Sanaria® PfSPZ Challenge with Pyrimethamine Chemoprophylaxis (PfSPZ-CVac Approach): Phase 1 Dose Escalation Trial to Determine Safety and Development of Protective Efficacy after Exposure to Only Pre-erythrocytic Stages of *Plasmodium falciparum*

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List of Abbreviations

AE	Adverse event
AES	Asexual erythrocytic stages
ALT	Alanine transaminase
ANC	Absolute neutrophil count
AP	Alkaline phosphatase
AST	Aspartate aminotransferase
β-hCG	β human choriogonadotropin
CBC	Complete blood count
CFR	Code of Federal Regulations
cGMP	Current Good Manufacturing Practices
CHD	Coronary heart disease
CHI	Center for human Immunology, Autoimmunity and Inflammation
CHMI	Controlled human malaria infection
CoA	Certificate of Analysis
CPS	Chemoprophylaxis with sporozoites
CQ	Chloroquine Phosphate
CRF	Case report form
CRIMSON	Clinical Research Information Management System of the NIAID
CRP	C reactive protein
CSP	Circumsporozoite protein
CVac	Chemoprophylaxis Vaccination
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
DVI	Direct Venous Inoculation
EC	Ethics Committee
ECG	Electrocardiogram
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme-linked immunosorbent spot assay
FDA	Food and Drug Administration
FWA	Federal Wide Assurance
GAP	Genetically attenuated parasites
GCP	Good Clinical Practice
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HRP2	histidine-rich protein 2
HSA	Human serum albumin
IB	Investigator's Brochure
ICS	Intracellular cytokine staining
ID	Intradermal
IFA	Immunofluorescence assay

IHI	Ifakara Health Institute
IM	Intramuscular
IND	Investigational New Drug
IRB	Institutional Review Board
ISI	Inhibition of sporozoite invasion
ITV	Infection treatment vaccine
IV	Intravenous
LDH	Lactate dehydrogenase
LMIV	Laboratory of Malaria Immunology and Vaccinology (of NIAID)
LMVR	Laboratory of Malaria Vector Research
N	Number (typically refers to subjects or participants)
NASBA	nucleic acid sequence–based amplification
NHLBI	National Heart, Lung and Blood Institute
NIAID	National Institute of Allergy and Infectious Diseases, NIH
NIH	National Institutes of Health
NOCI	New Onset of Chronic Illness
OCRPRO	Office of Clinical Research Policy and Regulatory Operations
OHRP	Office for Human Research Protections
OHSR	Office of Human Subjects Research
PATH	Program for Appropriate Technology in Health
PBS	Phosphate-buffered saline
PI	Principal Investigator
Pf	*Plasmodium falciparum*
Pf7G8	*Plasmodium falciparum, 7G8 strain*
PfNF54	*Plasmodium falciparum, NF54 strain*
PfAES	*Plasmodium falciparum* asexual erythrocytic stages
PfSPZ	*Plasmodium falciparum* sporozoites
PfSPZ-CVac	Sanaria® PfSPZ Challenge Chemoprophylaxis vaccination
pRBC	Packed Red Blood Cells
PYR	Pyrimethamine
qPCR	Quantitative polymerase chain reaction
Real time qPCR	Real time quantitative polymerase chain reaction (qPCR assay performed the same day)
RUNMC	Radboud University Nijmegen Medical Center
Sanaria® PfSPZ Challenge	Aseptic, purified, cryopreserved *Plasmodium falciparum* sporozoites used for controlled human malaria infection (CHMI)
SAE	Serious adverse event/serious adverse experience
SOP	Standard Operating Procedure
SPZ	Sporozoites
SUSARS	Serious and unexpected suspected adverse reactions
UP	Unanticipated Problem
WBC	White blood cell
WHO	World Health Organization

PROTOCOL SUMMARY

Full Title:	Sanaria® PfSPZ Challenge with Pyrimethamine Chemoprophylaxis (PfSPZ-CVac Approach): Phase 1 Dose Escalation Trial to Determine Safety and Development of Protective Efficacy after Exposure to Only Pre-erythrocytic Stages of *Plasmodium falciparum*
Short Title:	Dose Escalation PfSPZ-CVac
Clinical Phase:	1
IND Sponsor:	Sanaria (product); OCRPRO (clinical activities)
Conducted by:	Laboratory of Malaria Immunology and Vaccinology (LMIV)
Principal Investigators:	Patrick Duffy, MD (LMIV/NIAID/NIH)
Site	National Institute of Health (NIH), Bethesda, Maryland, USA
Sample Size:	N=67 to 71
Accrual Ceiling:	230
Study Population:	Healthy malaria-naïve US adults, 18-50 years of age
Accrual Period:	Approximately June 2017 to June 2020
Study Design:	This is a phase 1 study to investigate the safety, tolerability, and immunogenicity of direct venous inoculation (DVI) with three monthly doses of aseptic, purified, cryopreserved *Plasmodium falciparum* (Pf) sporozoites (Sanaria® PfSPZ Challenge (NF54)), combined with either pyrimethamine or chloroquine drug dosing, known as PfSPZ chemoprophylaxis vaccination (CVac). This study will also investigate the protective efficacy of pre-erythrocytic stage only parasite exposure (pyrimethamine arm; *Arm 2a*) against homologous (same strain as that used for vaccination, Pf NF54) controlled human malaria infection (CHMI;) and that of pre-erythrocytic stage only parasite exposure (pyrimethamine, *Arm 2b*) *AND* pre-erythrocytic and erythrocytic stage parasite exposure (chloroquine arm; *Arm 3*) against heterologous (different strain from that used during vaccination Pf 7G8) CHMI.

In brief:

- *Arm 1* is a pilot arm to determine dose of sporozoites and pyrimethamine for *Arm 2*
- *Arm 5* is a pilot arm to determine a safe dose of sporozoites under chloroquine prophylaxis for *Arm 3*
- *Arm 2* and *3* are main study arms under pyrimethamine only (*Arm 2*) and chloroquine only prophylaxis (*Arm 3*)
- *Arm 4* is the infectivity control arm for *Arm 2* and *3* during CHMI

The study is to occur in two parts, pilot study and main study. The pilot study will be divided into two, first an adaptive pilot study (n=8-12) to assess safety, tolerability and identify a PfSPZ-CVac pyrimethamine regimen that maximizes the PfSPZ Challenge dose while still preventing subpatent parasitemia. Second, a pilot study (n=6) to assess safety and tolerability of PfSPZ-CVac chloroquine with increasing dose of PfSPZ Challenge dose. The regimens selected in the pilot study will then be followed by the main study which is designed to assess safety, tolerability, immunogenicity, and protective efficacy of PfSPZ-CVac with pyrimethamine (against homologous and heterologous CHMI) and PfSPZ-CVac with chloroquine (against heterologous CHMI).

In the first pilot study (*Arm 1*), subjects will undergo PfSPZ-CVac with one exposure of Sanaria® PfSPZ Challenge (NF54) with pyrimethamine dosing on days 2, 3 post PfSPZ Challenge. The goal of the pilot study is to progressively increase the dose of PfSPZ Challenge (50,000; 100,000; 200,000) while evaluating safety and tolerability of these increasing PfSPZ doses. The dose of pyrimethamine may also be increased as needed if there is detectable subpatent parasitemia (via qPCR) in the preceding arm. Subjects will be treated with standard treatment doses of Malarone® at the end of their enrollment. Subjects from the pilot study will not join the main study nor undergo CHMI.
In the second pilot study (*Arm 5*), subjects will undergo PfSPZ-CVac with one exposure of Sanaria® PfSPZ Challenge (NF54) with chloroquine, loading dose

administered 2 days before PfSPZ Challenge and maintenance dose administered 5 days post PfSPZ Challenge. The goal of the pilot study is to progressively increase the dose of PfSPZ Challenge (100,000; then 200,000) while evaluating safety and tolerability of these increasing PfSPZ doses. Subjects will be treated with standard treatment doses of Malarone® at the end of their enrollment. Subjects from the pilot study will not join the main study nor undergo CHMI.

In the main study, subjects will be enrolled in *Arm 2a* (pyrimethamine only; homologous CHMI n=17), Arm 2b (pyrimethamine only; heterologous CHMI n=10) or *Arm 3* (chloroquine only; heterologous CHMI n=10). Subjects in the main study will receive three exposures of Sanaria® PfSPZ Challenge (NF54), separated by 4 weeks, with pyrimethamine or chloroquine coverage (PfSPZ-CVac). *Arm 2a* and *2b* will use the optimal doses of PfSPZ-CVac with pyrimethamine as determined in the pilot study. *Arm 3* will use the optimal dose of PfSPZ-CVac with chloroquine as determined in the pilot study. The goal is for *Arm 2a, 2b* and *3* to use the same highest number of PfSPZ Challenge if this is determined to be safe and tolerable in the pilot study.

Subjects in the main study together with non-immunized infectivity controls (*Arms 4a* and *4b*) will then undergo CHMI. *Arm 2a* and *Arm 4a* will undergo CHMI using Sanaria® PfSPZ Challenge NF54 whereas *Arm 2b, 3* and *Arm 4b* will undergo CHMI using Sanaria® PfSPZ Challenge 7G8.
Enrollment in the study was divided into three cohorts. High significant level of protection against heterologous CHMI was observed after enrollment of cohorts 1&2. As a result, the study will not complete enrollment in *Arms 2a* and *4a* that were planned to undergo homologous CHMI (see details in **section 3.3.2**).

Study Duration:

Start Date: Approximately June 2017
End Date: Approximately June 2020
Study subjects will be enrolled for a total of approximately 1 (pilot/controls) to 7 (main) months

Study Agent/ Intervention Description:

Pyrimethamine: ***Arms 1, 2a*** and ***2b*** will receive orally two to three single strength pills (for a total of 50-75 mg pyrimethamine per day. The dose of pyrimethamine will be determined in the pilot study, *Arm 1*) dosed 2

and 3 days post Sanaria® PfSPZ Challenge (NF54) during the PfSPZ-CVac immunization phase. *Arms 3* (chloroquine arm) and *4* (infectivity controls) will not receive pyrimethamine.

Chloroquine: ***Arm 3*** **and** ***5*** will receive a loading dose of approximately 1000 mg chloroquine (600 mg base) 2 days prior to Sanaria® PfSPZ Challenge (NF54). *Arm 5* will receive a second and last dose of chloroquine (500 mg salt, 300 mg base) 5 days post Sanaria® PfSPZ Challenge. After loading dose, *Arm 3* will receive 500 mg (300 mg base) weekly continuously with the last dose administered 5 days post 3rd Sanaria® PfSPZ Challenge injection (NF54). A total of 10 doses of chloroquine will be given to *Arm 3*. *Arms 1, 2*, and *4* will not receive chloroquine.

Sanaria® PfSPZ Challenge during CVac

Arms 1*:* 1 dose of 50,000 or 100,000 or 200,000 aseptic, purified, vialed, cryopreserved, fully infectious Sanaria® PfSPZ Challenge (NF54) will be administered by DVI to *Arms 1*. The first day of administration of Sanaria® PfSPZ Challenge (NF54) is Study Day 1 for each Arm.

Arms 5*:* 1 dose of 100,000 or 200,000 aseptic, purified, vialed, cryopreserved, fully infectious Sanaria® PfSPZ Challenge (NF54) will be administered by DVI to *Arms 5*. The first day of administration of Sanaria® PfSPZ Challenge (NF54) is Study Day 1 for each Arm

Arm 2*:* 3 doses of 200,000 aseptic, purified, vialed, cryopreserved, fully infectious Sanaria® PfSPZ Challenge (NF54) will be administered by DVI four weeks apart. The first day of administration of Sanaria® PfSPZ Challenge is Study Day 1 thus Sanaria® PfSPZ Challenge will be administered on Study Days 1, 29 and 57.

Arms 3*:* 3 doses of 200,000 aseptic, purified, vialed, cryopreserved, fully infectious Sanaria® PfSPZ Challenge (NF54) will be administered by DVI four weeks apart. The first day of administration of Sanaria® PfSPZ Challenge is Study Day 1 thus Sanaria® PfSPZ Challenge will be administered on Study Days 1, 29 and 57.

Sanaria® PfSPZ Challenge for CHMI:

Arms 2a, 4a**:** Sanaria® PfSPZ Challenge (NF54; dose: 3,200 PfSPZ) will be administered by DVI to assess

homologous protective efficacy by CHMI approximately 13 weeks after 3rd Sanaria® PfSPZ Challenge for *Arm 2a* (Study Day 147). Infectivity controls (*Arm 4a)* join the main study at CHMI. Neither *Arm 2a* nor *4a* will receive any antimalarial chemoprophylaxis during CHMI.

***Arms 2b, 3, 4b*:** Sanaria® PfSPZ Challenge (7G8; dose: 3,200 PfSPZ, if this dose is confirmed by ongoing studies) will be administered by DVI to assess heterologous protective efficacy by CHMI approximately 13 weeks after 3rd Sanaria® PfSPZ Challenge (NF54) for *Arm 2b* and *3* (Study Day 147). Infectivity controls (*Arm 4b)* join the main study at CHMI. Neither *Arm 2b* and *3* nor *4b* will receive any antimalarial chemoprophylaxis during CHMI.

Primary Objectives:

Safety

- To monitor the safety and tolerability of Sanaria® PfSPZ Challenge (NF54) via DVI with pyrimethamine (PfSPZ-CVac pyrimethamine) or chloroquine (PfSPZ-CVac chloroquine). (*Arms 1, 2, 3, 5)*
- To evaluate whether pyrimethamine on days 2, 3 prevents patent parasitemia post Sanaria® PfSPZ Challenge (NF54) via DVI at increasing PfSPZ Challenge doses (maximum of 200,000 PfSPZ). (*Arm 1, 2)*
- To evaluate whether weekly chloroquine prevents clinical malaria diagnosis requiring treatment with additional antimalaria post Sanaria® PfSPZ Challenge (NF54) via DVI at increasing PfSPZ Challenge doses (maximum of 200,000 PfSPZ). (*Arm 3, 5)*

Secondary Objectives:

Protective Efficacy

- To assess the protective efficacy of PfSPZ-CVac pyrimethamine against homologous (NF54) CHMI. (*Arm 2a and 4a)*
- To assess the protective efficacy of PfSPZ-CVac pyrimethamine or chloroquine against heterologous (7G8) CHMI. (*Arm 2b, 3 and 4b*)

Exploratory Objectives:	**Immunogenicity** • To assess the humoral and cell mediated immune responses to PfSPZ and to known pre-erythrocytic and blood stage antigens • To assess the humoral and cell mediated immune responses to novel pre-erythrocytic antigens • To assess the kinetics of subpatent parasitemia during PfSPZ-CVac • To describe changes in γδ T cells in malaria naïve individuals after CVac and CHMI • To explore new diagnostic testing platforms (qPCR, rapid diagnostics, ELISA)
Primary Endpoints:	**Safety** • Incidence and severity of local and systemic adverse events (AEs) and serious adverse events (SAEs) occurring after PfSPZ-CVac. (*Arms 1, 2, 3, 5)* • *P. falciparum* blood stage infection defined as detection of *P. falciparum* parasites by qPCR (real time NIH qPCR and sensitive retrospective LMIV qPCR) following Sanaria® PfSPZ Challenge. (*Arm 1, 2)* • Incidence of clinical malaria diagnosis occurring after PfSPZ-CVac – chloroquine requiring treatment with additional antimalaria *(Arms 3 and 5)*
Secondary Endpoints:	**Protective Efficacy** • *P. falciparum* blood stage infection defined as detection of at least 2 *P. falciparum* parasites by microscopic examination of 0.5 µL of blood or one positive real time NIH qPCR after **homologous** PfSPZ CHMI (NF54) via DVI. (*Arm 2a and 4a*) • *P. falciparum* blood stage infection defined as detection of at least 2 *P. falciparum* parasites by microscopic examination of 0.5 µL of blood or one positive real time NIH qPCR after **heterologous** PfSPZ CHMI (7G8) via DVI. (*Arm 2b, 3 and 4b*)
Exploratory Endpoints:	• Humoral immune responses after PfSPZ-CVac regimens by assessing antibodies to PfSPZ, Pf asexual erythrocytic stages (AES), and specific Pf sporozoite, liver and blood-stage antigens such as CSP, EXP-1, LSA-1, MSP-3,

MSP-1, AMA-1 in subjects with no patent and/or subpatent parasitemia and those with patent and/or subpatent parasitemia, among drug groups.

- Cellular immune responses after PfSPZ-CVac regimen to PfSPZ, PfAES, and specific Pf sporozoite, liver and blood-stage antigens, such as CSP, EXP-1, LSA-1, MSP-3, MSP-1, AMA-1, in subjects with no patent and/or subpatent parasitemia and those with patent and/or subpatent parasitemia, among drug groups.
- Subpatent parasitemia and degree of subpatent parasitemia by sensitive retrospective LMIV qPCR following Sanaria® PfSPZ-CVac pyrimethamine and Sanaria® PfSPZ-CVac chloroquine.
- Comparison of γδ T cells before and after Sanaria® PfSPZ-CVac and CHMI using *ex vivo* whole blood staining
- Comparison of time to positivity of various malaria diagnostic platforms (qPCR, rapid diagnostics, ELISA).

Précis

Human studies have shown that immunization by the bite *Plasmodium falciparum* (Pf) sporozoite(SPZ)-infected mosquitoes under drug coverage with chloroquine, an approach called chemoprophylaxis with sporozoites (CPS) or infection treatment vaccination (ITV), can provide high level, long term protection against homologous controlled human malaria infection (CHMI) (Roestenberg, McCall et al. 2009, Roestenberg, Teirlinck et al. 2011). The Sanaria® PfSPZ chemoprophylaxis vaccination (PfSPZ-CVac) approach duplicates this with an injectable SPZ regimen. In both approaches, whether mosquitoes or syringes are used for SPZ administration, when chloroquine is used as the chemoprophylactic agent, transient, limited, asexual erythrocytic stage is seen in the majority of participants. Thus the question remains whether the significant protective efficacy seen can be achieved with pre-erythrocytic (sporozoite/liver stage) exposure only.

Previously, we performed a phase 1 study to investigate the safety, tolerability, immunogenicity, and protective efficacy of Sanaria® PfSPZ-CVac with chloroquine (sporozoites, liver, and blood stage) or pyrimethamine with chloroquine (sporozoites and liver stage only) to further describe stage specific sterile protection (NIAID protocol #15-I-0169). In this study, we demonstrated that pyrimethamine is safe to administer, well tolerated, and can prevent subpatent and patent parasitemia (qPCR and blood smear negative) 100% of the time during Sanaria® PfSPZ-CVac. The study also duplicated the results previously reported with Sanaria® PfSPZ-CVac with chloroquine in terms of safety profile and protective efficacy against homologous CHMI. Although a combination of Sanaria® PfSPZ-CVac with pyrimethamine and PfSPZ Challenge at 51,200 PfSPZ did not provide a significant protection level against homologous CHMI, we demonstrated that some subjects did develop protective immunity without any evidence of blood stage exposure during PfSPZ-CVac.

Building on these results and taking the lessons learned from other pre-erythrocytic vaccine studies and models that have shown the importance of reaching a minimal antigen threshold required for the development of sterile immunity, this proposed study will assess the safety, tolerability, immunogenicity, and protective efficacy of increasing the dose of Sanaria® PfSPZ Challenge sporozoites while receiving the same, or, if needed to successfully prevent parasitemia, a higher dose of pyrimethamine. Unlike the first study, however, pyrimethamine will be administered by itself as the partner drug, and will not be co-administered with chloroquine. The efficacy of PfSPZ-CVac with pyrimethamine will be assessed against CHMI with homologous parasites (*Arm 2a*) and CHMI with heterologous parasites (*Arm 2b*). Additionally, we will explore the impact of increasing the dose of Sanaria® PfSPZ Challenge sporozoites while receiving chloroquine alone prophylaxis. The efficacy of PfSPZ-CVac with chloroquine will be assessed only against CHMI with heterologous parasites (*Arm 3*), as protection against homologous parasites has now been shown in two separate trials. It will thus be possible to compare the efficacy of the two partner drugs against heterologous CHMI. The results of this study will contribute to understanding the targets and mechanisms of immunity against Pf

malaria infection and how the degree of exposure to the parasite (pre-erythrocytic or erythrocytic stage only or both) impacts these responses and subsequent protective efficacy.

1 Introduction and Rationale

1.1 Malaria Epidemiology

According to the World Health Organization (WHO), global control efforts have resulted in a reduction in the number of deaths since 2000; however 438,000 people are estimated to have died due to malaria in 2015, 69% of whom are African children under the age of 5 (WHO 2015). Malaria-related morbidity and mortality have a major economic impact in endemic regions and present a significant health risk to non-immune travelers to endemic regions and military personnel deployed overseas. To stem the worldwide impact of this devastating disease, a safe and broadly effective malaria vaccine and improved antimalarial therapeutics are urgently required. Investigations into the development of sterilizing immunity against malaria infection (prevention of blood-stage parasitemia and clinical illness) are of great importance to guide malaria vaccine development efforts and enhance the efficacy of current subunit malaria vaccine candidates.

1.2 Malaria Life Cycle and Vaccine Strategies

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

Vaccination targeted toward the clinically silent liver-stage of infection would ideally provide sterile protective immunity, preventing progression to blood-stage infection and clinical disease, and transmission of the parasites to mosquitoes. This is the target of leading vaccine strategies including the partially effective recombinant circumsporozoite protein (CSP) based RTS,S vaccine (Mosquirix®), in which phase 3 trials were completed throughout Africa in 2015, and phase 4 and implementation studies are underway. Subunit vaccines of this type utilize conserved antigenic targets to elicit protection against sporozoite migration, hepatocyte infection and intrahepatocytic parasite replication. RTS, S has protected malaria-naïve adults against an experimental Pf CHMI and reduced malaria-associated episodes in children living in malaria endemic areas, but the level and length of immunity seen is relatively modest (Stoute, Kester et al. 1998, Agnandji, Lell et al. 2011, Rts 2015). Although follow up studies of RTS,S vaccine have shown decreases in clinical malaria in vaccinated children; at 6 months post vaccination follow up, the levels of antibodies between vaccinated and unvaccinated children were not significantly different and did not predict protection against clinical malaria in the following 12 months

(Campo, Sacarlal et al. 2014). The mechanism by which RTS,S and other SPZ and pre-erythrocytic vaccine strategies confer protective immunity is still under investigation.

In the absence of more effective subunit vaccines, malaria vaccine development has continued to emphasize strategies that increase the feasibility of whole organism vaccination to induce sterile immunity. Prior studies using mosquito bites or irradiated SPZ (including Sanaria® PfSPZ Vaccine) for vaccination have required a large number of SPZ for the development of protective immunity (Hoffman, Goh et al. 2002, Roestenberg, McCall et al. 2009, Roestenberg, Teirlinck et al. 2011, Seder, Chang et al. 2013). In 2009, a human study suggested that anti-infection immunity can be achieved with a much smaller parasite inoculum. Roestenberg, et al. demonstrated that wild- type PfSPZ administered 3 times by 12-15 infectious mosquito bites (a total of 36-45 infected mosquitoes) to human subjects receiving concurrent antimalarial prophylaxis with the blood- stage drug chloroquine can induce sterile protection against malaria infection upon subsequent challenge with homologous parasites (Roestenberg, McCall et al. 2009). This method of immunization by experimental Pf infection with PfSPZ in conjunction with antimalarial prophylaxis is referred to as chemoprophylaxis with SPZ (CPS), infection treatment vaccine (ITV), and chemoprophylaxis vaccination (PfSPZ-CVac) by different authors. We use the term CVac in this protocol to refer to this vaccination concept with PfSPZ Challenge by direct venous inoculation (DVI).

1.3 Rationale for Chemoprophylaxis Vaccination (CVac) with SPZ Regimens

In rodent models, sterile protection against malaria can be achieved by the inoculation of intact SPZ while treating the animals concomitantly with chloroquine (Beaudoin, Strome et al. 1977, Belnoue, Costa et al. 2004) a drug that kills parasites in the asexual blood stage but not in the pre-erythrocytic stages. Importantly, the protective efficacy of this approach requires fewer SPZ to achieve high-level protective efficacy than does the radiation-attenuated SPZ model (Belnoue, Costa et al. 2004). Furthermore, as noted above, in humans, fully infectious PfSPZ administered by mosquito bites to subjects taking chloroquine chemoprophylaxis also induced protection in 10/10 volunteers and this protection lasted for at least 28 months in 4 of 6 participants (Roestenberg, McCall et al. 2009, Roestenberg, Teirlinck et al. 2011). Although this approach requires far fewer mosquito bites, thus fewer SPZ, than the strategy using irradiated mosquitoes, it still suffers from the same logistical limitation that providing infectious mosquitoes at a scale large enough for mass vaccination campaigns is not practical. And since chloroquine is a blood-stage schizonticide, the degree to which the protective immune response induced by the chloroquine CVac model targets sporozoites, liver, or blood-stage antigens is unclear from the study of Roestenberg et al.

The concept that sterile protective immunity to Pf can be induced by parasite exposure limited to the pre-erythrocytic stages of the parasite life cycle is supported by experimental infection studies in animals and humans. In rodent malaria models, blood-stage parasites may modulate dendritic cell responses and suppress protective CD8+ T cell responses that target parasites infecting hepatocytes (Ocana-Morgner, Mota et al. 2003). Additionally, CVac pyrimethamine regimens in mice has been shown to induce sterile protective immunity (Friesen, Borrmann et al. 2011). It is important to further define whether sterile protective immunity to Pf can be induced in humans by wild-type (non-attenuated) SPZ immunizations when exposure is limited to SPZ and pre-erythrocytic stages of the parasite life cycle and by a low dose of SPZ inoculum.

We performed a human study that explored this question (NIAID protocol #15-I-0169) and showed that a CVac regimen using chloroquine weekly in addition to pyrimethamine can prevent subpatent parasitemia. Using quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) we showed that none of the participants in pyrimethamine + chloroquine arms (Arm 1a, 2) were exposed to blood stage parasites. Participants in the chloroquine only arm (Arm 3) all had detectable subpatent parasitemia after the first CVac, with fewer participants being parasitemic after CVac #2 and #3. In addition, this initial study showed that CVac with pyrimethamine can lead to protective immunity against homologous CHMI, as 2/9 subjects in the pyrimethamine + chloroquine arm (Arm 2) remained uninfected and 1 additional participant was significantly delayed.

Other antimalarial drugs with activity against pre-erythrocytic stage parasites that have been considered for CVac regimens include primaquine, proguanil, and azithromycin. Proguanil alone is not FDA approved for treatment or prophylaxis for malaria. In a recently completed human trial, we found that single dose (45 mg) primaquine is insufficient to prevent blood stage parasitemia after Pf SPZ exposure (Healy, Duffy et al. in preparation). Azithromycin is currently under study for PfSPZ-CVac and is active against liver stage parasites, however it allows non-infectious merozoites to emerge from the infected hepatocyte and did not prevent subpatent parasitemia during PfSPZ-CVac.

Natural infection with wild-type Pf does not normally induce sterile protection against pre-erythrocytic stage parasites, possibly because only low levels of pre-erythrocytic or liver-stage specific cellular immune responses are generated (Plebanski, Aidoo et al. 1997). But unlike natural infections with wild-type parasites, radiation-attenuated *P. falciparum* parasites that arrest during liver-stage development can induce sterile protection in humans, but >1000 infectious mosquito bites (>10^5 cumulative sporozoites) are required to achieve this effect (Hoffman 1997, Hoffman, Goh et al. 2002). One possible conclusion drawn from this observation is that sterile protective immunity requires substantial or extended antigenic exposure. But this observation also highlights the possibility of a broadly effective combination SPZ and liver stage vaccine.

As seen in prior studies using irradiated sporozoites, a high number of sporozoites is required to develop protective immunity (Seder, Chang et al. 2013). Although CVac models that lead to blood stage exposure require lower number of Pf SPZ (Bijker, Borrmann et al. 2015), the optimal number of SPZ during CVac approaches using SPZ and liver stage exposure only has not been established. With results from our previous study, it is possible that this number is higher than the three episodes of exposure to 51,200 Sanaria® PfSPZ Challenge but less than the 4 PfSPZ Vaccine vaccinations of 270,000 irradiated sporozoites required to achieve 73% vaccine efficacy (Ishizuka, Lyke et al. 2016).

The study being proposed here aims to increase by up to four fold the number of Sanaria® PfSPZ Challenge (NF54) that was used in the prior trial (up to 200,000 PfSPZ). Our study attempts to determine the optimal combination of pyrimethamine and dose of PfSPZ Challenge required to prevent the development of asexual erythrocytic stages of the malaria parasite and subsequently be evaluated for protective immunity.

We propose to evaluate an optimal regimen of pyrimethamine in combination with an increasing number of PfSPZ to determine whether protective efficacy can be achieved in humans only exposed to pre-erythrocytic parasite stages. Exploration of stage-specific immune responses involved in protective immunity to Pf and discovery of new target antigens is critical for future development of malaria vaccine strategies.

1.4 Previous Human Experience

1.4.1 Whole SPZ Vaccination Models

Experimental infection studies in the early 1970s with radiation attenuated parasites demonstrated that induction of sterile protective immunity is achievable by whole SPZ vaccination. However, because of the substantial number of infectious mosquito bites (>1000) required to achieve this effect, this vaccine approach was logistically prohibitive to implement on a large scale. However, evaluation of the response to vaccination in these early studies provided valuable insight on anti-infection immunity and allowed identification of several candidate immunogens included in later vaccine platforms (Hoffman, Goh et al. 2002).

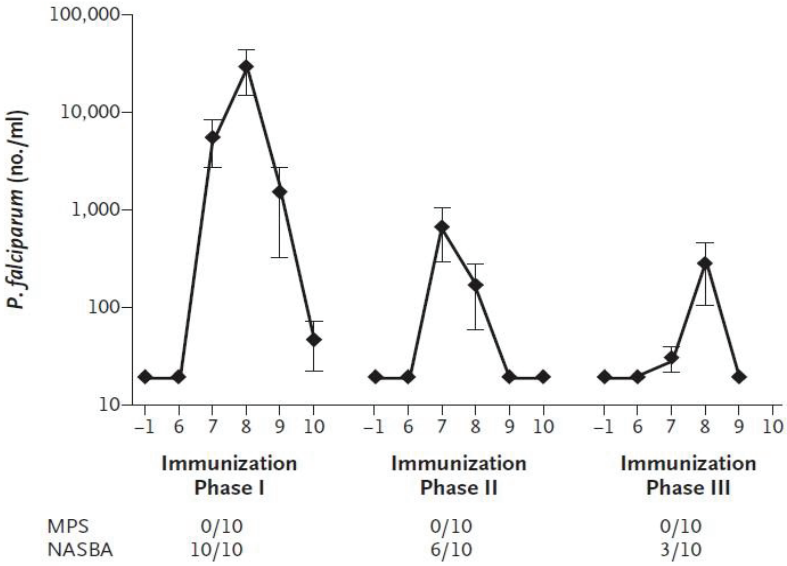
A recent variation on the whole SPZ vaccination concept achieves attenuation of the parasite through targeted disruption of genes critical to liver-stage development (Vaughan, Wang et al. 2010). Vaccination of mice with live, whole SPZ genetically attenuated parasites (GAP) that arrest during liver-stage development has been shown to confer durable and complete protection against subsequent challenge with wild-type (non-attenuated) sporozoites. A dose or genetic disruption required to achieve a sterile protective immune response in a Pf human GAP vaccine model is currently unknown, but initial dose-finding studies of the first GAP vaccine in humans have recently begun and will initially target a cumulative dose consistent with the irradiated SPZ model (Vaughan, Wang et al. 2010).

Recent human studies suggest that anti-infection immunity might be achieved with much smaller exposure or SPZ inoculum using wild type (non-attenuated) parasites in a CVac model. Roestenberg et al. have reported that human subjects who received a limited number of infected mosquito bites while receiving concurrent antimalarial prophylaxis were subsequently protected from development of blood-stage parasitemia upon challenge with homologous parasites. Subjects in this CVac model were exposed to three monthly episodes of experimental infection by the bites of 12-15 Pf infected mosquitoes while taking concurrent standard dose weekly suppressive prophylaxis with chloroquine. Four weeks after stopping chemoprophylaxis, subjects underwent subsequent challenge with homologous sporozoites by the bites of five infected mosquitoes and were completely protected from development of blood-stage parasitemia (Roestenberg, McCall et al. 2009). The total exposure over three episodes was 36 to 45 infected mosquito bites per subject, implying a one to two log-fold reduction in SPZ load required for induction of protective immunity compared to the irradiated SPZ model in humans. The degree to which this immune response targeted liver or blood-stage antigens is unknown. However, this approach to immunization does not protect against infection by DVI of Pf-infected erythrocytes, suggesting that immunity is primarily directed at the liver stages (Bijker, Bastiaens et al. 2013).

In addition, heterologous protection induced by CVac pyrimethamine and CVac chloroquine models is being explored. This is an important step in understanding the immune development and vaccine design as in endemic areas, people are exposed to multiple strains of Pf. Schats R et al. conducted a study (Clinicaltrials.gov Identifier: NCT01660854) in which they challenged subjects who had been immunized with NF54 infected mosquitoes (low to high dose) with NF135.C10 1 year after initial homologous challenge. Of the 13 subjects who were protected against homologous challenge, 2 were protected against this heterologous challenge. Those who were not protected against homologous challenge, were also not protected against heterologous challenge (Schats, Bijker et al. 2015). As with pre-erythrocytic stage vaccines, such as irradiated SPZ based vaccines, a higher number of sporozoites used for vaccination may be necessary. A vaccine that can induce protection against a various number of strains will be important in the goals to eliminate malaria.

Chloroquine is a blood-stage schizonticide frequently used for prophylaxis (suppressive therapy) against Pf malaria infection in non- immune populations with weekly oral dosing (1g [600 mg base] loading dose then 500 mg [300 mg base]). It has no activity on SPZ liver or early merozoite forms, but when used for prophylaxis it effectively kills intra-erythrocytic parasites early in the blood stage of infection preventing development of patent parasitemia and clinical disease (Yayon, Vande Waa et al. 1983). Accordingly, subjects in the first chloroquine CVac study were completely protected from development of patent parasitemia following each episode of experimental infection under chloroquine prophylaxis, although a brief subpatent parasitemia was detected by nucleic acid sequence–based amplification (NASBA) in progressively decreasing magnitude, duration and number of subjects following each of the three CVac infections (**Figure 1**).

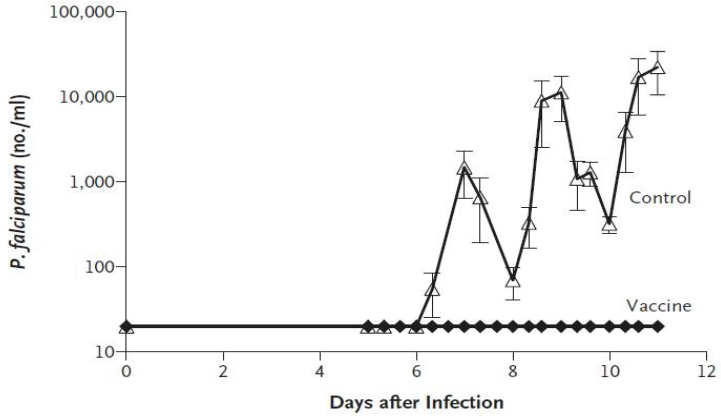
Figure 1: Mean Parasite Density Following *P. falciparum* Experimental Infection



The mean parasite density following *P. falciparum* experimental infection with concurrent chloroquine suppressive prophylaxis, as measured by nucleic acid sequence–based amplification (NASBA). The numbers of subjects who had positive results on peripheral blood smears for malarial parasites (MPS) and NASBA are shown below the graph. *Adapted from (Roestenberg, McCall et al. 2009)*

Following the challenge with homologous sporozoites in the absence of drug, subjects were monitored twice daily from day 6-21 for the development of patent and subpatent parasitemia. All subjects in the chloroquine CVac group were protected from development of both patent and subpatent parasitemia, whereas control subjects who received drug and uninfected mosquito bites during CVac developed a patent infection post-challenge (**Figure 2**).

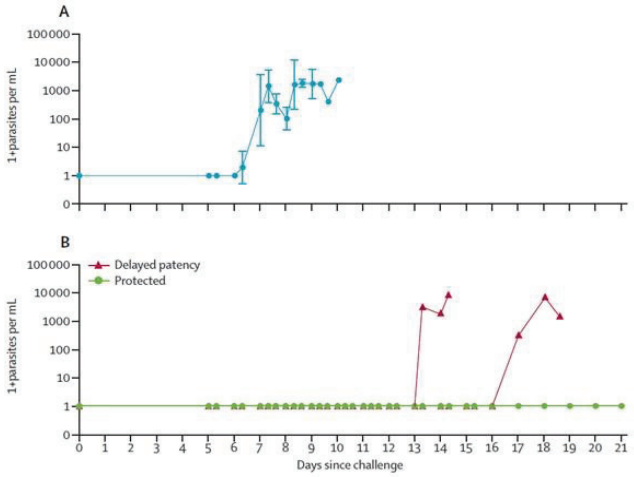
Figure 2: Mean Parasite Density in Control Group and Vaccinees Following *P falciparum* Challenge



The mean parasite density in control group (open triangles) and vacinees (diamonds) following *P. falciparum* challenge as determined by real-time polymerase-chain-reaction assay. The bars denote standard errors. *Adapted from (Roestenberg, McCall et al. 2009).*

Six of the previously immunized subjects from this study were rechallenged 28 months after the first controlled human malaria infection (CHMI) (Roestenberg, Teirlinck et al. 2011). Five newly recruited malaria-naïve subjects who served as infectivity controls developed patent parasitemia as previously reported. In contrast, four of the six vaccinees continued to have sterile protection, meaning both peripheral blood smear and real-time PCR evaluations remained negative (**Figure 3**). In the remaining two immunized subjects, patent parasitemia was delayed to 15 and 18 days post-challenge.

Figure 3: Parasite Density after Re-challenge with *P. falciparum*



Panel A shows the geometric mean (95% confidence interval) for parasite density in control subjects (n=5). Panel B shows individual plots for the six previously immunized subjects (n=6). Numbers of parasites were determined by real-time PCR. *Adapted from (Roestenberg, Teirlinck et al. 2011)*

In order to further dissect the role of pre-erythrocytic and erythrocytic stages in the development of protective immunity in a CVac model, Bijker EM, et al. as part of the above-described study, challenged immunized volunteers with stage specific parasites. Group 1 (n=9) were challenged by DVI of Pf infected erythrocytes. Group 2 (n=5) immunized volunteers were challenged by infected mosquito bites. Groups 3 and 4 were naïve subjects, infection controls for infected mosquito bites and DVI with erythrocytic stages respectively. 100% of volunteers in Group 2 were protected where as 0% of Group 1 were protected. In addition the group challenged by infected erythrocytes had pre-patent period similar to that of naïve controls (see **Table 1**). This study elucidated that the presence of erythrocytic stages during chloroquine CVac model may not have a significant role in development of protective immunity and that the developed immunity may be stage specific (Bijker, Bastiaens et al. 2013).

Table 1: Protection against blood stage versus sporozoites challenge after chloroquine CVac

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

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

^Adapted from Bijker EM, et al. 2013

To further characterize the immune response, Behet, M. et al. demonstrated that vaccinated volunteers developed IgG against circumsporozoite protein (CSP) that inhibited SPZ ability to glide and traverse in hepatocytes *in vitro*. In addition, compared to human liver-chimeric mice receiving pre-vaccination IgG, mice receiving post vaccination IgG had a 91% decrease pre-erythrocytic stages when infected with Pf SZP. This study demonstrated that CVac vaccination strategy results in development of inhibitory antibodies against CSP that may play a role in the development of protective immunity (Behet, Foquet et al. 2014).

In the above studies using chloroquine CVac, immunized volunteers were exposed to both erythrocytic and pre-erythrocytic blood stages, hence it is still unclear the role of each stage in the development of protective pre-erythrocytic stage immunity in this model.

