A Phase I Challenge Study to Evaluate Safety, Immunogenicity, and Efficacy of a Malaria Vaccine (rCSP adjuvanted with AP 10-602 [GLA-LSQ]), in Healthy Adults

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STATEMENT OF COMPLIANCE

The study trial will be carried out in accordance with Good Clinical Practice (GCP) and as required by the following:

- United States Code of Federal Regulations (CFR) 45 CFR Part 46: Protection of Human Subjects
- Food and Drug Administration (FDA) Regulations, as applicable: 21 CFR Part 50 (Protection of Human Subjects), 21 CFR Part 54 (Financial Disclosure by Clinical Investigators), 21 CFR Part 56 (Institutional Review Boards), 21 CFR Part 11, and 21 CFR Part 312 (Investigational New Drug Application), 21 CFR 812 (Investigational Device Exemptions)
- International Conference on Harmonisation: Good Clinical Practice (ICH E6); 62 Federal Register 25691 (1997); and future revisions
- Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, Report of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research
- National Institutes of Health (NIH) Office of Extramural Research, Research Involving Human Subjects, as applicable
- National Institute of Allergy and Infectious Diseases (NIAID) Clinical Terms of Award, as applicable
- Applicable Federal, State, and Local Regulations and Guidance

SIGNATURE PAGE

The signature below provides the necessary assurance that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH E6 Good Clinical Practice (GCP) guidelines.

I agree to conduct the study in compliance with GCP and applicable regulatory requirements.

I agree to conduct the study in accordance with the current protocol and will not make changes to the protocol without obtaining the sponsor's approval and IRB/IEC approval, except when necessary to protect the safety, rights, or welfare of subjects.

Site Investigator Signature:

Signed: _____ Date: _____

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Associate Professor of Pediatrics
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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
AESI	Adverse Event of Special Interest
BMF	Biologics Master File
BSC	Biological Safety Cabinet
CFR	Code of Federal Regulations
CHMI	Controlled Human Malaria Infection
CIOMS	Council for International Organizations of Medical Sciences
CONSORT	Consolidated Standards of Reporting Trials
CFR	Code of Federal Regulations
CRF	Case Report Form
CRO	Contract Research Organization
CSP	Circumsporozoite Protein
CSR	Clinical Study Report
CVD	Center for Vaccine Development
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
FWA	Federal wide Assurance
GCP	Good Clinical Practice
GCRC	General Clinical Research Center

GLA	Glucopyranosyl Lipid A
GLP	Good Laboratory Practice
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IDES	Internet Data Entry System
IDRI	Investigational Diseases Research Institute
IDS	Investigational Drug Service
IEC	Independent or Institutional Ethics Committee
IM	Intramuscular
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
JAMA	Journal of the American Medical Association
LSQ	Liposome *Quillaja saponaria* formulation
MedDRA ®	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MTG	Monothioglycerol
N	Number (typically refers to subjects)
NCI	National Cancer Institute, NIH, DHHS
NDA	New Drug Application
NEJM	New England Journal of Medicine
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS

NIH	National Institutes of Health
OCRA	Office of Clinical Research Affairs, DMID, NIAID, NIH, DHHS
OHRP	Office for Human Research Protections
ORA	Office of Regulatory Affairs, DMID, NIAID, NIH, DHHS
PBMC	Peripheral Blood Mononuclear Cells
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetics
PSC	Protein Sciences Corporation
QA	Quality Assurance
QC	Quality Control
QMP	Quality Management Plan
rCSP	recombinant Circumsporozoite Protein
RUNMC	Radboud University Nijmegen Medical Center
SAE	Serious Adverse Event/Serious Adverse Experience
SDCC	Statistical and Data Coordinating Center
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
UMB	University of Maryland, Baltimore
US	United States
USP	United States Pharmacopeial Convention
VTEU	Vaccine and Treatment Evaluation Units
WBC	White Blood Cells
WFI	Water For Injection
WHO	World Health Organization

PROTOCOL SUMMARY

Title:	A Phase I Challenge Study to Evaluate Safety, Immunogenicity, and Efficacy of a Malaria Vaccine (rCSP adjuvanted with AP 10-602 [GLA-LSQ]), in Healthy Adults
Phase:	1
Population:	56 healthy male and female subjects aged 18 to 45 years, inclusive.
Number of Sites:	Single Site - Center for Vaccine Development (CVD), University of Maryland School of Medicine, Baltimore, Maryland
Subject Participation Duration:	117 – 510 days, including screening
Description of Agent or Intervention:	Recombinant Circumsporozoite Protein (rCSP) unadjuvanted and adjuvanted with AP 10-602 [Glucopyranosyl Lipid A (GLA) in liposome *Quillaja saponaria* 21 formulation (LSQ)]
Objectives:	Primary: • To assess the safety and reactogenicity of candidate rCSP/ AP 10-602 [GLA-LSQ] malaria vaccine when administered intramuscularly on a 1, 29, and 85 day schedule to healthy malaria-naïve adults aged 18-45 years. Secondary: • To assess immunogenicity of rCSP/AP 10-602 [GLA-LSQ] malaria vaccine when administered intramuscularly on a 1, 29, and 85 day schedule. • To assess the preliminary efficacy of candidate rCSP/AP 10-602 [GLA-LSQ] malaria vaccine against infection with *Plasmodium falciparum* malaria (defined as *P. falciparum* asexual parasitemia or a delay in patency of infection >2 days versus unimmunized infectivity controls) under Controlled Human Malaria Infection (CHMI). Exploratory:

- To collect peripheral blood mononuclear cells (PBMC) to allow for future assessment of immune responses against circumsporozoite protein and related antigens at days 1, 29, 85, and 113 post-enrollment in groups 4 and 5

Description of Study Design:

This clinical trial is a phase I, single-site, dose escalation study of the recombinant circumsporozoite protein (rCSP) antigen, based on a full-length *P. falciparum* circumsporozoite protein produced via a novel *Pseudomonas fluorescens* expression platform, administered with AP 10-602 [Glucopyranosyl Lipid A-liposome-*Quillaja saponaria* 21 formulation (GLA-LSQ)], in 40 healthy adult volunteers. Ten healthy adult volunteers will receive rCSP alone. Six additional healthy adults will be recruited to serve as infectivity controls for CHMI. The clinical trial will be conducted under U.S. FDA IND at the CVD Outpatient Facility and at the University of Maryland Medical Center's General Clinical Research Center (GCRC).

