances in our understanding of pre-erythrocytic protective immunity, but more importantly this work represents the first steps toward determining whether an attenuated sporozoite vaccine will protect against the extremely diverse malaria parasites found in nature.

Approach

For vaccine and placebo groups, occurrence of infection with parasites with genotypes identical to the NF54 vaccine strain will be recorded. In addition to strict identity and non-identity with the vaccine strain, measures of genetic diversity between the NF54 vaccine strain and infections occurring in study participants will also be measured. Diversity will be measured using microsatellite maps, genome-wide single nucleotide polymorphism (SNP) typing platforms including a microarray that detects 33,000 *P. falciparum* SNPs developed by the UMD/CVD Division of Malaria Research, and/or by next-generation genome sequencing. Methods for microsatellite typing are robust and well established at the UMD Center for Vaccine Development[28;29]; methods for SNP typing and genome sequencing are also established in the UMD Division of Malaria Research in collaboration with the UMD Institute for Genome Sciences (C. Plowe, unpublished).

Parasite DNA extracted from leukocyte-depleted blood collected during clinical episodes and follow-up visits will be subjected to genome-wide genotyping and genome sequencing at the University of Maryland.

Analysis

Sieve analysis is a method for determining how vaccine efficacy varies according to genotype of the infecting pathogen, which has been used to measure strain-specific efficacy in HIV vaccine trials.[30] The "sieve" is the vaccine-induced immune barrier to infection. Sieve analysis involves identifying "holes" in the sieve, i.e. pathogen characteristics—such as DNA sequence divergence from the vaccine strain at a given locus—that allow it to pass through the barrier created by vaccine immunity. We used this method to measure strain-specific efficacy in a Phase 2 trial of a blood-stage malaria vaccine.[31;32] The analysis will focus on parasite genomes in vaccinated and control individuals during six months following presumptive artesunate treatment. We will estimate strain-specific odds ratios by calculating the odds of infection by a given allele in breakthrough infections in vaccinated individuals compared to the odds of infection by that strain in infected unvaccinated individuals. Vaccine relative risk ratios will be approximated by dividing the odds ratio for a given strain by the odds ratio for the vaccine strain.[32] We will perform this sieve analysis both genome-wide, but also focusing on candidate genes encoding pre-erythrocytic antigens that may contribute to whole sporozoite vaccine efficacy such as CSP, LSA1 and TRAP. "Strains" (or "variants") will be defined both at the level of individual amino acids and at the gene level in ordered categories of genetic distance between the gene sequence of the breakthrough infections compared to that of the vaccine strain. Loci with inflated vaccine relative risk ratios, after adjusting for multiple comparisons, will be considered to be under selection by the vaccine and thus represent the key antigens responsible for driving strain-specific protective efficacy. We will use a similar rationale on a genome-wide level to look for evidence of allele-specific efficacy and vaccine-induced selection in loci not determined *a priori* to be of interest. We will (i) identify regions of reduced polymorphism in parasites from breakthrough infections from vaccinated individuals compared to those sampled from infected unvaccinated individuals, and (ii) identify regions where average genetic distance to the vaccine strain is highest in parasites from breakthrough infections relative to the average observed for parasites sampled from infected unvaccinated individuals. We will use a sliding window approach, with optimal window and step size determined empirically from the data. Genes located in regions of interest will be carefully evaluated for function and expression profile.

Depending on the efficacy of the PfSPZ-CVac, the number of breakthrough infections from this first trial of PfSPZ-CVac in a malaria-exposed population may be too small to provide sufficient power for these genomic epidemiology analyses to detect statistically significant differences. Through a recently formed consortium to coordinate the clinical development of the PfSPZ Malaria Vaccine, we are forming a network of investigators around the world who plan to design and conduct trials to evaluate PfSPZ-CVac in malaria-exposed populations. Results from genomic analyses for this first trial will eventually be pooled with those of contemporaneous and subsequent trials to accomplish a larger sample size for these exploratory analyses.