1.4.1.1 Whole SPZ Vaccination Models – Pre-erythrocytic Stage Exposure

In order to dissect the role of various parasite stages to protective immunity, as noted prior, we conducted a study in which we used CVac with pyrimethamine to limit parasite exposure to pre-erythrocytic stages alone (NIAID protocol #15-I-0169). In that study, the main group (Arms 2 and 3: Arm 2, PYR+CQ, n=12; Arm 3, CQ only, n=6) received 3 immunization with PfSPZ-CVac (51,200 Sanaria® PfSPZ Challenge by DVI plus 50mg pyrimethamine on 2 and 3 days post injection and weekly chloroquine until 5 days after 3rd CVac (Arm 2) **or** plus weekly chloroquine only (Arm 3) before undergoing homologous CHMI with 3,200 Sanaria® PfSPZ Challenge via DVI. In Arm 3, 4/5 participants were protected as seen in above study. In Arm 2, 2/9 subjects remained uninfected and one subject (seen in red) was delayed which demonstrated that the CVac

with pyrimethamine approach may lead to protective immunity, even if the doses of PfSPZ Challenge were too low to induce high grade immunity (**Table 2**)

Table 2*:* CVac-PYR may lead to protective immunity following homologous CHMI

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

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

This proposed study will evaluate whether increasing number of Sanaria® PfSPZ Challenge will lead to protective immunity. The results of this study will help to evaluate the stage-specific exposure and immune response required for sterile protection following a complete CVac immunization series and subsequent homologous CHMI with Sanaria® PfSPZ Challenge.

1.4.2 Sanaria® PfSPZ Challenge (NF54)

To date, several trials have used Sanaria® PfSPZ Challenge produced by Sanaria® Inc. and are summarized here briefly:

1.4.2.1 Live (non-attenuated) PfSPZ (NF54 Sanaria® PfSPZ Challenge) Used for Controlled Human Malaria Infection (CHMI)

This product, Sanaria® PfSPZ Challenge, has now been used for CHMI in 15 different trials conducted in the USA, the Netherlands, the United Kingdom, Tanzania, Kenya, Germany, Gabon, Mali, and Spain (see below). Four hundred and fifty two (452) volunteers have received 456 doses of PfSPZ Challenge administered by the intradermal (ID), intramuscular (IM), intravenous (IV) and DVI routes to induce CHMI, and like PfSPZ Vaccine, it has been extremely well tolerated. 3,200 PfSPZ by DVI has been established as a gold standard regimen for inducing blood stage infection in malaria-naïve volunteers, and has infected 36/36 volunteers to date with a pre-patent period of 11.0-11.5 days, equivalent to the pre-patent period of the standard 5 mosquito bite CHMI (Mordmuller, Supan et al. 2015).

The availability of vialed Sanaria® PfSPZ Challenge has facilitated the broader use of CHMI, and therefore, has the potential to accelerate malaria vaccine development. Studies with CHMI in

endemic areas - to test the efficacy of new vaccine candidates - are now feasible at sites that do not have access to an insectary, enabling the malaria community to test new vaccine candidates directly in the target population in Africa.

In addition to ease of administration, the possibility to inject PfSPZ by needle and syringe offers further advantages compared to CHMI using infected mosquitoes:

- The number of sporozoites with which volunteers are inoculated can easily be calculated and predefined.
- The impact of variation in infectivity of mosquito-inoculated sporozoites when performing parallel clinical trials at multiple sites or sequential clinical trials at the same site with different lots of infected mosquitoes will be eliminated. Consequently, comparability will be increased substantially.
- Since challenges do not need to be administered in real time (as with mosquito bites), volunteers can be infected over an extended period with different doses, routes, and with varying concomitant medications.

1.4.2.2 CHMI using Intravenous Inoculation of NF54 Sanaria® PfSPZ Challenge in Malaria Naïve Subjects in Germany

The first volunteer received cryopreserved *P. falciparum* sporozoites by an indwelling intravenous catheter of Sanaria® PfSPZ Challenge at the Institute of Tropical Medicine Tübingen, Germany (ClinicalTrials.gov Identifier: NCT01624961) (Mordmuller, Supan et al. 2015). In a sequential dose-escalation trial 50, 200, 800, and 3,200 Sanaria® PfSPZ Challenge were administered IV until a dose that infected 9 out of 9 volunteers was established and, as a secondary endpoint, the geometric mean pre-patent period was 12 days or less (**Table 3**). A control group (Group 1) received 2,500 Sanaria® PfSPZ Challenge by intradermal injection to assess infectivity of the Sanaria® PfSPZ Challenge lot and to compare parasitological and clinical outcomes between sites.

The Sanaria® PfSPZ Challenge by DVI dose was escalated to 3,200 PfSPZ. Consequently, volunteers were recruited into all five groups as specified in **Table 3** below.

Table 3: Infection rate and pre-patent period

Group	Route	PfSPZ dose	Volunteers inoculated	Volunteers infected (%)	Days mean prepatency (ranges)
1	ID	2,500	6	4 (67)	13.6 (12.3 – 15.3)
2	IV	50	3	1 (33)	13.3 (N/A)
3	IV	200	3	1(33)	13.9 (N/A)
4	IV	800	9	7 (78)	11.7 (10.9-12.5)
5	IV	3,200	9	9 (100)	11.2 (10.5-12.5)

Sanaria® PfSPZ Challenge DVI was well tolerated in all 24 volunteers at all doses. Volunteers with parasitemia developed mild or moderate symptoms of malaria and were cured with standard antimalarial treatment.

In total 179 adverse events (AEs) occurred in 28 of the 30 volunteers: 162 Grade 1 AEs, 16 Grade 2 AEs and one Grade 3 AE as outlined in **Tables 4** and **5** below. No serious AE (SAE) occurred. Ten hours after injection one volunteer (800 PfSPZ group) experienced nausea, which was considered possibly related to PfSPZ injection. No local AE occurred after ID injection or IV of Sanaria® PfSPZ Challenge. Five volunteers had AEs that occurred during the 5 days after injection of Sanaria® PfSPZ Challenge, one of which was judged possibly related to the inoculation of Sanaria® PfSPZ Challenge. All other AEs occurred from day 5 after injection, and were possibly or probably related to the intervention. These AEs were likely to be caused by asexual parasite multiplication.

Table 4: Number of all Adverse Events for Groups 1-5

	Group 1 (ID 2500)	**Group 2 (IV50)**	**Group 3 (IV 200)**	**Group 4 (IV 800)**	**Group 5 (IV 3200)**
Adverse Events					
Total no. of AEs	36	23	10	39	71
SAEs	0	0	0	0	0
Total solicited AEs	30	17	7	36	62
Grade 1	22	16	6	35	58
Grade 2	8	1	1	0	4
Grade 3	0	0	0	1	0
Total unsolicited AEs	6	6	3	3	9
Grade 1	6	6	3	3	9
Grade 2 & 3	0	0	0	0	0

Table 5: Number of erythocytic stage adverse events and clinically significant laboratory abnormalities (Day 6 to Day 21)

Post- challenge AEs	Group 1 (ID 2500) 4/6 had parasitemia	Group 2 (IV 50) 1/3 had parasitemia	Group 3 (IV 200) 1/3 had parasitemia	Group 4 (IV 800) 7/9 had parasitemia	Group 5 (IV 3200) 9/9 had parasitemia
Abdominal pain	0	1	0	0	0
Anemia	0	0	0	0	1
ALT increase	0	1	0	0	0
AST increase	0	1	0	0	0
Back pain	0	1	0	0	0
Bilirubinuria	1	0	1	0	0
Chills	2	1	0	0	0
Common cold	0	0	0	0	1
Diarrhea	1	0	1	0	3
Dizziness	0	0	0	0	1
Fatigue	6	4	1	7	9
Fever	3	1	1	2	6
Feverish	0	0	0	0	1
Hemoglobinuria	0	0	1	0	0
Headache	4	8	1	13	18
Inflammation of the eye	0	0	0	0	1
Insomnia	1	1	1	0	0
Loss of appetite	0	0	0	0	2
Lymphopenia	0	0	0	1	0
Muscle ache	1	0	0	0	0
Myalgia	1	1	0	0	7
Nausea	0	2	0	2	6
Neck pain	0	2	0	0	0
Neutropenia	1	0	0	0	0
Stomach ache	1	1	0	0	0
Syncope	1	0	0	0	0
Tachycardia	3	0	3	3	8
TOTAL	29	22	10	28	64

1.4.2.3 CHMI using NF54 Sanaria® PfSPZ Challenge by DVI

A single center, varied dose, open label, controlled trial of Sanaria® PfSPZ Challenge with an escalated design in two parts, was conducted in Barcelona, Spain by the Barcelona Center for International Health Research (CRESIB) (NCT01771848) (Mordmuller, Supan et al. 2015). Part A determined a volume for IM administration of 2,500 Sanaria® PfSPZ Challenge was 10µL. Part B was designed to assess the effectiveness of administering different doses of Sanaria® PfSPZ Challenge (25,000 and 75,000 Sanaria® PfSPZ Challenge in 10 µL) intramuscularly and comparing to DVI administration of 3,200 Sanaria® PfSPZ Challenge, a dose that had been proven in a prior study to result in 100% infection. In part B, although 100% of volunteers were infected with geometric mean pre-patent period of 11-12 days, a higher number of Sanaria® PfSPZ Challenge administered IM was required to achieve a pre-patent period of less than 12 days that is seen in DVI administration as seen on **Table 6.**

Table 6:Summary of study design and infectivity outcomes

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
	N = 6	N = 6	N = 6	N = 6	N = 6	N = 6
Dose (number of PfSPZ & volume)	2,500 IM 10 µL	2,500 IM 50 µL	2,500 IM 250 µL	3,200 IV 500 µL	25,000 IM 10 µL	75,000 IM 10 µL
Number of volunteers who became thick smear positive (TS+)	4	1	3*	6	6	6
Listing of times to TS+ (days)	14, 15, 15, 12	14	16, 10*, 14	11, 10.4, 12.3, 10.9, 11.9, 12.2	12.4, 12.4, 13.3, 12.4, 11.1, 12	11.8, 11, 11, 11.4, 12, 12
Geometric mean time to TS+ (days)	13.9	14.0	13.1	11.4	12.2	11.5
Listing of parasite density at time of TS+ (parasites/µL blood)	10, 48, 6, 5	34	10, 4, 27	5, 1.25, 10, 8, 12, 14	4, 10, 5.8, 10, 3.75, 3.3	56, 0.83, 0.83, 8, 3.33, 6.6
Geometric mean parasite density at time of TS+ (parasites/µL blood)	11.0	34	10.3	6.6	5.5	4.4

* Volunteer BA001-022 from group 3, positive by microscopy on day 10 post CHMI was determined to be a false positive result by qPCR.

In total between part A and part B, 588 AEs occurred in 36 of the 36 volunteers: 379 Grade 1 AEs, 184 Grade 2 AEs and 25 Grade 3 AEs. There were no acute systemic allergic reactions. Only one volunteer presented a local AE following the injection. Most AEs recorded following challenge were consistent with symptoms associated with clinical malaria. No Grade 3 AEs were observed during the pre-erythrocytic phase (between challenge and day 5), nor during the erythrocytic phase (from Day 6 until Day 21 or day of treatment).

1.4.3 Using Sanaria® PfSPZ Challenge (NF54) for CVac

As previously described in detail, administration of PfSPZ via mosquito bites under chloroquine chemoprophylaxis induced high levels of long lasting protection in man, making it the currently most efficient way to induce sterile immunity against *P. falciparum* (Roestenberg, McCall et al. 2009, Roestenberg, Teirlinck et al. 2011). In its current format, unfortunately, the requirement of infectious mosquitoes precludes widespread use of this approach. However, the advent of vialed, viable sporozoites enabling delivery of PfSPZ via needle and syringe could avoid this logistical bottleneck. The previously described trials demonstrate that ID, IM, and IV administration of Sanaria® PfSPZ Challenge reliably infects both malaria naïve and malaria exposed individuals. We and others therefore consider further clinical studies exploring the usefulness of Sanaria® PfSPZ Challenge in the context of CVac highly warranted.

1.4.3.1 NF54 Sanaria® PfSPZ Challenge CVac under Chloroquine Prophylaxis

A randomized, placebo controlled, double-blinded, Sanaria® PfSPZ Challenge dose finding trial for CVac studies has been completed in Germany (NCT02115516) to identify the Sanaria® PfSPZ Challenge dose needed to replicate the results see in *Roestenberg et al.* In stage A, three groups (n=9 in each) received three CVac administrations 4 weeks apart by DVI with 3,200, 12,800 or 51,200 Sanaria® PfSPZ Challenge while under chloroquine prophylaxis for a total of 10 doses, starting with loading dose 2 days before the first Sanaria® PfSPZ Challenge and last dose given 5 days after the third Sanaria® PfSPZ Challenge DVI. A fourth group (n=5) received placebo injections. Nine to ten weeks after 3rd Sanaria® PfSPZ Challenge results show that upon homologous CHMI with 3,200 Sanaria® PfSPZ Challenge via DVI, 3/9 (3,200 PfSPZ-CVac), 6/9 (12,800 PfSPZ-CVac) and 9/9 (51,200 PfSPZ-CVac) of the subjects respectively were protected (Mordmuller, Surat et al. 2017). These results highlight the importance of higher antigen load required for vaccination efficacy, but significantly lower PfSPZ amounts required than needed to obtain 100% protection previously seen by Sanaria® PfSPZ Vaccine (irradiated *P.falciparum* sporozoites) (Seder, Chang et al. 2013).

Safety data from this study after unblinding show that adverse event (AE) between the four treatment groups were not significantly different in the frequencies of total adverse events [pain, tenderness, erythema/redness, and induration/swelling at the injection site; fever (°C), tachycardia, bradycardia, hypertension (systolic, diastolic), abnormal respiratory rate; nausea/vomiting, diarrhea, headache, fatigue, myalgia; abnormal laboratory tests (sodium, potassium glucose, bilirubin, creatinine, LDH, ALT, AST, white blood count differential component count including neutrophils, lymphocytes, monocytes, basophils, eosinophils, erythrocyte count, hemoglobin, hematocrit], as shown in **Figure 4**. There were also no significant differences between the three injections of Sanaria® PfSPZ Challenge (data not shown) in any of the groups.

Figure 4: Frequency of adverse events in all 4 groups after Sanaria® PfSPZ Challenge DVI

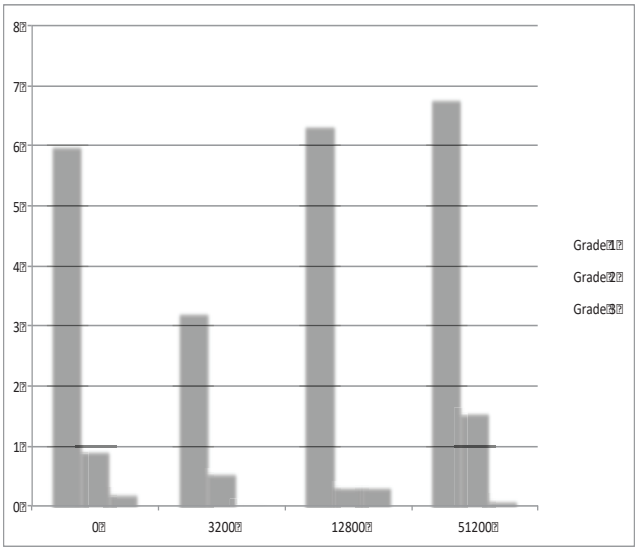


Figure 4. Total number of solicited and unsolicited adverse events (including abnormal signs and laboratory values) recorded per volunteer during administration of 3 doses of vaccine or placebo (y axis), in each PfSPZ dose group (x axis). The period covered is from day -3 to day 77.

Parasitemia was seen by qPCR in all PfSPZ-immunized volunteers after the first Sanaria® PfSPZ Challenge (except one of the group receiving 3200 PfSPZ), and in a decreasing number of volunteers after the second and third Sanaria® PfSPZ Challenge (**Table 7**). Parasitemia typically occurred during the six to ten day interval after PfSPZ Challenge administration, peaking on days 7 or 8.

Table 7: Summary of PfSPZ-CVac doses and number of qPCR positive subjects per CVac

PfSPZ Challenge dose per immunization	Number Subjects Positive by qPCR		
	Immunization 1	Immunization 2	Immunization 3
3,200	8 / 8*	9 / 9	6 / 9
12,800	9 / 9	8 / 9	7 / 9
51,200	9/ 9	8 / 9	3 / 9

* Technical difficulties precluded assessment of one volunteer's sample

The density of parasites increased with increasing doses of PfSPZ Challenge, approximately doubling with each four-fold increase in PfSPZ dose. The geometric mean parasite densities (and ranges) after the first immunizations in the low, medium and high dose groups in TÜCHMI-002 were, respectively, 462 (228-989), 711 (120-1,891) and 15,652 (5,146-36,404) parasites/mL (**Table 8**).

Table 8: Summary of PfSPZ-CVac doses and mean parasite density per CVac

	Geometric Mean Parasite Density (range)*		
Dose	Immunization 1	Immunization 2	Immunization 3
3,200	462 (228-989)	78 (2-1,236)	210 (31-2,170)
12, 800	711 (120-1,891)	195 (13-762)	57 (14-417)
51, 200	15,652 (5,146-36,404)	555 (63-3,089)	42 (11-126)

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

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

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

Thus, in summary, there was no clear indication that immunization with up to 51,200 PfSPZ of PfSPZ Challenge caused any excess of abnormal signs, symptoms or laboratory values relative to normal saline placebo, with the possible exception of one volunteer in the 51,200 PfSPZ group. In stage B of the study, three doses of 51,200 PfSPZ are being administered to 19 volunteers divided into three cohorts, and concurrently three doses of normal saline are being administered to 6 volunteers in blinded fashion (two per cohort). The timelines for the regimens were condensed relative to Stage A: 9 volunteers are receiving doses of PfSPZ Challenge on days 0, 14 and 28, and 9 volunteers are received doses of PfSPZ Challenge on days 0, 5 and 10. Half of the 10 volunteers on the four-week schedule are received 2g extended release azithromycin (Zithromax UNO, Pfizer) in addition to chloroquine with the first dose of PfSPZ Challenge. AE profiles were similar to those seen in Stage A. CHMI was conducted using PfSPZ Challenge administered by DVI 10-11 weeks after the last immunization, and 6/9 (67%) volunteers were protected after the 0, 14 and 28 day regimen, and 5/8 (63%) volunteers were protected after the 0, 5 and 10 day regimen. These results showed that the PfSPZ-CVac approach induced sterile immunity even after highly condensed regimens.

However, there was one unanticipated problem. One of the volunteers receiving the 0, 14 and 28 day regimen of Sanaria® PfSPZ Challenge experienced parasitemia by PCR on day 7, peaking on day 8 and then falling progressively on days 9 and 10, which is typical pattern previously seen. But then atypically, the subject showed increasing parasitemia on day 11, and was found to be blood smear positive on day 13, during which time the subject also experienced mild

symptoms of malaria (headache, sweating). The volunteer was treated with atovaquone/proguanil on days 13-15 and cleared the parasitemia. A retrospective review of the day 11 blood smear with more intensive searching revealed trace parasites. This subject's plasma chloroquine levels on day 13 were less than 2 ng/mL (certified limit of assay quantification 5 ng/mL) although trace levels of chloroquine were present. On questioning, the volunteer stated that he did swallow the chloroquine tablets. The team proposed a pharmacokinetic study of chloroquine absorption and metabolism in this volunteer to explain the apparent poor bioavailability. The volunteer initially agreed to undergo this study, and to take another dose of 10 mg/kg chloroquine with additional blood samplings to diagnose possible causes of the low plasma levels. However, when the pharmacokinetic study had been set up, the volunteer refused to participate. Therefore, the reason for the low chloroquine levels in this volunteer may never be definitively determined. The suspicion is that the volunteer kept the chloroquine tablets in his cheek and discarded them later. The small amount of absorption that would have taken place through the oral mucosa would explain the trace levels found on day 13.

The parasites from this volunteer were isolated on day 13 and tested *in vitro* for chloroquine resistance, and were found to be highly sensitive to chloroquine, consistent with all testing that has been performed on Sanaria® PfSPZ Challenge in the past.

As a result of this unanticipated event (inadequate levels of chloroquine), the IND Sponsor (Sanaria, Inc.) is recommending that chloroquine levels should be collected after chloroquine administration and prior to the anticipated peaking of parasitemia post PfSPZ Challenge, and this recommendation is followed in this protocol (see **Section 8.14**).

Although CVac-chloroquine with 51,200 NF54 Sanaria® PfSPZ Challenge in three successive 4-weekly vaccinations leads to 80-100% protective efficacy against homologous CHMI, it is likely that a higher dose of NF54 Sanaria® PfSPZ Challenge will be required to achieve protection against heterologous CHMI. As mosquitoes in endemic areas harbor multiple strains of Pf, it will be important to demonstrate that vaccine candidates can protect against heterologous strains.

Therefore, a phase 1, blinded, randomized, dose escalation trial to assess the safety and efficacy of NF54 Sanaria® PfSPZ Challenge DVI under chloroquine cover in healthy malaria-naïve adults in the United States (Clinicaltrials.gov Identifier: NCT02773979) is currently being conducted by Seattle Group Health. The trial has 3 groups with increasing PfSPZ Challenge doses from 51,200, 102,400 and 204,800 PfSPZ (NF54). Each group has 12 subjects randomized 3:1 (PfSPZ Challenge: normal saline) and receives 3 CVac vaccinations with a 7 day interval between each vaccination (vaccinations were done at 4 week intervals in our prior study NIAID protocol # 15-I-0169). Enrollment of successive groups for dose escalation is staggered by 3 week intervals, with each higher dose group being enrolled after the lower dose group has received all three CVac vaccinations. Currently, the initial group receiving 51,200 PfSPZ Challenge has completed the vaccination phase. Unexpectedly, subjects experienced a higher frequency and severity of AEs (**Table 9**) in comparison to other PfSPZ-CVac chloroquine studies with 51,200 PfSPZ Challenge dose given at 4 weeks interval [NIAID protocol # 15-I-0169, **Table 10**; (Mordmuller, Surat et al. 2017)**]** or at 5 or 14 day intervals (Mordmuller, Surat et al. 2017).All severe AEs, which included high grade fever, severe malaise, severe myalgia,

severe arthralgia and severe chills, happened approximately 8 days after receiving an injection with PfSPZ Challenge, and coincided with periods of transient parasitemia. Investigations are ongoing to understand this phenomenon. One of the theories is that there was exaggerated immune response due to the administration of a large number of sporozoites in the presence of blood stream parasites (parasitemia peaked on roughly the seventh and eighth days after each injection of PfSPZ Challenge). Since in this proposed study the vaccinations are at 4 week intervals, subjects will already have cleared any blood stage parasitemia by the time of the next injection. Thus we do not expect to see the same frequency and severity of AEs as the Seattle study. However, as a precaution, we propose to add a PfSPZ Challenge dose escalation pilot study to determine safety and tolerability of 100,000 and 200,000 PfSPZ Challenge under chloroquine prophylaxis before proceeding to the main study. Safety data from the pilot study will be used to determine which PfSPZ Challenge dose will be used in *Arm 3* of the main study.

***Table 9**:* List of AEs post PfSPZ-CVac with weekly 51,200 Sanaria® PfSPZ Challenge DVI under chloroquine in *NCT02773979*

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

Note: X (X) X% refers to Absolute number of AEs (Number of subjects with AEs) Percent of subjects with AEs
* vaccination happened on days 3, 10 and 17
** 1 subject did not receive Vax #3 but was followed for safety
*** Data only available for 6 subjects followed up to study day 25, 22 days post CVac #1
**** Malaria symptoms are expected around 7 days and beyond post injection corresponding to parasitemia

1.4.3.2 Sanaria® PfSPZ-CVac (NF54) using Chloroquine and Pyrimethamine Prophylaxis

As briefly summarized in **Section 1.4.1.1**, an open label, single center, phase 1 study was undertaken at the National Institutes of Health (NIH) Clinical Center starting in 2015 (NIAID protocol #15-I-0169, NCT02511054). The Arm 1a pilot regimen (n=2; dosing of 50 mg pyrimethamine on days 2, 3 post 51,200 PfSPZ Challenge via DVI while under weekly chloroquine chemoprophylaxis) successfully prevented patent and subpatent parasitemia (negative by blood smear and qPCR) in 2 of the 2 subjects enrolled following first vaccination (CVac #1), thus no further pilot regimens were explored. Arm 1a subjects completed 3 immunization with PfSPZ-CVac, 28 days apart, and underwent homologous CHMI, approximately 6 months later, with the main group.

The main group (Arms 2 [pyrimethamine + chloroquine, n=12]; Arm 3 [chloroquine only, n=6]) received 3 immunizations with PfSPZ-CVac, 28 days apart. Following approximately 12 weeks of drug washout, subjects then underwent homologous CHMI in June 2016.

To determine if limited exposure to the parasite, pre-erythrocytic stages only (pyrimethamine + chloroquine) results in sterile protection against homologous CHMI with 3,200 PfSPZ Challenge administered via DVI was conducted. In total 21 subjects were challenged (Arms 1a [n=2], 2 [n=9] and 3 [n=5] as well as Arm 4 [infectivity controls, n=5]).

1.4.3.2.1 CVac period

Overall vaccinations were well tolerated. The majority of AE's reported, 180/210 (86%) were mild (Grade 1) and mostly transient (laboratory abnormalities, insomnia, dizziness, nausea, diarrhea, headache, myalgia, fatigue) (**Table 10**).

There were a few reported Grade 2 AE's, 27/210 (13%), that all resolved; most commonly, diarrhea, headache, hemoglobin decrease, insomnia and fatigue. In total, three (3) Grade 3 AEs were reported: sleep disorder and acute gastroenteritis (unlikely related to any of the study products; both occurred in the same individual) and encephalopathy NOS (which was also reported as a SAE) that was determined to be possibly related to chloroquine (**Table 10**). All Grade 2 and 3 AEs and the SAE resolved without complication.

The following SAE was reported following CVac #3. A 23 year old previously healthy female who was enrolled in Arm 2 (pyrimethamine + chloroquine) presented with the acute onset of

nausea, vomiting, headache, tinnitus, fogginess, and confusion worsening over the course of a week concerning for encephalitis versus encephalopathy of unknown etiology. During the ensuing evaluation, she was diagnosed with an incidental Chiari I malformation with syrinx on magnetic resonance imaging. She was hospitalized for 4 days to facilitate prompt clinical evaluation and management. She was treated with intravenous acyclovir for presumed herpes simplex virus encephalitis while she underwent further evaluation. She was evaluated by NIH neurology, neurosurgery, and otolaryngology consulting services during this time. Unfortunately, cerebrospinal fluid could not be obtained due to the presence of a Chiari malformation however the rest of her work up which included negative malaria diagnostic testing (NIH diagnostic qPCR and blood smears), blood and urine cultures, urine toxicology, viral, rickettsial, and autoimmune causes of encephalitis were all unrevealing. She completed a 14 days course of intravenous acyclovir. Her symptoms improved over time with the main symptom of 'fogginess' fully resolving in 11 days. A grade 1 headache lingered and finally fully resolved in approximately 40 days from presentation. Due to the SAE, she was withdrawn from the study and treated with Malarone® per protocol. Convalescent viral titers were completed approximately 4 months post presentation but were also unrevealing of a diagnosis. Final diagnosis was encephalopathy of unknown etiology that may have been possibly related to one of the study products (chloroquine) or another etiology unrelated to study participation (such as viral).

Table 10: Summary of Adverse Events during Vaccination Period

	Arm 1a (PYR/CQ)				Arm 2 (PYR/CQ)				Arm 3 (CQ only)			
	CVac 1	CVac 2	CVac 3	**Total**	CVac 1	CVac 2	CVac 3	**Total**	CVac 1	CVac 2	CVac 3	**Total**
	N= 2	N= 2	N= 2	**N= 2**	N= 12	N= 12	N= 11	**N=12**	N= 6	N= 5	N= 5	**N= 6**
Total # AEs	**12 (2) 100%**	**1 (1) 50%**	**6 (2) 100%**	**19 (2) 100%**	**61 (12) 100%**	**23 (10) 83%**	**41 (9) 82%**	**125 (12) 100%**	**35 (6) 100%**	**14 (4) 80%**	**17 (5) 100%**	**66 (6) 100%**
Classification												
Local Reactogenicity	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**	1 (1) 8%	0 (0) 0%	0 (0) 0%	**1 (1) 8%**	1 (1) 17%	0 (0) 0%	0 (0) 0%	**1 (1) 17%**
Systemic Reactogenicity	5 (2) 100%	0 (0) 0%	2 (2) 100%	**7 (2) 100%**	33 (11) 92%	9 (6) 50%	14 (6)55%	**56 (12) 100%**	18 (6) 100%	10 (4) 80%	5 (3) 60%	**33 (6) 100%**
Laboratory Abnormalities	1 (1) 50%	1 (1) 50%	1 (1) 50%	**3 (1) 50%**	5 (4) 33%	4 (3) 25%	4 (2) 18%	**13 (8) 67%**	3 (2) 33%	1 (1) 20%	3 (1) 20%	**7 (2) 33%**
Unsolicited AEs	6 (2) 100%	0 (0) 0%	3 (2) 100%	**9 (2) 100%**	23 (10) 83%	10 (5) 42%	23 (7) 64%	**56 (11) 92%**	13 (5) 83%	3 (1) 20%	9 (5) 100%	**25 (5) 83%**
Severity and Relationship												
Grade 1	**12 (2) 100%**	**1 (1) 50%**	**6 (2) 100%**	**19 (2) 100%**	**53 (12) 100%**	**19 (9) 75%**	**33 (8) 73%**	**105 (12) 100%**	**30 (6) 100%**	**12 (3) 60%**	**14 (5) 100%**	**56 (6) 100%**
Related to PfSPZ Challenge	2 (2) 100%	0 (0) 0%	2 (1) 50%	**4 (2) 100%**	20 (9) 75%	5 (4) 33%	4 (2) 18%	**29 (9) 75%**	5 (4) 67%	1 (1) 20%	4 (4) 80%	**10 (4) 67%**
Related to Induced Malaria	1 (1) 50%	0 (0) 0%	0 (0) 0%	**1 (1) 50%**	6 (4) 33%	1 (1) 8%	7 (3) 27%	**14 (4) 33%**	4 (3) 50%	1 (1) 20%	1 (1) 20%	**6 (3) 50%**
Related to Chloroquine	4 (1) 50%	1 (1) 50%	1 (1) 50%	**6 (2) 100%**	13 (8) 67%	2 (2) 17%	14 (2) 18%	**29 (8) 67%**	4 (4) 67%	1 (1) 20%	1 (1) 20%	**6 (4) 67%**
Related to Pyrimethamine	3 (1) 50%	1 (1) 50%	0 (0) 0%	**4 (1) 50%**	11 (4) 33%	4 (3) 25%	7 (1) 9%	**22 (4) 33%**	N/A	N/A	N/A	**N/A**
Grade 2	**0 (0) 0%**	**0 (0) 0%**	**0 (0) 0%**	**0 (0) 0%**	**8 (7) 58%**	**4 (4) 33%**	**7 (6) 55%**	**19 (11) 92%**	**3 (3) 50%**	**2 (2) 40%**	**3 (2) 40%**	**8 (5) 83%**
Related to PfSPZ Challenge	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**	1 (1) 8%	1 (1) 8%	1 (1) 9%	**3 (3) 25%**	1 (1) 17%	0 (0) 0%	0 (0) 0%	**1 (1) 17%**
Related to Induced Malaria	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**	0 (0) 0%	1 (1) 8%	0 (0) 0%	**1 (1) 8%**	1 (1) 17%	1 (1) 20%	1 (1) 20%	**3 (1) 17%**
Related to Chloroquine	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**	2 (2) 17%	1 (1) 8%	1 (1) 9%	**4 (2) 17%**	1 (1) 17%	0 (0) 0%	0 (0) 0%	**1 (1) 17%**
Related to Pyrimethamine	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**	N/A	N/A	N/A	**N/A**
Grade 3	**0 (0) 0%**	**0 (0) 0%**	**0 (0) 0%**	**0 (0) 0%**	**0 (0) 0%**	**0 (0) 0%**	**1 (1) 9%**	**1 (1) 8%**	**2 (1) 17%**	**0 (0) 0%**	**0 (0) 0%**	**2 (1) 17%**
Related to PfSPZ Challenge	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**	0 (0) 0%	0 (0) 0%	1 (1) 9%	**1 (1) 8%**	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**
Related to Induced Malaria	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**
Related to Chloroquine	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**	0 (0) 0%	0 (0) 0%	1 (1) 9%	**1 (1) 8%**	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**
Related to Pyrimethamine	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**	N/A	N/A	N/A	**N/A**
Grade 4	**0 (0) 0%**	**0 (0) 0%**	**0 (0) 0%**	**0 (0) 0%**	**0 (0) 0%**	**0 (0) 0%**	**0 (0) 0%**	**0 (0) 0%**	**0 (0) 0%**	**0 (0) 0%**	**0 (0) 0%**	**0 (0) 0%**
Related to PfSPZ Challenge	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**
Related to Induced Malaria	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**
Related to Chloroquine	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**
Related to Pyrimethamine	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**	N/A	N/A	N/A	**N/A**

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

1.4.3.2.2 Homologous CHMI

A total of 21 subjects underwent CHMI with 3,200 PfSPZ Challenge (NF54) administered by DVI. In this study, two positive NIH Malaria Genus Species 4-plex real time polymerase chain reaction (NIH malaria real time qPCR) on separate days or a single peripheral thick blood smear were used for diagnosis and determination of treatment.

The majority of symptoms reported were solicited and expected as part of a malaria diagnosis with 70/103 (69%) being Grade 1 and most commonly reported were headache, fatigue, myalgia, and arthralgia. Twenty- one (21) of the 103 symptoms reported (20%) were Grade 2 which included headache, fatigue and myalgia. There were few Grade 3 AEs 12/103 (12%) of which the majority were transient laboratory abnormalities seen following the diagnosis of malaria and included decreases in white blood cell count, neutrophil count, lymphocyte count, and platelet count (**Table 11**); one (1) subject experienced Grade 3 increase in alanine transferase (ALT) and aspartate transferase (AST). Most subjects had mild malaria symptoms following diagnosis of malaria, however five (5) subjects (3 vaccinees and 2 controls) developed the final diagnosis of Grade 3 malaria due to either reported symptoms and/or laboratory abnormalities that made Grade 3 criteria, though these were often transiently Grade 3, reported after diagnosis and initiation of treatment, and all resolved without complication. However, one subject in Arm 1a developed Grade 3 symptoms (Grade 3 due to not being able to go to work that day) prior to the second positive PCR result being received and was treated per the investigator's discretion that morning based off symptoms and reported previous single positive NIH malaria real time PCR. Both the NIH malaria real time PCR and blood smear in this subject were reported positive a few hours later that day.

Of those subjects who had a reported positive blood smear during the study, the majority were reported after PCR diagnosis had been made, either at the timing of start of antimalarial treatment or day 1 post antimalarial treatment was initiated. Majority of subjects developed symptoms after starting treatment for malaria that required management with non-steroidal anti-inflammatory drugs (NSAIDs) and anti-nausea medications. None of the subjects required hospitalization or significant medical management for symptoms or treatment of malaria. All AEs fully resolved without complication.

Table 11: Summary of Reported Symptoms (Reactogenicity and AEs) in Arms 1-4 during CHMI

	CHMI				
	Arm 1a	**Arm 2**	**Arm 3**	**Arm 4**	**Total**
	N= 2	N= 9	N= 5	N= 5	**N= 21**
Total # AEs	**12(2)100%**	**36(9)100%**	**23 (5)100%**	**32(5)100%**	**103 (21) 100%**
Classification					
Local Reactogenicity	0 (0) 0%	0 (0) 0%	0 (0) 0%	1 (1) 20%	**1 (1) 5%**
Systemic Reactogenicity	5 (2) 100%	7 (6) 67%	10 (4) 80%	16 (5) 100%	**38 (17) 81%**
Laboratory Abnormalities	4 (2) 100%	20 (6) 67%	7 (2) 40%	11 (3) 60%	**42 (13) 62%**
Unsolicited AEs	3 (1) 50%	9 (4) 44%	6 (3) 60%	4 (2) 40%	**22 (10) 48%**
Severity and Relationship					
Grade 1	**9 (2) 100%**	**22 (9) 100%**	**22 (5) 100%**	**17 (5) 100%**	**70 (21) 100%**
Related to PfSPZ Challenge	2 (1) 50%	0 (0) 0%	5 (2) 40%	4 (1) 20%	**11 (4) 19%**
Related to Induced Malaria	4 (1) 50%	18 (6) 67%	6 (3) 60%	8 (4) 80%	**36 (14) 67%**
Grade 2	**1 (1) 50%**	**10 (5) 56%**	**0 (0) 0%**	**10 (3) 60%**	**21 (9) 43%**
Related to PfSPZ Challenge	0 (0) 0%	1 (1) 11%	0 (0) 0%	3 (2) 40%	**4 (3) 14%**
Related to Induced Malaria	1 (1) 50%	5 (4) 44%	0 (0) 0%	4 (2) 40%	**10 (7) 33%**
Grade 3	**2 (1) 50%**	**4 (1) 11%**	**1 (1) 20%**	**5 (2) 40%**	**12 (5) 24%**
Related to PfSPZ Challenge	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**
Related to Induced Malaria	2 (1) 50%	4 (1) 11%	1 (1) 20%	5 (2) 40%	**12 (5) 24%**
Grade 4					
Related to PfSPZ Challenge	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**
Related to Induced Malaria	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	**0 (0) 0%**

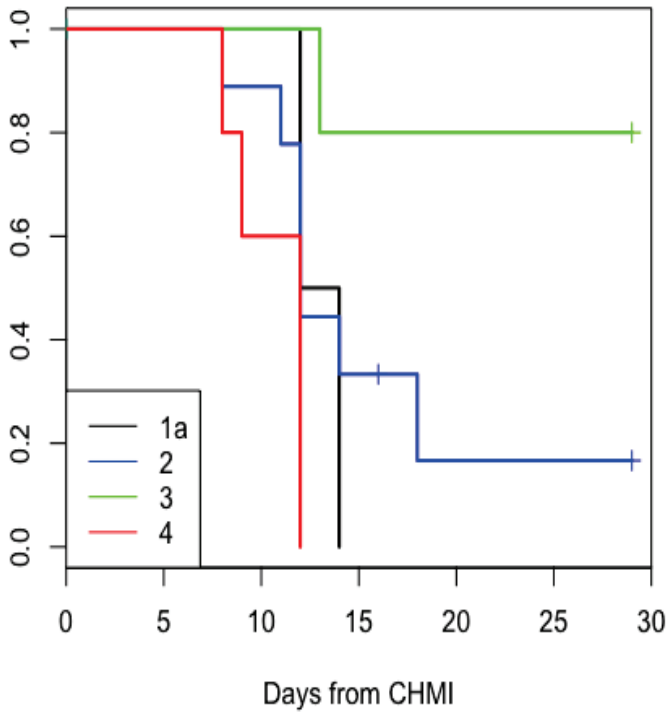
Note: X (X) X% refers to absolute number of AEs (Number of subjects with AEs) Percent of subjects with AEs

In this study, two positive NIH malaria real time qPCR (limit of detection ~500 parasites/mL) on separate days or a single peripheral thick blood smear were used for diagnosis and clinical decisions to treat the subjects. Although, the first NIH malaria real time qPCR detected parasitemia approximately 2 days prior to positive blood smears and before participants developed any symptoms, the second positive NIH malaria real time qPCR coincided with increased symptoms in most participants. Other studies, and our experience here, have shown that higher parasite number during CHMI is directly related to symptoms. Although most prior CHMI studies have used thick blood smears for malaria diagnosis, recently, in order to decrease adverse events related to clinical malaria, using qPCR has been suggested as a more sensitive measure (Walk, Schats et al. 2016).

1.4.3.2.3 Protective Efficacy Against Homologous CHMI

Final efficacy results by time to positivity are presented (as previously seen in **Table 2** and below in **Figure 5**). Briefly 2/2 subjects in pilot Arm 1a (pyrimethamine + chloroquine; 6 months post last PfSPZ-CVac to CHMI) were infected. In Arm 2 (pyrimethamine + chloroquine); 7/9 subjects were infected (1 additional subject was delayed, as seen in **Table 2**); 1/9 had negative PCR until day 16 post CHMI when the subject was withdrawn from study (note hash mark on survival curve at day 16). In Arm 3 (chloroquine only), 1/5 subjects was infected and in the infectivity control group, Arm 4, all 5/5 subjects were infected (**Table 2** and **Figure 5**). Arm 3 (chloroquine only) was significantly different in time to positivity and the binary endpoint from the infectivity controls however Arm 2 (pyrimethamine + chloroquine) was not significantly different in either endpoint when compared to the controls.