Healthy, malaria-naïve volunteers ages 18 to 45 years will be recruited into the study. Three dose-escalating groups of 10 participants each will receive 3 intramuscular (IM) doses of vaccine (days 1, 29, and 85) based on the rCSP component: 10µg rCSP + AP 10-602 [GLA-LSQ] (Group 1), 30µg rCSP + AP 10-602 [GLA-LSQ] (Group 2), and 60µg rCSP + AP 10-602 [GLA-LSQ] (Group 4). One additional group to receive 30µg rCSP without adjuvant will be enrolled (Group 3) on the same schedule as Group 2. Six volunteers will be recruited for malaria challenge (Group 6). Groups will be vaccinated in a stepwise manner following a "telescoped" design. For each group, two participants will be immunized first, if there are no safety concerns identified after 24 hours that trigger halting rules as per Section 9.5, then the other 8 volunteers will be immunized as per schedule. If safety and reactogenicity are acceptable after Group 1 dose 1, per "Group Halting Rules," Section 9.5, Group 1 will receive dose 2 and Groups 2 and 3 will each receive dose 1. If safety and reactogenicity are acceptable after Groups 2 and 3 receive dose 1, then Groups 2 and 3 will receive dose 2. Safety and reactogenicity data from groups 1-3 up to day 57 will then be reviewed by the SMC. The SMC will electronically review all available safety data for enrolled study Groups 1-3 including laboratory monitoring done 7 days after the second vaccination and make recommendation to proceed with the enrollment of Groups 4- 6. If immunogenicity analysis conducted 28 days post-2nd dose in Groups 1, 2 and 3 show promise (at least fourfold increase in geometric mean anti-CSP antibody or geometric mean anti-CSP titer of 20), the 10 volunteers in Group 5 will receive the lowest rCSP dose that gives this predefined immunogenic response ((either 10 or 30µg rCSP) + AP 10-602 [GLA-LSQ]), otherwise Group 5 will receive 60µg rCSP + AP 10-602 [GLA-LSQ]. Once dose is determined, Groups 4 and 5 will receive dose 1. If safety and reactogenicity are acceptable after Group 4 and Group 5 dose 1, then Groups 4 and 5 will receive dose 2. For Groups 1-5, if safety and reactogenicity are acceptable after dose 2, then each Group will receive dose 3. Both groups 4 and 5 will undergo CHMI together at 28 days post-3rd dose. For CHMI, Groups 4 and 5 will be joined by 6 volunteers to serve as infectivity controls (Group 6).

Subjects will be observed for adverse events, and vital signs will be measured before and 30 minutes after each vaccination. Participants will be telephoned the day following each vaccination to review symptoms. Once the first vaccinations for Group 1 are complete (10 volunteers receiving the 10µg rCSP + AP 10-602 [GLA-LSQ] dose) and the memory aid and one-week post-vaccination safety lab values are recorded, the PI and Independent Safety Monitor (ISM) will review the safety data and determine if the study will proceed to the next dose level. The same process will occur after first vaccinations for Groups 2-5 are completed. Volunteers will complete a memory aid for 7 days following each vaccination with rCSP+AP 10-602 [GLA/LSQ] and rCSP. Complete blood counts, alanine aminotransferase (ALT), and serum creatinine will be measured 7 and 28 days after each vaccination, and 84 days after the last vaccination. Participants will be contacted by telephone 12 months (365 days) after last vaccination to document any subsequent severe adverse events.

CHMI will occur at ~28 days post-3rd vaccination in Group 4 and 5, together with 6 infectivity controls (Group 6). Standardized procedures will be followed, including daily malaria diagnostics 8-18 days post-CHMI. Participants who do not test positive for malaria by 18 days post-CHMI will be followed every other day in clinic for malaria testing until 28 days post-CHMI.

Estimated Time to Complete Enrollment: Up to 4 weeks for each group.

Figure 1-1: Schematic of Study Design

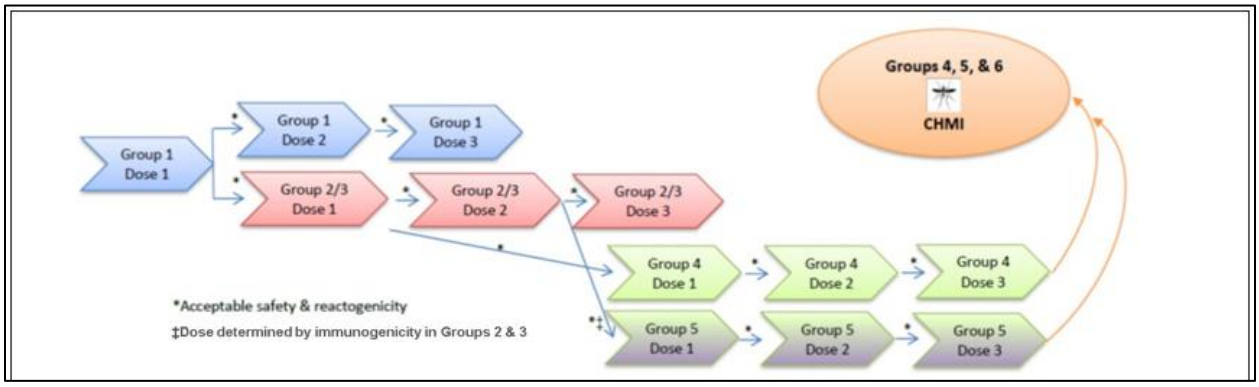

Table 1-1: Study Group Assignments

Group	Study Product	rCSP Dose Level (µg/dose)	AP 10-602 [GLA-LSQ] Dose Level (µg of GLA per dose/LSQ per dose)	Sample Size
1	rCSP/ AP 10-602 [GLA-LSQ]	10	5/2	10
2	rCSP/ AP 10-602 [GLA-LSQ]	30	5/2	10
3	rCSP	30	None	10
4	rCSP/ AP 10-602 [GLA-LSQ]	60	5/2	10
5	rCSP/ AP 10-602 [GLA-LSQ]	10, 30 or 60	5/2	10
6	None	None	None	6
				Total = 56

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

According to WHO estimates, about 428,000 people died from malaria in 2015 and more than 200 million cases are detected each year (1). Given the incidence of this disease, development of a malaria vaccine to prevent and ultimately eliminate the disease has been a priority.

The circumsporozoite protein (CSP) is the major surface protein of the sporozoite (the form injected by the mosquito that invades the liver at the beginning of the parasite life cycle) and forms a dense coat on the parasite's surface, which contains a region I at the NH2-terminal, a centrally-located repeat region, and the COOH-terminus contains a type I thrombospondin repeat region. CSP is one of the key targets recognized by the host immune system and has emerged as one of the prime candidates for an antimalarial vaccine. A recent Phase 3 clinical trial of an RTS,S/AS01 vaccine, a virus like particle consisting of a fragment (central repeat and C-terminal regions) of the CSP fused to a Hepatitis B surface antigen (developed by Glaxo Smith Kline), showed 30.1% vaccine efficacy in infants (2). Immunogenicity studies in various RTS,S/AS01 vaccine trials also established that a high level of anti-CSP antibody response correlated with reduced clinical malaria episodes (3). The partial protective efficacy of the RTS,S/AS01 vaccine has demonstrated the viability of a malaria vaccine based on CSP and suggests the need for further improvement of a CSP-based vaccine.

2.1.1 Safety of Controlled Human Malaria Infection

A select number of institutions employ the use of the CHMI technique as a tool to evaluate antimalarial drug and vaccine efficacy (4-7). To date, the centers with active challenge programs include The University of Maryland, Center for Vaccine Development, the Walter Reed Army Institute of Research (WRAIR), Naval Medical Research Center (NMRC), Oxford University and Nijmegen University. Both the Johns Hopkins School of Medicine and Seattle Biomedical Institute have conducted 1-2 malaria challenge trials each in recent years. WRAIR has successfully used a similar CHMI model in over 400 volunteers, with more than 300 patent malaria infections developing in the volunteers. They have experienced no deaths, episodes of severe malaria, anaphylactic emergencies, serious or unexpected complications or recrudescent malaria infections in any of these individuals (Kent Kester, MD, personal communication). The NMRC has a similarly robust experience with malaria challenge studies and an equivalent safety record (8, 9).