11.3.8 Analysis of gene expression profiling

In all participants, and participants by group, participants who are PCR-negative for malaria parasites from the day of first vaccination through the day of the second vaccination (expected to be approximately 30-60% of individuals in the group), gene expression profiling will be conducted at the University of Maryland, Institute for Genome Sciences. The data analysis will be carried out by Emmes according to the Statistical Analysis Plan that will be developed in collaboration with University of Maryland.

11.3.9 Analysis of protein arrays

P. falciparum protein array analysis will be carried out by Emmes according to the Statistical Analysis Plan that will be developed in collaboration with University of Maryland.

11.3.10 Missing Data

Every effort will be made to minimize missing data and collect all endpoints specified in this protocol. Subjects who discontinue treatment will be followed after treatment discontinuation for collection of all scheduled safety data with their consent. No adjustments for missing safety data will be performed for the primary analysis. The efficacy objectives of the study are to compare time to parasitemia between treatment arms and to compare proportions infected at six months post-vaccination between treatment arms. If parasitemia measurements are missing due to dropout, the survival analysis proposed will naturally adjust for the missing response variable. Thus, no additional adjustment for missingness is needed. Survival analysis assumes that the relationship between treatment arm and parasitemia does not differ between subjects who drop out and those who remain in the study. If dropout occurs, the validity of this assumption may be evaluated by comparing these subjects on other relevant covariates and/or through sensitivity analysis.

11.4 Study cohorts/datasets to be evaluated

Intent to Treat Cohort

The ‘Intention-to-Treat’ Cohort’ (ITT cohort) will include all participants randomized to the study vaccine or placebo, classified according to the randomization assignment. As exploratory analysis, an analysis of efficacy will be done on this cohort.

Per Protocol Cohort

The ‘Per Protocol Cohort’ will include all eligible participants randomized to study vaccine or placebo, and who received all assigned vaccinations with the study vaccine or placebo control. Participants will be classified according to the treatment (vaccine or placebo) received. Analysis of immunogenicity endpoints occurring after the last vaccination, as well as efficacy analysis, will be performed on this cohort.

Safety cohort

The ‘Safety Cohort’ will consist of all participants who have received at least one dose of study vaccine or placebo and for whom any data on safety are available. Participants will be classified according to the treatment received. The primary safety analysis will be done on this cohort.

The presentation of safety data will explore separately the adverse experiences among participants who received all vaccinations, among those who received only some and among those with protocol deviations related to receipt of study products.

Immunogenicity cohort

The ‘Immunogenicity Cohort’ will include all evaluable participants (i.e., those meeting all eligibility criteria, and who have received at least one vaccination with the study vaccine or placebo) for whom data concerning immunogenicity endpoint measures are available. This cohort will include participants for whom assay results are available for antibodies against at least one component antigen of the study vaccine antigen component after vaccination. Participants will be classified according to the treatment received. Immunogenicity analysis will be done using this cohort or the Per Protocol cohort, as appropriate.

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The participating study site will maintain appropriate medical and research records for this trial, in compliance with International Conference on Harmonisation (ICH) harmonised tripartite guideline E6(R1): Good Clinical Practice (GCP), Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects.

Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Examples of these original documents and data records include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, subjects' memory aid or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and participant files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial.

Electronic CRFs (eCRFs) will be supplied by The Emmes Corporation under contract to the NIAID, and a remote data entry system will be used. Data collection forms derived from the eCRFs will be made available on the project website as Source Document Workbooks (SDWs). The SDW for each participant will be maintained at the study site. All SDWs will be filled out completely and by appropriate study personnel. These data collection forms compiled as SDWs will serve as source documents.

The study site will permit authorized representatives of Sanaria and DMID, their designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress. These representatives will be permitted access to all source data.

13 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted study site quality management plan, the study site will conduct routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. The quality management plan is located in the University of Maryland Center for Vaccine Development Office of Regulatory Affairs and Quality Management. The protocol-specific quality management plan is in conjunction with the University of Maryland Center for Vaccine Development Office of Regulatory Affairs and Quality Management and describes the study site's internal quality management activities including how the data will be evaluated for compliance with the protocol, which documents will be reviewed, and methods of training staff.

Standard operating procedures (SOPs) will be used at all clinical and laboratory sites. Routine monitoring will be performed according to ICH/GCP (E6) (e.g., data monitoring). DMID-designated clinical monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements. The study site will provide direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing by DMID and Sanaria, and inspection by local and regulatory authorities.