Figure 5: Kaplan Meier curve comparing vaccinated groups and infective control



Arm 1a- Pilot with pyrimethamine + chloroquine; Arm 2 – pyrimethamine + chloroquine; Arm 3 – chloroquine only, Arm 4 – infective controls

1.4.4 Sanaria® PfSPZ Challenge (7G8)

Pf 7G8 infected mosquitoes have been used in a number of CHMI studies with the number of mosquitoes required to achieve 100% infectivity rate and safety profile similar to that observed in Pf NF54 infected mosquitoes (Egan, Hoffman et al. 1993, Heppner, Gordon et al. 1996, Epstein, Rao et al. 2007). Recently, Pf7G8-infected mosquitoes (created from same Working Cell Bank (WCB, BMF 15326; used to produce 7G8 PfSPZ Challenge) were used to infect a total of 10 control volunteers in a Sanaria-sponsored clinical trial of PfSPZ Vaccine under IND 14826 (Clinicaltrials.gov Identifier: NCT02215707). The five mosquito bite CHMI that is standard for Pf NF54 and the Pf 3D7 clone of Pf NF54 was found to be optimal for Pf 7G8, resulting in 100% infection rates, comparable pre-patent periods, similar parasite densities and analogous clinical effects (**Table 12, 13** and **14** below). Hence these data indicate that the *in vivo* biological profile of Pf 7G8 is highly similar to that of Pf NF54, and also to that of Pf 3D7.

Table 12: Results of CHMI#1 by exposure to the bites of PfSPZ (7G8)-infected mosquitoes in the WRAIR study described above

Vol #	# of Bites	Prepatent Period	Parasites/μL of blood at time of diagnosis	Days 0-5 AEs by grade	Days 6-18 AEs by grade
1051	5	9.76	54.55	1- Yes 2- No 3- No 4- No	1- Yes 2- Yes 3- No 4- No
1054	4 (+1 bite with SPZ rating of 1)	12.82	47.62	1- Yes 2- Yes 3- No 4- No	1- Yes 2- Yes 3- No 4- No
1055	5	9.99	36.36	1- No 2- No 3- No 4- No	1- Yes 2- No 3- No 4- No
1060	5	12.87	17.32	1- No 2- No 3- No 4- No	1- Yes 2- Yes 3- No 4- No
Summary		11.26 (geometric mean)	41.99 (median)	1- 2/4 2- 1/4 3- 0/4 4- 0/4	1- 4/4 2- 3/4 3- 0/4 4- 0/4

Table 13: Results of CHMI#2 by exposure to the bites of PfSPZ (7G8)-infected mosquitoes in the WRAIR study described above.

Vol #	# of Bites	Prepatent Period	Parasites/μL of blood at time of diagnosis	Days 0-5 AEs by grade	Days 6-18 AEs by grade
1064	5	11.74	10,757.58*	1- No 2- No 3- No 4- No	1- Yes 2- Yes 3- Yes 4- No
1066	5	9.81	72.73	1- No 2- No 3- No 4- No	1- Yes 2- Yes 3- Yes 4- No
1068	5	10.87	8.66	1- No 2- No 3- No 4- No	1- Yes 2- Yes 3- No 4- No
1069	5	10.91	54.55	1- No 2- No 3- No 4- No	1- Yes 2- Yes 3- No 4- No
1070	5	9.93	12.99	1- No 2- No 3- No 4- No	1- Yes 2- Yes 3- Yes 4- No
1074	5	10.90	36.36	1- No 2- No 3- No 4- No	1- Yes 2- No 3- No 4- No
Summary		10.67 (geometric mean)	45.46 (median)	1- 0/6 2- 0/6 3- 0/6 4- 0/6	1- 6/6 2- 5/6 3- 3/6 4- 0/6

*This volunteer first developed symptoms approximately one hour before the thick blood smear was taken. Treatment was initiated immediately after the thick blood smear was positive. The volunteer recovered uneventfully and was in normal condition at the 28- and 56-day follow up visits. Because the parasite density was so high at time of diagnosis, an investigation is in process. Based on the results, if necessary, a corrective and preventive action (CAPA) plan will be implemented.

Table 14: Results of CHMI by exposure to the bites of PfSPZ (3D7)-infected mosquitoes in the WRAIR study described above.

Vol #	# of Bites	Prepatent Period	Parasites/μL of blood at time of diagnosis	Days 0-5 AEs by grade	Days 6-18 AEs by grade
1065	5	10.92	36.4	1- Yes 2- No 3- No 4- No	1- Yes 2- Yes 3- No 4- No
1067	5	11.92	72.7	1- No 2- Yes 3- No 4- No	1- Yes 2- Yes 3- No 4- No
1071	5	12.84	56.3	1- No 2- No 3- No 4- No	1- Yes 2- Yes 3- No 4- No
1072	5	10.98	26.0	1- Yes 2- No 3- No 4- No	1- Yes 2- No 3- No 4- No
1073	5	11.23	8.7	1- No 2- No 3- No 4- No	1- Yes 2- Yes 3- Yes 4- No
1075	5	14.03	13.0	1- No 2- No 3- No 4- No	1- Yes 2- Yes 3- Yes 4- No
Summary		11.94 (geometric mean)	31.20 (median)	1- 2/6 2- 1/6 3- 0 4- 0	1- 6/6 2- 5/6 3- 2/6 4- 0/6

An initial study to assess the dose of 7G8 Sanaria® PfSPZ Challenge DVI required to achieve 100% infectivity was initiated in a MAVACHE study in Tubingen, Germany (Clinicaltrials.gov Identifier: NCT02704533). The study is being done in two phases. In phase 1, dose optimization in which malaria naïve subjects received 800, 1600 and 3,200 7G8 Sanaria® PfSPZ Challenge DVI (n=3 in each group). In phase 2, regimen verification will be done. Per verbal report from the study team, 3,200 7G8 Sanaria® PfSPZ Challenge DVI (similar to NF54 Sanaria® PfSPZ Challenge DVI) resulted in 100% infection (Hoffmann SL, personal communication).

Another study was recently completed at University of Maryland in Baltimore, MD (Clinicaltrials.gov Identifier: NCT02780154) to assess the safety and reactogenicity of 7G8 and NF54 Sanaria® PfSPZ Challenge DVI. In this trial, the dose of 7G8 Sanaria® PfSPZ Challenge DVI will be escalated through 800, 1600, 3,200 (n=9 in each group) and 4,800 (n=3) PfSPZ. Per verbal report 3,200 7G8 Sanaria® PfSPZ Challenge DVI resulted in 8/9 infected subjects (92% infection) (Richie T, personal communication). Safety data from both clinical trials will be available prior to CHMI with 7G8 Sanaria® PfSPZ Challenge DVI in this proposed study.

1.5 Clinical Trial Plan

The study proposed here by Sanaria and LMIV will assess PfSPZ-CVac using an increased number of Sanaria® PfSPZ Challenge (NF54) and pyrimethamine in attempt to determine the dose of Sanaria® PfSPZ Challenge required for development of protective immunity with this regimen.

The design of the proposed clinical trial entailed 2 phases, a pilot phase and a main phase.:
1) **Pilot trial phase** (COMPLETED) was divided in two additional parts
   a) Pyrimethamine only Arm (*Arm 1*): to assess safety and to ensure that the increased number of Sanaria® PfSPZ Challenge (50,000 or 100,000 or target dose 200,000) and pyrimethamine dosing regimen on days 2 and 3 (50 mg or 75 mg) will not result in subpatent parasitemia in any of the subjects (NIH malaria real time PCR and retrospective LMIV qPCR negative in 2 of 2 subjects);
   b) Chloroquine only Arm (*Arm 5*): to assess safety and to ensure that the increased number of Sanaria® PfSPZ Challenge (100,000 or target dose 200,000) while on weekly chloroquine regimen will not result in diagnosis of clinical malaria during CVac requiring treatment with another antimalaria medication

2) **Main trial phase** (ONGOING) will assess the safety of Sanaria® PfSPZ-CVac conducted with pyrimethamine alone or chloroquine alone with increased Sanaria® PfSPZ Challenge doses. In addition, the main arm will assess the protective efficacy against homologous challenge (NF54) for the pyrimethamine *Arm 2a* and heterologous challenge (7G8) for the pyrimethamine *Arm 2b* and chloroquine *Arm 3*. Subjects in the main study will receive three successive CVac administrations (NF54 Sanaria® PfSPZ Challenge + pyrimethamine OR chloroquine) at 4 week intervals followed by CHMI. Antibody and cellular immune responses and transcriptomic analyses will be assessed periodically after vaccination in order to identify correlates of protection**.**

2 Study Objectives

2.1 Primary Objective

The primary objectives of this study are twofold:

- **Safety:**
  - To monitor the safety and tolerability of Sanaria® PfSPZ Challenge (NF54) via DVI with pyrimethamine (PfSPZ-CVac pyrimethamine) or chloroquine (PfSPZ-CVac chloroquine). (*Arms 1, 2, 3, 5)*
  - To evaluate whether pyrimethamine on days 2, 3 prevents patent parasitemia post Sanaria® PfSPZ Challenge (NF54) via DVI at increasing PfSPZ Challenge doses (maximum 200,000 PfSPZ). (*Arm 1, 2)*
  - To evaluate whether weekly chloroquine prevents clinical malaria diagnosis requiring additional antimalaria medication post Sanaria® PfSPZ Challenge (NF54) via DVI at increasing PfSPZ Challenge doses (maximum of 200,000 PfSPZ). (*Arm 3, 5*)

2.2 Secondary Objective

The secondary objective of this study is:

Protective Efficacy:

- To assess the protective efficacy of PfSPZ-CVac pyrimethamine against **homologous** (NF54) CHMI. *(Arm 2a and 4a)*
- To assess the protective efficacy of PfSPZ-CVac pyrimethamine or chloroquine against **heterologous** (7G8) CHMI. *(Arm 2b, 3 and 4b)*

2.3 Exploratory Objectives

The exploratory objectives of this study are:

- To assess the humoral and cell mediated immune responses to PfSPZ and to known pre-erythrocytic and blood stage antigens
- To assess the humoral and cell mediated immune responses to novel pre-erythrocytic antigens
- To assess the kinetics of subpatent parasitemia during PfSPZ-CVac
- To describe changes in γδ T cells in malaria naïve individuals after CVac and CHMI
- To explore new diagnostic testing platforms (qPCR, rapid diagnostics, ELISA)

3 Study Design

3.1 Overall Design

This is a Phase 1 study to investigate the safety, immunogenicity, and protective efficacy following pre-erythrocytic stage only parasite exposure by DVI with aseptic, purified, cryopreserved Pf sporozoites (NF54 Sanaria® PfSPZ Challenge), under pyrimethamine treatment, to induce stage specific homologous and heterologous sterile protection. Additionally, we look to evaluate the heterologous protective efficacy of PfSPZ-CVac with chloroquine with increasing doses of PfSPZ Challenge during PfSPZ-CVac.

3.2 Pilot Study

The goal of the pilot study was to ensure increasing the number of Sanaria® PfSPZ Challenge (NF54) to 200,000 under pyrimethamine or chloroquine is safe and well tolerated. The pilot study occured in two parts:

1. Pyrimethamine pilot study (***Arm 1a, 1b and 1d***) assessed whether increasing the number of Sanaria® PfSPZ Challenge (NF54) to 200,000 while under pyrimethamine dosing will prevent the development of asexual erythrocytic stages of the malaria (qPCR negative). The highest number of Sanaria® PfSPZ Challenge administered by DVI in PfSPZ CVac with pyrimethamine to date is 51,200 PfSPZ (in NIAID protocol #15-I-0169). The goal of this study, is in a stepwise manner increase the number of sporozoites injected during Sanaria® PfSPZ-CVac to 200,000 PfSPZ while monitoring closely for safety, tolerability, and presence of detectable parasitemia (**Figure 6**).
    - ***Arm 1a*** (n=2) is designed to confirm that injecting 50,000 of Sanaria® PfSPZ Challenge with pyrimethamine on 2 and 3 days post DVI *without* chloroquine

prophylaxis will prevent the development of either subpatent parasitemia (negative retrospective LMIV qPCR result; a positive result is defined as two consecutive samples > 40 parasites/mL or a single positive retrospective LMIV qPCR ≥ 100 parasites/mL AND negative NIH malaria real time qPCR) in ≥1/2 subjects. In our previous study (NIAID protocol #15-I-0169), participants who received pyrimethamine and chloroquine did not develop subpatent parasitemia during PfSPZ-CVac whereas all those who received chloroquine alone were parasitemic post CVac #1.

Subjects will be defined as having definitively negative subpatent parasitemia if through Day 14 post injection all qPCRs have remained negative (NIH malaria real time qPCR AND retrospective LMIV qPCR). For safety monitoring, concurrent NIH malaria real time qPCR will be performed and participants will be treated with Malarone® for 3 consecutive days if they have one positive qPCR result regardless of symptoms. All pilot subjects (*Arm 1x*) who remain negative through day 14 post PfSPZ Challenge will be treated at the end of the study. Retrospective LMIV qPCR will be completed as outlined in **Section 8.13**.
The NIH malaria real time and LMIV retrospective qPCR results will determine which pilot arm will be assessed next as outlined in **Figure 6**.

- ***Arm 1b*** (n=2) 100,000 Sanaria® PfSPZ Challenge with 50 mg of pyrimethamine
- ***Arm 1c*** (n=2) 50,000 Sanaria® PfSPZ Challenge with 75 mg of pyrimethamine
- ***Arm 1d*** (n=2) 200,000 Sanaria® PfSPZ Challenge with 50 mg of pyrimethamine
- ***Arm 1e*** (n=2) 100,000 Sanaria® PfSPZ Challenge with 75 mg of pyrimethamine
- ***Arm 1f*** (n=2) 200,000 Sanaria® PfSPZ Challenge with 75 mg of pyrimethamine

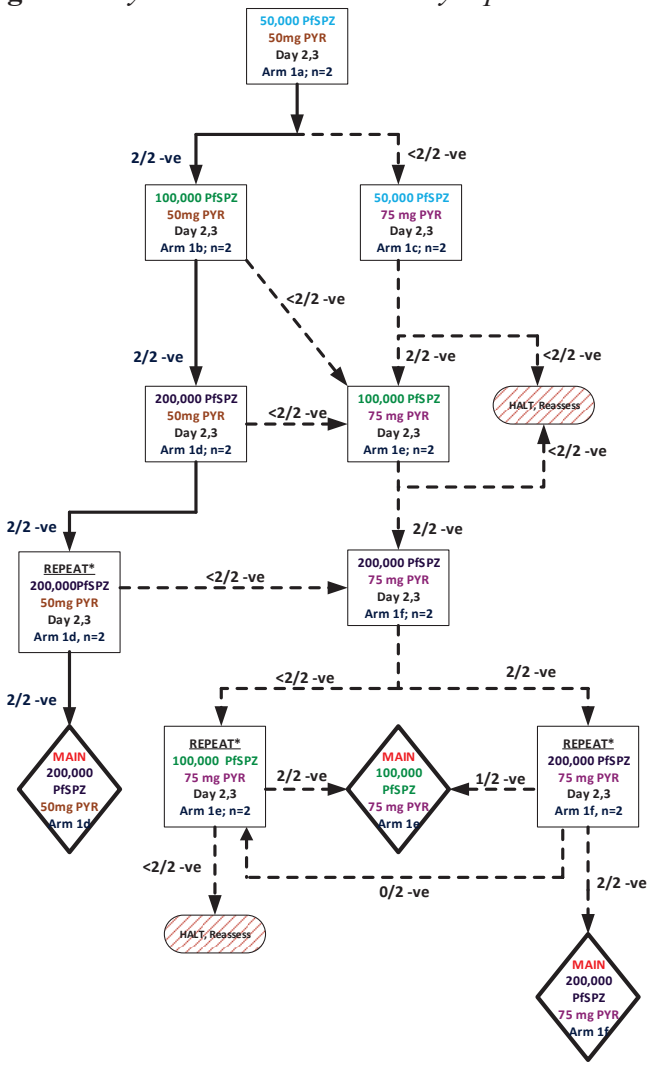
The ideal pilot pathway is doubling the number of Sanaria® PfSPZ Challenge stepwise while maintaining pyrimethamine dose at 50 mg without detection of subpatent parasitemia in 2/2 subjects per arm. Hence, if subjects in *Arm 1a* do not develop subpatent parasitemia (2/2 subjects with negative qPCRs until day 14 post DVI), *Arm 1b* will follow. If *Arm 1 b* meets criteria, it will be followed by *Arm 1d*. Before continuing with *Arm 1d* to the main arm, we will confirm the results with an additional 2 subjects to ensure that this regimen does prevent development of subpatent parasitemia. (**Figure 6 –** solid line pathway).

If 1 or both (≥1/2) subjects in *Arms 1a* are qPCR positive (less than 2/2 have negative qPCR), we will first increased the dose of pyrimethamine to 75 mg (*Arm 1c*) and maintain the dose of Sanaria® PfSPZ Challenge at 50,000 sporozoites (*Arm 1c*). If Arm 1c meets criteria, we will continue with doubling the number of sporozoites while maintaining the dose of pyrimethamine at 75 mg (**Figure 6** – dotted line pathway). If subpatent parasitemia is detected in either *Arm 1c* or *1e*, the study will halt for reassessment.

If the pilot reaches Arm *1f*, and subpatent parasitemia is not detected in 2/2 subjects, then 2 more subjects will be enrolled in Arm *1f* for confirmation. If the second group enrolled meets criteria, the regimen in Arm *1f* will be used for the main study. If 1/2 subjects develops subpatent parasitemia (1/2 negative qPCR), then we will step down the dose of Sanaria® PfSPZ Challenge and Arm *1e* regimen will be used for the main study. If both subjects in the second group of Arm *1f* develop subpatent parasitemia (0/2 negative LMIV qPCR), we will step down to Arm *1e* but repeat by enrolling 2 more subjects for confirmation before continuing to the main study. If any

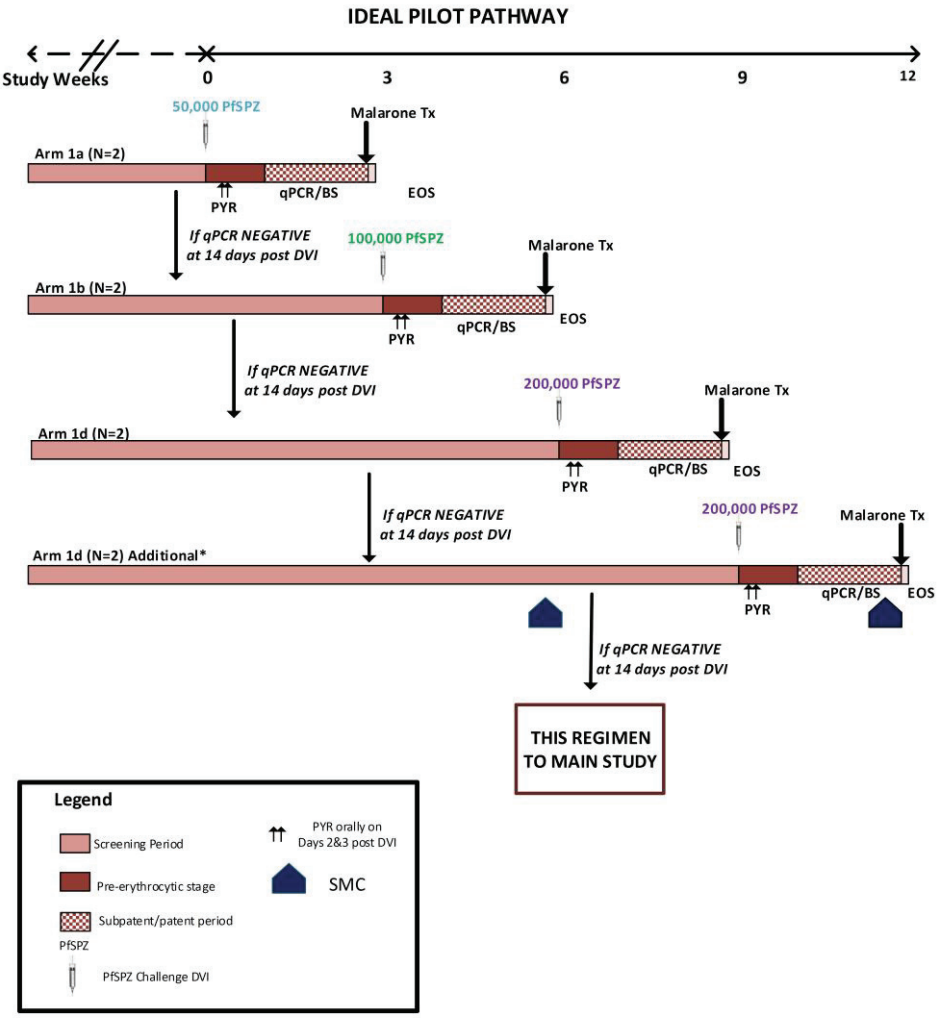
of the subjects in *Arm 1e* develop subpatent parasitemia (less than 2/2 negative qPCR), the study will halt for reassessment (**Figure 6**).

Figure 6: *Pyrimethamine Pilot Study Up-Down Schema*



Solid line (▬) = 50 mg of pyrimethamine; Dotted line (▬ ▬) = 75 mg pyrimethamine.
–ve = NEGATIVE
***** Two additional volunteers will be enrolled into the pilot study to confirm that the selected regimen does not result in development of subpatent parasitemia before the main study starts
Note: LMIV qPCR will be done daily from days 6-14 post DVI and the results will be used to determine the pilot path to be followed.

Figure 7*: Ideal Pyrimethamine Pilot Study Pathway Schema*



* Two additional volunteers will be enrolled into the pilot study to confirm that the selected regimen does not result in development of subpatent parasitemia before the main study starts

2) Chloroquine pilot study (***Arms 5a and 5b***) assessed safety and ensured that the increased number of PfSPZ of Sanaria® PfSPZ Challenge (100,000 or target dose 200,000) while on weekly chloroquine regimen will not result in diagnosis of clinical malaria requiring treatment with another antimalaria medication. The goal of this study, is in a stepwise manner increase the number of sporozoites injected during Sanaria® PfSPZ-CVac to 200,000 PfSPZ while monitoring closely for safety, tolerability, and development of clinical malaria requiring treatment with another antimalaria medication (**Figure 8**).

- ***Arm 5a*** (n=2) is designed to confirm that injecting 100,000 PfSPZ of Sanaria® PfSPZ Challenge with weekly chloroquine starting with loading dose 2 days prior to DVI and second and last dose 5 days post DVI will prevent the development of clinical malaria diagnosis requiring treatment with another antimalarial medication. Clinical malaria diagnosis is per definition outlined in LMIV SOP 25: Diagnosing Malaria; in which subject will be evaluated for

clinical malaria diagnosis requiring treatment if **clinically ill** and has a T>38.0°C without other symptoms ***or*** has two or more signs and symptoms (fever T>38.0°C, headache; chills; sweats; malaise; myalgia; arthralgia; nausea; vomiting; abdominal pain; diarrhea; low back pain; or non-musculoskeletal chest pain). If the subject meets these criteria, in discussion with the PI, a work up with a symptomatic blood smear and/or real-time NIH qPCR and blood smear set will be initiated. If the work up is positive and in agreement with the PI, the subject will be treated with Malarone® for 3 consecutive days and withdrawn from the study.

Subjects will be monitored daily with blood smears, routine qPCRs will not be done as it is expected that with PfSPZ-CVac with chloroquine, subpatent parasitemia is detectable. In our previous study (NIAID protocol #15-I-0169) in which subjects received 51,200 Sanaria® PfSPZ Challenge, subjects who received chloroquine only developed subpatent parasitemia (qPCR positive). None of the subjects developed patent parasitemia (positive blood smear). It is however expected that with higher numbers to 200, 000 PfSPZ of Sanaria® PfSPZ Challenge, subjects may develop patent parasitemia, thus the presence of parasitemia alone will not determine treatment with another antimalaria medication (Malarone®).

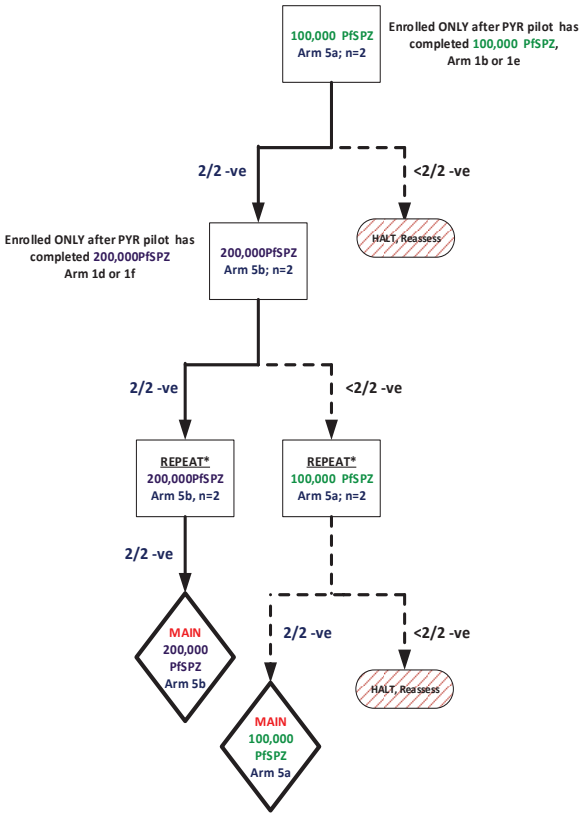
Subjects will be defined as not been diagnosed with clinical malaria if they are negative through day 12 post injection. All pilot subjects (*Arm 5x*) who remain negative through day 12 post PfSPZ Challenge will be treated with Malarone® for 3 consecutive days at the end of the study. Retrospective LMIV qPCR will be completed as outlined in **Section 8.13**.

If both subjects in *Arm 5a* do not develop clinical malaria requiring treatment with another antimalaria medication, then *Arm 5b* will be assessed. If both subjects in *Arm 5b* do not develop clinical malaria, two additional subjects will be enrolled into this Arm for confirmation. If 1 or both (≥1/2) subjects in *Arm 5a* develop clinical malaria then, we will not continue to *Arm 5b* and the chloroquine pilot will halt for reassessment. If 1 or both (≥1/2) subjects in *Arm 5b* develop clinical malaria requiring treatment with another antimalaria medication, then the regimen in *Arm 5a* will first be repeated in additional 2 subjects before being used in the main study (**Figure 8** and **9**).

- ***Arm 5b*** (n=2) 200,000 Sanaria® PfSPZ Challenge with weekly chloroquine (2 doses total)

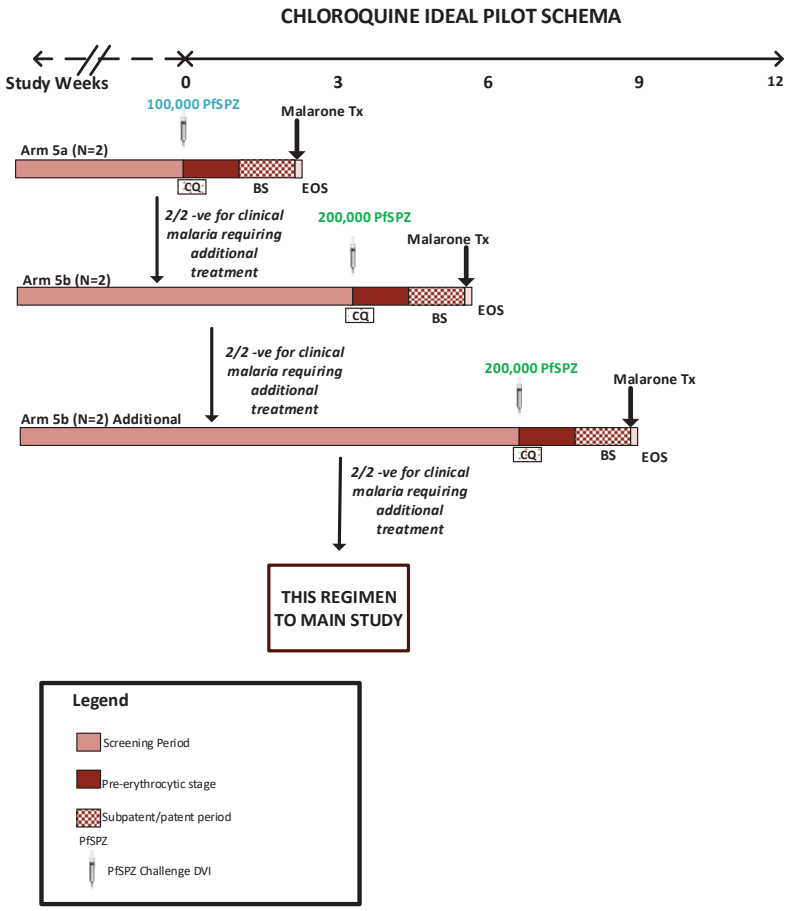
Since the goal of the study is to use the same dose of Sanaria® PfSPZ Challenge in both *Arms 2 and 3*, the chloroquine pilot (***Arm 5a***) will only be started after the pyrimethamine pilot has completed 100,000 Sanaria® PfSPZ Challenge Arm (***Arm 1b or 1e***). *Arm 5b* will only be enrolled after the pyrimethamine pilot has completed the first round of 200,000 Sanaria® PfSPZ Challenge (***Arm 1d*** or ***Arm 1f***) (**Figure 8**). If the chloroquine pilot study is unsuccessful to progress through 100,000 Sanaria® PfSPZ Challenge (*Arm 5a*), the study will halt for reassessment.

Figure 8:*Chloroquine Pilot Flow Chart*



Note: **Solid line** – Ideal pathway; **Dash line** – alternative pathway

***Figure 9**: Chloroquine Pilot Ideal Pathway*



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3.2.1 Pilot Study Outcomes

The pilot study was completed in October 2017 and the goal dose of 200, 000 PfSPZ Challenge SPZ was achieved in both the pyrimethamine group and the chloroquine group. A total of 13 volunteers completed the study, 1 volunteer is still being followed Grade 1 laboratory abnormality. The two regimens were found to be safe and well tolerated. Briefly, the safety results are summarized below:

In the pyrimethamine pilots (n=8), all doses from 50,000 to 200,000 PfSPZ Challenge SPZ were well tolerated. Most of the observed AEs were Grade 1 and many unlikely related to study participation. There was one transient Grade 3 AE of heat exhaustion in a participant who received 100,000 PfSPZ Challenge SPZ that was not related to study participation. A second Grade 3 AE of costochondritis, unrelated to study participation, occurred in a subject who received 200,000 PfSPZ Challenge SPZ. All AEs resolved without sequalae. Safety labs were also followed closely per protocol, there was one Grade 1 white count decreased and one Grade 1 ALT increased observed in one asymptomatic subject who received 200,000 PfSPZ Challenge SPZ. The WBC decreased has resolved, ALT increase is stable and being followed by the study team (***Table 15***).

Table 15: Summary of AEs by severity and frequency observed in pyrimethamine pilot study

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

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

In the chloroquine pilots (n=6), overall, there have been more reported AEs and related AEs compared to the pyrimethamine pilots.
However, the majority have remained Grade 1, expected AEs (***Table 16***). At the dose of 100,000 PfSPZ of PfSPZ Challenge, neither participant had a positive blood smear (0/2). At the dose of 200,000 PfSPZ of PfSPZ Challenge, the majority of individuals (3/4; 75%) had a single positive blood smear (symptomatic blood smear which is 15 passes versus 5 passes for routine asymptomatic read) with associated expected symptoms related to induced malaria such, some of which were Grade 3 (fever and myalgia) in severity. Symptoms improved quickly or resolved with administration of 1 dose of NSAIDs (ibuprofen or acetaminophen). All subjects recovered without sequalae and symptoms were at Grade 3 for less than 24 hours during the period of expected peak parasitemia. All CQ Arm subjects were LMIV research qPCR positive for at least 2 consecutive days (day 7 and 8 post PfSPZ Challenge) as expected, with those with the highest peak parasitemia being blood smear positive with associated Grade 3 symptoms.

All participants were treated with Malarone® per protocol and completed their study participation per protocol. The summary of the AEs is outlined below.

Table 16: Summary of AEs by severity and frequency observed in chloroquine pilot study

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

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

The results of the pilot study were reviewed by SMC per protocol and ad hoc and after the careful review, it was determined that administration with 200,000 PfSPZ Challenge SPZ with either pyrimethamine or chloroquine was safe and tolerable. The SMC recommended continuation to the main study at this dose with added monitoring of *Arm 3* (chloroquine only Arm) on the days of peak parasitemia. Additional clinic visits and pre-emptive administration of NSAIDs to *Arm 3* in the main study have thus been added to this protocol in order to increase tolerability of this regimen.

All participants were treated with Malarone® per protocol and completed their study participation per protocol.

3.3 Main Study

Since the pilot study has completed and the optimal regimens have been determined as outlined in **Section 3.2,** the main study can start. As outlined above in Section 3.2.1 the following regimens will be executed during the main phase:

- ***Arm 2a*** (n=17) and ***Arm 2b*** (n=10) will receive the selected regimen of Sanaria® PfSPZ Challenge (NF54 at 200,000) via DVI and pyrimethamine 50mg on 2, 3 days post PfSPZ Challenge without chloroquine prophylaxis. These participants will receive three successive PfSPZ-CVac immunizations administered approximately 4 weeks apart. Participants in *Arm 2* will be monitored in real time daily by NIH malaria real time qPCR on days 6-14 post DVI after the first CVac. NIH malaria real time qPCR will be done in real time from days 7-9 post DVI (most likely time period to detect parasitemia) during second and third CVacs. NIH malaria real time qPCR will be done at other times outside this window if clinically indicated. If there is a single positive NIH malaria real time qPCR detected during the first PfSPZ-CVac, NIH malaria real time qPCR will be done in real time from days 6-10 (and longer if CVac #1 qPCR positive greater than day 10) post DVI during second and third CVac immunizations for all subjects in this Arm.

  We did not observe subpatent parasitemia (negative by blood smear and qPCR) in the PfSPZ-CVac with pyrimethamine + chloroquine arms in protocol #15-I-0169, indicating the concurrent administration of chloroquine may not be needed. Thus the pyrimethamine arms in this study (Arm 1 and 2) will not receive chloroquine, but as an added safety measure, as outlined above, we will use NIH real time ("real time" meaning performed the day that the sample is drawn to provide immediate results) malaria qPCR for subpatent parasitemia monitoring during the pilot study and during PfSPZ-CVac of the main study in *Arm 2* only as outlined above (days 6-14 post DVI during CVac #1 and days 7-9 post DVI during CVac #2 and #3). If subpatent parasitemia is not detected during the first vaccination (NIH real time malaria qPCR and LMIV retrospective malaria qPCR are negative in ALL subjects at ALL timepoints), based on previous studies, it is expected that subpatent parasitemia will likely be prevented in subsequent vaccinations. If ≥ 1 subject has a positive NIH real time malaria qPCR during PfSPZ-CVac #1, then NIH real time malaria qPCR will be conducted from days 6-10 post DVI during CVac #2 and #3 for all subjects in this Arm (see **Appendix A**).

  Arm 3 (n=10) will receive the same number of Sanaria® PfSPZ Challenge determined for *Arm 2* (NF54 at 200,000) via DVI while under weekly chloroquine prophylaxis without pyrimethamine. These participants will receive three successive vaccinations administered approximately 4 weeks apart. These subjects will be monitored by daily blood smears from days 6-14 during CVac #1 and from days 6-10 during CVac #2 and #3. NIH real time malaria PCR will **not** be completed routinely in this arm during CVac unless clinically indicated given it is expected that Arm 3 will be qPCR positive on multiple days following CVac #1, #2, and even #3.

  As already noted, weekly chloroquine prophylaxis in the CVac model protected all subjects from development of patent parasitemia following CHMI (Roestenberg, McCall et al. 2009). More recently, chloroquine prophylaxis was used with Sanaria's injectable infectious PfSPZ (Sanaria® PfSPZ Challenge) and again, all subjects were protected (9/9)

using a regimen of three monthly injections of 51,200 PfSPZ (Mordmuller, Surat et al. 2017). In a LMIV protocol (NIAID Protocol #15-I-0169), these results were replicated in which 4/5 participants were protected against homologous CHMI.

In the proposed study, chloroquine prophylaxis will be similarly employed as the previous study (at standard dosing, unless clinically indicated, due to adverse events, to adjust to weight based dosing) in *Arm 3* subjects only. This chloroquine arm will serve as CVac phase positive controls as well as a comparison arm to assess differences in immunological responses induced by exposure to SPZ and liver stages (PfSPZ-CVac pyrimethamine) only versus SPZ, liver and blood stage exposures (PfSPZ-CVac chloroquine). Additionally, with the plan to undergo 7G8 challenge, *Arm 2b* and *Arm 3* serve to explore if increasing the PfSPZ Challenge exposure can result in heterologous protection.

As noted above, the pilot study was completed in November 2017 and the goal dose of PfSPZ Challenge of 200,000 NF54 SPZ was achieved in both the pyrimethamine group (pyrimethamine dosed at 50mg on days 2 and 3 post DVI) and the chloroquine group; thus, PfSPZ Challenge 200,000 NF54 SPZ dose will be administered in both *Arms 2* and *3* during CVac 1, 2 and 3. *Arm 3* will continue to have daily blood smears during prepatent period, but will also have closer monitoring (and more frequent blood smears if clinically indicated) with twice a day visits during peak parasitemia post CVac #1 (see **Appendix A**). In addition, all subjects in *Arm 3* will be given around the clock nonsteroidal anti-inflammatory drugs (NSAIDs) pre-emptively during expected peak parasitemia (days 7 and 8 post PfSPZ Challenge DVI) to improve tolerability.

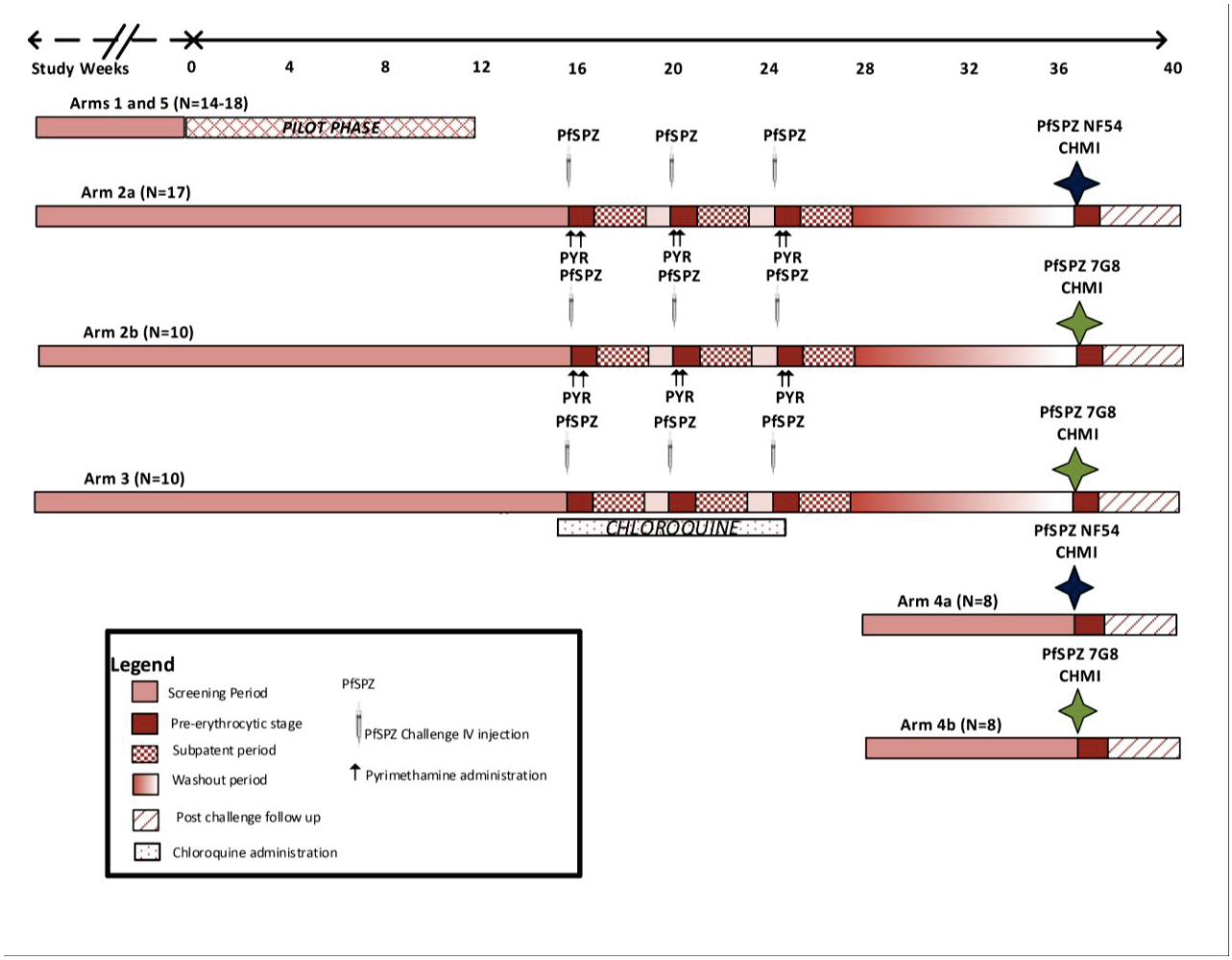
- ***Arm 4a*** (n=8) will serve as CHMI infectivity controls for *Arm 2a* and will only receive one dose of 3,200 Sanaria® PfSPZ Challenge (**NF54**) via DVI at CHMI.