In the cumulative published experience, involving ~1400 challenge events in combined published and unpublished series, no deaths have been reported. The collective experience has clearly established that the *in vivo* malaria challenge model can be safely employed to test the efficacy of drugs, subunit vaccines and whole parasite vaccines. Risks associated with malaria challenge include local inflammatory reactions, lymphadenitis, persistent pruritus and larger local reactions involving the whole forearm, allergic reactions to mosquito bites and the development of malaria infection. Additional risks include possible side effects of the anti-malarial medication taken. Artemether/lumefantrine (Coartem®) side effects include nausea, vomiting, arthralgia, asthenia, chills, dizziness, fatigue, headache, myalgia, palpitations, fever, and sleep disorder. Atovaquone/proguanil side effects include nausea, vomiting, abdominal pain, anorexia, diarrhea, headache, cough and rarely, anemia, oral ulcerations, insomnia, fever, edema, rash and alopecia. The study team extensively discusses these medications and their possible side effects in detail both as part of the informed consent process and before initiation of treatment for participants who become infected after malaria-infection challenge.

Table 2-1: Number of volunteers undergoing sporozoite challenge

Institute	Date	Strain	Volunteers (n)	SAE[b]
Army (WRAIR)	Since 1985	NF54	126	None
		3D7	815	None
		7G8	42	None
Navy (NMRC)	Since 1998	NF54	135	None
		other	Unknown	None
	Before 1998		Unknown	None
RUNMC[a]	Since 2001	NF54	92 challenged, 87 infected	2
Oxford		3D7	240	None
UMB-CVD[a]	Before 1991	NF54	40	None
	After 2008	NF54	243 challenged, 103	None
		7G8	infected	None

[a] RUNMC refers to Radboud University Nijmegen Medical Center and UMB-CVD refers to the University of Maryland, Baltimore, Center for Vaccine Development

[b] SAE refers to a serious adverse event that possibly, probably or definitely related to the CHMI

There is also the possibility of complications of malaria, which are seen during naturally acquired malaria when diagnosis and treatment are delayed and high levels of parasitemia develop. Under the carefully controlled conditions of this study, the chance of such complications is unlikely and the risk of death from malaria infection is very small.

As documented over the past 30 years, controlled human malaria infection (CHMI) via the bites of infected mosquitoes has been well tolerated. One male participant, who apparently did not

develop infection as evidenced by sequential negative PCR tests, had a myocardial infarction 2 days after treatment with chloroquine; in retrospect, this participant appears to have had a moderate risk of a coronary event within ten years (10, 11). In addition, a cardiac event was reported in a female participant in The Netherlands at Radboud University Nijmegen Medical Centre (RUNMC) (12). This 20-year-old participant received three doses of a recombinant malaria protein vaccine containing aluminum hydroxide. The volunteer became parasitemic on Day 11 after challenge (44 parasites/µl). Two days after completing a course of treatment with Riamet® (artemether/lumefantrine) for malaria infection, the participant was awoken from sleep with acute chest pain and required hospitalization (Day 16 post-challenge). A diagnosis of acute coronary syndrome with limited myocardial necrosis of the inferior wall was made based upon the pain, electrocardiogram (ECG) findings of <1mm ST segment elevation inferiorly and cardiac enzymes. A cardiac MRI was negative for evidence of atherosclerotic disease. The etiology of the event is unclear. Subsequent evaluation revealed low hemoglobin on the day of challenge (6.9 mmol/L (normal range 8-12 mmol/L)), and an abnormally elevated lumefantrine metabolite (desbutyl-lumefantrine) levels. Cholesterol, coagulopathy and autoantibody studies were normal or negative. No toxin screen was performed at the time of ER presentation but analysis of hair samples revealed no presence of illicit drugs. It is unclear whether the event was related to her malaria infection, the test vaccine that she received, the medicine used to treat malaria or other etiology. No reports of cardiovascular pathology or events have been reported with natural malaria and autopsy studies (n = 99, personal communication) reveal sequestration of parasites in all organs but no evidence of myocardial damage (13, 14). One episode of coronary spasm in a 17 year old male has been reported by Novartis after treatment of falciparum malaria with Coartem® (artemether-lumefantrine). A cannabis positive urine test was reported concomitantly (personal communication). The malaria challenge community worldwide has conducted extensive discussions and the collective opinion of this community (WRAIR, UMD, NMRC, Oxford, RUNMC) is that the RUNMC case was not related to the malaria challenge event. There have been no other published cases with complications resulting in severe disability or death. Furthermore, no hospitalization has been required in past studies for treatment due to failure to respond to an anti-malaria drug or for any other reason related to the study.

The CVD has extensive human clinical trials experience with irradiated sporozoite vaccination and CHMI from the late 1960s through to the present (8, 11, 15-18). The challenge methodology at the University of Maryland utilizes several procedures to diminish the effects of confounding clinical and laboratory variables and strives to improve the safety controlled human malaria infection in volunteers. Moreover, investigators at the CVD are part of a consortium of international scientists working with the World Health Organization to standardize CHMI methodologies and standard operating procedures (19, 20).

2.2 Rationale

One of the approaches to improve the efficacy of a CSP-based subunit vaccine is to utilize the full length protein since, for example, the N-terminus has been shown to contain the ligand binding domain and multiple human HLA-restricted epitopes. Further, it has been shown that natural immunity to the N-terminus of CSP is associated with protection (21). However, the expression, purification and scale-up of a properly folded full length CSP have presented great challenge. The recombinant CSP (rCSP) utilizes a novel *Pseudomonas fluorescens* expression platform from Pfenex, Inc., which yields soluble, full-length *P. falciparum* rCSP, followed by straightforward downstream processing due to rCSP being secreted into the periplasmic space of the *P. fluorescens* cell line. Pfenex, Inc. has established the process to produce a full length rCSP under cGMP compliance at a scalable level and have produced rCSP suitable for clinical usage.

As with most all subunit malaria vaccines, rCSP-based vaccines are designed to be administered with adjuvant to enhance the immunogenic response. AP 10-602 consists of Glucopyranosyl Lipid Adjuvant (GLA) in a liposomal formulation with the saponin liposome *Quillaja saponaria* QS-21 formulation (LSQ). In the proposed study, rCSP will be mixed with AP 10-602 [GLA-LSQ] before vaccination. The GLA is a synthetic monophosphoryl lipid A-like molecule, which is a Toll-like receptor 4 agonist that activates specific cells of the immune system. AP 10-602 [GLA-LSQ] was selected to be used in combination with rCSP to enhance the immune response of the antigen. The AP 10-602 [GLA-LSQ] adjuvant, as tested in preclinical and clinical studies, has been shown to be effective and safe. In addition, when rCSP is combined with AP 10-602 [GLA-LSQ], the rCSP vaccine + GLA-LSQ adjuvant shows promising biological activity in mice. This adjuvant system was developed and manufactured by the Infectious Diseases Research Institute (IDRI). Two clinical trials of vaccines containing AP 10-602 [GLA-LSQ] adjuvant are on-going and results are not yet publicly available [NCT02508376 AP 10-602 and NCT02647489]. Additionally, GLA and QS-21 have been used as adjuvants in separate clinical studies and no safety signal has been detected (22-24).