The Emmes Corporation and the study site data manager will implement QC procedures beginning with the data entry system and generate data QC checks that will be run on the database. Any missing data or data anomalies will be communicated to the study site for clarification/resolution.

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Ethical standard

Sanaria's PfSPZ Vaccine granted FDA IND #16889 and the study described in this protocol will be conducted according to current Good Clinical Practices (US 21 CFR Part 50-Protection of Human Subjects and Part 56-Institutional Review Boards, US 45 CFR 46, 21 CFR 312, the Declaration of Helsinki, and the applicable rules and regulations of Mali).

The Mali IRB (FWA00001769) will review and approve the protocol before study start. In addition, the study will be reviewed by DMID and the University of Maryland IRB. Documentation of the approval by these ethical review boards will be kept in the study site's regulatory file.

14.2 Institutional review board

All amendments will be submitted to the local IRB, the UMD IRB, as well as to Sanaria and DMID. No amendments will go into effect without written approval from the local IRB, the UMD IRB, Sanaria and DMID except when necessary to eliminate immediate hazards to the participants. Protocol deviations will also be reported to each IRB according to the policy of each IRB. CRFs and other source documents will be examined to determine whether missing data were not transcribed, unavailable or missing for unknown reasons and this information will be coded and documented in the database.

The investigators will inform all the IRBs and DMID of the following:

- All subsequent protocol amendments, informed consent form changes or revisions of other documents originally submitted for review
- Serious and/or unexpected AEs occurring during the study, where required
- New information, including any provided by Sanaria, that may affect adversely the safety of the participants or the conduct of the study
- An annual update and/or continuing review reports, as required
- A final report including SAE outcomes will be provided when the study has been completed.

14.3 Informed consent process

The principles of informed consent in the current edition of the Declaration of Helsinki will be implemented before any protocol-specified procedures or interventions are carried out.

Information will be given in both oral and written form whenever possible. The written consent documents will embody the elements of informed consent as described in the current edition of the Declaration of Helsinki, will adhere to the ICH Harmonised Tripartite Guideline for Good Clinical Practice and 45CFR46 and 21CFR50, and will also comply with applicable host country regulations. The oral consent process will be consistent with 45CFR46, 46.117, 21CFR50.27 and ICH E6 (R1) Section 4.8. Independent witnesses will be used to attest that illiterate potential participants have understood the contents of the informed consent document.

14.3.1 Screening and study informed consent

We have published detailed descriptions of the processes we use to obtain community "permission to enter" and individual informed consent [33]. We consider informed consent to be a dynamic, ongoing process, with continuous availability of investigators to answer any questions that arise in the course of the trial and to ensure that participants understand trial procedures. Should new data become available that could affect participant safety and/or willingness to continue in the study, informed consent would be obtained and documented again.

The extensive contact between the team of investigators and the population of Sikasso District has led to the development of mutual trust and the establishment of an ongoing informed consent process attempting to address issues related to interventional studies in resource-limited settings. Many discussions with local community leaders, heads of families and citizens through group meetings, and more limited group interviews have reviewed the need to obtain a written informed consent. The community has now become familiar with the informed consent process, including written, signed consent forms, which have been used for two malaria vaccine trials and several antimalarial drug studies at this study site, including a Phase 2 trial of MSP-LSP/Alum vaccine in 400 children (NCT01341704).

Before initiating the study in Bougoula Hameau, the senior investigators will visit local district government official, the mayor, the district heath staff and the Bougoula Hameau and other Sikasso District village chiefs. These are courtesy visits in which plans for the current study are explained and any questions are answered.

These individual meetings are followed by a larger community meeting attended by the above persons as well as numerous other local health care providers, traditional healers and notable citizens (including several respected women from the community). Planned studies are

explained in more detail, and ample time is given for carefully and thoroughly addressing all questions and concerns. Each presentation, question and response will be translated into local language(s) so that all present understand the entire discourse.