- ***Arm 4b*** (n=8) will serve as infectivity controls for *Arm 2b* and *3* and will only receive one dose of 3,200 (or other determined dose based on results from studies discussed in **Section 1.4.4**) Sanaria® PfSPZ Challenge (**7G8**) via DVI at CHMI.

Subjects in *Arms 1a, 1b, 1d (and if need Arms 1c, 1e and 1f)* will be considered enrolled upon receipt of Sanaria® PfSPZ Challenge. Subjects in *Arm 2*, *3, 4a*, and *4b,* will be considered enrolled into the study upon blood draw of first baseline research samples (approximately 2 days prior to administration of first dose of Sanaria® PfSPZ Challenge). Subjects in *Arms 5a and 5b* will be considered enrolled into the study upon receipt of loading dose of chloroquine (2 days prior to administration of first dose of Sanaria® PfSPZ Challenge). Enrolled subjects who withdraw prior to administration of first dose of Sanaria® PfSPZ Challenge, will be replaced. A Main study schema is represented in **Figure 10.**

Antibody and cellular immune responses and transcriptomic profiles will be assessed periodically after vaccination and in unvaccinated infection controls (*Arm 4a & 4b*) as per **Appendix A.**

***Figure 10*:** *Main Study Schema*



3.3.1 Preliminary results of the first cohort of the main phase

The main study began enrollment in January 2018. Due to logistical reasons, staffing and slow recruitment, only half of the planned participants were enrolled in the first cohort. The participants were split into all three Arms, the following number of subjects in each arm were enrolled and received at least one vaccination:

- ***Arm 2a*** (PfSPZ CVac with 200,000 PfSPZ Challenge (NF54) under PYR chemoprophylaxis; then homologous NF54 challenge; **n=8**)
- ***Arm 2b*** (PfSPZ CVac with 200,000 PfSPZ Challenge (NF54) under PYR chemoprophylaxis; then heterologous 7G8 challenge; **n=5**)
- ***Arm 3*** (PfSPZ CVac with 200,000 PfSPZ Challenge (NF54) under weekly CQ chemoprophylaxis, then heterologous 7G8 challenge; **n=5**)

In the pyrimethamine Arms, (*Arms 2a* and *2b, n=13*), vaccinations were safe and well tolerated, 93% of the reported AEs were Grade 1. The most commonly reported AEs were fatigue, palpitations, arthralgia, and myalgia. There were 4 Grade 2 AEs (myalgia, gastroenteritis, creatinine increased and hemoglobin decreased). There were 2 Grade 3 AEs, a gastroenteritis that

was deemed not related to the study and a neutrophil decrease that was considered related to the study.

NIH Malaria qPCRs were completed daily from days 6-14 post PfSPZ Challenge after first vaccination and from days 7-9 post PfSPZ Challenge after the 2nd and 3rd vaccinations. Participants were only monitored with blood smears on days 6 and 10 post PfSPZ Challenge injection after 2nd and 3rd vaccinations. All NIH malaria qPCRs (performed real time; used for safety follow up) and the LMIV qPCRs (performed retrospectively throughout pre-patency period; used to confirm no blood stage exposure) were negative at every timepoint (**Figure 11**). These results in a larger group of participants confirmed that the pyrimethamine regimen prevents the development of blood stage parasites even with higher number of sporozoites used for vaccination (200,000 PfSPZ Challenge NF54). Therefore subsequent participants enrolled in either *Arm 2a* or *2b* will be monitored by daily by real time qPCR on days 6-10 post DVI (rather than days 6-14) during 1st vaccination (**Appendix A**) .

In the chloroquine Arm, (*Arm 3, n=5)*, vaccinations were safe and well tolerated. The majority of reported AEs (96%) were Grade 1, expected AEs. There was a decrease in severity of AEs after vaccination #1 in the main phase compared to the chloroquine arms in the pilot phase of the study, likely due to addition of pre-emptive NSAIDs starting day 7 post vaccination. We also observed a decrease in frequency of AEs with each successive vaccination as has been observed in prior studies. There were no Grade 2 AEs reported. There were two Grade 3 SAEs. The first SAE was that of acute change in mental status due to acute anticholinergic poisoning after ingesting seeds of a *Datura stramonium* plant for recreational purposes. This episode was deemed to also be possibly related to chloroquine due to timing. The second SAE was that of acute spontaneous pneumothorax that occurred 59 days after third vaccination and was determined not to be related to study procedures.

Thick blood smears were completed daily from days 6-14 post PfSPZ Challenge after 1st vaccination and from days 6-10 post PfSPZ Challenge after 2nd and 3rd vaccination.
One blood smear was positive in one participant on day 8 post the first vaccination. Otherwise, all blood smears remained negative throughout. A sensitive RNA based LMIV qPCR was performed retrospectively throughout the pre-patency period to confirm no blood stage exposure. As expected, due to the action of chloroquine, all subjects had detectable subpatent parasitemia after the first vaccination (**Figure 11**). Fewer subjects had detectable subpatent parasitemia with subsequent vaccinations, likely reflecting the developing immunity. The decreasing frequency and density of parasitemia in second and third immunizations was expected as has been demonstrated in similar studies at LMIV and elsewhere. Therefore subsequent participants enrolled in *Arm 3* will be monitored daily by blood smear days 6-10 post DVI (rather than days 6-14) during 1st vaccination (**Appendix A**).

***Figure 11**: LMIV qPCR results during vaccination phase*

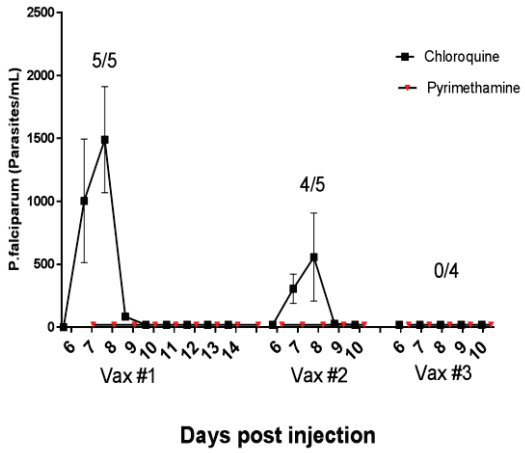


Figure 11: X/Y indicate number of participants with positive qPCR/Total participants in the chloroquine Arm. One participant in the chloroquine Arm was withdrawn prior to vaccination #3. None of the participants in the pyrimethamine Arms had a positive qPCR during vaccination.

CHMI was performed ~12 weeks post third vaccination with $3.2x10^3$ sporozoites of either PfSPZ Challenge NF54 (homologous, *Arm 2a,* n=8) or PfSPZ Challenge 7G8 (heterologous, *Arm 2b* n=4; *Arm 3* n=3). In addition, half of the originally planned unvaccinated controls were enrolled to undergo either PfSPZ Challenge NF54 (*Arm 4a*, n=4) or PfSPZ Challenge 7G8 (*Arm 4b*, n=4). CHMI was safe and well tolerated. The majority of reported AEs (92%) were Grade 1 expected AEs that were due to malaria symptoms. The most common reported AEs were fatigue, headache and myalgia. There were 8 Grade 2 AEs, commonly fatigue and decreased hemoglobin. There were no reported Grade 3 AEs or SAEs reported during CHMI. All AEs were transient and resolved without sequaelae.

Participants were monitored for malaria diagnosis with NIH malaria PCR daily starting 6 days post CHMI. All unvaccinated controls became infected with malaria within 12 days of CHMI. In the vaccinated groups, only 1/8 participant in *Arm 2a* (homologous CHMI) became infected. All other vaccinated participants remained uninfected throughout 28 days post CHMI per protocol (**Table 17**). Our preliminary results show, for the first time, that homologous and heterologous immunity can be achieved in the absence of blood stage exposure in a chemoprophylaxis vaccination model.

3.3.2 Rationale for staggered enrollment

Due to ongoing slow progress in recruitment, the study split the remainder of the main phase into two cohorts. The second cohort enrolled into heterologous Arms only (*Arms 2b and 3 n=10*). The third cohort was planned to be started at a later date, would enroll into the homologous Arm (*Arm 2a; n=9*). The order of the 2nd and 3rd cohorts was determined after reviewing the results of the first cohort and literature review as outlined below.

This study is a follow up study from a prior chemoprophylaxis vaccination with pyrimethamine that used 50, 000 sporozoites of PfSPZ Challenge NF54 for vaccination, NIAID protocol #15-I-0169. In that study, 1/9 participants was protected against homologous CHMI (**Section 1.4.3.2.1)**. One premise of this current study was that increasing number of sporozoites four fold (to 200,000 per vaccination), thus increasing the antigen dose, would increase the level of protective efficacy against homologous CHMI as had been observed with vaccinations with irradiated sporozoites. Our preliminary results in which 7/8 participants in *Arm 2a* remained uninfected after homologous CHMI show that indeed this higher dose of sporozoites may lead to significant increase in the level of protective efficacy observed.

***Table 17**: Results of CHMI for the first Cohort*

Arm	Number of subjects challenged	Number of subjects infected	Days post CHMI to diagnosis	Median days post CHMI to diagnosis	Positive Blood Smear	Percent UNinfected
2a; pyrimethamine with homologous CHMI (NF54)	8	1	12	12	0	87.5%
2b; pyrimethamine with heterologous CHMI (7G8)	4	0	N/A	N/A	0	100%
3; chloroquine with heterologous CHMI (7G8)	3	0	N/A	N/A	0	100%
4a; NF54 Control	4	4	9,11, 11, 11	11	1	0%
4b; 7G8 Control	4	4	9, 9, 12, 12	10.5	0	0%

The study is also designed to explore development of immunity against heterologous CHMI. Immunity against heterologous CHMI is a more stringent test of efficacy. In an earlier study malaria naïve participants were immunized by 5 doses of 2.7×10^5 PfSPZ Vaccine. CHMI performed three weeks after last vaccination showed 12/13 participants (92.3% [95% CI: 48.0, 99.8]) were protected against homologous CHMI while 4/5 participants (80.0% [95% CI: 10.4, 99.5]) were protected against heterologous CHMI. The difference in protective efficacy was more pronounced when CHMI was performed twenty four weeks post last immunization during which 7/10 participants (70.0% [95% CI: 17.3, 93.3]) were protected against homologous CHMI where as only 1/10 participants (10.0% [95% CI: –35.8, 45.6]) were protected against heterologous CHMI (Epstein, Paolino et al. 2017). This 5-dose regimen was also used in an LMIV study in an endemic area in which the hazard ratio for vaccine efficacy was 0. 712 (0. 528–0. 918) by proportional analysis (p=0.006) (Sissoko, Healy et al. 2017). In another study, malaria naïve individuals were immunized by bites of *Plasmodium falciparum* NF54 infected mosquitoes. CHMI by infected mosquitoes performed 14 weeks post last immunization also showed lower efficacy against heterologous CHMI (2/10 and 1/9 remained uninfected against two different strains, NF135.C10 and NF166.C8 respectively) compared to homologous CHMI (5/5 remained uninfected) (Walk, Reuling et al. 2017).

In this current study, some participants in the pyrimethamine Arm also underwent heterologous challenge with PfSPZ Challenge 7G8, *Arm 2b* (n=4). All participants in this group remained uninfected post CHMI. Although the numbers are small, this is an unprecedented result for a chemoprophylaxis vaccination study with or without blood stage exposure. These preliminary results indicate that this regimen has the potential to provide protection against heterologous CHMI and subsequent vaccine efficacy in the field.

In view of these preliminary results and existing limitations, for the second cohort of the main study, the plan was to first enroll into heterologous Arms, *Arm 2b* and *3*. At that juncture, evidence of development of protective efficacy of PfSPZ-CVac under pyrimethamine against **homologous** (NF54) CHMI had been demonstrated. However, there was more evidence that heterologous CHMI better predicts vaccine efficacy in field trials where heterogeneous strains circulate. Therefore Arms that would undergo **heterologous** (7G8) CHMI (*Arms 2b and 3*) were prioritized.

The number of participants planned to be enrolled in each Arm in the 2nd and 3rd cohort is outlined below (**Table 18**).

In the 2nd cohort:

- ***Arm 2b*** (PfSPZ CVac with 200,000 PfSPZ Challenge (NF54) under PYR chemoprophylaxis; then heterologous 7G8 challenge; **n=5**)
- ***Arm 3*** (PfSPZ CVac with 200,000 PfSPZ Challenge (NF54) under weekly CQ chemoprophylaxis, then heterologous 7G8 challenge; **n=5**)
- ***Arm 4b*** (Control participants for 7G8 CHMI **n=4**)

In the 3rd cohort:

- ***Arm 2a*** (PfSPZ CVac with 200,000 PfSPZ Challenge (NF54) under PYR chemoprophylaxis; then homologous NF54 challenge; **n=9**)
- ***Arm 4a*** (Control participants for NF54 CHMI **n=4**)

***Table 18**: Number of participants in each Arm during first, second and third cohort*

ARMS	Participants in 1st Cohort enrolled and received at least 1 vaccination (COMPLETED CHMI)	Participants in 2nd Cohort	Participants in 3rd Cohort	Total # of participants
2a, PYR +NF54	8 (8)	0	9	17
2b, PYR +7G8	5 (4)*	5	0	10
3, CQ +7G8	5 (3)#	5	0	10
4a, NF54 Control	4 (4)	0	4	8
4b, 7G8 Control	4 (4)	4	0	8

*One participant withdrew consent after 1 vaccination

Two participants were withdrawn due to SAEs prior to CHMI

3.3.3 Rationale for stopping the study prior to full enrollment

Cohort 2 was enrolled in September 2018 and completed the study in March 2019. Heterologous (7G8) CHMI was conducted approximately 12 weeks post the 3rd vaccination and showed that high protective efficacy can be induced by both regimens. In the vaccinated groups, 3/5 (60%) in *Arm 2b* and 3/3 (100%) participants in *Arm 3* remained uninfected post CHMI whereas in the control group, *Arm 4b*, 0/4 (0%) participants were protected. Combined results of cohorts 1 and 2 are shown in **Table 19**. With this high significant level of protection against heterologous CHMI, and since heterologous CHMI is a more stringent test of assessing development of immunity (see **section 3.3.2**), we can already fully answer the secondary objectives of the study. Therefore, the study will not complete enrollment in *Arms 2a* and *4a* to undergo homologous CHMI (cohort 3, **Table 18**).

Table 19: Combined Results of CHMI for the first and Second Cohort

Arm	***Number of subjects challenged***	***Number of subjects infected***	***Days post CHMI to diagnosis***	***Median days post CHMI to diagnosis***	***Positive Blood Smear***	***Estimated Vaccine Efficacy***	***95% CI***	***p- value***
2a; pyrimethamine with homologous CHMI (NF54)	*8*	*1*	*12*	*12*	*0*	*0.875*	*0.425, 1*	*0.003*
2b; pyrimethamine with heterologous CHMI (7G8)	*9*	*2*	*10, 10*	*10*	*0*	*0.778*	*0.398, 1*	*<0.001*
3; chloroquine with heterologous CHMI (7G8)	*6*	*0*	*N/A*	*N/A*	*N/A*	*1*	*0.541, 1*	*<0.001*
4a; NF54 Control	*4*	*4*	*9,11, 11, 11*	*11*	*1*	*N/A*	*N/A*	*N/A*
4b; 7G8 Control	*8*	*8*	*9, 9,9, 9, 10, 12, 12, 16*	*9.5*	*0*	*N/A*	*N/A*	*N/A*

4 Description of Investigational Products and Plan

4.1 Chloroquine Phosphate

Chloroquine has been widely used for treatment and prophylaxis of malaria since 1946 (Most, London et al. 1984). It was the treatment of choice for uncomplicated malaria for decades because it was safe, well tolerated, affordable and highly effective for the treatment of malaria. However, increasing spread of chloroquine-resistant Pf over the last two decades has severely limited its use (Wellems 2002). Chloroquine phosphate is U.S Food and Drug Administration (FDA) approved for suppressive treatment (prophylaxis) and for acute attacks of malaria due to *P. vivax, P. malariae, P. ovale,* and susceptible strains of Pf. Chloroquine is a blood-stage schizonticide, highly active against replicating forms of blood-stage drug-sensitive parasites. As such, it is routinely employed as first-line prophylaxis against development of patent parasitemia and clinical malaria in non-immune travelers to areas with chloroquine sensitive Pf. Under standard weekly dosing, as used in this study [1g (600 mg base) loading dose then 500mg (300mg base) weekly], routine monitoring in clinical practice for patent blood-stage infection (via blood smear or qPCR) is not typically conducted unless triggered by presentation for evaluation of malaria related symptoms. Weight based chloroquine dosing also has been approved for treatment and prevention of Pf malaria.

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

4.2 Pyrimethamine

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

The approved dosage of pyrimethamine for treatment of toxoplasmosis can be higher than utilized for malaria treatment or chemoprophylaxis (75-100 mg per day) and given for prolonged periods of time (weeks to months), usually in conjunction with sulfadoxine and folinic acid. With doses of pyrimethamine used for the treatment of toxoplasmosis, side effects such as anorexia and vomiting may occur, but can be minimized by giving the medication with meals.
Similar to our previous study, the plan is to utilize the dose of pyrimethamine at 50 mg orally for two days, as approved for adult dosage of acute malaria. If subpatent parasitemia is seen during the pilot phase, at most we will increase the pyrimethamine dose to 75 mg daily orally for two days, similar to pyrimethamine doses utilized in toxoplasmosis treatment, but for a significantly shorter period of time.

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

4.3 Sanaria® PfSPZ Challenge

Sanaria Inc has manufactured two strains of Sanaria® PfSPZ Challenge: NF54 and 7G8. The Sanaria® PfSPZ Challenge contains fully infectious PfSPZ purified from the salivary glands of *Anopheles stephensi* mosquitoes raised under aseptic conditions. The infectious PfSPZ are formulated in cryoprotectant to maintain potency for an extended period. Sanaria® PfSPZ Challenge (NF54) is known to be susceptible to chloroquine, pyrimethamine, atovaquone, artesunate, but not mefloquine. Sanaria® PfSPZ Challenge (7G8) is known to be susceptible to mefloquine, atovaquone, artemether and artesunate but not to chloroquine or pyrimethamine. More detailed Sanaria® PfSPZ Challenge product information can be found in **Section 7.1** and the Investigator's Brochure.

The clinical studies for Sanaria® PfSPZ Challenge are detailed in **Sections 1.4.2, 1.4.3,** and **1.4.4**.

4.4 Description of Intervention

The intervention in this study is induction of stage specific immunity (pre-erythrocytic immunity) to malaria following DVI with aseptic, purified, vialed, cryopreserved, fully infectious NF54 PfSPZ (referred to as Sanaria® PfSPZ Challenge) when exposure is limited to the SPZ and liver-stages of the parasite life cycle (as summarized in **Table 19**).

Pilot Study

- ***Pyrimethamine pilot [Arm 1a, 1b and 1d]*** will receive 1 dose of NF54 Sanaria® PfSPZ Challenge **AND** 2 doses of pyrimethamine (50 mg and if needed 75 mg) administered on 2 and 3 days post injection timed to eliminate parasites in the liver-stage of development, after hepatocyte invasion and development, but prior to full maturation and release into the bloodstream. This regimen is intended to prevent parasitemia detectable by qPCR (subpatent parasitemia) and by blood smear (patent parasitemia). Subjects will be followed daily with NIH real time malaria qPCR from day 6 through day 14 post PfSPZ Challenge. All subjects will be treated with standard, approved treatment dosing of Malarone® for three consecutive at the time of a single qPCR detection of parasites or at the conclusion of follow-up post PfSPZ-CVac. See **Section 3.2** for more details.

- ***Chloroquine pilot (Arms 5a and 5b):*** will receive 1 dose of NF54 Sanaria® PfSPZ Challenge **AND** chloroquine weekly dosing beginning 2 days prior to Sanaria® PfSPZ Challenge (1g (600 mg base) loading dose then 2nd and last dose of 500 mg (300 mg base) on day 5 after the third NF54 Sanaria® PfSPZ Challenge to prevent development of patent parasitemia detectable by thick blood smear and clinical malaria. Subjects will be followed daily with blood smears from day 6 through day 12 post PfSPZ Challenge. All subjects will be treated with standard, approved treatment dosing of Malarone® for three consecutive at the time of clinical malaria diagnosis or at the conclusion of follow-up post PfSPZ-CVac. See **Section 3.2** for more details.

Main study

- ***Arm 2a and 2b*** will receive three vaccinations, 28 days apart, with the 200,000 NF54 Sanaria® PfSPZ Challenge **AND** pyrimethamine, 50mg per dose, given on days 2 and 3 post injection. Pyrimethamine is timed to eliminate parasites in the liver-stage of development, after hepatocyte invasion and development, but prior to full maturation and release into the bloodstream. This group will not receive chloroquine. This regimen is intended to prevent parasitemia detectable by qPCR (subpatent parasitemia) and by blood smear (patent parasitemia) (see **Section 3.3** for more details). This group will not receive chloroquine.
- ***Arm 3*** will receive three vaccinations, 28 days apart, using 200,000 NF54 Sanaria® PfSPZ Challenge with continuous suppressive prophylaxis with the blood-stage antimalarial drug chloroquine [weekly dosing beginning 2 days prior to first Sanaria® PfSPZ Challenge (1g (600 mg base) loading dose then 500 mg (300 mg base) maintenance dosing weekly) thereafter] and continuing throughout CVac, last dose on day 5 after the third NF54 Sanaria® PfSPZ Challenge to prevent development of patent parasitemia detectable by thick blood smear and clinical malaria. This regimen will not prevent parasitemia detectable by qPCR, but will eliminate the blood stage parasites before they can replicate in erythrocytes. This group will not receive pyrimethamine.
- ***Arms 4a and 4b*** (*Infectivity Controls* for CHMI) will be the challenge control group. This group will join the study for administration of Sanaria® PfSPZ Challenge, NF54 and 7G8 respectively, at CHMI. This group will not receive chloroquine or pyrimethamine. Upon diagnosis with malaria infection per protocol (one positive NIH real time malaria qPCR or single positive blood smear) subjects will be treated with standard, approved treatment dosing of Malarone®**.**

Sanaria® PfSPZ Challenge (3,200 PfSPZ) produced by Sanaria®, Inc will be given to all enrolled and eligible subjects in the absence of any antimalarial drugs during CHMI. Subjects in *Arms 2a* and *4a* will be challenged with Sanaria® PfSPZ Challenge (NF54) whereas *Arms 2b, 3* and *4b* will be challenged with Sanaria® PfSPZ Challenge (7G8) (**Figure 10**). Subjects who develop patent parasitemia (one positive real time NIH qPCR OR 2 unambiguous parasites seen in a single thick blood smear) will be treated with a standard, FDA approved treatment dosing of Malarone® (1g atovaquone/400 mg proguanil hydrochloride) daily for three days. Subjects who demonstrate sterile immunity, meaning they do not develop a positive real time NIH qPCR or blood smear, will also be treated with Malarone® daily for three days at the end of the study, starting on day 27 post CHMI.

The chemoprophylactic agents used in the current study are pyrimethamine and chloroquine. Both pyrimethamine and chloroquine are commercially available Food and Drug Administration (FDA)-approved antimalarial drugs for treatment and prophylaxis of Pf. Chloroquine dosing used in the study is abbreviated from the standard weekly suppressive prophylaxis in non-immune populations (600 mg base loading dose, then 300 mg base weekly maintenance dose for a total of 4 weeks after Pf exposure) given that in this study the day of exposure to SPZ is precisely known, there is no risk of *P. vivax* co-infection, and the biology of the Pf NF54 parasite is well characterized. Thus the loading dose will be administered 2 days prior to NF54 Sanaria® PfSPZ Challenge DVI and continued weekly until 5 days after the third PfSPZ Challenge. In prior studies, administering the

last dose of chloroquine in this manner has been sufficient to eliminate parasitemia in all study subjects during CVac, as described in **Section 1.4.3.1** (Mordmuller, Surat et al. 2017**)** and observed in our previous study, NIAID protocol #15-I-0169 as described in **Section 1.4.3.2.1.**

Pyrimethamine dosing in this study will be daily dosing of 50 mg for two consecutive days dosed on days 2 and 3 after NF54 Sanaria® PfSPZ Challenge DVI. This uses the FDA recommended dosing regimen for treatment of malaria due to susceptible parasites. In *Arms 2a* and *2b*, where PfSPZ Challenge will be administered three times, there will be a total of 6 doses of pyrimethamine received during the study. Treatment with pyrimethamine on 2 and 3 days post NF54 Sanaria® PfSPZ Challenge DVI is chosen to successfully maximize antigenic exposure of liver-stage parasites to the host while preventing maturation and release into the bloodstream, thus achieving the aims of the study.

Table 20: Chemoprophylaxis regimen and assignment

Intervention	Arm Assignment	Dosing
Antimalarials		
Pyrimethamine	Arm 1[1]: n=8-12 Arm 2a: n=17 Arm 2b: n=10	Two single strength pills (50 mg pyrimethamine total) given2, 3 days following each administration of Sanaria® PfSPZ Challenge by DVI during PfSPZ-CVac immunization. In total, 2 daily doses given for Arms 1 and 6 daily doses given for Arm 2.
Chloroquine	Arm 3: n=10 Arm 5: n=2-6	Loading dose of approximately 1 g chloroquine phosphate (600 mg chloroquine base) 2 days prior to first administration of NF54 Sanaria® PfSPZ Challenge by DVI. Then 500 mg (300 mg base) weekly until 5 days after 1st injection (*Arm 5*, 2 doses total) OR until 5 days after 3rd injection (*Arm 3*, 10 doses total).
P. falciparum sporozoites (PfSPZ), non-attenuated		
Sanaria® PfSPZ Challenge (NF54) administered by DVI for ***CVac***	Arm 1[1]: n= 8-12 Arm 2a: n=17 Arm 2b: n=10 Arm 3: n=10 Arm 5: n=2-6	Aseptic, purified, vialed, cryopreserved, non-attenuated PfSPZ (NF54 Sanaria® PfSPZ Challenge) administered by DVI of either **50,000, 100,000** or **200,000** PfSPZ in pilot arms (Arm 5 will not receive 50,000 dose). **200,000** PfSPZ dose will be administered to *Arm 2a, 2b* and *Arm 3*.
Sanaria® PfSPZ Challenge (NF54) administered by DVI for ***CHMI***	Arm 2a: n=17 Arm 4a: n=8	Aseptic, purified, vialed, cryopreserved, non-attenuated PfSPZ (NF54 Sanaria® PfSPZ Challenge) administered by DVI of **3,200 PfSPZ**
Sanaria® PfSPZ Challenge (7G8) administered by DVI for ***CHMI***	Arm 2b: n=10 Arm 3: n=10 Arm 4b: n=8	Aseptic, purified, vialed, cryopreserved, non-attenuated PfSPZ (7G8 Sanaria® PfSPZ Challenge) administered by DVI of **3,200 PfSPZ** (this dose may be adjusted depending on the results of other studies; if adjusted an amendment will be submitted)

[1] Arm 1 represents pilot phase with administration of 50 mg pyrimethamine and 50,000 Sanaria® PfSPZ Challenge during CVac. Depending on whether primary Pf infection objective is achieved or not, arms 1b to 1f may be followed as outlined in **Section 3.1.** The target regimen is Arm 1d. All pilot subjects only receive 1 CVac and will not undergo homologous challenge via CHMI per **Appendix A.**

4.5 Presumptive Antimalarial Treatment

Subjects who develop patent parasitemia (defined as one positive real time NIH qPCR or a single positive blood smear) following CHMI (all Arms) or PfSPZ-CVac (*Arms 1* and *2*) will be immediately treated with a standard, FDA-approved treatment dosing of Malarone® (1 g atovaquone/ 400 mg proguanil hydrochloride) daily for three consecutive days. Treated subjects will continue to be monitored with daily visits and blood smears until three consecutive daily blood smears are negative and any residual symptoms of malaria are mild or resolved.

Subjects who remain real time NIH qPCR and blood smear negative will be treated empirically with a standard dose of Malarone® at 27 days after CHMI with Sanaria® PfSPZ Challenge, at 14 days after Sanaria® PfSPZ-CVac (*Arm 1*) and at 12 days after Sanaria® PfSPZ-CVac (*Arm 5*). Subjects will return to the clinical site for two additional consecutive days to receive the second and third doses of Malarone® under direct observation by study staff.

5 Study Endpoints

5.1.1 Primary Endpoints

Safety

- Incidence and severity of local and systemic adverse events (AEs) and serious adverse events (SAEs) occurring after PfSPZ-CVac. (*Arms 1, 2, 3,5)*
- *P. falciparum* blood stage infection defined as detection of *P. falciparum* parasites by qPCR (real time NIH qPCR and sensitive retrospective LMIV qPCR) following Sanaria® PfSPZ Challenge. (*Arm 1, 2)*
- Incidence of clinical malaria diagnosis occurring after PfSPZ-CVac – chloroquine requiring treatment with additional antimalaria *(Arms 3 and 5)*

5.1.2 Secondary Endpoints

Protective Efficacy

- *P. falciparum* blood stage infection defined as detection of at least 2 *P. falciparum* parasites by microscopic examination of 0.5 µL of blood or one positive real time NIH qPCR after **homologous** PfSPZ CHMI (NF54) via DVI. (*Arm 2a and 4a*)
- *P. falciparum* blood stage infection defined as detection of at least 2 *P. falciparum* parasites by microscopic examination of 0.5 µL of blood or one positive real time NIH qPCR after **heterologous** PfSPZ CHMI (7G8) via DVI. (*Arm 2b, 3 and 4b*)

5.1.3 Exploratory Endpoints

- Humoral immune responses after PfSPZ-CVac regimens by assessing antibodies to

PfSPZ, Pf asexual erythrocytic stages (AES), and specific Pf sporozoite, liver and blood-stage antigens such as CSP, EXP-1, LSA-1, MSP-3, MSP-1, AMA-1 in subjects with no patent and/or subpatent parasitemia and those with patent and/or subpatent parasitemia, among drug groups.

- Cellular immune responses after PfSPZ-CVac regimen to PfSPZ, PfAES, and specific Pf sporozoite, liver and blood-stage antigens, such as CSP, EXP-1, LSA-1, MSP-3, MSP-1, AMA-1, in subjects with no patent and/or subpatent parasitemia and those with patent and/or subpatent parasitemia, among drug groups.
- Subpatent parasitemia and degree of subpatent parasitemia by sensitive retrospective LMIV qPCR following Sanaria® PfSPZ-CVac pyrimethamine and Sanaria® PfSPZ-CVac chloroquine.
- Comparison of γδ T cells before and after Sanaria® PfSPZ-CVac and CHMI using *ex vivo* whole blood staining
- Comparison of time to positivity of various malaria diagnostic platforms (qPCR, rapid diagnostics, ELISA).

5.2 Sample Size and Estimated Duration of Study

A total of up to 67 subjects are expected to be enrolled in this trial, if the pyrimethamine and chloroquine ideal pilot pathway is followed in which only four pilot arms will be conducted (**Figures 6, 7**). Subjects in the pilot arms and control arm will be monitored actively for about 1 month whereas subjects in the main arms will be actively monitored for approximately 7 months. Up to 200 subjects will be screened to accommodate possible screen failures.

6 Study Population

6.1 Description of Population and Site

The study will be conducted at the NIH Clinical Center (outpatient facilities) in Bethesda, Maryland. NIH Clinical Center has successfully supported healthy volunteer subject clinical trials, including malaria vaccine and controlled human malaria infection trials. Healthy malaria-naïve subjects will be recruited from the surrounding community.

6.2 Recruitment

Healthy adult male and non-pregnant female subjects will be recruited from a variety of sources including those previously screened or enrolled in other vaccine trials at the NIH clinical center or by the use of an Institutional Review Board (IRB)-approved screening protocol and study- specific print or media advertising. After an initial phone screen (using an IRB approved Phone Screen), a screening visit will be scheduled.

During the screening process, which may require more than one visit, the subject will read the consent form, be encouraged to ask questions, and then complete a written comprehension evaluation questionnaire (Malaria Comprehension Exam). The questionnaire is used to identify the areas of the study and consent that the subject may not fully understand. The person administering consent reviews the answers with the subject. If the subject gets a question wrong, the person administering the consent reviews the portion of the consent form that relates to that particular

question with the subject. The subject may either sign the consent form during the screening visit, or return after further consideration.

6.3 Participation of NIH Staff

NIH staff and members of their immediate families may participate in this protocol. We will follow the Guidelines for the Inclusion of NIH staff in NIH Research Studies and will give each staff member a copy of the "NIH information sheet on Staff Research Participation."

For NIH staff:

- Neither participation nor refusal to participate will have an effect, either beneficial or adverse, on the participant's employment or work situation.
- The NIH information sheet regarding NIH staff research participation will be distributed to all potential subjects who are NIH staff.
- The staff subject's privacy and confidentiality will be preserved in accordance with NIH Clinical Center and NIAID policies, which define the scope and limitations of the protections.
- For NIH staff subjects, consent will be obtained by an individual independent of the staff's team. Those in a supervisory position to any staff and co-workers of the staff will not obtain consent.
- The importance of maintaining confidentiality when obtaining potentially sensitive and private information from co-workers or subordinates will be reviewed with the study staff at least annually and more often if warranted.

6.4 Inclusion Criteria

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

1. Age ≥ 18 and ≤ 50 years.
2. In good general health and without clinically significant medical history
3. Malaria comprehension exam completed, passed (a score of ≥80% or per investigator's discretion) and reviewed prior to enrollment
4. Reliable access to the clinical trial center and availability to participate for duration of study
5. Females of childbearing potential must be willing to use reliable contraception (as defined below) from 21 days prior to study day -2 to 28 days following last Sanaria® PfSPZ Challenge exposure

    - **Subject to the judgment and discretion of the PI**, female participants who meet ANY ONE of the criteria listed immediately below, may not be required to take any additional measures to avoid pregnancy. Such participants will be counseled on risks at the time of consent and at appropriate points (e.g. when pregnancy testing occurs) during the study:

- Females who have had their uterus, and/or BOTH ovaries removed
- Females who have had BOTH fallopian tubes surgically 'tied' or removed
- Females who are above the age of 45 and have spontaneously had no menses at any point during the past 12 or more consecutive months (i.e. have reached menopause)
- Females who, in the conservative and reasonable judgment of the PI (e.g. due to sexual orientation or serious life choice (such as being celibate clergy or transgender), during the entire trial will NOT participate in any potentially reproductive sexual contact
- Females who, in the conservative and reasonable judgment of the PI, are in a monogamous stable relationship with a male who has undergone vasectomy at least 4 months prior or another procedure/medical condition that deems the male sterile

- **Subject to the judgment and discretion of the PI**, female participants who DO NOT meet ANY of the criteria listed above, will be appropriately counseled on reproductive risks and pregnancy avoidance, and will be required to adhere to the following measures and agree to 2 methods of pregnancy prevention as noted below:

CATEGORY 1:

- a highly effective hormonal method to prevent pregnancy [e.g. CONSISTENT, CONTINUOUS use of contraceptive pill, patch, ring, implant or injection], and/or
- IUD or equivalent

IN ADDITION TO

CATEGORY 2:

- a barrier method to be used at the time of potentially reproductive sexual activity (e.g. [male/female condom, 'cap,' or diaphragm] + spermicide).

6.5 Exclusion Criteria

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

1. Currently is breast-feeding (if female).
2. Pregnancy as determined by a positive urine or serum human choriogonadotropin (β-hCG) test at any point during the study (if female).
3. Recent travel to a malaria endemic area within 5 years of enrollment (Endemic areas are defined per the CDC website. Factors such as but not limited to use of antimalaria prophylaxis during travel, length of stay, activities during the travel, history of illnesses within 30 days of travel will be considered to determine the likelihood that the subject was exposed to malaria)
4. Planned travel to a malaria endemic area (as defined by the Center for Disease Control) during the study period

5. Reported history of confirmed malaria diagnosis on peripheral blood smear or by clinical history in the past 10 years.
6. Hemoglobin, WBC, platelets, ALT, and creatinine outside of local lab normal range (subjects may be included at the investigator's discretion for 'not clinically significant' values outside of normal range)
7. Abnormal urinalysis as defined by positive urine glucose, protein, and red blood cells. Subject can be included if investigator determine the abnormality is "not clinically significant".
8. BMI < 17 or BMI > 35
9. Anticipated use during the study period, or use within the following periods prior to enrollment:
    a. Investigational malaria vaccine within the last five years
    b. Malaria chemoprophylaxis within 6 months
    c. Chronic systemic immunosuppressive medications (>14 days) within 6 months (e.g.cytotoxic medications, oral/parental corticosteroids >0.5 mg/kg/day prednisone or equivalent). Corticosteroid nasal spray for allergic rhinitis and topical corticosteroids for mild, uncomplicated dermatitis are allowed.
    d. Blood products or immunoglobulins within 6 months
    e. Systemic antibiotics with antimalarial effects within 30 days (such as clindamycin, doxycycline)
    f. Investigational or non-registered product or vaccine within 30 days
    g. Receipt of a live vaccine within 28 days or a killed vaccine within the 14 days prior to Sanaria® PfSPZ Challenge
    h. Medications known to interact with pyrimethamine and/or chloroquine (for the main and pilot study participants ONLY); atovaquone, proguanil (ALL participants)
10. Reported history of:
    a. Sickle cell disease, sickle cell trait, or other hemoglobinopathies
    b. Splenectomy or functional asplenia
    c. Systemic anaphylaxis
    d. Any allergic reactions to study drugs (pyrimethamine, chloroquine) or NSAIDs, atovaquone, proguanil
    e. Documented history of chronic or active neurologic disease (including seizures, uncontrolled migraine headaches)
    f. Psoriasis or porphyria
    g. Ocular diseases including retinopathy or visual field defects
11. Clinically significant medical condition, physical examination findings, other clinically significant abnormal laboratory results, or past medical history that may have clinically significant implications for current health status and participation in the study in the opinion of the Investigator. A clinically significant condition or process includes but is not limited to:
    a. A process that would affect the immune response, or requires medication that affects the immune response
    b. Any contraindication to repeated phlebotomy
    c. A condition or process in which signs or symptoms could be confused with reactions to malaria challenge and/or infection, including dermatologic abnormalities at the site of sporozoite inoculation

d. A chronic or subclinical condition which could be exacerbated by administration of any of the PfSPZ-CVac components or malaria infection

12. History of, or known active cardiac disease including: (1) prior myocardial infarction (heart attack); (2) angina pectoris; (3) congestive heart failure; (4) valvular heart disease; (5) cardiomyopathy; (6) pericarditis; (7) stroke or transient ischemic attack; (8) exertional chest pain or shortness of breath; or (9) other heart conditions under the care of a doctor
13. Clinically significant ECG findings, as determined by the expert study cardiologist
14. Moderate or high risk for coronary heart disease (CHD) based on NHANES I cardiovascular risk assessment (**Appendix B)**
15. Acute illness at the time of enrollment
16. Infection with HIV, Hepatitis B, Hepatitis C
17. Psychiatric condition that precludes compliance with the protocol including but not limited to:
    a. Psychosis within the past 3 years
    b. Ongoing risk for suicide, or history of suicide attempt or gesture within the past 3 years
18. Suspected or known current alcohol or drug abuse as defined by the American Psychiatric Association in the DSM V at the discretion of the PI
19. Clinical trial staff and/or Sanaria® Inc. employees with direct involvement in the conduct of the trial are excluded from participation.
20. Participating in other clinical trials involving investigational interventions or off label medication use during the study period (excluding participation in the optional long term follow up visits). Participation in other trials such as observational or imaging studies will be discussed with the investigators.
21. Any other finding that, in the judgment of the Investigator, would interfere with, or serve as a contraindication to, protocol adherence, assessment of safety or reactogenicity, or a subject's ability to give informed consent, or increase the risk of having an adverse outcome from participating in the study

7 Study Agents

7.1 Sanaria® PfSPZ Challenge

7.1.1 Sanaria® PfSPZ Challenge (NF54)

Sanaria® PfSPZ Challenge consists of aseptic, cryopreserved *P. falciparum* produced by the biotechnology company; Sanaria Inc. (Rockville, MD, USA) (Hoffman, Billingsley et al. 2010, Epstein, Tewari et al. 2011, Roestenberg, Bijker et al. 2013). In brief, manufacture includes the production, under traditional environmental conditions, of eggs from a colony of *A. stephensi* mosquitoes housed in a controlled environmental chamber. Surface disinfection of the eggs is performed by exposure to chemical agents in a Class II biosafety cabinet. From this point forward, all materials and products are handled using aseptic methods to ensure that contaminating microorganisms are not introduced to and carried through the process. Surface- disinfected eggs are inoculated into sterile, vented flasks containing aseptic growth medium. The eggs hatch and develop into pupae, which are transferred to an adult mosquito container where the adult mosquitoes emerge. These adult mosquitoes, which have been raised under aseptic conditions, are

fed Pf gametocyte-infected blood in an aseptic, High-Security Insectary. The *P. falciparum* gametocyte-infected blood produced from cultures of the Pf isolate NF54 (Pf NF54) derived from a Working Cell Bank of the well- characterized Pf isolate NF54. Infected adult mosquitoes are maintained under aseptic conditions. Pf SPZ migrate to the salivary glands. The salivary glands from the Pf SPZ infected mosquitoes are removed by hand dissection under aseptic conditions. Salivary glands are then triturated to release the *P. falciparum* sporozoites. The sporozoites are purified, counted, and, at a specified concentration, cryopreserved. Cryopreservation commences with the addition of cryoprotective additives to the purified sporozoites to produce the Sanaria® PfSPZ Challenge product. Sanaria® PfSPZ Challenge is dispensed into screw-cap vials and stored in liquid nitrogen vapor phase (LNVP) at 150°C to -196°C. All the procedures are described in more detail in the investigator's brochure.