The rCSP candidate malaria vaccine antigen will be administered in conjunction with the AP 10-602 [GLA-LSQ] adjuvant via intramuscular injection in this first-in-human phase 1 clinical trial to assess the safety, immunogenicity, and preliminary efficacy of rCSP/AP 10-602[GLA-LSQ] in healthy subjects against controlled human malaria infection (CHMI) with *P. falciparum* malaria. The rationale to allow for administration of a low dose to Group 5 is based on evidence from another CSP-based vaccine that lower doses might translate to better protection against CHMI (25). The study is designed to compare the lowest dose of rCSP that provides a minimum threshold of anti-CSP antibody (a fourfold increase in geometric mean anti-CSP antibody or a

geometric mean anti-CSP titer of $\geq$ 20) to the high dose of 60 micrograms of rCSP. If, however, the lower doses of rCSP (10 or 30µg) do not provide this minimum threshold immune response, the high dose of 60 µg rCSP will be administered to both Groups 5 and 6.

In CHMI trials, a group of vaccinated volunteers and a group of unimmunized infectivity controls are inoculated with *P. falciparum* sporozoites via the bite of infected female Anopheline mosquitoes in well-controlled laboratory settings. Participants are then actively followed for bloodstream malaria infection and are treated when positive. Vaccine efficacy is calculated by comparing malaria attack rates in vaccinees compared to infectivity controls. The use of CHMI is accepted as a powerful tool for investigating malaria vaccine efficacy, and to support or refute further clinical development (26).

2.2.1 Preclinical Investigations

The rCSP administered with adjuvant GLA-LSQ is immunogenic in animals as measured by ELISA for antibody titers and ELISPOT for T cell responses, and is shown to be efficacious by demonstrating 90% or greater protection at 20ug doses of rCSP in a malaria parasite challenged mouse model, which utilized recombinant rodent malaria parasites (*P. berghei*) with a *P. falciparum* CSP insert as the challenge parasites (27).

2.2.1.1 Toxicity Study of rCSP/GLA-LSQ in New Zealand White Rabbits

A repeat-dose Good Laboratory Practice (GLP) toxicity study entitled "Repeat-dose Toxicity, Local Tolerance, and Immunogenicity of Recombinant Circumsporozoite Protein with Glucopyranosyl Lipid Adjuvant Liposome-QS-21 (GLA-LSQ) in Rabbits," was conducted to assess the local tolerance, toxicity and immunogenicity. The rCSP antigen, the adjuvant (GLA-LSQ), the test article (rCSP with GLA-LSQ), and the control article (Sodium Chloride for Injection, USP) were administered by intramuscular injection on Days 0, 14, 28, and 42 to male and female New Zealand White Hra:(NZW)SPF rabbits during a 70 day study period.

When administered via intramuscular injection of rCSP and/or GLA-LSQ to rabbits at doses of 10 µg GLA-20 µg QS-21 (adjuvant only); 60 µg rCSP (the vaccine antigen only); and the vaccine 30 µg rCSP/5 µg GLA-10 µg QS-21 and 60 µg rCSP/10 µg GLA-20 µg QS-21 were safe and well tolerated. There were no deaths. There were no test article-related body weight changes, body temperature changes, changes in food consumption or ophthalmic changes attributed to the adjuvant, drug product, or test article. Although there were instances of mild redness and swelling

at the site of injection (predominately within 24 hours after injection), there was no greater incidence in any one group. There were slight elevations in CRP and statistically significant increases in fibrinogen corresponding to skeletal muscle inflammation predominately in the adjuvant groups.

Minimal microscopic injection site observations of vasculitis, hemorrhage and inflammation within the subcutis, and skeletal muscle were noted on Day 44. Groups containing adjuvant (Groups 2, 4, 5) also contained animals with minimal perineuronal vasculitis, hemorrhage and/or inflammation suggesting travel along perineuronal fascial planes. Lesions were most common in the injection site used on Day 42 and quickly dissipated as time progressed. On Day 70, there were findings in 5 animals (2 saline, 2 adjuvant, and 1 test article 60 µg rCSP/10 µg GLA-20 µg QS-21) that were unlikely due to test article and/or adjuvant since they were minimal in severity, sporadic in occurrence and did not rise above control. Thus, the GLP toxicity study supports a clinical trial design of three injections of rCSP/GLA-LSQ for up to a 60ug dose of rCSP with or without GLA-LSQ adjuvant containing up to 10 µg of GLA and 20 µg of QS-21.

Administration of rCSP in combination with GLA-LSQ adjuvant resulted in anti-rCSP antibody titers that were detected at Day 44 and increased slightly at Day 70 at the highest dose level. Statistical analysis of the various study factors indicated that titers varied significantly across all successive time periods. In addition, titers varied significantly as a function of dose. While all the rCSP dosing groups differed in titer from the no rCSP control groups (groups 1 and 2), a significant difference in titer was only observed between the unadjuvanted group (group 3) and the high dose adjuvanted group (group 5) at the Day 70 time point.

Given the immunogenicity, efficacy, and the safety profiles of the rCSP/GLA-LSQ in animal models, the rCSP/GLA-LSQ vaccine is proposed to be tested in a first-in-human study.

2.2.1.2 Preclinical Investigations of GLA-LSQ Adjuvant

Multiple nonclinical studies in mice have demonstrated the immunostimulatory properties of GLA-LSQ adjuvant with no significant safety signals.

2.2.2 Clinical Investigations

The rCSP vaccine antigen has not been tested in humans. Furthermore, the combination of the rCSP and the AP 10-602 [GLA-LSQ] adjuvant has not been administered to humans. The proposed clinical trial of the rCSP/AP 10-602 [GLA-LSQ] vaccine candidate will be the first in humans.

However, one Phase 1 study of a malaria vaccine that uses the AP 10-602 [GLA-LSQ] adjuvant is ongoing (NCT02647489). Individually, GLA and QS-21 have been used as adjuvants in various clinical studies (22-24).

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

The rCSP vaccine antigen has never been administered to humans. Possible local vaccine reactions may include pain, tenderness, swelling, erythema, and induration at the injection site. It is possible that systemic reactions such as fever, chills, myalgia, arthralgia, nausea, vomiting, headache, dizziness, malaise and fatigue, may also occur.

The adjuvant AP 10-602 [GLA-LSQ] is being administered to humans in other clinical trials as noted in Section 2.2.2, and no known safety signal has been reported.

As with the administration of any vaccine, and regardless of the precautions taken, the risk of a serious, or even life-threatening, allergic reaction is possible. Emergency equipment and supplies, including epinephrine, diphenhydramine, and prednisone, should be available to treat acute allergic symptoms as per standard medical procedures. As with all research, there is the remote possibility of risks that are unknown or that cannot be foreseen based on what is currently known about the product.

No interactions of the rCSP with other products are known. Immunosuppressive agents are likely to interfere with the development of an immune response; however, topical application of over the counter anti-inflammatory products may alleviate some of the symptoms of local reactions without interference with the immune response.

Animal reproductive studies have not been conducted with the rCSP/GLA-LSQ vaccine. It is not known whether the vaccine can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity. Vaccination might result in unknown risks to the fetus. Consequently, women should avoid becoming pregnant for up to 30 days after the most recent vaccination or for 30 days after CHMI. It is also not known if antibodies induced by the vaccine are excreted in breast milk.

In groups undergoing CHMI, there is a risk that the participant will develop malaria. Because therapy will be initiated with the detection of parasitemia by qPCR, dangerously high levels or prolonged duration of parasitemias are not likely to occur. However, when infections become symptomatic, most volunteers can be expected to experience mild malaria symptoms that

commonly include headache, myalgia, chest pain, fever, chills, sweats, nausea, vomiting, and diarrhea. Typical onset of symptoms and signs of malaria occur approximately 9 days post-inoculation.