Once this group of community leaders has expressed their approval of the planned study, they disseminate information to their various constituencies, so that when potential recruits are approached by study staff they are already generally aware of the nature of the impending study. The investigators do not consider this process to constitute “community consent” in addition to or in lieu of individual informed consent, but rather a community “permission to enter” that is a necessary prerequisite to conducting any study in a tight-knit and highly organized traditional rural community.[33]

After community meetings have been held, adults 18-45 years old from the Sikasso District and neighbor villages will be invited to the vaccinology unit based at Bougoula Hameau village to receive more information about the study. Before initiating screening and informed consent, the study team meets to review the oral translation of the consent forms into the relevant local languages and dialects (which are spoken and not generally written) word by word, until there is consensus that the individuals responsible for giving consent in each language are conveying as accurately as possible the exact content of the consent form. These sessions are led by senior investigators and include all clinical co-investigators who will be administering informed consent, as well as local guides who are collectively fluent in all of the local languages and dialects. The French version of the consent form is translated orally into local languages and dialects repeatedly until all investigators reach consensus on the oral translations.

A period of approximately 45 days is allotted for screening and recruitment to allow plenty of time for participants to consider their decision about participating and to discuss their participation with family members and others in the community. At the times of screening and recruitment, the consent forms are read to participants who speak French, and orally translated into the language of choice of each participant. Bambara is the main language spoken in Sikasso District and has written form. Other languages used in the area, including Fulani, largely do not have written forms. Generally, neither the local population nor the study staff uses the written forms of these mainly oral languages. Most of this rural population is illiterate; therefore, with the exception of those whose preferred language is French, informed consent will be administered by oral translation of the text in presence of a witness. In all cases, the investigator will give the participants ample opportunity to inquire about the details of the study and to ask any questions before dating and signing the consent forms, including the opportunity to take a copy of the consent form home to review with family members or others before returning on a later day with their decision. All illiterate individuals will have the study and consent forms explained to them point by point by the interviewer in the presence of a witness who will sign the consent form. Witnesses will have no association with the conduct of the study

and will not be related to the study participant. Witnesses receive training that emphasizes protection of confidentiality, and assist with ensuring full and accurate oral translation of the information on the consent forms. Individual consent to participate in research studies is given freely, and is not subject to approval by village elders or others. Investigators will carefully explain to potential participants that they may withdraw their participation from the study at any point in the future.

Informed consent will be documented by the use of a written consent form approved by the IRBs and signed or thumb printed and dated by the participant, and by the person who conducted the informed consent discussion. Thumb printing will be used for illiterate persons, who are expected to constitute the majority of participants. The consent form will be translated orally into native languages from the French written version of the consent form. Consent will be administered by a study clinician who is fluent in French and will either be fluent in the local language of the participant or use a translator. A witness will assist during the procedure. After the participant clearly states that she/he has understood what was explained and agrees to participate in the study, the consent forms will be completed. The participant will be asked if she/he prefers to thumbprint or to sign. In the case of the thumbprint option, the distal end of her/his left thumb will be applied to a stamp inker and then firmly applied to the space on the consent forms reserved for thumbprints.

The signature/thumbprint confirms that the consent is based on information that has been understood. Each participant’s signed informed consent form is kept on file by the investigator for possible inspection by regulatory authorities. The participant will receive a copy of the signed and dated written informed consent forms and any other written information provided by the investigator, and will receive copies of any signed and dated consent form updates and any amendments to the written information.

Since the vast majority of study participants do not use telephones, fax or mail, contact information is provided in terms of local physicians and study site staff who can be visited directly and who can themselves reach the investigators directly or by telephone.

14.3.2 Compensation

To compensate the participant for time lost to income generating activities as a result of participating in the study, each will be given either 130 kg of rice, worth about $100 USD, or cash equivalent in local currency, or a combination of these two methods of compensation, according to local ethics standards over the course of the entire study. Each participant will receive either 5 kg of rice, worth about $3.35 USD, or cash equivalent in local currency, according to local ethics standards after each scheduled visit.

14.4 Exclusion of Women, Minorities, and Children (Special Populations)

The PfSPZ-CVac has been tested in several Phase 1 clinical trials in adults in the United States, Germany, and Spain. Due to the limited amount of safety data available, it is felt that it is more ethical to perform this Phase 1 study first in adults who can give full, informed independent consent. Pregnant and breastfeeding women will also be excluded from participation due to theoretical risks to the developing fetus. Following this study, assuming the vaccine is shown to be safe and immunogenic in this study population, further studies in children and women of childbearing potential are anticipated.