7.1.2 Sanaria® PfSPZ Challenge (7G8)

PfSPZ Challenge (7G8) is manufactured and characterized by essentially the same methods as NF54 PfSPZ Challenge except that is comprised of PfSPZ from the 7G8 clone of *P. falciparum*. The *P. falciparum* gametocyte-infected blood is produced from cultures of the Pf isolate 7G8 (Pf 7G8) derived from a Working Cell Bank of the well-characterized Pf 7G8.The 7G8 clone of Pf (Pf 7G8) was derived from a Brazilian isolate, IMTM22, obtained from a 12 year old male near Manaus, Brazil on 12 March 1980. The isolate was cryopreserved and adapted to continuous culture. After 19 weeks of continuous culture, a sample was cloned by limiting dilution. The clone 7G8 was selected for ability to produce microgametes, exflagellate, and infect *A. freeborni* resulting in oocysts and sporozoites. It was tested for drug sensitivity by the Division of Experimental Therapeutics, Walter Reed Army Institute of Research (WRAIR) and was found to be resistant to chloroquine, and sensitive to mefloquine.

Pf 7G8 has a different genomic sequence than does Pf NF54 and a distinct microsatellite map. Thus, if Pf 7G8 is used to assess protective immunity by CHMI in subjects immunized with Sanaria® PfSPZ Challenge (NF54), it can assess the capacity of Sanaria® PfSPZ Challenge (NF54) to protect against heterologous parasites. All the procedures are described in more detail in the investigator's brochure.

7.1.3 Phosphate buffered saline and human serum albumin diluent

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

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

7.1.4 Storage and Handling of Sanaria® PfSPZ Challenge

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

7.2 Malarone®, Pyrimethamine and Chloroquine

Chloroquine, pyrimethamine, and Malarone® are a commercially available antimalarial drugs. Package inserts for all three drugs are provided.
Chloroquine phosphate is supplied and manufactured in tablets of 250 mg or 500 mg (equivalent to 150 or 300 mg chloroquine phosphate base). Based on availability of chloroquine phosphate in the U.S., chloroquine phosphate from Europe may be obtained under approval from the FDA, which is supplied and manufactured in tablets of 250 mg (equivalent to 155 mg chloroquine phosphate base).
Pyrimethamine is manufactured in tablets of 25 mg.
Malarone® is manufactured in tablets of 250mg atovaquone/ 100 mg proguanil hydrochloride.

7.2.1 Malarone®, Pyrimethamine and Chloroquine Packaging and Labeling

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

Malarone®, though not a drug under study but a drug that all participants will receive, will be maintained in the same manner as pyrimethamine and chloroquine.

7.2.2 Malarone®, Pyrimethamine and Chloroquine Accountability

7.2.2.1 Receipt

Pyrimethamine, chloroquine, and Malarone® tablets will be purchased from commercial sources and provided by the NIH Clinical Center Pharmacy. Drug accountability will be managed as outlined in the Pharmacy Manual.

7.2.2.2 Preparation and Administration

Pyrimethamine, chloroquine, and Malarone® will be provided as tablets for oral administration with food. Administration is under direct observation by study staff according to dosing parameters. Individual doses of pyrimethamine, chloroquine, and Malarone® will be prepared by study staff as described in the Pharmacy Manual.

7.2.2.3 Storage and Handling

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

7.2.2.4 Return of Study Product

Final accountability of drug supplies will be performed at the conclusion of the study. Final disposition of any remaining pyrimethamine, chloroquine, and Malarone® will be determined and documented.

8 Study Procedures

After signing the informed consent, study procedures are conducted prior to and following enrollment on all subjects (unless otherwise noted) at the time points indicated in **Appendix A**. Associated laboratory assays and blood volumes are also specified in **Appendix A**.

8.1 Screening

The purpose of the screening visit is to determine subject eligibility for study participation. Subjects who are diagnosed with a medical condition during the screening process (e.g., test positive for hepatitis B, hepatitis C, or HIV) will be notified and referred for medical care, reportable positive results will be reported to the Maryland Department of Health.

The following screening procedures and evaluations for this study must be completed within 56 days prior to study enrollment.

- Explain the study and Informed Consent to the subject. Ensure the subject has signed the Informed Consent prior to any study procedures and receives a signed copy of the Informed Consent.
- Ensure the subject has successfully completed the Malaria Comprehension Exam (scoring ≥ 80% correct and those questions answered in error reviewed)
- Ensure that HIV pre-test counseling has been performed and ensure that the subject has agreed to HIV testing (required by Maryland state law).
- Elicit a complete medical history, including menstrual and contraceptive history and/or history of surgical sterility for females.
- Pregnancy prevention counseling will be performed.
- Administer a complete physical examination, including vital signs (height, weight, blood pressure, temperature, respirations, and pulse).
- Complete NHANES I Classification (see **Appendix B**)
- Obtain blood samples for complete blood count (CBC) with differential and platelet count, ALT, Creatinine, Hepatitis B surface antigen, Hepatitis C antibody, and HIV antibody.
- Obtain urine or blood for pregnancy testing

- Obtain urine for urinalysis testing for protein and blood.
- Obtain electrocardiogram (ECG), review, initial and date. ECG to be transmitted to study cardiologist for final read.

Note: Laboratory studies and ECG completed under a different NIH protocol can be used for screening purposes as long as they are within 56 days prior to study enrollment.

8.2 Malaria Comprehension Exam

To ensure the subject fully comprehends key concepts related to the study and to highlight areas that may need additional discussion or clarification, a Malaria Comprehension Exam will be administered to the subject before signing informed consent. All incorrect responses will be reviewed with the subject, and he or she must verbalize understanding of all incorrect responses. A score of ≥ 80% correct is required for enrollment. For subjects scoring less than 80%, study staff may choose to review study details again with subject and reassess comprehension with a repeat Malaria Comprehension Exam. At the discretion of the Investigator, any subject whose comprehension is questionable, regardless of score, may be excluded from enrollment. Discussions of understanding will be documented in the subject's source documentation.

8.3 Assignment to Groups

Arm 1a, 1b and 1d (and Pilot *Arms 1c, 1e and 1f* if necessary) as well as *Arm 5a* and *5b* will be filled first and on an available basis. *Arm 2a, 2b* and *3* will be enrolled following completion of the Pilot Arms and will be randomized for enrollment into *Arm 2a, 2b* or *3*.

Subjects for *Arm 4a* and *4b* will be randomized for enrollment into *Arms 4a* or *4b*.

8.4 Study Schedule

The study schedule is outlined below. Details of specific laboratory samples and approximate amounts of blood drawn are summarized in **Appendix A**.

Note:

1. To ensure safety and compliance of enrolled subjects, all study drug administration (chloroquine, pyrimethamine and Malarone®) will be completed under directly observed therapy. The subject will swallow the study drugs with liquid and study staff will inspect the mouth to verify that the entire dose of medication was swallowed.
2. Although some doses of NSAIDs in *Arm 3* may be given in clinic, directly observed therapy is not required.
3. A complete physical exam will be performed during the screening visit. During ALL other visits, physical exam will only be performed as clinically indicated.

Chemoprophylaxis Vaccination for the pyrimethamine (Arm 1) and chloroquine (Arm 5) PILOT studies

Study Day -2 [Arm 5 ONLY] (Enrollment/ Chloroquine Load)

1. Verify that Informed Consent and up-to-date version of the Informed Consent was

obtained.

2. Ensure that all inclusion criteria are met and exclusion criteria are not met and Eligibility Checklist completed and signed.
3. Ensure that CBC with differential, ALT, and creatinine measurements from screening tests are within protocol-defined limits (see Exclusion Criteria).
4. Ensure that Hepatitis B, Hepatitis C, and HIV testing are negative from screening visit.
5. During the indicated physical examination, study staff will educate the subject regarding signs and symptoms of potential AEs and indications for contacting the site.
6. For females, obtain a urine or serum sample for β-hCG (pregnancy) testing. Ensure that the test is negative before administering chloroquine load dose; a positive test will exclude the subject from the trial.
7. Record vital signs (blood pressure, temperature, heart rate).
8. Provide chloroquine loading dose (approximately 1 gram) by directly observed therapy **(Arm 5 only).**
    a. Observe subject for at least 30 minutes following chloroquine loading dose to evaluate for immediate adverse reactions. (Only required after the first dose)
9. Record AEs and concomitant medications, if applicable

Study Day 1 [Arm 1 and 5](Enrollment for Arm 1/ Sanaria® PfSPZ Challenge #1)

1. Verify that Informed Consent and up-to-date version of the Informed Consent was obtained.
2. Ensure that all inclusion criteria are met and exclusion criteria are not met and Eligibility Checklist completed and signed.
3. Ensure that CBC with differential, ALT, and creatinine measurements from screening tests are within protocol-defined limits (see Exclusion Criteria).
4. Ensure that Hepatitis B, Hepatitis C, and HIV testing are negative from screening visit.
5. During the indicated physical examination, study staff will educate the subject regarding signs and symptoms of potential AEs and indications for contacting the site.
6. Obtain approximately 10 mL of blood for baseline labs including: retrospective LMIV qPCR, safety labs, and research labs.
    - **Arm 1 only:** Obtain approximately additional 10ml of blood for research labs.
    - **Arm 5 only**: Obtain approximately 3ml of blood and 1 ml of urine for research labs.
7. For females, obtain a urine or serum sample for β-hCG (pregnancy) testing. Ensure that the test is negative before Sanaria® PfSPZ Challenge; a positive test will exclude the subject from the trial.
8. Record AEs and concomitant medications, if applicable.
9. Verify that no contraindications for Sanaria® PfSPZ Challenge or deferral of Sanaria® PfSPZ Challenge have been met.
10. Confirm emergency contact information and complete medical emergency release form
11. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
12. Educate the subject regarding signs and symptoms of potential adverse events (AEs), clinical malaria, and indications for use of antipyretics if fever, headache, or malaise occurs.
13. For females, ensure continued agreement and compliance with pregnancy prevention

before Sanaria® PfSPZ Challenge.

14. Record vital signs (blood pressure, temperature, and heart rate).
15. Review Inclusion/Exclusion criteria and confirm final eligibility.
16. Administer Sanaria® PfSPZ Challenge via DVI.
    a. Observe subject for **at least 30 minutes** after to evaluate for immediate adverse reactions
17. Dispense diary, thermometer and ruler to subject and demonstrate use and assess understanding of use.
18. Review instructions for contacting the site to report adverse experiences and/or inquire about study procedures.
19. Study number will be the same as screening number.
20. Provide subject with Do's and Don't's handout.

Study Day 2 [Arm 1 and 5] (Phone Follow-Up; 1 Day after Sanaria® PfSPZ Challenge #1)

1. Perform basic history and AE review (including DVI site), emphasizing review of any acute complaints.
2. Complete diary review.
3. Record AEs and concomitant medications, if applicable.
4. Provide pregnancy prevention counseling for female subjects.

Study Day 3 [Arm 1 ONLY] (pyrimethamine dose; 2 Days after Sanaria® PfSPZ Challenge #1)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review.
4. Record AEs and concomitant medications, if applicable.
5. Provide pregnancy prevention counseling for female subjects.
6. Provide pyrimethamine dose by directly observed therapy **(Arm 1 only)**
   ***Note**:* 2 tablets (50mg) for *Arms 1a, 1b* and *1d*;
   3 tablets (75mg) for *Arms 1c, 1e* and *1f*
7. Observe subject for at least 30 minutes following pyrimethamine to evaluate for immediate adverse reactions (Only required after the first dose)

Study Day 4 [Arm 1 and 5] (pyrimethamine dose (Arm 1 only); 3 Days after Sanaria® PfSPZ Challenge #1)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review.
4. Record AEs and concomitant medications, if applicable.
5. Provide pregnancy prevention counseling for female subjects.
6. Obtain approximately 7 mL of blood for safety labs and research labs.
7. Provide pyrimethamine dose by directly observed therapy **(Arm 1 only)**
   ***Note**:* 2 tablets (50mg) for *Arms 1a, 1b* and *1d*;
   3 tablets for (75mg) *Arms 1c, 1e* and *1f*

Study Day 6 [Arm 5 ONLY] (Chloroquine dose; 5 Days after Sanaria® PfSPZ Challenge #1)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review.
4. Record AEs and concomitant medications, if applicable.
5. Provide pregnancy prevention counseling for female subjects.
6. Provide chloroquine dose (approximately 500 mg) by directly observed therapy **(Arm 5 only)**. *Note*: if a subject has problems related to tolerability of chloroquine, a lower weight-based dose (as defined in the PI) would be clinically appropriate for maintenance dosing, this adjustment may be made on a case by case basis.

*****NOTE for all visits between Study Day: 7 - 14:** If a subject develops malaria defined in Arm 1 as 1 positive NIH malaria qPCR and in Arm 5 as Clinical malaria as outlined by LMIV SOP 25.

1. Administer Malarone® dose (4 tablets) by directly observed therapy.
2. Observe subject for at least 30 minutes following Malarone® dose to evaluate for immediate adverse reactions. (Only required after the first dose)
3. Complete Malarone® dose treatment for 3 consecutive days
4. Arm 1: Do not repeat NIH malaria qPCR until end of study, study day 29

Study Day 7 [Arm 1 and 5] (Clinic Follow-Up; 6 Days after Sanaria® PfSPZ Challenge #1)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review.
4. Complete malaria symptom review.
5. Record AEs and concomitant medications, if applicable.
6. Provide pregnancy prevention counseling for female subjects.
7. **Arm 1 ONLY**: Obtain approximately 4 mL of blood for real time NIH and retrospective LMIV qPCR. Blood smear completed if indicated.
   Arm 5 ONLY: Obtain approximately 3 mL of blood for retrospective LMIV qPCR and Blood smear.
8. Confirm subject and emergency contact information to follow up malaria diagnostic result.

Study Day 8 [Arm 1 and 5] (Clinic Follow-Up; 7 Days after Sanaria® PfSPZ Challenge #1)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review.
4. Complete malaria symptom review.
5. Record AEs and concomitant medications, if applicable.
6. Provide pregnancy prevention counseling for female subjects.
7. **Arm 1 ONLY**: Obtain approximately 11 mL of blood for safety labs, real time NIH and retrospective LMIV qPCR, and research labs. Blood smear completed if indicated.
   Arm 5 ONLY: Obtain approximately 10 mL of blood for safety labs, retrospective LMIV qPCR, blood smear and research labs.
8. Confirm subject contact information to follow up malaria diagnostic result.

Note:
Arm 1 will have daily prepatent follow up until Study Day 14, Malarone treatment (unless otherwise indicated) will start Study Day 15
Arm 5 will have daily prepatent follow up until Study Day 12, Malarone treatment (unless otherwise indicated) will start Study Day 13

Study Day 9 – 14 [Arm 1 ONLY]; 9 – 12 [Arm 5 ONLY] (Clinic Follow-Up; 8 – 13; 8-11 Days after Sanaria® PfSPZ Challenge #1)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review. **Complete and Collect diary on Study Day 11**.
4. Complete malaria symptom review.
5. Record AEs and concomitant medications, if applicable.
6. Provide pregnancy prevention counseling for female subjects.
7. **Arm 1 ONLY** Obtain approximately 3 mL of blood for real time NIH and retrospective LMIV qPCR. Blood smear completed if indicated.
   Arm 5 ONLY: Obtain approximately 3 mL of blood for retrospective LMIV qPCR and Blood smear.
8. Confirm subject contact information.

Study Day 15 (Arm 1); Day 13 (Arm 5) (Treatment visit; 14; 12 Days after Sanaria® PfSPZ Challenge #1)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete malaria symptom review.
4. Record AEs and concomitant medications, if applicable.
5. Provide pregnancy prevention counseling for female subjects.
6. **Arm 1 ONLY**: Obtain approximately 14 mL of blood for real time NIH and retrospective LMIV qPCR, and research labs. Blood smear completed if indicated.
   Arm 5 ONLY: Obtain approximately 3 ml of blood for retrospective LMIV qPCR and Blood smear.
7. Administer Malarone® dose (4 tablets) by directly observed therapy.
   a. Observe subject for at least 30 minutes following Malarone® dose to evaluate for immediate adverse reactions. (Only required after the first dose)
8. Confirm subject contact information to follow up malaria diagnostic result.

Study Day 16 (Arm 1); Day 14 (Arm 5) (Treatment visit; 15; 13 Days after Sanaria® PfSPZ Challenge #1)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Provide pregnancy prevention counseling for female subjects

5. **Arm 1 ONLY**: Obtain approximately 3 mL of blood for real time NIH and retrospective LMIV qPCR. Blood smear completed if indicated.
   Arm 5 ONLY: Obtain approximately 3 mL of blood for retrospective LMIV qPCR, blood smear and research labs.
6. Administer Malarone® dose (4 tablets) by directly observed therapy
7. Confirm subject contact information

Study Day 17 (Arm 1); Day 15 (Arm 5) (Treatment visit; 16; 14 Days after Sanaria® PfSPZ Challenge #1)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Provide pregnancy prevention counseling for female subjects.
5. **Arm 1 ONLY**: Obtain approximately 10 mL of blood for safety, real time NIH and retrospective LMIV qPCR. Blood smear completed if indicated.
   Arm 5 ONLY: Obtain approximately 10 mL of blood for safety, retrospective LMIV qPCR and Blood smear.
6. Administer Malarone® dose (4 tablets) by directly observed therapy
7. Confirm subject contact information

Study Day 29 [Arm 1 and 5] (Final visit; 28 Days after Sanaria® PfSPZ Challenge #1)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Provide pregnancy prevention counseling for female subjects.
5. Obtain approximately 10 mL of blood for real time NIH qPCR and retrospective LMIV qPCR, and safety labs.
6. Confirm subject contact information

Chemoprophylaxis Vaccination for the MAIN study, Arms 2 and 3
Note: Subjects will be vaccinated by NF54 Sanaria® PfSPZ Challenge DVI

Study Day -7 (Day of Enrollment, Baseline labs #1; 7 days prior to Sanaria® PfSPZ Challenge)

1. Verify that Informed Consent and up-to-date version of the Informed Consent was obtained.
2. Ensure that all inclusion criteria are met and exclusion criteria are not met and Eligibility Checklist completed and signed.
3. Ensure that CBC with differential, ALT, and creatinine measurements from screening tests are within protocol-defined limits (see Exclusion Criteria).
4. Ensure that Hepatitis B, Hepatitis C, and HIV testing are negative from screening visit.
5. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
6. During the physical examination, study staff will educate the subject regarding signs and symptoms of potential AEs, clinical malaria, and indications for use of antipyretics if fever,

headache, or malaise occurs.
7. Record vital signs (blood pressure, temperature, heart rate).
8. For females, ensure continued agreement and compliance with pregnancy prevention
9. Obtain approximately 57 mL of blood for research labs.
10. Record AEs and concomitant medications, if applicable
11. Enrollment study number will be the same as screening number.

Note**: **If screening tests were done outside the 56 days window of enrollment, they will be repeated during this visit

Study Day -2 (Baseline labs #2; chloroquine load; 2 days prior to Sanaria® PfSPZ Challenge)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Study staff will educate the subject regarding signs and symptoms of potential AEs and indications for contacting the site.
3. Obtain approximately 51 mL of blood for baseline labs including: retrospective LMIV qPCR, safety labs, and research labs.
    a. Obtain additional 1 mL of blood for drug assays **(Arm 2 only)**
4. For females, obtain a urine or serum sample for β-hCG (pregnancy) testing. Ensure that the test is negative before administering chloroquine; a positive test will exclude the subject from the trial. Pregnancy prevention counseling will be performed.
5. Record vital signs (blood pressure, temperature, heart rate).
6. Provide chloroquine loading dose (approximately 1 gram) by directly observed therapy **(Arm 3 only).**
    a. Observe subject for at least 30 minutes following chloroquine loading dose to evaluate for immediate adverse reactions. (Only required after the first dose)
7. Record AEs and concomitant medications, if applicable

Study Day 1 (Sanaria® PfSPZ Challenge #1)

1. Verify that no contraindications for Sanaria® PfSPZ Challenge or deferral of Sanaria® PfSPZ Challenge have been met.
2. Confirm emergency contact information and complete medical emergency release form
3. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
4. During the physical examination, study staff will educate the subject regarding signs and symptoms of potential adverse events (AEs), clinical malaria, and indications for use of antipyretics if fever, headache, or malaise occurs.
5. For females, obtain a urine or serum sample for β-hCG (pregnancy) testing. Ensure that the test is negative before Sanaria® PfSPZ Challenge; a positive test will exclude the subject from the trial.
6. For females, ensure continued agreement and compliance with pregnancy prevention before Sanaria® PfSPZ Challenge.
7. Obtain approximately 3ml of blood and 3 ml of urine for drug assays **(Arm 3 only)**
8. Record vital signs (blood pressure, temperature, and heart rate).
9. Review Inclusion/Exclusion criteria and confirm final eligibility.
10. Administer Sanaria® PfSPZ Challenge via DVI.

a. Observe subject for at least 30 minutes after to evaluate for immediate adverse reactions.

11. Record AEs and concomitant medications, if applicable.
12. Review instructions for contacting the site to report adverse experiences and/or inquire about study procedures.
13. Dispense diary, thermometer and ruler to subject and demonstrate use and assess understanding of use.

Study Day 2 (Phone Follow-Up; 1 Day after Sanaria® PfSPZ Challenge #1)

1. Perform basic history and AE review (including DVI site), emphasizing review of any acute complaints.
2. Complete diary review.
3. Record AEs and concomitant medications, if applicable.
4. Provide pregnancy prevention counseling for female subjects.

Study Day 3 (pyrimethamine dose; 2 Days after Sanaria® PfSPZ Challenge #1)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review.
4. Record AEs and concomitant medications, if applicable.
5. Provide pregnancy prevention counseling for female subjects.
6. Provide pyrimethamine dose by directly observed therapy

   Note for Arm 2 only*:*

   2 tablets if 50mg dose
7. Observe subject for at least 30 minutes following pyrimethamine to evaluate for immediate adverse reactions (Only required after the first dose)
8. Record AEs and concomitant medications, if applicable.

Study Day 4 (pyrimethamine dose; 3 Days after Sanaria® PfSPZ Challenge #1)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review.
4. Record AEs and concomitant medications, if applicable.
5. Provide pregnancy prevention counseling for female subjects.
6. Obtain approximately 45 mL of blood for safety labs and research labs.
7. Provide pyrimethamine dose by directly observed therapy

Note for Arm 2 only*:*

2 tablets if 50mg dose

Study Day 6 (chloroquine dose; 5 Days after Sanaria® PfSPZ Challenge #1)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review.
4. Record AEs and concomitant medications, if applicable.
5. Provide pregnancy prevention counseling for female subjects.
6. Provide chloroquine dose (approximately 500 mg) by directly observed therapy **(Arm 3**

only). *Note*: if a subject has problems related to tolerability of chloroquine, a lower weight-based dose (as defined in the PI) would be clinically appropriate for maintenance dosing, this adjustment may be made on a case by case basis.

Study Day 7 (Clinic Follow-Up; 6 Days after Sanaria® PfSPZ Challenge #1)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review.
4. Complete malaria symptom review.
5. Record AEs and concomitant medications, if applicable.
6. Provide pregnancy prevention counseling for female subjects.
7. Obtain approximately 3 mL of blood for malaria infection assays (see below).
    - **Arm 2**: real time NIH and retrospective LMIV qPCR. Blood smear if indicated
    - **Arm 3**: retrospective LMIV qPCR and blood smear. Real time NIH PCR if indicated.
    - Obtain additional approximately 1 mL of blood for drug assays. **(Arm 2 only)**
8. Confirm subject and emergency contact information to follow up malaria diagnostic result.

Study Day 8 (Clinic Follow-Up; 7 Days after Sanaria® PfSPZ Challenge #1)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review.
4. Complete malaria symptom review.
5. Record AEs and concomitant medications, if applicable.
6. Provide pregnancy prevention counseling for female subjects.
7. Obtain approximately 59 mL of blood for safety labs, malaria infection assays (see below) and research labs.
    - **Arm 2**: real time NIH and retrospective LMIV qPCR. Blood smear if indicated.
    - **Arm 3**: retrospective LMIV qPCR and blood smear. Real time NIH PCR if indicated.
8. Confirm subject contact information to follow up malaria diagnostic result.
9. **Arm 3 ONLY**:
    a. Administer at least Ibuprofen 400 mg (or another NSAID if indicated) from the outpatient prescription
    b. Provide the remainder of outpatient prescription of NSAIDs for the subject to take home
    c. Instruct subject to bring NSAIDs prescription to all study Day 8 and 9 clinic visits

Study Day 8 PM; [Arm 3 ONLY] (PM Clinic Follow-Up; 7 Days after Sanaria® PfSPZ Challenge #1)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete malaria symptom review.

4. Record AEs and concomitant medications, if applicable.
5. Provide pregnancy prevention counseling for female subjects.
6. Confirm subject contact information to follow up malaria diagnostic result.
7. Provide at least Ibuprofen 400mg dose (or another NSAID is indicated)

Study Day 9 (Clinic Follow-Up; 8 Days after Sanaria® PfSPZ Challenge #1)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review.
4. Complete malaria symptom review.
5. Record AEs and concomitant medications, if applicable.
6. Provide pregnancy prevention counseling for female subjects.
7. Obtain approximately 3 mL of blood for malaria infection assays (see below).
    - **Arm 2**: real time NIH and retrospective LMIV qPCR. Blood smear if indicated.
    - **Arm 3**: retrospective LMIV qPCR and blood smear. Real time NIH PCR if indicated.
8. Confirm subject contact information to follow up malaria diagnostic result.
9. **Arm 3 ONLY**: Administer at least Ibuprofen 400 mg dose (or another NSAID if indicated) from the outpatient prescription

Study Day 9 PM; [Arm 3 ONLY] (PM Clinic Follow-Up; 8 Days after Sanaria® PfSPZ Challenge #1)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete malaria symptom review.
4. Record AEs and concomitant medications, if applicable.
5. Provide pregnancy prevention counseling for female subjects.
6. Confirm subject contact information to follow up malaria diagnostic result.
7. Provide at least Ibuprofen 400mg dose (or another NSAID is indicated)

Study Day 10 (Clinic Follow-Up; 9 Days after Sanaria® PfSPZ Challenge #1)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review.
4. Complete malaria symptom review.
5. Record AEs and concomitant medications, if applicable.
6. Provide pregnancy prevention counseling for female subjects.
7. Obtain approximately 3 mL of blood for malaria infection assays (see below).
    - **Arm 2**: real time NIH and retrospective LMIV qPCR. Blood smear if indicated.
    - **Arm 3**: retrospective LMIV qPCR and blood smear. Real time NIH PCR if indicated.

8. Confirm subject contact information to follow up malaria diagnostic result.

Study Day 11 (Clinic Follow-Up; 10 Days after Sanaria® PfSPZ Challenge #1)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review, enter information for today's visit in diary, and collect diary (window for diary collection +2 days)
4. Complete malaria symptom review.
5. Record AEs and concomitant medications, if applicable.
6. Obtain approximately 3 mL of blood for malaria infection assays (see below).
    - **Arm 2**: real time NIH and retrospective LMIV qPCR. Blood smear if indicated.
    - **Arm 3**: retrospective LMIV qPCR and blood smear. Real time NIH PCR if indicated.
    - Obtain additional approximately 1 mL of blood for drug assays. **(Arm 2 only)**
7. Confirm subject contact information to follow up malaria diagnostic result.

Study Day 13 (chloroquine dose; 12 Days after Sanaria® PfSPZ Challenge #1)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete malaria symptom review.
4. Record AEs and concomitant medications, if applicable.
5. Provide pregnancy prevention counseling for female subjects.
6. Provide chloroquine dose (approximately 500 mg by directly observed therapy **(Arm 3 only)**. *Note:* if a subject has issues related to tolerability of chloroquine and a lower weight-based dose (as defined in the PI) would be clinically appropriate for maintenance dosing, this adjustment may be made on a case by case basis.
7. Confirm subject contact information to follow up malaria diagnostic result.

Study Day 15 (14 Days after Sanaria® PfSPZ Challenge #1)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete malaria symptom review.
4. Record AEs and concomitant medications, if applicable.
5. Provide pregnancy prevention counseling for female subjects
6. Obtain approximately 53 mL of blood for research labs.
7. Confirm subject contact information to follow up malaria diagnostic result.

Study Day 20 (chloroquine dose; 19 Days after Sanaria® PfSPZ Challenge #1)

1. Perform basic history and focused physical examination, emphasizing examination of any

acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Provide pregnancy prevention counseling for female subjects.
5. Provide chloroquine dose (approximately 500mg) by directly observed therapy **(Arm 3 only)**. *Note*: if a subject has issues related to tolerability of chloroquine and a lower weight-based dose (as defined in the PI) would be clinically appropriate for maintenance dosing, this adjustment may be made on a case by case basis.

Study Day 27 (chloroquine dose; 26 Days after Sanaria® PfSPZ Challenge #1)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Provide pregnancy prevention counseling for female subjects.
5. Obtain approximately 24 mL of blood for safety labs, retrospective LMIV qPCR, and research labs.
    a. Obtain additional approximately 1 mL of blood for drug assays **(Arm 2 only).**
6. Provide chloroquine dose (approximately 500 mg) by directly observed therapy **(Arm 3 only).** *Note*: if a subject has issues related to tolerability of chloroquine and a lower weight-based dose (as defined in the PI) would be clinically appropriate for maintenance dosing, this adjustment may be made on a case by case basis.

Study Day 29 (Sanaria® PfSPZ Challenge #2)

1. Verify that no contraindications for Sanaria® PfSPZ Challenge or deferral of Sanaria® PfSPZ Challenge have been met.
2. Confirm emergency contact information.
3. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
4. During the physical examination, study staff will educate the subject regarding signs and symptoms of potential adverse events (AEs), clinical malaria, and indications for use of antipyretics if fever, headache, or malaise occurs.
5. For females, obtain a urine or serum sample for β-hCG (pregnancy) testing. Ensure that the test is negative before Sanaria® PfSPZ Challenge; a positive test will exclude the subject from the trial.
6. For females, ensure continued agreement and compliance with pregnancy prevention before Sanaria® PfSPZ Challenge.
7. Record vital signs (blood pressure, temperature, and heart rate).
8. Review Inclusion/Exclusion criteria and confirm eligibility.
9. Administer Sanaria® PfSPZ Challenge via DVI.
    a. Observe subject for at least 30 minutes after administration to evaluate for immediate adverse reactions.
10. Record AEs and concomitant medications, if applicable.
11. Dispense diary, thermometer, and ruler to subject and demonstrate use and assess understanding of use.
12. Review instructions for contacting the site to report adverse experiences and/or inquire about study procedures.

Study Day 31 (pyrimethamine dose; 2 Days after Sanaria® PfSPZ Challenge #2)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review.
4. Record AEs and concomitant medications, if applicable.
5. Provide pregnancy prevention counseling for female subjects.
6. Provide pyrimethamine dose by directly observed therapy

   Note for Arm 2 only*:*

   2 tablets if 50mg dose

Study Day 32 (pyrimethamine dose; 3 Days after Sanaria® PfSPZ Challenge #2)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review.
4. Record AEs and concomitant medications, if applicable.
5. Provide pregnancy prevention counseling for female subjects.
6. Obtain approximately 55 mL of blood for safety labs and research labs.
7. Provide pyrimethamine dose by directly observed therapy

   Note for Arm 2 only*:*

   2 tablets if 50mg dose

Study Day 34 (chloroquine dose, 5 Days after Sanaria® PfSPZ Challenge #2)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review.
4. Record AEs and concomitant medications, if applicable.
5. Provide pregnancy prevention counseling for female subjects.
6. Provide chloroquine dose (approximately 500 mg) by directly observed therapy **(Arm 3 only).** *Note*: if a subject has issues related to tolerability of chloroquine and a lower weight-based dose (as defined in the PI) would be clinically appropriate for maintenance dosing, this adjustment may be made on a case by case basis.

Study Day 35 (Clinic Follow-Up; 6 Days after Sanaria® PfSPZ Challenge #2)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review.
4. Complete malaria symptom review.
5. Record AEs and concomitant medications, if applicable.
6. Obtain approximately 3 mL of blood for malaria infection assays (see below) and research labs:

- **Arm 2**: retrospective LMIV qPCR and blood smear. Real time NIH PCR if indicated.
- **Arm 3**: retrospective LMIV qPCR and blood smear. Real time NIH PCR if indicated.
- Obtain additional approximately 1 mL of blood for drug assays **(Arm 2 only).**

7. Confirm subject contact information to follow up malaria diagnostic result..

Study Day 36 (Clinic Follow-Up; 7 Days after Sanaria® PfSPZ Challenge #2)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review.
4. Complete malaria symptom review
5. Record AEs and concomitant medications, if applicable.
6. Provide pregnancy prevention counseling for female subjects
7. Obtain approximately 59 mL of blood for safety labs, malaria infection assays (see below), and research labs.
    - **Arm 2**: real time NIH and retrospective LMIV qPCR.Blood smear if indicated.
    - **Arm 3**: retrospective LMIV qPCR and blood smear. Real time NIH PCR if indicated.
8. Confirm subject contact information to follow up malaria diagnostic result.

Study Day 36 PM [Arm 3 ONLY] (PM Phone Follow-Up; 7 Days after Sanaria® PfSPZ Challenge #2)

1. Perform basic history and AE review (including DVI site), emphasizing review of any acute complaints.
2. Complete malaria symptom review.
3. Record AEs and concomitant medications, if applicable.
4. Provide pregnancy prevention counseling for female subjects.

Study Day 37 (Clinic Follow-Up; 8 Days after Sanaria® PfSPZ Challenge #2)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate)
3. Complete diary review.
4. Complete malaria symptom review
5. Record AEs and concomitant medications, if applicable.
6. Provide pregnancy prevention counseling for female subjects
7. Obtain approximately 3 mL of blood for malaria infection assays (see below) and research labs:
    - **Arm 2**: real time NIH and retrospective LMIV qPCR. Blood smear if indicated.
    - **Arm 3**: retrospective LMIV qPCR and blood smear. Real time NIH PCR if indicated.
8. Confirm subject contact information to follow up malaria diagnostic result.

Study Day 37 PM [Arm 3 ONLY] (PM Phone Follow-Up; 8 Days after Sanaria® PfSPZ Challenge #2)

1. Perform basic history and AE review (including DVI site), emphasizing review of any acute complaints.
2. Complete malaria symptom review.
3. Record AEs and concomitant medications, if applicable.
4. Provide pregnancy prevention counseling for female subjects.

Study Day 38 (Clinic Follow-Up; 9 Days after Sanaria® PfSPZ Challenge #2)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, heart rate, and respiratory rate).
3. Complete diary review.
4. Complete malaria symptom review.
5. Record AEs and concomitant medications, if applicable.
6. Provide pregnancy prevention counseling for female subjects.
7. Obtain approximately 3 mL of blood for malaria infection assays (see below) and research labs:
    - **Arm 2**: real time NIH and retrospective LMIV qPCR. Blood smear if indicated.
    - **Arm 3**: retrospective LMIV qPCR and blood smear. Real time NIH PCR if indicated.
8. Confirm subject contact information to follow up malaria diagnostic result.

Study Day 39 (Clinic Follow-Up 10 Days after Sanaria® PfSPZ Challenge #2)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review, enter information for today's visit in diary, and collect diary (window for diary collection "+2" days) .
4. Complete malaria symptom review.
5. Record AEs and concomitant medications, if applicable.
6. Provide pregnancy prevention counseling for female subjects.
7. Obtain approximately 3 mL of blood for malaria infection assays(see below) and research labs:
    - **Arm 2**: retrospective LMIV qPCR and blood smear. Real time NIH PCR if indicated.
    - **Arm 3**: retrospective LMIV qPCR and blood smear. Real time NIH PCR if indicated.
    - Obtain additional approximately 1 mL of blood for drug assays **(Arm 2 only).**
8. Confirm subject contact information to follow up malaria diagnostic result.

Study Day 41 (chloroquine dose; 12 Days after Sanaria® PfSPZ Challenge #2)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete malaria symptom review.
4. Record AEs and concomitant medications, if applicable.
5. Provide pregnancy prevention counseling for female subjects.
6. Provide chloroquine dose (approximately 500 mg) by directly observed therapy **(Arm 3 only).** *Note*: if a subject has issues related to tolerability of chloroquine and a lower weight-based dose (as defined in the PI) would be clinically appropriate for maintenance dosing, this adjustment may be made on a case by case basis.

Study Day 43 (Clinic Follow-Up; 14 Days after Sanaria® PfSPZ Challenge #2)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete malaria symptom review.
4. Record AEs and concomitant medications, if applicable.
5. Provide pregnancy prevention counseling for female subjects.
6. Obtain approximately 52 mL of blood for research labs.

Study Day 48 (chloroquine dose; 19 Days after Sanaria® PfSPZ Challenge #2)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Provide pregnancy prevention counseling for female subjects.
5. Provide chloroquine dose (approximately 500 mg) by directly observed therapy **(Arm 3 only).** *Note*: if a subject has issues related to tolerability of chloroquine and a lower weight-based dose (as defined in the PI) would be clinically appropriate for maintenance dosing, this adjustment may be made on a case by case basis.

Study Day 55 (chloroquine dose; 26 Days after Sanaria® PfSPZ Challenge #2)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Provide pregnancy prevention counseling for female subjects.
4. Obtain approximately 24 mL of blood for safety labs, retrospective LMIV qPCR, and research labs.
    a. Obtain additional approximately 1 ml of blood for drug assays. **(Arm 2 only)**
5. Provide chloroquine dose (approximately 500 mg) by directly observed therapy **(Arm 3 only).** *Note*: if a subject has issues related to tolerability of chloroquine and a lower weight-based dose (as defined in the PI) would be clinically appropriate for maintenance dosing, this adjustment may be made on a case by case basis.