Artemether/lumefantrine (Coartem®) side effects include nausea, vomiting, arthralgia, asthenia, chills, dizziness, fatigue, headache, myalgia, palpitations, fever, and sleep disorder. Atovaquone/proguanil side effects include nausea, vomiting, abdominal pain, anorexia, diarrhea, headache, cough and rarely, anemia, oral ulcerations, insomnia, fever, edema, rash and alopecia.

2.3.2 Known Potential Benefits

There are no direct benefits to participants from participating in the study.

3 OBJECTIVES AND OUTCOME MEASURES

3.1 Study Objectives

3.1.1 Primary Objective

- To assess the safety and reactogenicity of candidate rCSP/AP 10-602 [GLA-LSQ] malaria vaccine when administered intramuscularly on a 1, 29, and 85 day schedule to healthy malaria-naïve adults aged 18-45 years.

3.1.2 Secondary Objectives

- To assess immunogenicity of rCSP/AP 10-602 [GLA-LSQ] malaria vaccine when administered intramuscularly on a 1, 29, and 85 day schedule.

- To assess the preliminary efficacy of candidate rCSP/AP 10-602 [GLA-LSQ] malaria vaccine against infection with *Plasmodium falciparum* malaria (defined as *P. falciparum* asexual parasitemia or a delay in patency of infection >2 days versus unimmunized infectivity controls) under Controlled Human Malaria Infection (CHMI).

3.1.3 Exploratory Objectives

- To collect peripheral blood mononuclear cells (PBMC) to allow for future assessment of immune responses against circumsporozoite protein and related antigens at days 1, 29, 85, and 113 post-enrollment in groups 4 and 5

3.2 Study Outcome Measures

3.2.1 Primary Outcome Measures

- Occurrence of solicited local reactions within 7 days following vaccination (day of vaccination and 7 subsequent days)
- Occurrence of solicited systemic reactions within 7 days following vaccination (day of vaccination and 7 subsequent days)

- Occurrence of severe (Grade 3) laboratory AE considered related to vaccination within 7 days following vaccination (day of vaccination and 7 subsequent days), according to the MedDRA classification.
- Occurrence of unsolicited AEs considered related to vaccination and that are severe (Grade 3) within 28 days following vaccination (day of vaccination and 28 subsequent days), according to the Medical Dictionary for Regulatory Activities (MedDRA classification)
- Occurrence of serious adverse events (SAEs) considered related to vaccination within 28 days following vaccination (day of vaccination and 28 subsequent days), according to the MedDRA classification
- Occurrence of SAEs at any point during the planned participant follow-up period, according to the MedDRA classification
- Occurrence of AESIs at any point during the planned participant follow-up period, according to the MedDRA classification

3.2.2 Secondary Outcome Measures

- Antibody titers against the malaria circumsporozoite antigen at days 1, 8, 29, 36, 57, 85, 92, 113, 169 and 253 post-enrollment in groups 1-5.
- Presence of *P. falciparum* asexual parasitemia following experimental malaria challenge.
- Time to *P. falciparum* asexual parasitemia following experimental malaria challenge.

3.2.3 Exploratory Outcome Measures

- Collection of samples to allow for future assessment of immune responses against the malaria circumsporozoite antigen and related antigens at days 1, 29, 85, and 113 post-enrollment in groups 4 and 5.

The treatment endpoint is defined as “two qPCR positive tests or a thick blood smear positive for malaria”. The time to *P. falciparum* asexual parasitemia is defined as the time until the treatment endpoint is met. This will be the time to the second qPCR positive test for those who met the treatment endpoint through two qPCR tests, and the time to the positive thick blood smear for

those who met the treatment endpoint that way. Similarly, the presence of asexual parasitemia is defined as meeting the treatment endpoint.

4 STUDY DESIGN

This clinical trial is a phase I, single-site, dose escalation study of the recombinant circumsporozoite protein (rCSP) antigen, based on a full-length *P. falciparum* circumsporozoite protein produced via a novel *Pseudomonas fluorescens* expression platform, administered with AP 10-602 [Glucopyranosyl Lipid A (GLA) in liposome *Quillaja saponaria* formulation (LSQ)], in 40 healthy adult volunteers. Ten volunteers will receive rCSP alone. Six additional healthy adults will be recruited to serve as infectivity controls for CHMI. The clinical trial will be conducted under U.S. FDA IND at the CVD Outpatient Facility and the University of Maryland Medical Center's General Clinical Research Center.

Healthy, malaria-naïve volunteers ages 18 to 45 years will be recruited into the study. Three dose-escalating groups of 10 participants each will receive 3 intramuscular (IM) doses of vaccine (days 1, 29, 85) based on the rCSP component: 10µg rCSP + AP 10-602 [GLA-LSQ] (Group 1), 30µg rCSP + AP 10-602 [GLA-LSQ] (Group 2), and 60µg rCSP + AP 10-602 [GLA-LSQ] (Group 4). One additional group to receive 30µg rCSP without adjuvant will be enrolled (Group 3) on the same schedule as Group 2. Six volunteers will be recruited for malaria challenge (Group 6). Groups will be vaccinated in a stepwise manner following a "telescoped" design. For each group, two participants will be immunized first; if there are no safety concerns identified after 24 hours that trigger halting rules as per Section 9.5, then the other 8 volunteers will be immunized as per schedule. If safety and reactogenicity are acceptable after Group 1 dose 1, Group 1 will receive dose 2 and Groups 2 and 3 will each receive dose 1. If safety and reactogenicity are acceptable after Groups 2 and 3 receive dose 1, then Groups 2 and 3 will receive dose 2. Safety and reactogenicity data from groups 1-3 up to day 57 will then be reviewed by the SMC. The SMC will electronically review all available safety data for enrolled study Groups 1-3 including laboratory monitoring done 7 days after second vaccination and make recommendation to proceed with the enrollment of Groups 4- 6.

The dose for Group 5 will depend on immunogenicity analysis from groups 1, 2 and 3. If immunogenicity analysis conducted 28 days post-2nd dose in Groups 1, 2 and 3 show promise (at least fourfold increase in geometric mean anti-CSP antibody or geometric mean anti-CSP titer of 20), the 10 volunteers in Group 5 will receive the lowest rCSP dose that gives this predefined immunogenic response ((either 10 or 30µg rCSP) + AP 10-602 [GLA-LSQ]), otherwise, Group 5 will receive 60µg rCSP + AP 10-602 [GLA-LSQ]. Once the dose is determined, Groups 4 and 5, will receive dose 1. If safety and reactogenicity are acceptable after Group 4 and Group 5 dose 1, then both Groups 4 and 5 will receive dose 2. For Groups 1-5, if safety and reactogenicity are acceptable after dose 2, then each Group will receive dose 3. Both groups 4 and 5 will undergo

CHMI together at 28 days post-3rd dose. For CHMI, Groups 4 and 5 will be joined by 6 volunteers to serve as infectivity controls (Group 6).