14.5 Subject confidentiality

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participating subjects.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

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

Participants will be assigned a unique participant ID number. All results will be keyed to this number. Study records will only be available to staff members and will be kept locked at the study site conforming to the investigators' SOPs. Following the conclusion of the study, all records will be maintained on site for a minimum of two years, after which they will be stored long-term in the data storage facilities in Bamako. All records will be retained in locked metal boxes for at least two years after a marketing application is approved for PfSPZ-CVac; or, if an application is not approved for PfSPZ-CVac, until two years after shipment and delivery of the drug for investigational use is discontinued and the FDA has been so notified. After either of these conditions has been met with permission of Sanaria and DMID, records will be destroyed. Representatives of Sanaria, the FDA, and DMID may review these records.

14.6 Study Discontinuation

The study may be discontinued at any time by DMID, the U.S. Food and Drug Administration, the institutional review board, or the investigators. In the event that the study is discontinued before completion, all participants who received study product will be asked to follow up with the study team for debriefing. Study team personnel will be available for questions or follow-up should evaluation be needed.

When the study ends, participants will be instructed to seek routine medical care offered at the local health clinics and at Sikasso district hospital. AEs that are ongoing at the time of study discontinuation will be followed by study staff to resolution, or, if a chronic condition has developed, until the end of the study follow-up period. As the PfSPZ-CVac is not a licensed product, it will not be offered to placebo recipients at the time of study discontinuation.

14.7 Future use of stored specimens

If residual nucleic acids, sera and cells are available following the serological and CMI assays described in this protocol, additional immunological and in vitro studies may be performed on those samples for which permission was expressly granted for preserving samples for future studies at the time of informed consent at study enrollment. These assays may include antibody epitope mapping, determination of response to other allelic forms of key parasite antigens, the ability of participant sera to interfere with in vitro parasite growth or invasion in an antigen-specific fashion, differential recognition of *P. falciparum* proteins pre- and post-vaccination and in protected and non-protected individuals, and sequence and expression analyses of human and parasite nucleic acids. Additional research questions to be asked for cells include antigen-specific cytokine induction as measured by ELISpot, flow cytometry, or both, or additional analysis to determine specificity of lymphoproliferation responses. These immunological studies will be limited to immune responses to malaria and mosquito antigens unless specific permission for additional studies is obtained from the relevant IRBs. Future use specimens will be kept at the DMID clinical repository. Samples from participants who did not grant permission to preserve samples will be discarded after the primary and secondary analyses described in this protocol have been completed. Study participants will have the right to withdraw their permission for further use of their samples at any time during and after the study. There will be no limitations on future use of cultured parasite lineages originally derived from clinical samples but which no longer have any human materials present.

15 DATA HANDLING AND RECORD KEEPING

The principal investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

Case report forms (CRFs) will serve as source documents. Only information that cannot be collected initially onto CRFs (namely, clinical laboratory test results and AE medical records) will first be collected onto separate source documents before transcription into CRFs. The information in the CRF will then be entered directly into the Internet data system.

CRFs derived from the eCRF will be provided and maintained for recording data for each participant enrolled in the study. Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained.

DMID and/or its designee will provide guidance to investigators on making corrections to the source documents and eCRF.

A copy of the cleaned and locked database will be provided to DMID, Sanaria and the Principal Investigator at the end of the study.

15.1 Data management responsibilities

All source documents and laboratory reports must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. AEs must be graded, assessed for severity and causality, and reviewed by the study site principal investigator or designee.

Data collection is the responsibility of the clinical trial staff at the study site under the supervision of the principal investigator. During the study, the investigator must maintain complete and accurate documentation for the study.

The Emmes Corporation will serve as the Statistical and Data Coordinating Center for this study and, in collaboration with the study data manager, will be responsible for data management, quality review, analysis, and reporting of the study data.