Study Day 57 (Sanaria® PfSPZ Challenge #3)

1. Verify that no contraindications for Sanaria® PfSPZ Challenge or deferral of Sanaria® PfSPZ Challenge have been met.

2. Confirm emergency contact information.
3. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
4. During the physical examination, study staff will educate the subject regarding signs and symptoms of potential AEs, clinical malaria, and indications for use of antipyretics if fever, headache, or malaise occurs.
5. For females, obtain a urine or serum sample for β-hCG (pregnancy) testing. Ensure that the test is negative before Sanaria® PfSPZ Challenge; a positive test will exclude the subject from the trial.
6. For females, ensure continued agreement and compliance with pregnancy prevention before Sanaria® PfSPZ Challenge.
7. Record vital signs (blood pressure, temperature,
8. and heart rate).
9. Review Inclusion/Exclusion criteria and confirm final eligibility.
10. Administer Sanaria® PfSPZ Challenge via DVI.
    a. Observe subject for at least 30 minutes after administration to evaluate for immediate adverse reactions.
11. Record AEs and concomitant medications, if applicable.
12. Dispense diary, thermometer and ruler to subject and demonstrate use and assess understanding of use.
13. Review instructions for contacting the site to report adverse experiences and/or inquire about study procedures.

Study Day 59 (pyrimethamine dose; 2 Days after Sanaria® PfSPZ Challenge #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review.
4. Record AEs and concomitant medications, if applicable.
5. Provide pregnancy prevention counseling for female subjects.
6. Provide pyrimethamine dose by directly observed therapy

***Note for Arm 2 only**:*

2 tablets if 50mg dose

Study Day 60 (pyrimethamine dose; 3 Days after Sanaria® PfSPZ Challenge #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review
4. Record AEs and concomitant medications, if applicable.
5. Provide pregnancy prevention counseling for female subjects
6. Obtain approximately 55 mL of blood for safety labs and research labs.
7. Provide pyrimethamine dose by directly observed therapy

***Note for Arm 2 only**:*

2 tablets if 50mg dose

Study 62 (chloroquine dose; 5 Days after Sanaria® PfSPZ Challenge #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review.
4. Record AEs and concomitant medications, if applicable.
5. Provide pregnancy prevention counseling for female subjects.
6. Provide chloroquine dose (500 mg) by directly observed therapy **(Arm 3 only).** *Note*: if a subject has issues related to tolerability of chloroquine and a lower weight-based dose (as defined in the PI) would be clinically appropriate for maintenance dosing, this adjustment may be made on a case by case basis.

Study Day 63 (Clinic Follow-Up; 6 Days after Sanaria® PfSPZ Challenge #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review.
4. Complete malaria symptom review.
5. Record AEs and concomitant medications, if applicable.
6. Provide pregnancy prevention counseling for female subjects.
7. Obtain approximately 3 mL of blood for malaria infection assays (see below) and research labs.
    - **Arm 2**: retrospective LMIV qPCR and blood smear. Real time NIH PCR if indicated.
    - **Arm 3**: retrospective LMIV qPCR and blood smear. Real time NIH PCR if indicated.

      Obtain additional approximately 1 mL of blood for drug assays **(Arm 2 only).**
8. Confirm subject contact information to follow up malaria diagnostic result.

Study Day 64 (Clinic Follow-Up; 7 Days after Sanaria® PfSPZ Challenge #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review.
4. Complete malaria symptom review.
5. Record AEs and concomitant medications, if applicable.
6. Provide pregnancy prevention counseling for female subjects.
7. Obtain approximately 59 mL of blood for malaria infection assays (see below), safety and research labs.
    - **Arm 2**: real time NIH and retrospective LMIV qPCR. .Blood smear if indicated.
    - **Arm 3**: retrospective LMIV qPCR and blood smear. Real time NIH PCR if indicated.
8. Confirm subject contact information to follow up malaria diagnostic result.

Study Day 64 PM [Arm 3 ONLY] (PM Phone Follow-Up; 7 Days after Sanaria® PfSPZ Challenge #3)

1. Perform basic history and AE review (including DVI site), emphasizing review of any acute

complaints.
2. Complete malaria symptom review.
3. Record AEs and concomitant medications, if applicable.
4. Provide pregnancy prevention counseling for female subjects

Study Day 65 (Clinic Follow-Up; 8 Days after Sanaria® PfSPZ Challenge #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review.
4. Complete malaria symptom review.
5. Record AEs and concomitant medications, if applicable.
6. Provide pregnancy prevention counseling for female subjects.
7. Obtain approximately 3 mL of blood for malaria infection assays (see below) and research labs.
    - **Arm 2**: real time NIH and retrospective LMIV qPCR. .Blood smear if indicated.
    - **Arm 3**: retrospective LMIV qPCR and blood smear. Real time NIH PCR if indicated.
8. Confirm subject contact information to follow up malaria diagnostic result.

Study Day 65 PM [Arm 3 ONLY] (PM Phone Follow-Up; 8 Days after Sanaria® PfSPZ Challenge #3)

1. Perform basic history and AE review (including DVI site), emphasizing review of any acute complaints.
2. Complete malaria symptom review.
3. Record AEs and concomitant medications, if applicable.
4. Provide pregnancy prevention counseling for female subjects

Study Day 66 (Clinic Follow-Up; 9 Days after Sanaria® PfSPZ Challenge #3 via DVI)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review.
4. Complete malaria symptom review.
5. Record AEs and concomitant medications, if applicable.
6. Provide pregnancy prevention counseling for female subjects.
7. Obtain approximately 3 mL of blood for malaria infection assays (see below) and research labs
    - **Arm 2**: real time NIH and retrospective LMIV qPCR. .Blood smear if indicated.
    - **Arm 3**: retrospective LMIV qPCR and blood smear. Real time NIH PCR if indicated.
8. Confirm subject contact information to follow up malaria diagnostic result.

Study Day 67 (Clinic Follow-Up; 10 Days after Sanaria® PfSPZ Challenge #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).

3. Complete diary review, enter information for today's visit in diary, and collect diary (window for diary collection "+2" days).
4. Complete malaria symptom review.
5. Record AEs and concomitant medications, if applicable.
6. Provide pregnancy prevention counseling for female subjects.
7. Obtain approximately 3 mL of blood for malaria infection assays (see below) and research labs

    - **Arm 2**: retrospective LMIV qPCR and blood smear. Real time NIH PCR if indicated.
    - **Arm 3**: retrospective LMIV qPCR and blood smear. Real time NIH PCR if indicated.
    - Obtain additional approximately 1 mL of blood for drug assays **(Arm 2 only).**
8. Confirm subject contact information to follow up malaria diagnostic result.

Study Day 71 (Clinic Follow-Up; 14 Days after Sanaria® PfSPZ Challenge #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete malaria symptom review.
4. Record AEs and concomitant medications, if applicable.
5. Provide pregnancy prevention counseling for female subjects.
6. Obtain approximately 52 mL of blood for research labs.

Study Day 84 (Clinic Follow-Up; 27 Days after Sanaria® PfSPZ Challenge #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Provide pregnancy prevention counseling for female subjects.
4. Record AEs and concomitant medications, if applicable.
5. Obtain approximately 15 mL of blood for research labs.

Study Day 112 (Phone Follow Up; 55 Days after Sanaria® PfSPZ Challenge #3**)**

1. Perform basic history and AE review, emphasizing review of any acute or new chronic complaints.
2. Record AEs and concomitant medications, if applicable. Provide pregnancy prevention counseling for female subjects.
3. Provide pregnancy prevention counseling for female subjects.

Study Day 141 (Pre Challenge Visit; 84 Days after Sanaria® PfSPZ Challenge #3**)**

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Provide pregnancy prevention counseling for female subjects.

5. Obtain approximately 72 mL of blood for safety labs, malaria infection assays- retrospective LMIV qPCR and research labs.
    a. Obtain additional approximately 1 ml of blood for drug assays. **(Arm 2 only**)

Note: This blood draw can be split into two separate draws within the visit window and prior to Study Day 147 (Day of CHMI).

Controlled Human Malaria Infection (CHMI) Arms 2a, 2b, 3, 4a and 4b

Study Day -7--Arm 4a and 4b ONLY (Day of Enrollment, Baseline #1 labs; 7 days prior to Sanaria® PfSPZ Challenge)

1. Verify that Informed Consent and up-to-date version of the Informed Consent was obtained.
2. Ensure that all inclusion criteria are met and exclusion criteria are not met and Eligibility Checklist completed and signed.
3. Ensure that CBC with differential, ALT, and creatinine measurements from screening tests are within protocol-defined limits (see Exclusion Criteria).
4. Ensure that Hepatitis B, Hepatitis C, and HIV testing are negative from screening visit.
5. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
6. During the physical examination, study staff will educate the subject regarding signs and symptoms of potential AEs, clinical malaria, and indications for use of antipyretics if fever, headache, or malaise occurs.
7. Record vital signs (blood pressure, temperature, heart rate).
8. For females, ensure continued agreement and compliance with pregnancy prevention
9. Obtain approximately 47 mL of blood for research labs.
10. Record AEs and concomitant medications, if applicable
11. Enrollment study number will be the same as screening number.

Study Day -2 Arm 4a and 4b ONLY (Baseline labs #2; 2 days prior to Sanaria® PfSPZ Challenge)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Study staff will educate the subject regarding signs and symptoms of potential AEs and indications for contacting the site.
3. Obtain approximately 51 mL of blood for baseline labs including: safety labs, retrospective LMIV qPCR, and research labs.
4. For females, ensure continued agreement and compliance with pregnancy prevention
5. Record vital signs (blood pressure, temperature, heart rate).
6. Record AEs and concomitant medications, if applicable.

Study Day 147 Arms 2a, 2b &3, Study Day 1 Arm 4a and 4b (CHMI with Sanaria® PfSPZ Challenge)

NOTE: *Arm 2a* and *4a* will receive homologous CHMI with NF54 Sanaria® PfSPZ Challenge
***Arm 2b, 3* and *4b* will receive heterologous CHMI with 7G8 Sanaria® PfSPZ Challenge**

1. Verify that no contraindications for Sanaria® PfSPZ Challenge or deferral of Sanaria® PfSPZ Challenge have been met.
2. Confirm emergency contact information and complete Medical Emergency Release Form (Arm 4a and 4b only).

3. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
4. During the physical examination, study staff will educate the subject regarding signs and symptoms of potential AEs and indications for use of antipyretics if fever, headache, or malaise occurs.
5. For females, obtain a urine or serum sample for β-hCG (pregnancy) testing. Ensure that the test is negative before vaccinating; a positive test will exclude the subject from the trial.
6. For females, ensure continued agreement and compliance with pregnancy prevention before vaccinating.
7. Record vital signs (blood pressure, temperature, and heart rate).
8. Review Inclusion/Exclusion criteria and confirm final eligibility.
9. Administer Sanaria® PfSPZ Challenge via DVI.
    a. Observe subject for at least 30 minutes after administration to evaluate for immediate adverse reactions.
7. Record AEs and concomitant medications, if applicable.
8. Dispense diary, thermometer and ruler to subject and demonstrate use and assess understanding of use.
9. Review instructions for contacting the site to report adverse experiences and/or inquire about study procedures.
10. Provide subject with Do's and Don't's handout.

Study Day 148 Arms 2a, 2b &3, Study Day 2 Arm 4a and 4b (Phone visit; 1 Day after Sanaria® PfSPZ Challenge)

1. Perform basic history and AE review (including DVI site), emphasizing review of any acute complaints.
2. Complete diary review.
3. Record AEs and concomitant medications, if applicable.
4. Pregnancy prevention counseling for females.

Study Day 150 Arms 2a, 2b &3, Study Day 4 Arm 4a and 4b (3 Days after Sanaria® PfSPZ Challenge)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review.
4. Record AEs and concomitant medications, if applicable.
5. Provide pregnancy prevention counseling for female subjects.
6. Obtain approximately 55 mL of blood for safety labs and research labs.

Study Day 152 Arms 2a, 2b &3, Study Day 6 Arm 4a and 4b (Phone visit; 5 Days after Sanaria® PfSPZ Challenge)

1. Perform basic history and AE review (including DVI site), emphasizing review of any acute complaints.
2. Complete diary review.
3. Record AEs and concomitant medications, if applicable.
4. Pregnancy prevention counseling for females.

Study Day 153 Arms 2a, 2b &3, Study Day 7 Arm 4a and 4b (Clinic Follow-Up; 6 Days after Sanaria® PfSPZ Challenge)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review.
4. Complete malaria symptom review.
5. Record AEs and concomitant medications, if applicable.
6. Provide pregnancy prevention counseling for female subjects.
7. Obtain approximately 3 mL of blood for malaria infection assays (see below)
    a. Real time NIH and retrospective LMIV qPCR. Blood smear if indicated.
8. Confirm subject's emergency contact information to follow up real time NIH qPCR and blood smear results.

Study Day 154 Arms 2a, 2b &3, Study Day 8 Arm 4a and 4b (Clinic Follow-Up; 7 Days after Sanaria® PfSPZ Challenge)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review.
4. Complete malaria symptom review.
5. Record AEs and concomitant medications, if applicable.
6. Provide pregnancy prevention counseling for female subjects.
7. Obtain approximately 58 mL of blood for safety labs, malaria infection assays (see below) and research labs
    a. Real time NIH and retrospective LMIV qPCR. Blood smear if indicated.
    .
8. Confirm subject's emergency contact information to follow up real time NIH qPCR and blood smear results.
9. If a subject is diagnosed with malaria infection,
    a. Administer Malarone® dose (4 tablets) by directly observed therapy.
    b. Observe subject for at least 30 minutes following first Malarone® dose to evaluate for immediate adverse reactions.
    c. Complete Malarone® dose treatment for 3 consecutive days.
    d. Following 3rd (final) Malarone® dose, subject's next visit will be Study Day 176 for Arms 2 &3 and Study Day 30 for Arm 4 to complete final visit and lab draws.

Study Day 155-160 Arms 2a, 2b &3, Study Day 9-14 Arm 4a and 4b (Clinic Follow-Up; 8-13 Days after Sanaria® PfSPZ Challenge)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete diary review (For Study Day 157 – Arms 2a, 2b,&3; Study Day 11-Arms 4a &4b enter information for the day's visit in the diary and collect diary (diary collection has a

window of +2 days).
4. Complete malaria symptom review.
5. Record AEs and concomitant medications, if applicable.
6. Provide pregnancy prevention counseling for female subjects.
7. Obtain approximately 3 mL of blood for malaria infection assays (see below)
    a. Real time NIH and retrospective LMIV qPCR. Blood smear if indicated.
8. Confirm subject's emergency contact information to follow up real time NIH qPCR and blood smear results.
9. If a subject is diagnosed with malaria infection,
    a. Administer Malarone® dose (4 tablets) by directly observed therapy.
    b. Observe subject for at least 30 minutes following first Malarone® dose to evaluate for immediate adverse reactions.
    c. Complete Malarone® dose treatment for 3 consecutive days.
    d. Following final Malarone® dose, subject's next visit will be Study Day 176 for Arms 2 &3 and Study Day 30 for Arm 4 to complete final visit and lab draws.

Study Day 161 Arms 2a, 2b &3, Study Day 15 Arm 4a and 4b (Clinic Follow-Up; 14 Days after Sanaria® PfSPZ Challenge)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete malaria symptom review.
4. Record AEs and concomitant medications, if applicable.
5. Provide pregnancy prevention counseling for female subjects.
6. Obtain approximately 62 mL of blood for safety labs, malaria infection assays and research labs.
    a. Real time NIH and retrospective LMIV qPCR. Blood smear if indicated.
7. Confirm subject's emergency contact information to follow up real time NIH qPCR and blood smear results.
8. If a subject is diagnosed with malaria infection,
    a) Administer Malarone® dose (4 tablets) by directly observed therapy.
    b) Observe subject for at least 30 minutes following first Malarone® dose to evaluate for immediate adverse reactions.
    c) Complete Malarone® dose treatment for 3 consecutive days.
    d) Following final Malarone® dose, subject's next visit will be Study Day 176 for Arms 2 &3 and Study Day 30 for Arm 4 to complete final visit and lab draws.

Study Day 162-168, 170, 172 Arms 2a, 2b &3, Study Day 16-22, 24, 26 Arm 4a and 4b (Clinic Follow-Up; 15 – 21, 23, 25,Days after Sanaria® PfSPZ Challenge)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete malaria symptom review.
4. Record AEs and concomitant medications, if applicable.
5. Provide pregnancy prevention counseling for female subjects.

6. Obtain approximately 3 mL of blood malaria infection assays (see below).
    a. Real time NIH and retrospective LMIV qPCR. Blood smear if indicated.
7. Confirm subject's emergency contact information to follow up real time NIH qPCR and blood smear results.
8. If a subject is diagnosed with malaria infection,
    a. Administer Malarone® dose (4 tablets) by directly observed therapy.
    b. Observe subject for at least 30 minutes following first Malarone® dose to evaluate for immediate adverse reactions.
    c. Complete Malarone® dose treatment for 3 consecutive days.
    d. Following final Malarone® dose, subject's next visit will be Study Day 176 for Arms 2 &3 and Study Day 30 for Arm 4 to complete final visit and lab draws.

Study Day 174-175 Arms 2a, 2b &3, Study Day 28-29 Arm 4a and 4b (Treatment Visit; 27- 28 Days after Sanaria® PfSPZ Challenge)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Complete malaria symptom review.
4. Record AEs and concomitant medications, if applicable.
5. Obtain approximately 3 mL of blood for malaria infection assays (see below).
    a. Real time NIH and retrospective LMIV qPCR. Blood smear if indicated.

6. ALL subjects that have been real time NIH qPCR and blood smear NEGATIVE receive Malarone® dose (4 tablets) by directly observed therapy on Study Day 174 and Day 175 for Arm 2 &3 and Study Day 28 and Day 29 for Arm 4.
7. Observe subject for at least 30 minutes following first Malarone® dose to evaluate for immediate adverse reactions only upon receipt of the first dose of Malarone®.

Study Day 176 Arms 2a, 2b &3, Study Day 30 Arm 4a and 4b (Final Visit, Treatment; 29 Days after Sanaria® PfSPZ Challenge) – **ALL subjects (Arms 2, 3 & 4) return for lab draw on this visit.**

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Subjects that have been real time NIH qPCR and blood smear NEGATIVE receive 3rd dose of Malarone® dose (4 tablets) by directly observed therapy. In addition to above subjects, rest of Arm 2, 3, & 4 subjects return for LAB draw on this day.
5. Obtain approximately 73 mL of blood for safety labs, malaria infection assays (see below) and research labs
    a. Real time NIH and retrospective LMIV qPCR. Blood smear if indicated.
    b. Obtain additional approximately 1 ml of blood for drug assays. (Arm 2 only)

    Note: This blood draw can be split into two separate draws occurring after receipt of first dose of Malarone®.

8.5 Large Volume Blood Draw

Blood samples will be drawn at multiple time points throughout the study as outlined in **Appendix A**. Throughout the study, there are three large volume draws. The first occurs before Sanaria® PfSPZ Challenge #1, the second before CHMI, and the last at the end of the study. In addition, there are multiple medium volume blood draws, 2 baseline samples and throughout vaccination and CHMI period that will be used for deep immunological studies at the Center for Human Immunology (CHI). These time points are identified as crucial to obtain the maximum amount of sample as the current trial presents an opportunity to determine the frequency, function and phenotype of the cellular and humoral immune system required for conferring protection against *Plasmodium* infection. We anticipate that the levels of antigen specific cells will be less than 1% of the total cells collected. Hence, the large blood draws at baseline, before CHMI, and after CHMI are required to thoroughly dissect the (protective) immune response. The samples will also serve as a Biobank that can be used for studies on future technological platforms as they become available.

No more than 80 mL of blood will be drawn at a single time point and the total amount of blood drawn is not to exceed 10.5 mL/Kg or 550 mL whichever is smaller in a 56 day period (as defined as the amount of blood allowed to be drawn under the M95-9: guidelines of blood drawn for research purposes in the clinical center). Subjects will be offered to split the large blood draw in two days if desired. If blood volume collection is limited, specimen collection will be prioritized for safety and malaria assays (blood smears, real time NIH qPCR), followed by secondary endpoint assessments (retrospective LMIV qPCR, immunology labs), and lastly long term storage.

8.6 Symptom Memory Enhancement Card (Diary)

Subjects will be asked to keep daily symptom memory enhancement diary (IRB approved paper or electronic diary) for recording oral temperature once during the day, as well as solicited questions regarding and signs/symptoms the subject has experienced that may be related to Sanaria® PfSPZ Challenge, malaria infection subsequent to Sanaria® PfSPZ Challenge, chloroquine, and pyrimethamine. In the pilot study and the main study, subjects will complete a daily symptom memory enhancement diary through 10 days post Sanaria® PfSPZ Challenge. The type of information (solicited symptoms: direct question of known possible side effects of the product; unsolicited: open-ended questioning such as “do you have any other symptoms”), duration of collected information, and format information is captured are summarized below in **Section 12.3**.

The size of any injection-site reaction will be measured using a standardized plastic measurement tool and recorded in the memory enhancement diary. Clinical site staff will review these diaries with the subjects and capture reported symptoms in CRIMSON. The memory enhancement diaries will be collected by the study staff or electronically.

8.7 Photographs of Rash or Injection Site Reactions

If a subject develops a rash or injection site reaction, photographs may be taken by the subject or NIH division of medical arts. These photographs will not include the subject's face or any identifying scars, marks, or tattoos.

8.8 Indications for Deferral of Sanaria® PfSPZ Challenge

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

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

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

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

8.9 Treatments that Could Potentially Interfere with Vaccine-induced Immunity

Treatment with any of the following medications during the study may exclude a subject from receiving further doses of Sanaria® PfSPZ Challenge. However, the subject will be encouraged to remain in the study for the duration of the study for safety evaluations.

- Licensed vaccine in the 2-week period (4 weeks for live vaccines) prior to and following each vaccination.
- Receipt of immunoglobulins and/or any blood products up to 3 months prior to the first Sanaria® PfSPZ Challenge through 30 days after administration of the last Sanaria® PfSPZ Challenge.

- Chronic oral or intravenous administration (≥14 days) of immunosuppressive doses of steroids, i.e., prednisone >10 mg per day, immunosuppressants or other immune-modifying drugs from each day of Sanaria® PfSPZ Challenge to 2 weeks following each Sanaria® PfSPZ Challenge.

- Any investigational drug or investigational vaccine during the study period.

- Required surgical removal of the spleen or the development of a hematologic or other disease that would interfere with normal immunity.

- Receipt of antibiotics with antimalarial activities during the study period. Specifically, antibiotic use will be assessed on a case by case basis with the study Sponsor and if needed, Safety Monitoring Committee, given the variable half lives of antibiotics with antimalarial activities.

8.10 Clinical Laboratory Testing

Using standard techniques, the clinical laboratory will perform the following tests:

1. Complete blood count (CBC) plus white blood cell differential and platelet count.

   The following CBC parameters will be assessed for safety throughout the trial: WBC, absolute neutrophil count (ANC), hemoglobin, and platelet count.

2. Serum creatinine (Cr).
3. Alanine aminotransferase (ALT)
4. HbsAg test (ELISA, PCR if indicated).
5. HCV test (ELISA, PCR if indicated).
6. HIV test (ELISA, Western Blot if indicated).
7. Urine dipstick/urinalysis (at screening only).
8. Urine and/or serum pregnancy testing (β-hCG) at screening and prior to every Sanaria® PfSPZ Challenge via DVI or as clinically indicated.
9. Malaria real time NIH qPCR at the NIH Clinical Center (NIH Malaria Genus Species (4-plex) PCR).

Note: Laboratory studies completed under a different NIH protocol can be used for screening purposes as long as they are within 56 days prior to study enrollment.

8.11 Electrocardiograms

Electrocardiograms (12-lead ECGs) will be performed during screening and as clinically indicated throughout the study in the cardiology department and read by study cardiologist or representative. Subjects with clinically significant abnormalities will be excluded from the study.

Note: ECG completed under a different NIH protocol can be used for screening purposes as long as they are within 56 days prior to study enrollment.

8.12 Malaria Blood Smears

The gold standard for malaria diagnosis and evaluation of vaccine efficacy endpoints is the detection of malaria parasites on Giemsa-stained thick blood films. Blood smears are prepared in duplicate according to standard malaria challenge procedures and evaluated by trained study microscopists, and the results reported to the study Principal Investigator within 24 hours (usually within 6 hours), and is prioritized to be read immediately if a subject is symptomatic. At least 0.5µl are scanned for the presence of malaria parasites. This method allows for detection of a parasite density of approximately 3 parasites/µl and early diagnosis, often before subjects become symptomatic for malaria.

For symptomatic subjects, (including all subjects during CHMI and Arm 2a/2b subjects during CVac #2 and #3; for Arm 3 only when clinically ill),at least 1.5µl are evaluated.

Blood smears are considered positive if at least two unambiguous parasites per slide are identified and confirmed by a second microscopist. Blood smears will be performed as outlined in **Appendix A** and as noted below.

1. *Arm 1* (pyrimethamine pilot): blood smears will only routinely be done for symptomatic subjects.
2. *Arm 5* (chloroquine pilot): routine daily blood smears from days 6-12 post injection.
3. *Arms 2a & 2b*:
    a. CVac #1: blood smears will only routinely be done for symptomatic subjects.
    b. CVac #2 & #3: routine daily blood smears on day 6 and day 10 post injection regardless of symptoms.
4. *Arm 3:*
    a. CVac #1: routine daily blood smears from days 6-10 post injection.
    b. CVac #2 &3: routine daily blood smears from days 6-10 post injection.

5. CHMI (*Arms 2a, 2b, 3, 4a, 4b*): blood smears will only be done for symptomatic subjects OR if real time NIH qPCR cannot be resulted on the same day.

    Note:

    1. Regardless on timing, at any time point post PfSPZ Challenge injection, if clinically indicated, a blood smear may be completed and subjects evaluated and managed per protocol and LMIV malaria diagnosis SOP.
    2. A positive blood smear will be followed daily until 3 consecutive negative blood smears have been recorded.

8.13 qPCR (Retrospective LMIV and Real Time NIH)

While detection of parasites on thick blood smears remains the most common primary endpoint in human challenge trials, both PCR- and nucleic acid sequence-based amplification (NASBA)-based methods have been increasingly used to support blood smear data in malaria vaccine trials (Walther, Dunachie et al. 2005, Webster, Dunachie et al. 2005, Walther, Thompson et al. 2006, Roestenberg, McCall et al. 2009). These research molecular assays have significantly increased sensitivity for detection of *P. falciparum* blood-stage infection approaching 20 parasites/mL, often resulting in detection 2-4 days earlier than by paired thick blood smears (Hermsen, Telgt et al. 2001, Schneider, Schoone et al. 2004, Andrews, Andersen et al. 2005). Quantification of parasite density by these methods allows evaluation of parasite growth curves for assessing the utility of partially-effective vaccine candidates. LMIV has also developed a retrospective LMIV qPCR that detects 18s of Pf with a lowest detection threshold of approximately 40 parasites/mL that will be used during PfSPZ-CVac immunization period and during the CHMI period for comparison to the real time NIH qPCR performed by the NIH molecular diagnostics department and to other malaria challenge sites performing qPCR.

In the past few years, qPCR detection of malaria infection has become more acceptable parasite detection tool. The molecular diagnostics department, part of the clinical laboratory, at the NIH has developed a NIH Malaria Genus Species (4-plex) PCR with a sensitivity of 500 parasites/mL of whole blood (0.5 parasites/µl), detecting parasitemia approximately 2 days before clinical

symptoms develop. This assay has been externally validated and is the malaria molecular assay at the NIH clinical center currently.

In this study, retrospective LMIV qPCR will be used for evaluation of endpoints, specifically to characterize the presence and kinetics of subpatent parasitemia following Sanaria® PfSPZ Challenge exposure and pyrimethamine dosing during CVac. Retrospective LMIV qPCR will be completed in batches during days 6-14 in the pilot and first CVac in the main study; then 6-10 in the second and third CVac in the main arms post Sanaria® PfSPZ Challenge. Retrospective LMIV qPCR will also be completed daily starting on day 6 post DVI following PfSPZ CHMI. If a blood smear is clinically indicated during the CVac time period of the study, a retrospective LMIV qPCR will also be collected at this time.

NIH Malaria Genus Species 4-plex qPCR will be used for malaria diagnosis and treatment decisions during the pilot, first CVac for Arm 2, and following Sanaria® PfSPZ CHMI (all Arms) and for any visits for which a subject presents symptomatic and requires rapid qPCR results. One positive NIH real time malaria qPCR result or a single peripheral thick blood smear during pilot study, at any time for Arm 2, and at any time post-CHMI will be used for diagnosis and clinical decisions to treat the subject. Subjects in Arm 3 are expected to have evidence of blood stage exposure given they are only on chloroquine, thus the presence of asexual parasites alone in these subjects will not warrant automatic treatment during PfSPZ-CVac. The NIH qPCR will be performed as outlined in **Appendix A.**

- *Arm 1* (pyrimethamine pilot): daily real time NIH qPCR from day 6-14 post injection
- *Arm 5* (chloroquine pilot): NIH real time qPCR will only be done if clinically indicated.
- *Arm 2*:
  - CVac #1: daily real time NIH qPCR from day 6-10 post injection
  - CVac #2 &3: daily real time NIH qPCR from days 7-9 post injection
- *Arm 3*: NIH real time qPCR will only be done if clinically indicated.
- CHMI (*Arms 2, 3, 4a, 4b*): daily real time NIH qPCR from day 6 post injection until diagnosis, then at the end of study.

Any blood left over from qPCR sampling time points may be sent to collaborators with established malaria qPCR or point of care diagnostics for further quantification of parasite loads, validation of assay results, and inter-assay comparison.

8.14 Drug Assays (Chloroquine, Desethylchloroquine, and Pyrimethamine)

Chloroquine levels will be sent for *Arm 3* and 5 only on study Day 1 (approximately 2 days after loading dose) and will be analyzed in real time by NMS Labs (Willow Grove, PA) or by another laboratory with an equivalent capability. The results of the chloroquine levels will be available by day 7 post administration of Sanaria® PfSPZ Challenge (Study Day 8). If the chloroquine level is <10 ng/mL in the serum and undetectable in the urine, the subject will be treated with Malarone® and withdrawn from the study regardless of their clinical presentation. If the subject has a lower than expected chloroquine level, but one that is detectable above 10 ng/mL by the blood assay, that subject will continue to be followed per protocol with daily histories, diary symptom review, blood smears and qPCR assays, as we would expect chloroquine to still be effective in clearing

parasitemia. If clinically indicated, the subject will have additional thick blood smears and/or real time NIH PCRs completed. The decision to withdraw the subject and treat the subject with Malarone® will be based primarily on the clinical presentation and parasitology results, but the specific chloroquine level will be strongly taken into consideration. If there appears to be an issue with the chloroquine testing (multiple subjects having undetectable levels) repeat samples will be sent immediately for testing from all enrolled subjects at risk of parasitemia (depending on timing). In the protocol #15-I-0169 in which subjects were dosed with chloroquine similarly, all subjects (n=20) had detectable chloroquine levels above 10 ng/mL.

Demonstrating that a subject has adequate chloroquine levels after the first administration will rule out poor absorption, unusual metabolism, or surreptitious non-compliance (e.g., purposeful regurgitation). For this reason, there are no plans to re-test chloroquine levels after subsequent doses of chloroquine. However, individual subjects may be retested at any point based on clinical need.
For the majority of sampling time points, whole blood levels will be obtained by venous draw and stored on filter paper until analysis is performed following the last study visit or sooner if clinically indicated. Pyrimethamine (Arm 2 only) and chloroquine (Arm 3 only) levels will be tested retrospectively by high performance liquid chromatography/tandem mass spectrometry throughout the study as outlined in **Appendix A**.

8.15 Immunology Laboratories

As indicated in the objectives, assays will be conducted to assess immunogenicity in addition to safety as described above. Laboratory assays to assess immune response to Sanaria® PfSPZ Challenge will be performed at the Laboratory of Malaria Immunology and Vaccinology, NIAID, NIH, Sanaria Inc, NIH CHI, and Antigen Discovery, Inc. according to standard laboratory procedures.

These assays may include but are not limited to:

1. ELISA for antibodies to *P. falciparum* liver stage and blood stage antigens (PfCSP, PfMSP-1, PfAMA-1, PfLSA-1, PfMSP-5, PfEBA-175, EXP-1)
2. (IFN-γ) ELISPOT assay and multi-parameter flow cytometry with intracellular cytokine staining on peripheral blood mononuclear cells in *P. falciparum* liver- stage antigens (CSP, LSA-1) pre-/post- Sanaria® PfSPZ Challenge administration
3. B and T cells studies to analyze immunologic responses
4. Immunofluorescence assay for antibodies to Pf sporozoites, asexual, and sexual erythrocytic stage parasites
5. Inhibition of sporozoite invasion assay for antibodies exhibiting functional activity against sporozoites
6. Protein microarrays

In addition Sanaria Inc will assess antibodies to whole PfSPZ by immunofluorescence assay (IFA) and inhibition of sporozoite invasion assay (ISI) and to asexual erythocytic stage parasites by IFA as described (Epstein, Tewari et al. 2011, Seder, Chang et al. 2013).

Laboratory assays to assess immune responses to novel pre-erythrocytic antigens will be performed in the PEVA Consortium laboratories at the Laboratory of Malaria Immunology and Vaccinology,

NIAID, NIH according to standard laboratory procedures. The target proteins are novel antigens that confer protection against liver stage malaria in rodent malaria models (*P. yoelii*, *P. berghei*) according to vaccination studies conducted by Seattle BioMed and LMIV. The novel antigens to be used for these laboratory assays include PFL1995c, PFE0305w, LISP1 (PF14_0179), SAP1 (PF11_0480), MAL7P1.164, PF14_0113, using the identifiers in the PlasmoDB database (www.plasmodb.org). These antigens were initially selected on the basis of their gene expression during early liver stage development of *P. falciparum*, and preliminary indicating that these antigens are immunologically recognized by individuals previously exposed to *P. falciparum*. The potential utility of these antigens as pre-erythrocytic vaccines has been supported by animal studies, wherein orthologues of these genes incorporated in DNA vaccines induce protective immunity in mice that significantly reduces the liver stage development of *P. berghei* and *P. yoelii* parasites. The assays included in this study can confirm that individuals receiving CVac with pyrimethamine develop immune responses to pre-erythrocytic antigens, and can provide additional data by which to assess the potential for these antigens to be developed as subunit vaccines to prevent infection. The long term objective of the PEVA consortium is to identify antigens that individually, or in combination with CSP or other antigens, will induce a high level of pre-erythrocytic immunity that provides protective immunity against *P. falciparum*.

The assays to be performed include:

1. Binding ELISA for antibodies to *P. falciparum* pre-erythrocytic antigens (PFL1995c, PFE0305w, LISP1 (PF14_0179), SAP1 (PF11_0480), MAL7P1.164, PF14_0113)
2. IFN-γ ELISPOT/Intracellular cytokine staining (ICS) assay on peripheral blood mononuclear cells in *P. falciparum* pre-erythrocytic antigens (PFL1995c, PFE0305w, LISP1(PF14_0179), SAP1 (PF11_0480), MAL7P1.164, PF14_0113) pre-/post-Sanaria ®PfSPZ Challenge administration

The Center for Human Immunology will collaborate with LMIV to assess the innate and humoral immune responses after vaccination and CHMI using but not limited to flow cytometry, Somalogic, Luminex and RNA-Seq.

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

Intended Use: Samples and data collected under this protocol will be used to study malaria and related diseases and possible adverse reactions to PfSPZ, chloroquine, or pyrimethamine. Genetic testing may be performed in accordance with the genetic testing information that is included in the study informed consent.

Storage: Access to stored research samples will be limited using either a locked room or a locked freezer. Temporary storage of samples may occur at the LMIV laboratory in Building 10 at the NIH Clinical Center, prior to shipment to LMIV. Samples will be stored at the LMIV in Rockville, MD or at LMIV's designated repository, Thermo Scientific, Rockville, MD. Samples and data will be stored using codes assigned by the investigators or their designees. Data will be kept in password-protected computers. Only investigators or their designees will have access to the samples and data.

Tracking: Samples will be tracked using a sample-tracking software program, e.g., Freezerworks.

Disposition at the Completion of the Protocol: In the future, other investigators (both at the NIH and outside) may wish to study these samples and/or data. In that case, IRB approval must be

sought prior to any sharing of samples and/or data. Any clinical information shared about the sample with or without patient identifiers would similarly require prior IRB approval.
At the completion of the protocol (termination), samples and data will either be destroyed or, after IRB approval, transferred to another existing protocol.
Reporting the Loss or Destruction of Samples/Specimens/Data to the IRB: Any loss or unanticipated destruction of samples (for example, due to freezer malfunction) or data (for example, misplacing a printout of data with identifiers) that meets the definition of Protocol Deviation and/or compromises the scientific integrity of the data collected for the study, will be reported to the IRB.
Consent to allow long term storage of study samples is a part of the inclusion criteria for this study. However, subjects may decide at any point not to have their samples stored. In this case, the principal investigator will destroy all known remaining samples and report what was done to both the subject and to the IRB. This decision will not affect the subject's participation in this protocol or any other protocols at NIH.

10 Retention of Specimens for Future Use

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

At the completion of the protocol (termination), samples and data will either be destroyed, or transferred to another existing protocol, "Research Use of Human Specimens", NIAID Protocol #08-I-N064. In the future, other investigators may wish to study these samples and/or data. If so, the National Institutes of Health (NIH) may send samples to the other investigators. In that case, IRB approval must be sought prior to any sharing of samples. Any clinical information shared about the sample with or without patient identifiers would similarly require prior IRB approval. The research use of stored, unlinked, or unidentified samples (for example, as a standard for immunological analyses) may be exempt from the need for prospective IRB review and approval. Exemption requests will be submitted in writing to the NIH Office of Human Subjects Research Protections (OHSRP), which is authorized to determine whether a research activity is exempt.

11 Data Sharing Plan

In NIH's view, all data should be considered for data sharing. Data should be made as widely and freely available as possible while safeguarding the privacy of subjects, and protecting confidential and proprietary data. We recognize that the public dissemination of our scientific results can facilitate the creation of collaborative efforts with domestic and international collaborators.

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

12 Assessment of Safety

12.1 Documenting, Recording, and Reporting Adverse Events

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

- immediately documented in the subject's medical record/source document,
- recorded on the electronic database, and
- reported as outlined below (e.g., IND Sponsor, IRB, FDA).

A study clinician will be available during the study period and will be available to the study subjects at all times. Should a subject call on a study clinician to report an AE, it will be discussed with the PI and documented, recorded, and reported appropriately.

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

12.2 Definitions

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

Adverse Reaction (AR)

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

Suspected Adverse Reaction (SAR)
An adverse event for which there is a reasonable possibility that the investigational agent caused the adverse event. 'Reasonable possibility' means that there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction which implies a high degree of certainty.

Serious Adverse Event (SAE)
A Serious Adverse Event (SAE) is defined as any adverse event that

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

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

Unexpected Adverse Event
An AE is considered unexpected if it is not listed in the Investigator's Brochure or Package Insert (for marketed products) or is not listed at the specificity or severity that has been observed. It is the responsibility of the IND Sponsor to make this determination.

Serious and Unexpected Suspected Adverse Reaction (SUSAR)
A SUSAR is a Suspected Adverse Reaction that is both serious and unexpected.

Protocol Deviation
Any change, divergence, or departure from the IRB approved study procedures in a research protocol. Protocol deviations are designated as serious or non-serious and further characterized as

1. Those that occur because a member of the research team deviates from the protocol.
2. Those that are identified before they occur, but cannot be prevented.
3. Those that are discovered after they occur.

Serious Protocol Deviation
A deviation that meets the definition of a Serious Adverse Event or compromises the safety, welfare or rights of subjects or others.

Non-compliance

The failure to comply with applicable NIH HRPP policies, IRB requirements, or regulatory requirements for the protection of human subjects. Non-compliance is further characterized as:

1. Serious: Non-compliance that
    a. Increases risks, or causes harm, to participants
    b. Decrease potential benefits to participants
    c. Compromises the integrity of the NIH-HRPP
    d. Invalidates the study data
2. Continuing: Non-compliance that is recurring
3. Minor: Non-compliance that, is neither serious nor continuing.

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

1. unexpected in terms of nature, severity, or frequency in relation to
    a. the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents; and

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

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

Unanticipated problem that is not an Adverse Event (UPnonAE): An unanticipated problem that does not fit the definition of an adverse event, but which may, in the opinion of the investigator, involve risk to the subject, affect others in the research study, or significantly impact the integrity of research data. These events may involve a greater risk of social or economic harm to subjects or others rather than physical/psychological harm. Such events would be considered a non-serious UP. Examples of an UPnonAE include a breach of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

New Onset of Chronic Illness (NOCI)
The new onset of chronic illness is defined as a diagnosis of a new medical condition that is chronic in nature, including those potentially controllable by medication (e.g., diabetes, asthma). Any NOCI will be recorded in the same manner as unsolicited AEs.