Table 4-1: Study Group Assignments

Group	Study Product	rCSP Dose Level (µg/dose)	AP 10-602 Dose Level (µg of GLA per dose/LSQ per dose)	Sample Size
1	rCSP/AP 10-602	10	5/2	10
2	rCSP/AP 10-602	30	5/2	10
3	rCSP	30	None	10
4	rCSP/AP 10-602	60	5/2	10
5	rCSP/AP 10-602	10 or 30 or 60	5/2	10
6	None	None	None	6
				Total = 56

Subjects will be observed for adverse events, and vital signs will be measured before and 30 minutes after each vaccination. Participants will be telephoned the day following each vaccination to review symptoms. Once the first vaccinations for Group 1 are complete (10 volunteers receiving the 10µg rCSP + AP 10-602 [GLA-LSQ] dose) and the memory aid and one-week post-vaccination safety lab values are recorded, the PI and independent safety monitor (ISM) will review the safety data and to determine if the study will proceed to the next dose level. The PI and ISM will permit dose escalation if the following have not been recorded: any serious adverse event, any grade 3 related adverse event, or any grade 3 reactogenicity in 2 or more participants. The same process will occur after first vaccinations for Groups 2-5 are completed. Volunteers will complete a memory aid for 7 days following each vaccination with rCSP/AP 10-602 [GLA-LSQ] and rCSP. Complete blood counts, alanine aminotransferase (ALT), and serum creatinine will be measured 7 and 28 days after each vaccination, and 84 days after the last vaccination. Participants will be contacted by telephone 365 days after last vaccination to document any subsequent serious adverse events.

CHMI will occur at ~28 days post-3rd vaccination in Group 4 and 5, together with 6 infectivity controls (Group 6). Subjects will be observed for adverse events, and vital signs will be measured before and 30 minutes after CHMI. Standardized procedures will be followed, including daily visits for history, physical exam, and malaria diagnostics (qPCR with thick blood smear as backup if needed) 8-18 days post-CHMI. Hemoglobin, WBC, platelets, ALT, and serum creatinine will be measured if a participant tests positive for malaria. Participants who do not test positive for

malaria by 18 days post-CHMI will be followed every other day in clinic for malaria testing until 28 days post-CHMI and will not be treated for malaria if they continue to test negative at 28 days post-CHMI.

4.1 Substudies

The study team designed a substudy to evaluate the epitope mapping of vaccine-induced antibodies on a diversity peptide microarray, protocol DMID 17-0081. For this analysis, 2 mL of blood will be collected before first vaccination and at Day 113 for Groups 1 through 5 and at screening and day 29 for infectivity controls.

At present, no other substudies are planned, but if immunogenicity and/or CHMI results are favorable, it is possible that immunology substudies will be planned for remaining serum samples. These substudies would potentially include analysis of antibody subclass and avidity.

5 STUDY ENROLLMENT AND WITHDRAWAL

The study population will be enrolled from a CVD database of volunteers from the greater Baltimore-Washington metropolitan area and will include 56 U.S. malaria-naïve, volunteers aged 18-45 years. The sample size of 56 was chosen so that at least 8 vaccinated participants from each of the vaccine groups will be evaluable with regard to safety, immunogenicity, and in the case of groups 4 and 5, preliminary efficacy, after accounting for potential loss to follow-up. For malaria challenge controls, a sample size of 5 is standard for a single malaria challenge (per consensus document by WHO working group on standardization of design and conduct of *P. falciparum* CHMI), so 6 are included as controls in case of a single dropout. While two different doses of the vaccine will be tested , given the finding in prior RTS,S trials that a higher dose may not result in the best efficacy,(28) if anti-CSP antibodies measured by ELISA do not show an appropriate rise (at least fourfold increase in geometric mean anti-CSP antibody or geometric mean anti-CSP titer of 20), Group 5 will receive the same dose as Group 4 to increase the number of subjects for efficacy evaluation at that dose level and these groups will be analyzed together. The goal of the study is to compare the preliminary efficacy of this vaccine to that of RTS,S and having a larger sample size will allow for better comparisons. Indeed, prior studies of RTS,S in malaria naïve adults showed a range of 0 to 85.7% depending on dose and adjuvant system when the sample size was less than or equal to 10 versus an efficacy range of 30 to 62.5% (for standard dose regimens) depending on dose and adjuvant system for sample sizes greater than 10 (3, 25). Increasing our sample size will result in better precision for this comparison. Enrollment of each group will occur over a 1-2 month period and the target population will reflect the community at large at the study site. Information regarding study materials will be mailed to potential subjects who have previously participated in vaccine trials or expressed willingness to participate at the enrollment sites and have signed an authorization form that they are willing to be contacted for future studies. This has been done with Institutional Review Board (IRB) approval. Educational meetings will be offered to the public both within and outside the university system for the purposes of informing individuals of the study. Every effort will be made to achieve a balanced participant population while avoiding the enrollment of vulnerable populations. Careful screening will be conducted to avoid enrollment of individuals with substance abuse or psychiatric difficulties. Efforts to retain study participants include, but are not limited to: development of professional rapport with potential participants, obtaining valid contact and backup contact information from potential participants, and telephone reminder calls and/or email reminders for upcoming study visits.

Due to the occurrence of cardiovascular events concurrent with previous CHMI trials (described in **Section 2.1.1**), screening for cardiovascular disease risks and sub-clinical cardiovascular disease will be performed for participants planned for inclusion in groups 4, 5 and 6 to undergo CHMI, similar to those instituted in challenge centers worldwide. These participants will be assessed according to the non-invasive criteria for assessing cardiac risk provided by Gaziano in 2008 (29) Participants falling into either of the two “low risk” categories defined as $\leq$5% risk for fatal or non-fatal cardiovascular event within 5 years and >5% risk but $\leq$10% risk for fatal or non-fatal

cardiovascular event within 5 years, are eligible for inclusion. It is generally accepted as reasonable to exclude from challenge participants falling into the three "moderate risk" or "high risk" categories. Importantly, no evidence of cardiovascular compromise has been established as a result of the malaria challenge to date. In addition, a screening electrocardiogram will be obtained for groups 4, 5 and 6 to exclude individuals with abnormal findings as listed in the exclusion criteria. Volunteers will be closely examined and questioned at all follow-ups for presence of cardiovascular-related signs or symptoms. Signs or symptoms of an event suggestive of a cardiac etiology will prompt a cardiology work-up.

The Center for Vaccine Development (CVD) will be the site for volunteer enrollment and malaria challenge events. Vaccinations will occur at the General Clinical Research Center (GCRC) due to its proximity to the investigational drug service. The post-vaccination and post-CHMI follow-up will be performed at the CVD outpatient facilities.

5.1 Subject Inclusion Criteria

1. Healthy adults (males and non-pregnant, non-lactating females) between the ages of 18 and 45 years, inclusive.
2. Able and willing to participate for the duration of the study.
3. Able and willing to provide written (not proxy) informed consent.
4. Provides informed consent and correctly answers $\geq$ 70% on the post consent quiz before any study procedures and is available for all study visits.
5. Females of childbearing potential and males must agree to practice highly effective contraception.*
6. Is in good health, as determined by vital signs (heart rate, blood pressure, oral temperature); medical history; normal laboratory ranges listed in Appendix C†; and a physical examination.
7. Agree not to travel to a malaria endemic region during the entire course of the trial.
8. Willing to avoid non-study related blood donation for the duration of participation in the study or until at least 1 year after receiving the last investigational vaccine, whichever is longer.
9. Able to understand and comply with planned study procedures including daily outpatient follow-up visits beginning 5 days after malaria challenge‡
10. Willing to avoid non-study related blood donation for 3 years (30) following *P. falciparum* challenge‡

* Contraception must be practiced from 30 days before the time of enrollment until at least 30 days following the third vaccine dose for groups 1, 2 and 3, and the malaria challenge event for groups 4, 5 and 6 (such as double barrier methods (condoms plus foam or spermicide, diaphragm plus foam or spermicide), licensed intrauterine devices (IUDs), intravaginal or intra/transdermal or oral hormonal methods initiated at least 30 days before inoculation or challenge, documented surgical sterilization via tubal ligation the essure procedure or hysterectomy, abstinence or a vasectomized partner). The contraceptive method should remain unchanged throughout the study participation.