15.2 Data capture methods

Clinical data (including AEs, concomitant medications, and reactogenicity data) and clinical laboratory data will be entered into a 21 CFR Part 11-compliant Internet Data Entry System (IDES) provided by The Emmes Corporation. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

15.3 Types of data

Safety assessments will be based on solicited reactogenicity reports collected from review of AE and SAE reports. Reactogenicity will be summarized by severity across the entire observation period and into local and systemic symptoms. All AEs will be MedDRA® coded for Preferred Term and System Organ Class. The rate of AEs in aggregate, and by MedDRA® codes, will be computed for each vaccine group.

The number of SAEs is likely to be small in this study and will be reported by a detailed listing showing the type, MedDRA® coding, relevant dates (vaccination and AE), severity, and outcome for each event. The list will be by vaccine group. In the event that the number of all AEs is small, they will also be listed with the additional attribution of seriousness and with laboratory and clinical assessments.

An individual forms grid, which indicates the current status of forms submission for each participant is provided as part of The Emmes Corporation's Advantage eClinical℠ internet data entry system.

15.4 Timing/reports

Primary data analysis will occur after the primary study endpoint is reached 24 weeks after presumptive artesunate treatment (study day 245), after which time study participants will continue to be followed for safety, immunogenicity and extended efficacy analysis. Results of this primary data analysis will be available for public release. While it is possible that the study blind may be compromised due to public release of data, the benefit of primary data analysis availability is of great importance for malaria vaccine development. To minimize the potential for compromising the study blind after public release of data, the primary data analysis report will not include unblinded individual listings by participant. Furthermore, individual-level treatment assignments will not be provided to study team members responsible for participant follow-up

physical examinations and laboratory evaluations, nor to study participants themselves, through the end of the study (study day 413).

Analyses of preliminary safety and immunogenicity data may occur after the 12-day follow-up visits have been completed following all three injections.

Coding of AEs will be according to the MedDRA classification and will be managed by the statistical data and coordinating center as AE data is entered into the online database.

15.5 Study records retention

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of DMID. It is the responsibility of DMID to inform the investigator when these documents no longer need to be retained.

15.6 Protocol deviations

A protocol deviation is any noncompliance with the clinical trial protocol, Good Clinical Practice (GCP), or Manual of Procedures requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the study site and implemented promptly.

These practices are consistent with ICH E6:

4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3

5.1 Quality Assurance and Quality Control, section 5.1.1

5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the study site to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All deviations must be promptly reported to DMID, via The Emmes Corporation’s IDES or via the TRI/ICON DMID-Clinical Research Operations and Management Support (CROMS) email (protocoldeviations@dmidcroms.com), web- (www.dmidcroms.com) or fax-based system (1-215-699-6288).

All deviations from the protocol must be addressed in study subject source documents. A completed copy of the DMID Protocol Deviation Form (TRI/ICON DMID-CROMS or IDES form) must be maintained in the regulatory file, as well as in the subject's source document. Protocol deviations must be sent to the local IRB/IEC per their guidelines. The study site PI/study staff is responsible for knowing and adhering to their IRB/IEC requirements.

16 PUBLICATION POLICY

Following completion of the study, the investigator is expected to publish the results of this research in a scientific journal. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. It is the responsibility of DMID to register this trial in an acceptable registry. Any clinical trial starting enrollment after 01 July 2005 must be registered on or before patient enrollment.

The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., Phase I trials), would be exempt from this policy.

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18 APPENDIX A: NORMAL LAB VALUES

MRTC Laboratories

Lab Test	**Normal Range**
Hemoglobin, Female	9.1-13.8 gm/dL
Hemoglobin, Male	10.8-15.8 gm/dL
Platelets, Female	144,000-413,000/mm^3
Platelets, Male	114,000-335,000/mm^3
WBCs	3,600-9,000/mm^3
Creatinine, Female	≤ 71.8 µmol/L
Creatinine, Male	48-98 µmol/L
Alanine Aminotransferase (ALT)	3 – 41 IU/L
Urine pregnancy	Negative
Hepatitis B Surface Antigen	Negative
Hepatitis C Antibody	Negative
HIV	Negative
Hemoglobin electrophoresis or High-Performance Liquid Chromatography	No hemoglobin SS present