12.3 Investigator Assessment of Adverse Events

If a diagnosis is clinically evident (or subsequently determined), the diagnosis rather than the individual signs and symptoms or lab abnormalities (except for malaria, when in addition each laboratory value will be separately recorded) will be recorded as the AE. Assessment of safety will

include clinical observation and monitoring of hematological, chemical, and immunologic parameters. Subjects will be closely monitored for at least 30 minutes following the first dose of any medication and after each Sanaria® PfSPZ Challenge. Additionally, subjects will return to the clinic for clinical assessments, almost daily from Day 1- 14 after Sanaria® PfSPZ Challenge via DVI and then weekly for two more weeks before the subsequent administration of Sanaria® PfSPZ Challenge (**Appendix A**). The pilot arm will only receive one injection of Sanaria® PfSPZ Challenge.

The type of information (solicited symptoms: direct question of known possible side effects of the product; unsolicited: open-ended questioning such as "do you have any other symptoms"), duration of collected information, and format information is captured are summarized below:

Sanaria® PfSPZ Challenge (local reactogenicity related to the injection – solicited questions includes pain/tenderness, redness, swelling/induration, bruising, and pruritus at the injection site):

- Collected for 6 days post Sanaria® PfSPZ Challenge
- Collected from the subject via daily symptom memory enhancement cards (diary) and solicited questions at each study visit (telephone and clinic follow up)

Sanaria® PfSPZ Challenge (systemic reactogenicity related to the injection – solicited questions includes rash, urticaria, pruritus, edema, headache, fever, chills, malaise, myalgia, arthralgia, palpitations, shortness of breath, dizziness/vertigo and non-musculoskeletal chest pain):

- Collected for 6 days post Sanaria® PfSPZ Challenge
- Collected from the subject via daily symptom memory enhancement cards (diary) and solicited questions at each study visit (telephone and clinic follow up)

Sanaria® PfSPZ Challenge (systemic symptoms related to parasitemia/malaria infection – solicited questions includes headache, fever, chills, rigors, sweats, malaise, dizziness, palpitations, shortness of breath, myalgia, arthralgia, nausea, vomiting, abdominal pain, diarrhea, back pain, non-musculoskeletal chest pain):

- Collected from 6 days post Sanaria® PfSPZ Challenge to 14 days post Sanaria® PfSPZ Challenge during the PfSPZ-CVac phase (via solicited questions at study visits)
- Collected from 6 days post Sanaria® PfSPZ Challenge to 27 days post Sanaria® PfSPZ Challenge during the CHMI phase (via solicited questions at study visits)
- Collected from the subject via daily symptom memory enhancement cards (diary) (Collected through Day 10 post Sanaria® PfSPZ Challenge for the pilot and main Arms).

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

- Collected for 6 days post chloroquine administration
- Collected from the subject via daily symptom memory enhancement cards (diary)

when dispensed post Sanaria® PfSPZ Challenge and solicited questions at each study visit (telephone and clinic follow up) until 6 days post last chloroquine administration

Pyrimethamine (systemic symptoms related to pyrimethamine– solicited questions includes nausea, vomiting, diarrhea, abdominal pain, anorexia, rash)

- Collected for 6 days post pyrimethamine administration
- Collected from the subject via daily symptom memory enhancement cards (diary) when dispensed post Sanaria® PfSPZ Challenge and solicited questions at each study visit (telephone and clinic follow up) until 6 days post last pyrimethamine administration

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

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

All local and systemic reactions will be captured on the appropriate source documents and CRIMSON Data System. Those assessed as serious will be further reported on the Sponsor's SAE/UP report form. AEs judged to be possibly, probably, or definitely related to the study product will be followed to adequate resolution. All concomitant medications will be collected through day 27 after Sanaria® PfSPZ Challenge; new chronic medications will be collected for the remainder of the study.

12.3.1 Adverse Event Definitions

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

Solicited adverse events will be captured by subject daily symptom memory enhancement cards (diary) and/or by direct questioning during the study. Solicited adverse events to be recorded as endpoints for this study are provided in **Table 21**. Solicited adverse events that are possibly related to chloroquine and/or pyrimethamine will be collected for 6 days following each drug administration. Solicited adverse events that are possibly related to SPZ injection will be captured daily for 6 days (local and systemic symptoms). Symptoms related to malaria secondary to Sanaria® PfSPZ Challenge will be solicited from 6 to 14 days (systemic

symptoms) following Sanaria® PfSPZ Challenge during CVac and from 6 to 27 days following Sanaria® PfSPZ Challenge during CHMI.

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

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

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

Table 21: Solicited Adverse Events

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

12.3.2 Severity

Severity of AEs will be assessed by the investigator in **Appendix C**. AEs not included in the **Appendix C** will be graded for severity using the followings definitions as seen in **Table 22**.

Table 22: Definitions for Severity of AE Grading

Severity	Definition
Grade 1 (Mild)	No interference with activity, may use 1 dose of an over the counter medication
Grade 2 (Moderate)	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity
Grade 3 (Severe)	Activities of daily living limited to <50% of baseline, medical evaluation/therapy required
Grade 4 (Life-Threatening)	Extreme limitation in activity, significant assistance required; immediate medical intervention or therapy required to prevent death
Grade 5	Death

12.3.3 Causality

Causality (likelihood that the event is related to the study agent) will be assessed considering the factors listed under the following categories:

Definitely Related

- reasonable temporal relationship
- follows a known response pattern
- clear evidence to suggest a causal relationship
- there is no alternative etiology

Probably Related

- reasonable temporal relationship
- follows a suspected response pattern (based on similar agents)
- no evidence of a more likely alternative etiology

Possibly Related

- reasonable temporal relationship
- little evidence for a more likely alternative etiology

Unlikely Related

- does not have a reasonable temporal relationship

OR

- good evidence for a more likely alternative etiology

Not Related

- does not have a temporal relationship

OR

- definitely due to an alternative etiology

Note: Other factors will also be considered for each causality category when appropriate. Causality assessment is based on available information at the time of the assessment of the AE. The investigator may revise the causality assessment as additional information becomes available. The degree of certainty with which an AE can be attributed to administration of the study vaccine will be determined by how well the event can be understood in terms of one or more of the following:

- The event being temporally related with vaccination or reproduced on re-vaccination
- A reaction of similar nature having previously been observed with this type of vaccine and/or formulation
- The event having been reported in the literature for similar types of vaccines
- Whether or not there is another identifiable cause

All local (injection-site) reactions will be considered causally related to vaccination.
Reports will further classify AEs as follows:

- Related - all AEs that are assessed as definitely, probably, or possibly related
- Unrelated - all AEs assessed as unlikely or definitely not related

When reporting to regulatory authorities and IRBs is needed, AE relationship will be determined as noted above as **related** (including possibly, probably, or definitely) or **unrelated** (including unlikely or not).

12.4 Investigator Reporting Responsibilities to the Sponsor

12.4.1 Adverse Events

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

12.4.2 Serious Adverse Events

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

Sanaria, Inc.
SAE Fax: 240-306-0596

Individuals:
1. Stephen L. Hoffman, M.D.
Tel: 240-403-2701 (office)
Tel: 240-299-3178 (mobile)
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2. Thomas L Richie, M.D., Ph.D.
Tel: 240-403-2727 (office)
Tel: 301-466-7943 (mobile)
Email: trichie@sanaria.com

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

12.4.3 Unanticipated Problems

All Unanticipated Problems that are also adverse events will be reported to the IND Sponsor on the NIH Problem Report Form sent by fax or e-mail attachment no later than 7 calendar days of site awareness of the event.
Unanticipated Problems that are not AEs will also be reported to the IND Sponsor.

12.4.4 Pregnancy

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

- Withdrawn from the study but continue in follow-up for safety.
- PI will discuss with sponsor regarding the timing of the pregnancy from last dose of Sanaria® PfSPZ Challenge to determine whether treatment is warranted. If treatment is required, administration of Malarone® (4 tablets) daily x 3 days will be administered. Malarone® is pregnancy category C
- Report to IRB as an informational item.
- Advice research subject to notify the obstetrician of study product exposure (infectious SPZ, chloroquine, pyrimethamine, Malarone®).
- Report to IND Sponsor.

12.5 Reporting Procedures to the IRB

Assessment of Safety
AEs and other reportable events are defined in Policy 801: Reporting Research Events.

Reporting Procedures
Unanticipated problems, non-compliance, and other reportable events will be reported to the IRB according to Policy 801.

Reporting to the NIAID Clinical Director
The principal investigator will report UPs, major protocol deviations, and deaths to the NIAID Clinical Director according to institutional timelines.

12.6 Follow-up of Adverse Events and Serious Adverse Events

AEs that occur following enrollment of the subject (or AEs related to study procedures after signing the informed consent and before enrollment) are followed until the final outcome is known or until the end of the study follow-up period.
SAEs that have not resolved by the end of the follow-up period will be followed until final outcome is known. If it is not possible to obtain a final outcome for an SAE (e.g., the subject is lost to follow-up), the reason a final outcome could not be obtained will be recorded by the investigator on the AE electronic record and the SERF.
SAEs that occur after the study follow-up period (1 month following the CHMI) that are reported to and assessed by the Investigator to be possibly, probably, or definitely related must be reported to the IND Sponsor as described above.

12.7 Sponsor's Reporting Responsibilities

Serious and unexpected suspected adverse reactions (SUSARs) as defined in 21 CFR 312.32 and determined by the IND Sponsor will be reported to FDA. Sanaria Inc. will also report any relevant safety findings, including AEs that are also UPs, to other clinical trial sites where related Sanaria products are being studied.
The IND Sponsor will also submit an IND Annual Report of the progress of the investigation to the FDA as defined in 21 CFR 312.33.

12.8 Pausing Criteria for Entire Study Population

The PI will closely monitor study data as they become available and will make determinations regarding the presence and grading of AEs. The AEs will be evaluated with regard to the known complications associated with administration of PfSPZ-CVac, chloroquine, or pyrimethamine components.

12.8.1 Parasitemia and Malaria Symptoms

LMIV conducted a CVac study (NIAID protocol #15-I-0169) in which patent parasitemia (positive blood smear) was not detected in any participant during CVac. Subpatent parasitemia (positive qPCR) was not detected in any participant in Arm 2 (pyrimethamine and chloroquine) but was detected in Arm 3 (chloroquine only). In the proposed study, it will be the first time that a CVac study with increasing dose of Sanaria® PfSPZ Challenge with pyrimethamine alone is conducted.

During the **pyrimethamine pilot study (*Arms 1a-1e*)**, the participants will be monitored closely with NIH real time malaria qPCR and a single positive NIH real time malaria qPCR will result in

halting for the individual person and treatment with Malarone® per protocol. A positive NIH malaria qPCR and/or blood smear during PfSPZ-CVac immunization will therefore not necessarily provide a halt for the study as a whole, nor will it be reported as an unanticipated problem. However, if a positive NIH malaria real time qPCR and/or blood smear occurs, the subject's information, including results and any additional clinical or laboratory information will be sent with a Notification Report to the IND Sponsor for review.

The study may pause for further review during the pilot if subpatent parasitemia is detected in any subjects in *Arms 1c* and *1e* as outlined in **Section 3.2** and **Figure 6**.

For **Arm 2a and 2b (pyrimethamine only),** a positive NIH real time malaria qPCR (during CVac #1) and/or blood smear will result in treatment with Malarone® for the individual person and it will not be reported as an unanticipated problem. The subject will be withdrawn from the study. If a NIH malaria qPCR and/or positive blood smear occurs, the subject's information, including results and any additional clinical or laboratory information will be sent with a Notification Report to the IND Sponsor for review.

Again, ***individual subjects*** in **Arm 2** (**pyrimethamine only**) will be treated with another antimalarial medication (Malarone®) and withdrawn if the following occurs:

1. A positive NIH real time malaria qPCR
2. A positive blood smear
3. At the PI's discretion (for example, if non-compliance with pyrimethamine ingestion is suspected, or if concerning signs or symptoms develop)

For **Arm 3 and 5** (**chloroquine only),** participants receiving chloroquine only have presented with patent parasitemia in other CVac/CPS studies during CVac phase, including positive blood smears at the peak of expected parasitemia. In previous study (NIAID protocol #15-I-0169), participants in chloroquine only arm did not develop positive blood smears although they all did have positive LMIV qPCR during CVac. However, in the current study, due to higher doses of PfSPZ Challenge, a positive blood smear may occur. Therefore, for *Arm 3 and 5*, a positive blood smear during PfSPZ-CVac immunization will not necessarily result in a halt for an individual participant or the study as a whole, nor will it be reported as an unanticipated problem.

During the completed pilot phase of this study, in the chloroquine pilot (Arm 5b; 200,000 PfSPZ Challenge), we did have 3 out of the 4 subjects experience a single positive blood smear during the expected time period (during day 8 and 9 post PfSPZ Challenge respectively). These positive blood smears were associated with clinical symptoms, including preceding Grade 3 symptoms, that resulted in two separate study halts for further SMC review. All subjects, at the time of their positive blood smear, no longer had Grade 3 symptoms and were clinically well appearing, thus per protocol did not meet criteria for re-treatment with another antimalarial. With the SMC's agreement, the study resumed in both instances. The SMC recommended closer follow up of Arm 3 subjects during peak parasitemia period to improve tolerability of the 200,000 PfSPZ Challenge dose. This amendment institutes these recommended changes.

As conducted in the pilot phase, during the main phase of the study, in *Arm 3* (chloroquine-only arm) subject will be re-evaluated within 12 hours of a positive blood smear. ***Individual subjects***

will be re-treated with another antimalarial medication (Malarone®) and withdrawn if the following occurs:

1. A positive blood smear outside of the range of expected peak parasitemia (on or after day 10 post PfSPZ Challenge) regardless of symptoms (maximum expected peak parasitemia is 7-9 days post PfSPZ Challenge).
2. A subject with a positive blood smear at any time point and Grade 3 or greater symptoms (NOT laboratory values) lasting longer than 48 hours despite adequate symptomatic management (e.g non-steroidal anti-inflammatory drugs)
3. At the PI's discretion (for example, if non-compliance with chloroquine ingestion is suspected, or if concerning signs or symptoms develop)

Note: Further immunization of ***the entire Arm 3*** will be temporarily halted if any of these criteria are met.

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

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

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

- Four or more subjects experience a positive NIH real time malaria PCR in *Arm 2*
  OR

- Two or more subjects experience positive blood smears outside of the expected peak parasitemia (on or after day 10 post PfSPZ Challenge) in *Arm 3*

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

12.8.2 Reactogenicity

If a study product (Sanaria® PfSPZ Challenge, chloroquine, pyrimethamine) is considered unacceptably reactogenic (as described in the following criteria), the study will be halted. No new enrollments and no further vaccinations will be administered by the Investigators until reviewed by the SMC and study IND Sponsor. A report of SMC recommendations will be submitted to the IRB.

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

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

The IRB, the NIAID, the FDA, or other government agencies may discontinue the study at any time. Subsequent review of serious, unexpected, and related AEs by the SMC or IRB, the IND sponsor, the FDA, and other regulatory authorities may also result in suspension of further administration of immunizations at the clinical site (in case of the SMC, a recommendation of suspension to the Sponsor). The FDA, other regulatory authorities, and the study sponsor(s) retain the authority to suspend additional enrollment and administration of immunizations for the entire study as applicable.

12.8.3 Reporting of Study Halting

If a halting requirement is met, a description of the adverse event(s) or safety issue must be reported by the Investigator by fax or email within one business day to the IND Sponsor. The IND Sponsor is responsible for notifying the SMC within one business day of being informed by the PI. The LMIV Investigator will inform the IRB that a halting rule has been met.

12.8.4 Resumption of a Halted Study

The IND Sponsor, in collaboration with the PI and the SMC will determine if it is safe to resume the study. The IND Sponsor will notify the PI of this decision. The conditions for resumption of the study will be defined in this notification. The PI will notify the IRB(s) of the decision to resume the study.

12.9 Pausing Criteria for a Subject or Group

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

- A subject experiences an SAE or ≥ 2 or more Grade 3 or greater AEs that is/are unexpected (as determined by the IND Sponsor) and is/are possibly, probably, or definitely related to the study agent; **or**
- Any safety issue that the Site Investigator determines should pause administration of the study agent to a single participant or to all participants in a specific group.

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

12.9.1 Reporting of Pausing for a Participant or Group

If a pausing requirement is met, a description of the AE(s) or safety issue must be reported by the PI by fax or email within 1 business day to the IND Sponsor, the IRB, and SMC.
The PI must inform the IRB that a pausing rule has been met according to their requirements. The IND Sponsor will notify all sites of related Sanaria trials that the study has been paused.

12.9.2 Resumption of a Paused Study

The IND Sponsor in collaboration with the PI and the SMC will determine if it is safe to resume administration of the study agent to the participant/group. The IND Sponsor will notify the PI of this decision. The PI will notify their IRB of the decision to resume administration of the study agent prior to resumption.

12.10 Withdrawal Criteria for an Individual Participant

A subject will not be considered to have completed the trial if any of the following reasons apply:

1. *Research terminated by Sponsor or Investigator* – applies to the situation where the entire study is terminated by the Sponsor or Investigator, or other regulatory authority for any reason.
2. *Withdrawal of consent* – applies to a subject who withdraws consent to participate in the study for any reason.

3. *Noncompliant with protocol* – applies to a subject who does not comply with protocol-specific visits or evaluations, on a consistent basis, such that adequate follow-up is not possible and the subject's safety would be compromised by continuing in the trial. This also applies to a subject who is lost to follow-up and is not reachable by telephone or other means of communication and cannot be located.
4. *Developed an AE* – applies to a subject who is withdrawn from study due to an AE, serious or otherwise. Any grade 3 or greater AE that is assessed as possibly, probably, or definitely related to study products (other than Grade 3 local reactions lasting <72 hours, systemic reactions lasting <24 hours or those that occur 7 days after PfSPZ Challenge and assessed to be related to induced malaria lasting <48 hours) will result in withdrawal of the subject from further vaccinations. Subjects may also be withdrawn for any AE that would cause continued participation in the study to not be in the best interest of the subject, as per the investigator's judgment. Any subject who is withdrawn from the study because of an AE related to study agent will be followed for safety until at least resolution of that AE and will be encouraged to remain in the safety evaluation for the duration of the study.
5. *HIV/Hepatitis Infection* – If a subject acquires HIV infections during the course of the study, then they meet exclusion criteria for the study.
6. *Pregnancy* – If the subject becomes pregnant during the course of the study, they will be withdrawn for safety purposes and followed for the course of their pregnancy and for 3 months post pregnancy outcome.
7. *Inability to tolerate study product* – inability to tolerate any component of the study product individually or in combination (chloroquine, pyrimethamine, Sanaria® PfSPZ Challenge).
8. *Other* – is used when previous categories do not apply and a written explanation is required.

If a subject withdraws or is withdrawn prior to completion of the study, the reason for this decision will be recorded in the source documents and CRIMSON Data System. Any subject who has received at least 1 dose of Sanaria® PfSPZ Challenge will be strongly encouraged to remain in the safety evaluation for the duration of the study (followed at least one more visit post last Sanaria® PfSPZ Challenge). In particular, rigorous follow-up for the development of malaria infection including the institution of presumptive treatment may be required in order to assure the subject's safety. The subject's data will be included in the safety and immunogenicity analysis. If a subject fails to complete all planned Sanaria® PfSPZ Challenges because of an AE or SAE, the subject will be followed until resolution or stabilization of the event. If a subject withdraws, the investigator will make a reasonable effort to determine the reason.

12.11 Replacement of Withdrawn Subjects

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

12.12 Safety Oversight

12.12.1 Safety Monitoring Committee (SMC)

As agreed with OCRPRO, for this study a SMC chartered by the IND Sponsor, Sanaria Inc. will be used instead of the NIAID Intramural DSMB. The SMC will review the study twice during the pilot phase: 1) after the data have been collected on the first two volunteers receiving the 100,000 PfSPZ dose 2) after the pilot study is complete and the trial is ready to move to the main study. The SMC will also review the study at least once during the main Sanaria® PfSPZ-CVac portion of the study, prior to Sanaria® PfSPZ Challenge CHMI. The Board may convene additional reviews as necessary, and will issue recommendations concerning continuation, modification, or termination of the study. All SAEs will be reported by the PI to the IND Sponsor as outlined in **Section 10.4.2**. All reportable events (SAEs, UPs, pregnancies) as defined in **Section 10.4** will be reported by the Sponsor to the SMC within one day of being notified of the event.

The SMC will review the study data to evaluate the safety, efficacy, study progress, and conduct of the study. The Sponsor will notify the SMC at the time pausing criteria are met and obtain a recommendation concerning continuation, modification, or termination of the study. The PI will submit the written SMC summary reports with recommendations to the IRB(s).

13 Clinical Monitoring

13.1 Site Monitoring Plan

As per ICH-GCP 5.18 and FDA 21 CFR 312.50, clinical protocols are required to be adequately monitored by the study Sponsor. The Office of Clinical Research Policy and Regulatory Operations (OCRPRO) will provide oversight and monitor the compliance of this trial. This study monitoring will be conducted according to the "NIAID Intramural Clinical Monitoring Guidelines." Monitors under contract to the NIAID/OCRPRO will visit the clinical site to monitor aspects of the trial in accordance with appropriate regulations. The objectives of a monitoring visit will be:

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

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

A specific monitoring plan will be discussed with the Principal Investigator, study staff, and IND Sponsor prior to enrollment. The plan will outline the frequency of monitoring visits based on such factors as study enrollment, data collection status and regulatory obligations.

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

14 Statistical Considerations

14.1 General Pilot Study

The pilot is designed to find the largest NF54 Sanaria® PfSPZ Challenge dose that will not cause blood stage parasitemia in most subjects.

14.2 General Main Study

The main phase is designed to investigate the safety profile of the selected NF54 Sanaria® PfSPZ Challenge with concurrent chemoprophylaxis of pyrimethamine or chloroquine treatment in healthy, malaria-naïve US adults. As well, we wish to determine if this dose along with concurrent chemoprophylaxis of pyrimethamine can induce protective efficacy against homologous challenge and if this NF54 Sanaria® PfSPZ Challenge dose along with current chloroquine treatment can induce protective efficacy against heterologous challenge.

14.3 Primary Objectives

The primary objectives of this study are twofold:

- **Safety:** To monitor the safety and tolerability of NF54 Sanaria® PfSPZ Challenge under concurrent chemoprophylaxis with pyrimethamine treatment or chloroquine treatment in healthy, malaria-naïve US adults

- **Prevention of Infection** *(Pilot Phase Only):* To evaluate whether increasing the dose of Sanaria® PfSPZ Challenge (to a maximum of 200, 000 PfSPZ) under pyrimethamine chemoprophylaxis in humans will not result in the development of asexual erythrocytic stage parasitemia assessed by sensitive retrospective LMIV qPCR after DVI of Sanaria® PfSPZ Challenge in healthy, malaria-naïve adults.

14.4 Secondary Objective

The secondary objectives of this study includes assessing the protective efficacy of PfSPZ-CVac pyrimethamine against homologous CHMI with Sanaria® PfSPZ Challenge.

Table 23 shows the decision rule for rejecting the null hypothesis that there is no significant difference between *Arm 2a* (Pyrimethamine only) and the concurrently challenged controls (*Arm 4a*, n=8). Based on previous data, the team expects that the true infection rate among controls should be very high (≥99%). For example, if 13 participants from *Arm 2a* are challenged, and all 8 of the controls become infected, we will reject the null hypothesis if and only if 10 or fewer of the

13in *Arm 2a* participants become infected. If the number of infections in the challenged *Arm 2a* participants exceed the numbers in the table below, we will not reject the null hypothesis.

Table 23: Decision Rule:

The infection rate in any of the active arms (*Arm 2a, 2b, 3)* will be judged to be significantly different from the controls if the number of infections among the controls and infections among the challenged participants in that arm is less than or equal to the numbers in the table.

	Number infected out of 8 controls			
Number of participants challenged in Arm 2a, 2b or 3	**8/8**	**7/8**	**6/8**	**5/8**
17	**≤11**	**≤6**	**≤5**	**≤3**
16	**≤10**	**≤6**	**≤5**	**≤2**
15	**≤9**	**≤6**	**≤4**	**≤2**
14	**≤8**	**≤6**	**≤3**	**≤2**
13	**≤8**	**≤5**	**≤3**	**≤2**
12	**≤7**	**≤5**	**≤1**	**≤1**
11	**≤6**	**≤4**	**≤3**	0
10	**≤6**	**≤3**	**≤2**	**≤1**
9	**≤5**	**≤3**	**≤2**	**≤1**
8	**≤4**	**≤3**	**≤1**	0

The rule above was chosen to maximize the probability of declaring a significant difference if all 8 of the controls become infected, as this is considered the most likely scenario, while controlling the one-sided type 1 error rate at 0.025, conditioned on the number of *Arm 2a, 2b or 3* participants challenged.

14.5 Exploratory Objectives

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

14.6 Sample Size and Power Calculations

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

Table 24 describes the probability of break through parasitemia or infection during the pilot phase. For a group of two participants, there is a 75% chance of observing at least 1 infection if the true rate of such an event is 50% or more; the probability of observing at least 1 event among four participants at this level is 94% for this same true rate of 50%. Therefore, under the assumption that higher doses are more likely to cause infection, if we observe 4 or more subjects at higher doses without infection, we have good power to detect a 50% infection rate at the lower level.

Table 24: *Probability of observing 0 and 2 or more break through parasitemia, among arms of size 2 and 4, for different true event rates*

True event rate (%)	Pr(0/2)	Pr(2/2)	Pr(0/4)	Pr(2/4)
1	0.98	<0.01	0.96	<0.01
5	0.90	<0.01	0.81	0.01
10	0.81	0.01	0.66	0.05
20	0.64	0.04	0.41	0.18
30	0.49	0.09	0.24	0.35
50	0.25	0.25	0.06	0.69

14.6.2 Power calculations for Main Phase

14.6.2.1 Power calculations for primary objective (safety)

The goal of the safety evaluation for this study is to identify safety concerns associated with product administration. The ability of the study to detect serious adverse events (SAEs) can be expressed by the true event rate above which at least 1 SAE would likely be observed and the true event rate below which no events would likely be observed. Specifically, for *Arm 2a* (n =17), there is a 90% chance of observing at least 1 event if the true rate of such an event is 13% or more; and there is a greater than 90% chance of observing no events if the true rate is 0.5% or less. For *Arm 2b* and *3* (n =10 each), there is a 90% chance of observing at least 1 event if the true rate of such an event is 20% or more; and there is a 90% chance of observing no events if the true rate is 1.0% or less.

Probabilities of observing 0, and 2 or more events among Arms of size 17 and 10 are presented in **Table 25** for a range of possible true adverse event rates. These calculations provide a more complete picture of the sensitivity of this study design to identify potential safety problems with the vaccine.

Table 25: Probability of observing 0 events and 2 or more events, among arms of size 17 and 10, for different true event rates

True event rate (%)	Pr(0/17)	Pr(2+/17)	Pr(0/10)	Pr(2+/10)
1	0.843	0.012	0.904	0.012
5	0.418	0.208	0.599	0.208
10	0.167	0.518	0.349	0.518
20	0.023	0.882	0.107	0.882
30	0.002	0.981	0.028	0.981
50	<0.001	>0.99	0.001	>0.99

An alternative way of describing the statistical properties of the study design is in terms of the 95% confidence interval for the true rate of an adverse event based on the observed data **Table 26** shows the 2-sided 95% confidence intervals for the probability of an event based on a particular observed rate. If none of the 17 participants in *Arm 2a* experience a safety event, the 95% 2-sided upper confidence bound for the true rate of such events in the total vaccinated population is 0.2. For *Arm 2a or 3* (n = 10), the 2-sided upper confidence bound for this rate is 0.31.

Table 26: Two-sided 95% confidence intervals based on observing a particular rate of safety endpoints for arms of size 17 and 10

Observed event rate	**95% Confidence interval (%)**
0/17	(0.00, 0.20)
1/17	(0.001, 0.29)
2/17	(0.01, 0.36)
0/10	(0.00, 0.31)
1/10	(0.003, 0.45)

14.6.2.2 Power calculations for secondary objective (efficacy)

We expect dropout from *Arms 2* and *3* of approximately 30% before challenge. We expect no dropout among the control arms because they are enrolled just before challenge. Power calculations were considered for a range of numbers of people in *Arm 2* and *3* that make it to challenge.

We assume approximately 13 people from *Arm 2a* are challenged, and compared to 8 controls. The decision rule described in **Table 23** will have good power (>80%) to detect a difference between the arms if the true Vaccine Efficacy (VE), defined as 1- the probability of infection in the treatment group divided by the probability of infection in the controls, is at least 50%. This provides power calculations for the comparison of *Arms 2a* and *4a*.

We assume at least 7 people from *Arm 2b* and *3* are challenged, and compared to 8 controls. Power to detect a VE of at least 70% for heterologous challenge comparing *Arms 2b* and *4b* AND *Arms 3* and *4b*, will be greater than 85%.

14.7 Randomization

Randomization for *Arms 2* and *3* will be completed at the time of enrollment. For the second cohort, subjects will be randomized into *Arms 2b* and *3*. Subjects will also be randomized into enrollment into *Arm 4a* or *4b* in the first cohort only. In the second cohort, control subjects will join *Arm 4b* directly.. *Arms 1*, *and 5* will not be randomized. Subjects and investigators will be aware of *Arm* assignments. The third cohort will not be enrolled.

15 Human Subject Protections and Ethical Obligations

This research will be conducted in compliance with the protocol, Good Clinical Practices (GCP), and all applicable regulatory requirements.

15.1 Institutional Review Board

A copy of the protocol, informed consent forms, and other information to be completed by subjects, such as questionnaires, and any proposed advertising/recruitment materials or letters to the subjects will be submitted to the reviewing IRBs for written approval. The investigator must submit and obtain approval from the IRBs for all subsequent amendments to the protocol, informed consent documents, and other study documentation referenced above. The investigator will be responsible for obtaining IRB approval of the annual Continuing Review throughout the duration of the study. The investigators will notify the reviewing IRBs of protocol deviations and SAEs as specified in the relevant sections of the protocol.

15.2 Informed Consent Process

Informed consent is a process where information is presented to enable persons to voluntarily decide whether or not to participate as a research subject. It is an on-going conversation between the human research subject and the researchers which begins before consent is given and continues until the end of the subject's involvement in the research. Discussions about the research will provide essential information about the study and include: purpose, duration, experimental

procedures, alternatives, risks and benefits. Subjects will be given the opportunity to ask questions and have them answered.

The subjects will sign the informed consent document prior to undergoing any research procedures. The subjects may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the subjects for their records. The researcher will document the signing of the consent form in the subject's medical record. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

15.3 Justification for Exclusion of Children

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

15.4 Subject Confidentiality

All records will be kept confidential to the extent provided by federal, state and local law. The study monitors and other authorized representatives of the Sponsor may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records. Records will be kept locked and all computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by IRB, the FDA, the NIAID, the OHRP, Sanaria, Inc., or the sponsor's designee.

15.5 Risks

Risks to the subjects are associated with venipuncture, malaria infection, Sanaria® PfSPZ Challenge, Chloroquine, Pyrimethamine, and large volume blood drawing. These risks are outlined below:

15.5.1 Venipuncture

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

15.5.2 Sanaria® PfSPZ Challenge via DVI

Possible local reactions include pain, swelling, erythema, induration, limitation of limb movement for several days, lymphadenopathy, or pruritus at the injection site. Systemic reactions such as fever, chills, headache, fatigue, malaise, myalgia, and joint pain may also possibly occur, with some reactions moderate or severe.

Other than mild and transient local (site of administration) reactions, the listed adverse reactions remain theoretical.

In Equatorial Guinea two serious adverse events possibly related to PfSPZ Vaccine have been described. One volunteer who received PfSPZ Vaccine had a miscarriage at 10 weeks after getting pregnant while participating in a PfSPZ Vaccine trial. On vaccination day, the subject had a

negative urine pregnancy test. She was started on contraceptive measures on the same day she received the vaccine and was advised to additionally use a barrier method.

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

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

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

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

15.5.3 Malaria Symptoms after Administration of PfSPZ-CVac

In a PfSPZ-CVac study by LMIV (#15-I-0169) where subjects were injected with PfSPZ Challenge by DVI while under chloroquine chemoprophylaxis, all participants reported solicited or unsolicited symptoms that were recorded as AEs at least once during immunization phase. There was one SAE reported of encephalopathy NOS that was either possibly related to chloroquine or viral infection as described in **Section 1.8.2.1**. None of the participants developed patent parasitemia.

Similar frequency AEs have been reported in other studies during CVac with chloroquine. In the Roestenberg study where subjects were exposed to experimental infection by the bites of Pf-infected mosquitoes while under concurrent chloroquine prophylaxis, all subjects reported solicited or unsolicited symptoms that were recorded as AEs at least once during the PfSPZ-CVac immunization phase, although there were no statistically significant differences in AE frequency between the 10 vaccinees and 5 controls. There were no serious adverse events at any time and no evidence of blood-stage parasites (patent parasitemia) in any of the subjects at any time during the post-challenge follow- up period, though following that study, there have been reports of patent parasitemia at multiple centers in subjects during CVac/CPS while on chloroquine prophylaxis.

In the study of PfSPZ-CVac in Tübingen, all subjects developed transient parasitemia after the first PfSPZ Challenge injection. As in Nijmegen, there were no significant differences between the AE

profiles of vaccinees experiencing this subpatent parasitemia and the blinded placebo recipients. There was one subject with a low grade fever and concurrently the highest subpatent parasitemia recorded in the first stage of the study that the investigators determined may have been related. In the second stage of the study, there was one subject that did not develop therapeutic levels of chloroquine and was subsequently treated and withdrawn from the study.

15.5.4 Malaria Symptoms and Infection after PfSPZ CHMI

In the Roestenberg study, after subsequent challenge of vaccinees with a homologous NF54 strain of *P. falciparum* following the three CVac infections, the clinical course was similar to those in previously reported challenge studies without CVac.

In the LMIV CVac study (#15-I-0169), malaria symptoms in unprotected vaccinees was similar to that of unvaccinated controls with comparable severity to controls in other CVac studies. Protected participants developed mostly mild symptoms consistent with other CVac studies (**Table 10**).

As with any clinical investigation, even with extensive precautions, there is always a risk of serious, or even life-threatening allergic reactions to administration of the investigational product. These risks are minimized by entry criteria that exclude subjects with a potential for adverse reaction to the malaria challenge, infection or study drugs. Likewise, subjects will be closely monitored during the administration of Sanaria® PfSPZ Challenge and/or drugs and in the immediate period following administration by clinical staff trained and equipped to respond immediately to acute systemic reactions including anaphylaxis and clinical malaria.

15.5.4.1 Cardiac Abnormalities

There is no specific known cardiac risk for healthy subjects associated with CHMI or the antimalarial drugs at the proposed dosing used in this study. Likewise, cardiac abnormalities in uncomplicated clinical malaria are extremely rare (Ehrhardt, Mockenhaupt et al. 2005)and routine cardiac monitoring of subjects with severe malaria is not required even in subjects receiving treatment with antimalarials with known effects on cardiac electrical conduction (Bethell, Phuong et al. 1996, Bregani, Tien et al. 2004)

A challenge trial subject participating in a malaria vaccine trial in the Netherlands had an unexplained cardiac event following receipt of an investigational vaccine, malaria challenge, infection and treatment with artemether/lumefantrine (Nieman, de Mast et al. 2009). It is thought the temporal association of the event to malaria challenge was likely circumstantial (Lyke, Laurens et al. 2010). The definitive etiology of the event remains unknown and the subject recovered without sequelae.

Another cardiac event in a malaria vaccine trial participant in the Netherlands was reported (van Meer, Bastiaens et al. 2014). The trial subject received a different test malaria vaccine than the subject reported in 2009. The subject was observed (on day 13) after challenge to have changes in a blood test suggesting a heart muscle problem. Later that day the subject experienced chest pain and reported a heavy feeling in his left arm. After further evaluation the subject was diagnosed with myocarditis. It is not known whether this illness was related to malaria infection, the test vaccine that the subject received, the medicine used to treat malaria,

a viral infection unrelated to the study or something else. Myocarditis resolved without treatment.

Cardiac events, such as coronary vasospasm or myocarditis, are not associated with uncomplicated natural *P. falciparum* infection or experimental infection in the cumulative experience in over 1300 volunteers (Ehrhardt, Mockenhaupt et al. 2005) at three centers worldwide. However, in order to minimize the potential risk for cardiac abnormalities during the study, cardiac risk assessment will be conducted as part of the screening process based on the NHANES I study criteria as defined in **Appendix B** (Gaziano, Young et al. 2008**)** and screening electrocardiogram and subjects with clinically significant abnormalities on ECG or elevated risk (> low risk of a coronary event over the next 5 years) will be excluded from the study.

15.5.4.2 Pregnancy

Malaria infection can have adverse effects on both the pregnant mother and fetus. Women who are pregnant, nursing or plan to become pregnant during the study are excluded from the study.

Pregnancy testing is performed during screening and throughout the study including prior to each Sanaria® PfSPZ Challenge DVI. If clinical history indicates recent sexual activity that may lead to pregnancy, a serum β-HCG test will be sent and results reviewed by a study investigator prior to the subject continuing in the study. Pregnancy prevention counseling and compliance is reinforced at every clinic visit (see **Appendix A**) throughout the study.

15.5.5 Medications used in the study

The antimalarial medications administered in the study (pyrimethamine, chloroquine, Malarone®) are generally well tolerated in the dosing regimens to be used, although common side effects (e.g., nausea, vomiting, diarrhea, headaches, sleep disturbance) are anticipated to occur in some subjects. Potential subjects with known sensitivity or contraindications to the antimalarials administered in this study are excluded from participation. Subjects in the study will be closely monitored for adverse drug reactions and where possible, attempts will be made to minimize anticipated side effects (e.g. dosing of medications with food). Specific toxicities for pyrimethamine, chloroquine and Malarone® are noted below and further outlined in the package insert for each drug.

15.5.5.1 Chloroquine Phosphate

The most commonly reported side effects of chloroquine dosing include gastrointestinal disturbance, headache, dizziness, blurred vision, insomnia, tinnitus, and pruritus, but generally these effects do not require discontinuation of the drug. High doses of chloroquine, as used to treat rheumatic diseases (>3. 5 mg/kg/day over 1 year or more), have been associated with retinopathy, although generally requires a cumulative dose exceeding 100 grams (Salako 1984) and thus is extremely unlikely at doses used for routine weekly malaria prophylaxis especially for the limited duration in this study. Chloroquine is reported to exacerbate psoriasis; therefore subjects with a history of psoriasis are excluded.

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

In the current study, there was one SAE (acute change in mental status) that occurred during the main phase of the first cohort in a participant enrolled in *Arm 3*; 3 weeks after second vaccination. The SAE was determined to be due to acute anticholinergic poisoning after participant ingested *Datura stramonium* seeds for recreational purposes. Due to concurrent chloroquine prophylaxis use and unknown potentiation on effects of *Datura stramonium* during concurrent use, this episode was deemed possibly related to chloroquine. The participant recovered without sequaelae and was taken off study.

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

15.5.5.2 Pyrimethamine

The most commonly reported side effects of pyrimethamine dosing include vomiting and anorexia but generally these effects do not require discontinuation of the drug and can be minimized by giving the medication with food. These sides effects may increase with pyrimethamine dose but usually disappears promptly upon reduction of dosage. Prolonged and higher doses of pyrimethamine, as used to treat toxoplasmosis, may produce megaloblastic anemia, leukopenia, thrombocytopenia, pancytopenia, atrophic glossitis, hematuria, and disorders of cardiac rhythms though this is extremely unlikely at doses used for this study. Hematologic effects, however, may also occur at low doses in certain individuals, thus hematological parameters will be closely monitored.