† hemoglobin, white blood cell count, platelet count, glucose (random), serum alanine aminotransferase (ALT), serum creatinine, urine protein and urine blood

‡ Groups 4, 5 and 6 only

5.2 Subject Exclusion Criteria

1. Any history of malaria infection, or travel to a malaria endemic region within 6 months before first vaccination.
2. History of long-term residence (≥5 years) in an area known to have significant transmission of *P. falciparum*.
3. Positive serology for human immunodeficiency virus (HIV), hepatitis C virus (HCV), or hepatitis B surface antigen (HBsAg).
4. Positive sickle cell screening test or known hemoglobinopathy. ‡
5. Current or recent (within the last four weeks) treatment with parenteral or oral corticosteroids (intranasal or inhaled steroids are acceptable), or other immunosuppressive agents, or chemotherapy.
6. History of splenectomy.
7. Screening laboratory values outside protocol-specified acceptable normal ranges as noted in Appendix C, except hematuria>1+ detected during menses for females. †
8. Vaccination with a live vaccine within the past 30 days or with a nonreplicating, inactivated, or subunit vaccine within the last 14 days.
9. Known hypersensitivity to components of the vaccine for groups 1, 2, 3, 4 and 5; or to the adjuvant for groups 1, 2, 4 and 5.
10. History of acute or chronic medical conditions including, but not limited to, disorders of the liver, kidney, lung, heart, nervous system, or other metabolic or autoimmune/inflammatory conditions.
11. History of anaphylaxis or severe hypersensitivity reaction.
12. History of Guillain-Barré syndrome or severe adverse reaction to any vaccination.

13. Severe asthma, as defined by an emergency room visit or hospitalization within the last 12 months.
14. Pregnant or breastfeeding women or women who plan to become pregnant before day 115 in groups 1, 2 and 3; or before 30 days post-malaria challenge in groups 4, 5 and 6.
15. Concurrent participation in other investigational protocols or receipt of an investigational product within the previous 30 days.
16. Planned receipt of an investigational product within 28 days following the last vaccination dose or malaria challenge.
17. Any condition that, in the opinion of the investigator, would affect a participant's ability to understand or comply with the study protocol or would jeopardize a participant's safety or rights.
18. History of previous receipt of a candidate malaria vaccine or a vaccine containing the GLA-LSQ adjuvant.
19. Use or planned use of any drug with anti-malarial activity** 30 days before, or after malaria challenge. ‡
20. Planned surgery 30 days before or after vaccination or malaria challenge.
21. History of drug or alcohol abuse within the last five years.
22. Receipt of blood or blood products in the previous six months or donation of a unit of blood within two months before screening.
23. History of schizophrenia, bipolar disorder or other psychiatric condition that makes study compliance difficult. ‡‡
24. History of diabetes mellitus with the exception of pregnancy-induced diabetes that has resolved
25. Has evidence of increased cardiovascular disease risk (defined as > 10%, 5 year risk) as determined by the method of Gaziano (29). ‡ ***
26. Abnormal screening ECG. ††, ‡
27. Known hypersensitivity to mosquito bites, artemether-lumefantrine or atovaquone-proguanil. ‡
28. Anticipated medication use during the 28-day post-challenge period that are known to interact with artemether/lumefantrine or atovaquone/proguanil, such as cimetidine, metoclopramide, antacids, and kaolin. ‡
29. Previous participation in a CHMI study

‡ Groups 4, 5 and 6 only

† For females who are menstruating, urinalysis frequently tests positive for blood and is not an indicator of poor health status or increased risk.

‡‡ Subjects with psychoses or history of suicide attempt or gesture in the 3 years before study entry, ongoing risk for suicide.

** Medications with antimalarial activity include trimethoprim-sulfamethoxazole, azithromycin, erythromycin, tetracycline, doxycycline, minocycline, clindamycin, ciprofloxacin, levofloxacin, norfloxacin and rifampin

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

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

5.3 Treatment Assignment Procedures

5.3.1 Randomization Procedures

This study is open-label. No randomization is planned for this protocol.

5.3.2 Masking Procedures

Although the study is open-label, laboratory personnel who are conducting immunogenicity studies and who perform asexual parasitemia detection post-CHMI will be blinded to study group assignment. Samples provided to the lab for immunogenicity analyses will be blinded to participant ID and visit number.

5.3.3 Reasons for Withdrawal

Participants are free to withdraw from the study at any time. Participants who have received vaccine or who developed an adverse event or serious adverse event will be encouraged to remain in the study to be followed for safety purposes. A study participant will be discontinued from receiving further investigational product if any clinical adverse event (AE), intercurrent illness, or other medical condition or situation occurs that meets the exclusion criteria (to be reviewed before each vaccination and before malaria challenge), or if continued participation in the study would not be in the best interest of the participant, unless doing so would harm the participant in the opinion of the investigator.

Therefore, a study subject will be discontinued from participation in the study for:

- Development of any exclusion criteria;
- Pregnancy or breastfeeding;
- Request by subject to terminate participation;
- Requirement for prohibited concomitant medication or treatment;
- Treatment-related toxicity;
- Failure to adhere to the requirements of the protocol;
- Loss to follow-up;
- Request of primary care provider;
- At the request of the IRB/Ethics committee, NIH NIAID, or the FDA
- The subject's well-being, based on the opinion of the investigator

Because a true intent-to-treat analysis requires the inclusion of all participants enrolled to the extent possible, this requires an intent-to-treat design in which all participants are followed according to the prespecified schedule with primary, and perhaps secondary, outcome assessments, regardless of compliance, adverse effects, or other post-enrollment observations—participant refusal excepted.

5.3.4 Handling of Withdrawals and Discontinuation of Study Product Administration

If, for safety reasons a participant is deemed by the investigators to be not eligible to receive the study product as per protocol, he/she will discontinue subsequent vaccinations and administration of CHMI study product and be followed for safety, reactogenicity and immunogenicity, including specimen collections at the study termination visit as allowed. Treatment for possible incubating malaria infection will be administered if the participant withdraws/is withdrawn after CHMI but before antimalarial treatment administration. Efforts to continue follow-up (with the subject's consent) for safety, reactogenicity and immunogenicity will be made. If voluntary withdrawal occurs, the subject will be asked for permission to continue scheduled evaluations, complete an end-of-study evaluation, and be given appropriate care under medical supervision until the symptoms of any adverse event resolve or until the subject's condition becomes stable. Participants who discontinue the vaccinations or terminate their study participation early will not be replaced.

It is vital to collect safety data on any subject discontinued because of an AE or SAE, and every effort will be made to undertake protocol-specified safety follow-up procedures.

5.3.5 Subject Replacement

Subjects who withdraw or are terminated will not be replaced.

5.3.6 Termination of Study

The study may be discontinued due to the development of laboratory toxicities, study closure due to Safety Monitoring Committee review, at the discretion of the U.S. Food and Drug Administration or DMID, or due to natural disaster. If the study is prematurely terminated by the sponsor, any regulatory authority, or the investigator for any reason, the investigator will promptly inform the study subjects and assure appropriate therapy or follow-up for the subjects, as necessary. The investigator will provide a detailed written explanation of the termination to the IRB/IEC. If the clinical trial is halted and is unable to resume, a formal letter will be sent to the Food and Drug Administration (FDA) by the sponsor explaining the reasons for cessation of the study.