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

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

15.5.5.3 Malarone® (Atovaquone/proguanil)

Though not a part of the investigational product/regimen, all subjects will receive empiric treatment with Malarone® prior to completion of the trial. The most commonly reported side effects of Malarone® dosing include gastrointestinal disturbance, sleeping problems (insomnia), strange dreams, dizziness, depression, loss of appetite, fever, rash, cough and laboratory abnormalities such as anemia, neutropenia, transaminitis, hyponatremia or increase in amylase, but generally these effects do not require discontinuation of the drug. Malarone® has occasionally been associated with severe hypersensitivity reactions (such as Stevens-Johnson syndrome, erythema multiforme, and anaphylaxis)

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

15.5.5.4 Ibuprofen

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

Ibuprofen likewise is not a part of the investigational product/regimen, but will be provided to participants in the chloroquine arm (*Arm 3*) to mitigate typical symptoms of malaria infection such as headache, myalgia, fever and chills. The most commonly reported side effects of ibuprofen include abdominal discomfort, heartburn, nausea, skin rash, dizziness, and tinnitus (ringing in the ears). Less frequent side effects include headache, nervousness, itching, edema (fluid retention), constipation, diarrhea, decreased appetite, flatulence, and vomiting. Ibuprofen has rarely been associated with abnormal hepatic function tests, decreased creatinine clearance, leukopenia, thrombocytopenia, allergic rhinitis, anaphylaxis, aseptic meningitis, blurred vision, cardiac arrhythmia, confusion, conjunctivitis, cystitis, depression, drowsiness, duodenal ulcer, erythema multiforme, gastric ulcers, gastritis, gastrointestinal hemorrhage, hallucinations, hearing loss, hematuria, hypertension, insomnia, jaundice, palpitations, pancreatitis, peripheral neuropathy, Stevens-Johnson syndrome, tachycardia, and urticaria.

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

15.6 Risk to the Community

Malaria is not contagious and cannot be spread by person-to-person contact. Subjects are routinely diagnosed and treated before the infective form of the parasite (gametocyte) appears in the blood.

15.7 Benefits

Subjects will not receive any direct benefit from participation in this study. It is hoped that information gained in this study will contribute to the development of a safe and effective malaria vaccine.

15.8 Compensation

Subjects will be compensated for time and travel to participate in this trial. Compensation is pro-rated based upon completion of specific visits and is not contingent upon completing the entire study. A compensation schedule is provided below (see **Table 27).** Compensation for interim visits to follow up clinical or laboratory abnormalities will be provided at the Investigator's discretion, up to $50. If a subject withdraws from the trial, compensation will be made based on the last completed visit. Information about compensation, including the amount and schedule of payment(s) and applicable reporting to the IRS, will also be described in the informed consent form.

Table 27: Compensation Table

Pilot Study Arms PfSPZ-CVac					
Study Activity	Compensation	**Arm 1** Number of Visits	**Arm 1** Compensation Total	**Arm 5** Number of Visits	**Arm 5** Compensation Total
Screening Visit	$100	1	$100	1	$100
Sanaria® PfSPZ Challenge CVac	$200	1	$200	1	$200
Telephone Follow-Up	$10	1	$10	1	$10
Clinic Visit and Blood draw (<60ml)	$50	9	$450	8	$400
Clinic Visit, Drug Dose with/without Blood draw (<60ml), and	$75	5	$375	5	$375
Completed Diary (counted as visit)	$10	1	$10	1	$10
Total for Pilot Study	**N/A**	**18**	**$1,145**	**17**	**1095**

Main Study Arms PfSPZ-CVac					
Study Activity	Compensation Amount	Arm 2a, 2b Number of Visits	Arm 2a, 2b Compensation Total	Arm 3 Number of Visits	Arm 3 Compensation Total
Screening Visit	$100	1	$100	1	$100
Sanaria® PfSPZ Challenge CVac	$200	3	$600	3	$600
Telephone Follow-Up	$10	2	$20	6	$60
Clinic Visit	$40	6	$240	5	$200
Clinic Visit and Blood draw (<60 ml)	$50	26	$1,300	25	$1,250
Clinic Visit, Blood draw (<60 mL), and Drug Dose	$75	3	$225	4	$300
Clinic Visit, Blood draw (>60ml), and Drug Dose	$100	1	$100	1	$100
Clinic Visit and Drug Dose	$75	3	$225	6	$450
Completed Diary	$10	3	$30	3	$30
Total for CVAC	**N/A**	**48**	**$2,840**	**54**	**$3,090**

CHMI					
Study Activity	Compensation Amount	Arm 2a, 2b, 3 Number of Visits	Arm 2a, 2b, 3 Compensation Total	Arm 4a, 4b Number of Visits	Arm 4a, 4b Compensation Total
Screening Visit	$100	0	$0	1	$100
Sanaria® PfSPZ Challenge	$200	1	$200	1	$200
Telephone Follow-Up	$10	2	$20	2	$20
Clinic Visit and Blood Draw (<60ml)	$50	18	$900	20	$1,000
Clinic Visit and Blood Draw (>60 ml)	$100	1	$100	1	$100
Clinic Visit, Blood Draw (>60 ml) and Drug Dose	$100	1	$100	1	$100
Clinic Visit, Blood Draw (<60 ml) and Drug Dose	$75	2	$150	2	$150
Completed Diary	$14	1	$14	1	$14
Total CHMI	**N/A**	**26**	**$1,484**	**29**	**$1,684**

[1] Specification of participants visits are based on Appendix A, actual visits calculated are subject to change, based on clinical information; however, compensation per visit will follow the guidelines in this table

[2] Clinic follow up visits are reimbursed by visits completed. If a subject is diagnosed with malaria early this total amount may be less (maximum amount presented here).

[3] If enrolled in Arm 4 total compensation starts at CHMI – all subjects in Arm 4a and 4b will at least be compensated $1000 for their participation.

[4] Subjects that require blood draw only (no clinic visit) will be compensated $25

16 Data Handling and Record Keeping

16.1 Source Documentation

Complete source documentation (laboratory test reports, hospital or medical records, progress notes, observations, subject diaries, etc.) is required for every study subject for the duration of the study. The subject's research record must record his/her participation in the clinical trial, the treatment received (with doses and frequency) or other concomitant medications or interventions administered, as well as any adverse reactions experienced during the trial. Selected data from source documentation and subject symptom memory enhancement cards (diaries) for subjects enrolled in the study will be entered into the CRIMSON Data System. The data entry is to be completed on an ongoing basis during the study. Data entered into CRIMSON shall be performed by authorized individuals. Corrections to the data system shall be tracked electronically (password protected) with time, date, individual making the correction, and what was changed. Source documentation should support the data collected in CRIMSON**,** and must be signed and dated by the person recording and/or reviewing the data. The Investigator is responsible for the accuracy, completeness and timeliness of the data reported to the Sponsor in the CRIMSON Data System. All data entered into CRIMSON should be reviewed by the Investigator and signed as required with written or electronic signature, as appropriate. Data reported in CRIMSON should be consistent with source documents or the discrepancies should be explained. Source documentation will be made available for review or audit by the Sponsor, OCRPRO or their designees and any applicable Federal authorities.

16.2 Retention of Study Records

The investigator is responsible for retaining all essential documents listed in the ICH Good Clinical Practice Guideline. Study records will be maintained by the PI for a minimum of 5 to 7 years and in compliance with institutional, IRB, state, and federal medical records retention requirements, whichever is longest. All stored records will be kept confidential to the extent required by federal, state, and local law.

Should the investigator wish to assign the study records to another party and/or move them to another location, the investigator will provide written notification of such intent to OCRPRO/NIAID with the name of the person who will accept responsibility for the transferred records and/or their new location. NIAID will be notified in writing and written OCRPRO/NIAID permission shall be obtained by the site prior to destruction or relocation of research records.

16.3 Protocol Revisions

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

Appendix A: Clinical Evaluation and Laboratory Procedures

Appendix A: Clinic and Laboratory Procedures For Arms 1a, 1b, 1c, 1d, 1e and 1f																			
		Clinic visits	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
		Study Day (Pilot Study)	-56	1	2	3	4	7	8	9	10	11	12	13	14	15	16	17	29
		Days post-PfSPZ Cvac	-56	0	1	2	3	6	7	8	9	10	11	12	13	14	15	16	28
		Visit windows (days)		+/-3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+/-3
			Screen	Enroll/PfSPZ CVac #1	Phone f/u	PYR dose	PYR dose	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	TX	TX	TX	Final visit
Complete medical history/ physical			X																
Informed consent			X																
Malaria Comprehension Exam			X																
Pre-test/Post-test HIV counseling			X																
Pregnancy Prevention Counseling			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Interim clinical evaluation				X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
AE/SAE assessment				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Conmed review			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Diary				X	X	X	X	X	X	X	X	X							
PYR Dose						X	X												
Treatment with Malarone																X	X	X	
PfSPZ				X															
LABORATORY PROCEDURES	Designated Laboratory	Tube Type																	
Screening/Safety Labs																			
CBC with differential	NIH Clinical Center	EDTA	3	3			3		3									3	3
ALT, Cr		SST	4	4			4		4									4	4
Hepatitis B, C, HIV testing		SST	8																
EKG		Cardiology	X																
Urine dipstick or UA		Urine Container	X																
Urine/Serum Pregnancy Test (females only)		Urine Container or Lithium Hep	X	X															
Malaria Infection Assays																			
Real time NIH PCR[1]	NIH Clinical Center	EDTA						1	1	1	1	1	1	1	1	1	1	1	1
Retrospective LMIV qPCR	LMIV	EDTA		2				2	2	2	2	2	2	2	2	2	2	2	2
Peripheral Blood Smear[2]	NIH Clinical Center/LMIV	EDTA (pediatric)						(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	
Research Assays																			
Cellular Assays[3]	CHI	NaHep		10												10			
Ex vivo & PYR levels (research)	LMIV/WRAIR	EDTA (pediatric)		1			1		1							1			
Daily total			15	20	0	0	8	3	11	3	3	3	3	3	3	14	3	10	10
Cumulative total Pilot			15	35	35	35	43	46	57	60	63	66	69	72	75	89	92	102	112

[1] After first positive NIH PCR (malaria diagnosis), PCR will NOT be repeated until final visit, study day 29.

[2] 1 mL of for blood smear only for symptomatic subjects. A POSITIVE blood smear will be followed daily until 3 consecutive NEGATIVE blood smears have been recorded

[3] 10mL will be used for optimizing cellular assays (planned to be completed during the Main Phase) on Study Days 1 and 15

Appendix A: Clinic and Laboratory Procedures For Arm 5 (chloroquine pilot)

		Clinic visits	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
		Study Day (Pilot Study)	-56	-2	1	2	4	6	7	8	9	10	11	12	13	14	15	29
		Days post-PfSPZ Cvac	-56	-2	0	1	3	5	6	7	8	9	10	11	12	13	14	28
		Visit windows (days)			0	0	0	-1	0	0	0	0	0	0	0	0	0	+/-3
			Screen	CQ dose	Enroll/PfSPZ CVac #1	Phone f/u	Clinic f/u	CQ dose	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	TX	TX	TX	Final visit
Complete medical history/ physical			X															
Informed consent			X															
Malaria Comprehension Exam			X															
Pre-test/Post-test HIV counseling			X															
Pregnancy Prevention Counseling			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Interim clinical evaluation				X	X		X	X	X	X	X	X	X	X	X	X	X	X
AE/SAE assessment				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Conmed review			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Diary					X	X	X	X	X	X	X	X	X					
CQ dose				X				X										
Treatment with Malarone															X	X	X	
PfSPZ					X													
LABORATORY PROCEDURES	Designated Laboratory	Tube Type																
Screening/Safety Labs																		
CBC with differential	NIH Clinical Center	EDTA	3		3		3			3							3	3
ALT, Cr		SST	4		4		4			4							4	4
Hepatitis B, C, HIV testing		SST	8															
EKG		Cardiology	X															
Urine dipstick or UA		Urine Container	X															
Urine/Serum Pregnancy Test (females only)		Urine Container or Lithium Hep	X	X	X													
Malaria Infection Assays																		
Real time NIH PCR[1]	NIH Clinical Center	EDTA																1
Retrospective LMIV qPCR	LMIV	EDTA			2				2	2	2	2	2	2	2	2	2	2
Peripheral Blood Smear[1]	NIH Clinical Center/LMIV	EDTA (pediatric)							1	1	1	1	1	1	1	1	1	
Research Assays																		
Ex vivo	LMIV	EDTA (pediatric)			1		1			1							1	
Drug Assays																		
Plasma CQ levels (CQ, DECQ)	PA Lab/NMS	EDTA			3													
Urine CQ levels (CQ, DECQ)	PA Lab/NMS	Urine Container			X													
Daily total			15		13	0	8	0	3	11	3	3	3	3	3	3	11	10
Cumulative total Pilot			15		28	28	36	36	39	50	53	56	59	62	65	68	79	89

[1]A POSITIVE blood smear will be followed daily until 3 consecutive NEGATIVE blood smears have been recorded

Appendix A: Clinic and Laboratory Procedures For Arm 2 and 3 (PfSPZ CVac #1)

		Clinic visits	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
		Study Day (Main Study)	-56	-7	-2	1	2	3	4	6	7	8	8	9	9	10	11	13	15	20	27
		Days post-PfSPZ Cvac	-56	-7	-2	0	1	2	3	5	6	7	7	8	8	9	10	12	14	19	26
		Visit windows (days)		+/-5	+/-1	-1 to +3	0	0	0	+/-1	0	0	0	0	0	0	0	0	0	+/-2	+/-2
			Screen	Enrollment/ Baseline #1	Baseline #2	PfSPZ CVac #1	Phone f/u	Clinic f/u	Clinic f/u	Clinic f/u	Clinic F/U; Pre-pat	AM Clinic F/U; Pre-pat	PMClinic F/U; Pre-pat[4]	AM Clinic F/U; Pre-pat	PM Clinic F/U; Pre-pat[4]	Clinic F/U; Pre-pat	Clinic F/U; Pre-pat	Clinic F/U; Pre-pat	Clinic F/U; Pre-pat	Clinic f/u	Clinic f/u
CLINICAL PROCEDURES																					
Complete medical history/ physical			X																		
Informed consent			X																		
Malaria Comprehension Exam			X																		
Pre-test/Post-test HIV counseling			X																		
Pregnancy Prevention Counseling			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Interim clinical evaluation				X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
AE/SAE assessment				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Conmed review			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Diary[1]						X	X	X	X	X	X	X		X		X	X				
CQ dosing (Arm 3 ONLY)					X					X								X		X	X
Ibuprofen dosing (Arm 3 ONLY)																					
PYR Dose (Arm 2 ONLY)								X	X												
Treatment with Malarone																					
PfSPZ						X															
LABORATORY PROCEDURES	Designated Laboratory	Tube Type																			
Screening/Safety Labs																					
CBC with differential	NIH Clinical Center	EDTA	3		3				3			3									3
ALT, Cr		SST	4		4				4			4									4
Hepatitis B, C, HIV testing		SST	8																		
EKG		Cardiology	X																		
Urine dipstick or UA		Urine Container	X																		
Urine/Serum Pregnancy Test (females only)		Urine Container or Lithium Hep	X		X	X															
Malaria Infection Assay																					
Real time NIH PCR (Arm 2 only)[2]	NIH Clinical Center	EDTA									1	1		1		1	1				
Retrospective LMIV qPCR	LMIV	EDTA			2						2	2		2		2	2				2
Peripheral Blood Smear[3]	NIH Clinical Center/LMIV	EDTA (pediatric)									1	1		1		1	1				
Drug Assays																					
PYR levels (Arm 2 only)	WRAIR	EDTA (pediatric)			1						1						1				1
Plasma CQ levels (CQ, DECQ - Arm 3 only)	PA Lab/NMS	EDTA				3															
Urine CQ levels (CQ, DECQ - Arm 3 only)	PA Lab/NMS	Urine Container				X															
Research Assays																					
Humoral Assays (LMIV)	LMIV/CHI	SST			4														4		4
Humoral Assays (CHI)		SST		4	4				4			4							4		
Cellular Assays (CHI)		NaHep		30	30				30			30							30		
Cellular Assays (LMIV)		NaHep		20								10							10		10
Transcriptional Assays (CHI)		PAXgene		2.5	2.5				2.5			2.5							2.5		
Ex vivo		EDTA (pediatric)			1				1			1							1		1
Daily total			15	56.5	51.5	3	0	0	44.5	0	5	58.5	0	4	0	4	5	0	51.5	0	25
Study cumulative total			15	71.5	123	126	126	126	170.5	170.5	175.5	234	234	238	238	242	247	247	298.5	298.5	323.5

[1] Diary collection window +2 days

[2] After first positive NIH PCR (malaria diagnosis), PCR will NOT be repeated until Study day 27 unless clinically indicated.

[3] Arm 3 only will have blood smears daily on study day 7-11; resulted same day. Arm 2 will only have blood smear if symptomatic. A POSITIVE blood smear will be followed daily until 3 consecutive NEGATIVE blood smears have been recorded

[4] Arm 3 will have a clinic visit with medications. Arm 2 will have a visit ONLY if clinically indicated.

Appendix A: Clinic and Laboratory Procedures For Arm 2 and 3 (PfSPZ CVac #2)

		Clinic visits	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
		Study Day (Main Study)	29	31	32	34	35	36	36	37	37	38	39	41	43	48	55
		Days post-PfSPZ Cvac	0	2	3	5	6	7	7	8	8	9	10	12	14	19	26
		Visit windows (days)	-1 to +3	0	0	+/-1	0	0	0	0	0	0	0	+/-1	+/-1	+/-2	+/-2
			PfSPZ CVac #2	Clinic f/u	Clinic f/u	Clinic f/u	Clinic F/U; Pre-pat	AM Clinic F/U; Pre-pat	PM Phone [4] F/U; Pre-pat	AM Clinic F/U; Pre-pat	PM Phone [4] F/U; Pre-pat	Clinic F/U; Pre-pat	Clinic F/U; Pre-pat	Clinic f/u	Clinic f/u	Clinic f/u	Clinic f/u
CLINICAL PROCEDURES																	
Complete medical history/ physical																	
Informed consent																	
Malaria Comprehension Exam																	
Pre-test/Post-test HIV counseling																	
Pregnancy Prevention Counseling			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Interim clinical evaluation			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
AE/SAE assessment			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Conmed review			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Diary[1]			X	X	X	X	X	X		X		X	X				
CQ dosing (Arm 3 ONLY)						X								X		X	X
PYR Dose (Arm 2 ONLY)				X	X												
Treatment with Malarone																	
PfSPZ			X														
LABORATORY PROCEDURES	Designated Laboratory	Tube Type															
Screening/Safety Labs																	
CBC with differential	NIH Clinical Center	EDTA			3			3									3
ALT, Cr		SST			4			4									4
Urine/Serum Pregnancy Test (females only)		Urine Container or Lithium Hep	X														
Malaria Infection Assays																	
Real time NIH PCR (Arm 2 only)[2]	NIH Clinical Center	EDTA					(X)	1		1		1	(X)				
Retrospective LMIV qPCR	LMIV	EDTA					2	2		2		2	2				2
Peripheral Blood Smear[3]	NIH Clinical Center/LMIV	EDTA (pediatric)					1	1		1		1	1				
Drug Assays																	
PYR levels (Arm 2 only)	WRAIR	EDTA (pediatric)					1						1				1
Research Assays																	
Humoral Assays (LMIV)	LMIV/CHI	SST													4		4
Humoral Assays (CHI)		SST			4			4							4		
Cellular Assays (CHI)		NaHep			30			30							30		
Cellular Assays (LMIV)		NaHep			10			10							10		10
Transcriptional Assays (CHI)		PAXgene			2.5			2.5							2.5		
Ex vivo		EDTA (pediatric)			1			1							1		1
Daily total			0	0	54.5	0	4	58.5	0	4	0	4	4	0	51.5	0	25
Study cumulative total			339.5	339.5	394	394	398	456.5	456.5	460.5	460.5	464.5	468.5	468.5	520	520	545

[1] Diary collection window +2 days

[2] After first positive NIH PCR (malaria diagnosis), PCR will NOT be repeated until Study day 27 unless clinically indicated.

[3] For study days 36, 37, 38, Arm 2 will ONLY have blood smear if symptomatic or if PCR cannot be resulted in real time. Blood smears will be resulted on the same day. A POSITIVE blood smear will be followed daily until 3 consecutive NEGATIVE blood smears have been recorded

[4] Arm 3 will have a phone visit. Arm 2 will have a visit ONLY if clinically indicated.

Appendix A: Clinic and Laboratory Procedures For Arm 2 and 3 (PfSPZ CVac #3)

		Clinic visits	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
		Study Day (Main Study)	57	59	60	62	63	64	64	65	65	66	67	71	84	112	141
		Days post-PfSPZ Cvac	0	2	3	5	6	7	7	8	8	9	10	14	27	55	84
		Visit windows (days)	-1 to +3	0	0	+/-1	0	0	0	0	0	0	0	+/-2	+/-2	+/-7	+/-7
			PfSPZ CVac #3	Clinic f/u	Clinic f/u	Clinic f/u	Clinic F/U; Pre-pat	AM Clinic F/U; Pre-pat	PM Phone [4] F/U; Pre-pat	AM Clinic F/U; Pre-pat	PM Phone [4] F/U; Pre-pat	Clinic F/U; Pre-pat	Clinic F/U; Pre-pat	Clinic f/u	Clinic f/u	Phone f/u	Pre-chall Visit
CLINICAL PROCEDURES																	
Complete medical history/ physical																	
Informed consent																	
Malaria Comprehension Exam																	
Pre-test/Post-test HIV counseling																	
Pregnancy Prevention Counseling			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Interim clinical evaluation			X	X	X	X	X	X	X	X	X	X	X	X	X		X
AE/SAE assessment			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Conmed review			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Diary[1]			X	X	X	X	X	X		X		X	X				
CQ dosing (Arm 3 ONLY)						X											
PYR Dose (Arm 2 ONLY)				X	X												
Treatment with Malarone																	
PfSPZ			X														
LABORATORY PROCEDURES	Designated Laboratory	Tube Type															
Screening/Safety Labs																	
CBC with differential	NIH Clinical Center	EDTA			3			3									3
ALT, Cr		SST			4			4									4
Urine/Serum Pregnancy Test (females only)		Urine Container or Lithium Hep	X														
Malaria Infection Assays																	
Real time NIH PCR (Arm 2 only) [2]	NIH Clinical Center	EDTA					(X)	1		1		1	(X)				
Retrospective LMIV qPCR	LMIV	EDTA					2	2		2		2	2				2
Peripheral Blood Smear[3]	NIH Clinical Center/LMI	EDTA (pediatric)					1	1		1		1	1				
Drug Assays																	
PYR level, (Arm 2 only)	WRAIR	EDTA (pediatric)					1						1				1
Research Assays																	
Humoral Assays (LMIV)	LMIV/CHI	SST												4	4		4
Humoral Assays (CHI)		SST			4			4						4			4
Cellular Assays (CHI)		NaHep			30			30						30			30
Cellular Assays (LMIV)		NaHep			10			10						10	10		20
Transcriptional Assays (CHI)		PAXgene			2.5			2.5						2.5			2.5
Ex vivo		EDTA (pediatric)			1			1						1	1		1
Daily total			0	0	54.5	0	4	58.5		4		4	4	51.5	15	0	71.5
Study cumulative total			545	545	599.5	599.5	603.5	662		666		670	674	725.5	740.5	740.5	812

[1] Diary collection window +2 days

[2] NIH PCR will be resulted within 24 - 36 hours

[3] For study days 64, 65, 66; Arm 2 will ONLY have blood smear if symptomatic or if PCR cannot be resulted in real time. Blood smears will be resulted on the same day. A POSITIVE blood smear will be followed daily until 3 consecutive NEGATIVE blood smears have been recorded

[4] Arm 3 will have a phone visit. Arm 2 will have a visit ONLY if clinically indicated.

Appendix A: Clinic and Laboratory Procedures For Arms 2 and 3 (CHMI)

		Clinic visits	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70
		Study Day (Main Study)	147	148	150	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	170	172	174	175	176
		Days post-PfSPZ CHMI	0	1	3	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	23	25	27	28	29
		Visit windows (days)	"+/-7"	"0"	"+1"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"+/-1"	"+/-1"	"+/-2"	"+/-2"	"+/-2"
			PfSPZ Chall	Phone f/u	Clinic f/u	Phone f/u	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u	Clinic f/u	Tx	Tx	Final Visit/Tx[4]
CLINICAL PROCEDURES																											
Complete medical history/ physical																											
Informed consent																											
Malaria Comprehension Exam																											
Pre-test/Post-test HIV counseling																											
Pregnancy Prevention Counseling			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Interim clinical evaluation			X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
AE/SAE assessment			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Conmed review			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Diary[1]			X	X	X	X	X	X	X	X	X																
Treatment with Malarone							(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	X
PfSPZ			X																								
LABORATORY PROCEDURES	Designated Laboratory	Tube Type																									
Screening/Safety Labs																											
CBC with differential	NIH Clinical Center	EDTA			3			3							3												3
ALT, Cr		SST			4			4							4												4
Urine/Serum Pregnancy Test (females only)		Urine Container or Lithium Hep	X																								
Malaria Infection Assays																											
Real time NIH PCR[2]	NIH Clinical Center	EDTA					1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Retrospective LMIV qPCR	LMIV	EDTA					2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Peripheral Blood Smear[3]	NIH Clinical Center/LMIV	EDTA (pediatric)					(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Drug Assays																											
PYR levels (research)	WRAIR	EDTA (pediatric)																									1
Research Assays																											
Humoral Assays (LMIV)	LMIV/CHI	SST													4												4
Humoral Assays (CHI)		SST			4			4							4												4
Cellular Assays (CHI)		NaHep			30			30							30												30
Cellular Assays (LMIV)		NaHep			10			10							10												20
Transcriptional Assays (CHI)		PAXgene			2.5			2.5							2.5												2.5
Ex vivo		EDTA (pediatric)			1			1							1												1
Daily total			0	0	54.5	0	3	57.5	3	3	3	3	3	3	61.5	3	3	3	3	3	3	3	3	3	3	3	72.5
Study cumulative total			812	812	866.5	866.5	869.5	927	930	933	936	939	942	945	1006.5	1009.5	1012.5	1015.5	1018.5	1021.5	1024.5	1027.5	1030.5	1033.5	1036.5	1039.5	1109

[1] Diary collection window +2 days

[2] After first positive NIH PCR (malaria diagnosis), subsequent samples will NOT be drawn until End of Study visit

[3] Blood smear will only be done in symptomatic subjects OR if a NIH qPCR cannot be resulted on the same day. A POSITIVE blood smear will be followed daily until 3 consecutive NEGATIVE blood smears have been recorded

[4] Final treatment visit labs can be drawn after the first dose of malarone has been received. Large blood draw can be split into two separate draws.

Appendix A: Clinic and Laboratory Procedures For Arms 4 (CHMI)

	Clinic visits		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
	Study Day (Arm 4)		-56	-7	-2	1	2	4	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	24	26	28	29	30
	Days post-PfSPZ CHMI		-56	-7	-2	0	1	3	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	23	25	27	28	29
	Visit windows (days)			"+/-5"	"+/-1"	0	"0"	"+1"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"0"	"+/-1"	"+/-1"	"+/-2"	"+/-2"	"+/-2"
			Screen	Enrollment/Baseline #1	Baseline #2	PfSPZ Chall	Phone f/u	Clinic f/u	Phone f/u	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u; Pre-pat	Clinic f/u	Clinic f/u	Tx	Tx	Final Visit/Tx[4]
CLINICAL PROCEDURES																														
Complete medical history/ physical			X																											
Informed consent			X																											
Malaria Comprehension Exam			X																											
Pre-test/Post-test HIV counseling			X																											
Pregnancy Prevention Counseling			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Interim clinical evaluation				X	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
AE/SAE assessment				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Conmed review			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Diary[1]						X	X	X	X	X	X	X	X	X																
Treatment with Malarone										(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	X	X
PfSPZ						X																								
LABORATORY PROCEDURES	Designated Laboratory	Tube Type																												
Screening/Safety Labs																														
CBC with differential	NIH Clinical Center	EDTA	3		3			3			3							3												3
ALT, Cr		SST	4		4			4			4							4												4
Hepatitis B, C, HIV testing		SST	8																											
EKG		Cardiology	X																											
Urine dipstick or UA		Urine Container	X																											
Urine/Serum Pregnancy Test (females only)		Urine Container or Lithium Hep	X			X																								
Malaria Infection Assays																														
Real time NIH PCR[2]	NIH Clinical Center	EDTA								1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Retrospective LMIV qPCR	LMIV	EDTA			2					2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Peripheral Blood Smear[3]	NIH Clinical Center/LMIV	EDTA (pediatric)								(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Research Assays																														
Humoral Assays (LMIV)	LMIV/CHI	SST			4													4												4
Humoral Assays (CHI)		SST		4	4			4			4							4												4
Cellular Assays (CHI)		NaHep		30	30			30			30							30												30
Cellular Assays (LMIV)		NaHep		10				10			10							10												20
Transcriptional Assays (CHI)		PAXgene		2.5	2.5			2.5			2.5							2.5												2.5
Ex vivo		EDTA (pediatric)			1			1			1							1												1
Daily total			15	46.5	50.5	0	0	54.5	0	3	57.5	3	3	3	3	3	3	61.5	3	3	3	3	3	3	3	3	3	3	71.5
56 day cumulative total			15	61.5	112	112	112	166.5	166.5	169.5	227	230	233	236	239	242	245	306.5	309.5	312.5	315.5	318.5	321.5	324.5	327.5	330.5	333.5	336.5	339.5	411

[1] Diary collection window +2 days

[2] After first positive NIH PCR (malaria diagnosis), subsequent samples will NOT be drawn until End of Study visit

[3] Blood smear will only be done in symptomatic subjects OR if a NIH qPCR cannot be resulted on the same day. A POSITIVE blood smear will be followed daily until 3 consecutive NEGATIVE blood smears have been recorded

[4] Final treatment visit labs can be drawn after the first dose of malarone has been received, Large blood draw can be split into two separate draws.

Appendix B: Cardiovascular Risk Assessment

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

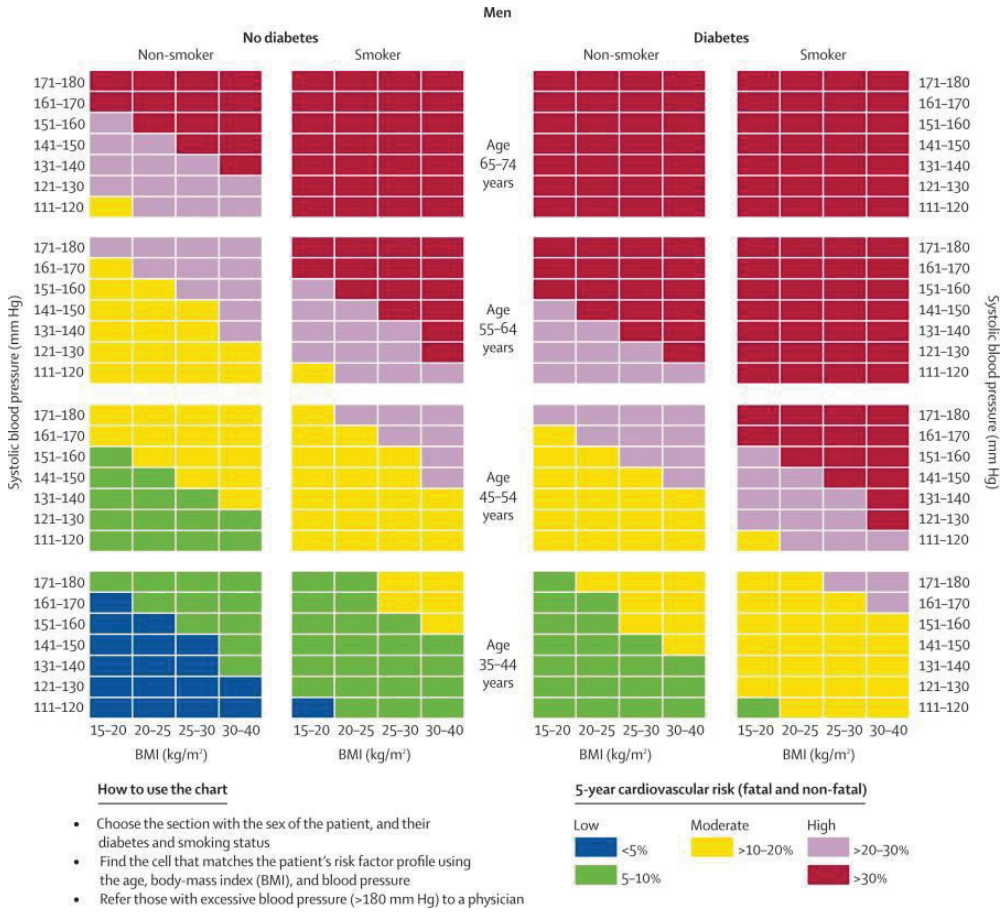
NHANES cardiovascular risk assessment includes the following assessments:

- Evaluation of **risk factors:** Calculated BMI [weight (kg)/height (m^2)], measured Systolic Blood Pressure, smoking status and known diabetes status as reported by the subject on review of medical history
- Evaluation of **5-year cardiovascular risk** using ***Figure 11*** (females) or ***Figure 12*** (males): Low, Moderate, High

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

Figure 12: NHANES I Classification for Women

Figure 13: NHANES I Classification for Men



Appendix C: Toxicity Table

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

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Erythema/Redness *	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Induration/Swelling **	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis
Bruising	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Pruritus	No interference with activity	Requiring PO or topical treatment > 24 hours or IV medications or steroids for ≤24 hours	Requiring IV medication or steroids for >24 hours	ER visit or hospitalization

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

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

Vital Signs * /Systemic adverse events	Mild (Grade 1)	Moderate(Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fever (°C) ** (°F) **	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	39.0 – 40 102.1 – 104	> 40 > 104
Tachycardia - beats per minute	101 – 115	116 – 130	> 130	ER visit or hospitalization for arrhythmia
Bradycardia - beats per minute***	50 – 54	45 – 49	< 45	ER visit or hospitalization for arrhythmia
Hypertension (systolic) - mm Hg	141 – 150	151 – 155	> 155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) - mm Hg	91 – 95	96 – 100	> 100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	< 80	ER visit or hospitalization for hypotensive shock
Shortness of breath	Shortness of breath with running or doing more than ordinary effort****	Shortness of breath on doing ordinary effort	Shortness of doing less than ordinary effort	Shortness of breath at rest
Palpitations	No or minimal interference with usual activities	Greater than minimal interference with usual activities or with associated symptoms	Inability to perform usual activities	ER visit or hospitalizatio n
Photosensitivity/ Rash	Localized rash with no medical intervention indicated	Localized rash with medical intervention indicated	Generalized rash with medical intervention indicated	ER visit or hospitalizatio n
Abdominal Pain	No or minimal interference with usual activities	Greater than minimal interference with usual activities	Inability to perform usual activities	ER visit or hospitalizatio n
Anorexia	Transient (< 24 hours) or intermittent anorexia with no or minimal interference with oral intake	Persistent anorexia resulting in decreased oral intake for 24 – 48 hours	Persistent anorexia resulting in minimal oral intake for > 48 hours OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Weight loss	5 to <10% from baseline, nutritional supplement not required	10 to <20% from baseline, nutritional supplement required	≥ 20% from baseline, invasive intervention for nutritional support required	Life-threatening consequences

Vital Signs * /Systemic adverse events	Mild (Grade 1)	Moderate(Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Back Pain	No or minimal interference with usual activities	Greater than minimal interference with usual activities	Inability to perform usual activities	ER visit or hospitalizatio n
Chills/Sweats/ Rigors	No or minimal interference with usual activities	Greater than minimal interference with usual activities	Inability to perform usual activities	ER visit or hospitalizatio n
Generalized Pruritus	Itching causing no or minimal interference with usual activities	Greater than minimal interference with usual activities	Inability to perform usual activities	ER visit or hospitalizatio n
Generalized Edema	Localized edema causing no or minimal interference with usual activities	Greater than minimal interference with usual activities	Inability to perform usual activities	ER visit or hospitalizatio n
Dizziness/Vertigo	Dizziness/vertigo causing minimal interference and above baseline	Dizziness/vertigo causing greater than minimal interference with usual social &	Dizziness/vertigo causing inability to perform usual social & functional activities	Disabling dizziness/vert igo causing inability to
Change in Vision	Change in vision causing minimal interference and above baseline	Difficulty seeing causing greater than minimal interference with usual social & functional activities	Difficulty seeing (diplopia, blurriness) causing inability to perform usual social & functional activities	Disabling change in vision (visual field loss, blindness) causing inability to perform basic
Sleep disorder (Insomnia, or Difficulty Sleeping, Drowsiness, etc)	Difficulty sleepingSleep disorder causing minimal interference and above baseline	Sleep disorder Difficulty sleeping causing greater than minimal interference with usual social & functional activities	Sleep disorder Difficulty sleeping causing inability to perform usual social & functional activities	Disabling Sleep disorder insomnia causing inability to perform basic self-care functions
Nausea/vomiting	No interference with activity or 1 – 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalizatio n for hypotensive shock

Vital Signs * /Systemic adverse events	Mild (Grade 1)	Moderate(Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue/Malaise	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Arthralgia	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Muscle weakness	No interference with activity	Greater than minimal interference with usual activities	Inability to perform usual activities	ER visit or hospitalization
Urticaria	No interference with activity	Requiring PO or topical treatment > 24 hours or IV medications or steroids for ≤24 hours	Requiring IV medication or steroids for >24 hours	ER visit or hospitalization
Tinnitus	Ringing in your ears causing no or minimal interference with usual activities	Greater than minimal interference with usual activities secondary to ringing in your ears	Inability to perform usual activities secondary to ringing in your ears.	ER visit or hospitalization
Peripheral Neuropathy	Tingling in your hands or feet causing no or minimal interference with usual activities	Tingling in your hands or feet causing greater than minimal interference with usual activities	Inability to perform usual activities due to tingling or pain in your hands or feet	ER visit or hospitalization

Vital Signs * /Systemic adverse events	Mild (Grade 1)	Moderate(Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Anxiety	No interference with activity	Repeated use of anti-anxiety medication > 24 hours or some interference with activity	Prevents daily activity	ER visit or hospitalization
Chest Pain (non-musculoskeletal)	Transient (< 24 hours) or intermittent chest pain with no or minimal interference	Persistent chest pain resulting in greater than minimal interference with usual activities	Persistent chest pain resulting in inability to perform usual activities secondary to chest pain	ER visit or hospitalization
Diarrhea	2 – 3 loose stools or < 400 gms/24 hours	4 – 5 stools or 400 – 800 gms/24 hours	6 or more watery stools or > 800gms/24 hours or requires outpatient IV hydration	ER visit or hospitalization

* Subject should be at rest for all vital sign measurements.
** Oral temperature; no recent hot or cold beverages or smoking.
*** When resting heart rate is between 60 – 100 beats per minute. Use clinical judgment when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.
**** Ordinary effort is that of the person himself / herself regards as previous effort of tolerance and usual life style (New York Heart Association definition)

Serum*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)**
Creatinine – mg/dL	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	> 2.5 or requires dialysis
Liver Function Tests – ALT, AST increase by factor	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	> 10 x ULN

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.
** The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example, a low sodium value that falls within a grade 3 parameter (125-127 mE/L) should be recorded as a grade 4 hyponatremia event if the subject had a new seizure associated with the low sodium value.
***ULN" is the upper limit of the normal range.
^Values adjusted according to the NIH clinical laboratory normal values

Hematology *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin (Female) - gm/dL	10.0 -11.0	8.5 - 9.9	7.5 – 8.4	< 7.5
Hemoglobin (Male) - gm/dL	12.0 – 13.0	10.0 - 11.9	8.0 - 9.9	< 8.0
WBC Increase - cell/mm^3	10,800 – 15,000	15,001 – 20,000	20,001 – 25, 000	> 25,000
WBC Decrease - cell/mm^3	2,500-3500	1,500 – 2499	1,000 – 1,499	< 1,000
Lymphocytes Decrease - cell/mm^3	750 – 1,000	500 – 749	250 – 499	< 250
Neutrophils Decrease - cell/mm^3	1000-1499	750-999	500 – 749	< 500
Platelets Decreased - cell/mm^3	125000-140000	100000-124000	25,000 – 99000	< 25,000

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.
** "ULN" is the upper limit of the normal range.
^Values adjusted according to the NIH clinical laboratory normal values

Urine*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Protein	Trace	1+	2+	Hospitalization or Dialysis
Glucose	Trace	1+	2+	Hospitalization for Hyperglycemia
Blood (microscopic) – red blood cells per microliter (rbc/µL)	17 – 30	31– 60	> 60 and/or gross blood	Hospitalization or packed red blood cells (PRBC) transfusion

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.
^Values adjusted according to the NIH clinical laboratory normal values

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