6 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

6.1 Study Product Description

rCSP Malaria Vaccine Antigen

The rCSP vaccine antigen is a full-length recombinant circumsporozoite *P. falciparum* protein expressed in a soluble form in the *Pseudomonas fluorescens* cell line. The CSP is composed of three regions: an amino terminus that binds heparin-sulfate proteoglycans, a four-amino-acid repeat region (NANP), and carboxy-terminus that contains a thrombospondin-like Type I region domain (31). The rCSP was manufactured by Ajinomoto Althea, Inc. for the DMID.

AP 10-602 Adjuvant

AP 10-602 is a nanoliposomal formulation containing the immunological adjuvant Glucopyranosyl Lipid A (GLA). GLA is a synthetic monophosphoryl lipid A-like molecule, which is a Toll-like receptor 4 agonist that activates specific cells of the immune system. The molecular formula for GLA is $C_{96}H_{184}N_3O_{22}P$ (MW:1,762.311). The AP 10-602 adjuvant was developed, manufactured, and supplied to DMID by the Infectious Disease Research Institute (IDRI), Seattle, Washington.

6.1.1 Acquisition

rCSP Malaria Vaccine Antigen

The rCSP malaria vaccine antigen will be produced by Ajinomoto Althea, Inc. but be supplied to the clinical site by DMID via Fisher Bioservices

AP 10-602 [GLA-LSQ] Adjuvant

The AP 10-602 [GLA-LSQ] adjuvant will be supplied by IDRI.

Sterile Water for Injection, USP (Diluent for rCSP Malaria Vaccine)

The sterile water for injection, USP (WFI) will be supplied by the DMID Clinical Material Services, Fisher BioServices.

The rCSP antigen, the AP 10-602 adjuvant, and the WFI will be transferred to the following address:

DMID-Clinical Material Services (CMS)
Fisher BioServices
20439 Seneca Meadows Parkway
Germantown, MD 20876
Tel: (240) 477-1350
Fax: (240) 477-1360
Email: DMID.CMS@ThermoFisher.com

The rCSP malaria vaccine antigen, AP 10-602 [GLA-LSQ] adjuvant, and WFI diluent will be shipped to the clinical research site from the DMID CMS upon request and approval by DMID.

6.1.2 Formulation, Packaging, and Labeling

rCSP Malaria Vaccine Antigen

The rCSP malaria vaccine antigen is filled as 0.6 mL in a 3 mL Type I borosilicate glass vial. The rCSP is formulated as 1.0 mg/mL recombinant full-length *P. falciparum* circumsporozoite protein (CSP) in PBS, pH 6.7, buffer containing 0.5 M L-arginine HCL and 1 mM monothioglycerol (MTG). The L-arginine and MTG are added to suppress aggregation and the formation of disulfide-stabilized dimers presumably due to the presence of an unpaired cysteine at the N-terminus of the full-length rCSP. The rCSP appears as a clear, colorless liquid with no particles when thawed.

AP 10-602 Adjuvant

AP 10-602 is a nanoliposomal formulation that includes QS 21, a saponin. AP 10-602 is vialed in 2 mL Type I borosilicate glass vials with 0.4mL volume. The AP 10-602 adjuvant appears as a milky-white liquid. The clinical dose for the AP 10-602 adjuvant is 5 µg GLA + 2 µg QS-21.

Sterile Water for Injection, USP

The sterile water for injection (WFI), USP is nonpyrogenic and contains no bacteriostatic, antimicrobial agent, or added buffer. This product will be used to dilute the vaccine (rCSP) and will be supplied as a single-dose vial.

Study products (rCSP and AP 10-602) will be labeled with the statement “Caution, New drug - Limited by Federal Law to investigational use”.

6.1.3 Product Storage and Stability

rCSP Malaria Vaccine Antigen
The rCSP is stored at ≤ -70°C.

AP 10-602 Adjuvant
The AP 10-602 Adjuvant is stored at 2°C - 8°C. Do Not Freeze.

Sterile Water For Injection, USP
The sterile WFI vials are stored at 20°C to 25°C (68°F to 77°F) [See USP Controlled Room Temperature]

The temperature of the storage unit must be recorded daily (excluding non-business days and holidays as applicable), continuously monitored and recorded during the duration of this trial per the VTEU site's standard operating procedures (SOP), and documentation will be maintained. If the temperature fluctuates outside of the required range, the affected study product(s) must be quarantined at the correct storage temperature and labeled as 'Do Not Use' (until further notice). The research pharmacist must alert the site principal investigator and study coordinator, if the temperature fluctuates outside of the required range. In the event the temperature fluctuates outside of the required range, the affected study product(s) must not be administered. The site principal investigator or responsible person should immediately contact the DMID Product Support Team (see MOP for contact information) for further instructions before any additional study vaccinations are administered. Based on the information collected, DMID and/or the manufacturer will determine whether the affected study product(s) can be used. If it cannot be used, the site will receive specific instructions on how to return the affected study product(s) to the DMID CMS or destroy it on site. Additional instructions for quarantine are provided in the protocol-specific MOP.

6.2 Dosage, Preparation and Administration of Study Intervention/Investigational Product

6.2.1 Dosage

Table 6-1: Doses for Study Group Assignments

Group	Study Product	rCSP Dose	AP 10-602 [GLA-LSQ] Dose Level in µg (µg of GLA per	Total Dose Volume

			assigned dose/LSQ per assigned dose)	
1	rCSP/ AP 10-602 [GLA-LSQ]	10µg	5µg/2 µg	0.5mL
2	rCSP/ AP 10-602 [GLA-LSQ]	30µg	5µg/2 µg	0.5mL
3	rCSP	30µg	None	0.5mL
4	rCSP/ AP 10-602 [GLA-LSQ]	60µg	5µg/2 µg	0.5mL
5	rCSP/ AP 10-602 [GLA-LSQ]	10 or 30 or 60µg	5µg/2 µg	0.5mL
6	None	None	None	None

6.2.2 Preparation

See the protocol-specific Manual of Procedures (MOP) for detailed information on the preparation, labeling, and storage of study product for each group. Study product preparation will be performed by the VTEU site's research pharmacist on the same day of study vaccine administration.

Visually inspect the study products upon receipt and prior to use. If the study product(s) appear(s) to have been damaged, contaminated or discolored, contain visible particulate matter or if there are any concerns regarding its integrity, do NOT use the affected study product(s). The affected study product(s) must be quarantined as per storage requirements and labeled as 'Do Not Use' (until further notice). The site principal investigator or responsible person should immediately contact the DMID Product Support Team (see MOP for contact information) and DMID Clinical Project Manager for further instructions before any additional study vaccinations are administered. Based on the information collected, DMID and/or the manufacturer will determine whether the affected study product(s) can be used. If it cannot be used, the site will receive specific instructions on how to return the affected study product(s) to the DMID CMS or destroy it on site. If the study product is unusable, study personnel will use another vial from the study supply. Replacement vials may be requested by contacting DMID. Additional instructions for quarantine and DMID contact information are provided in the protocol-specific MOP.

The Site Research Pharmacist will prepare the study vaccine on the same day of vaccination. Using aseptic technique, preparation of study vaccine must be performed under a biological safety cabinet (BSC) or laminar flow hood.

For Groups 1, 2, 4 and 5, the rCSP will be appropriately formulated and mixed with diluted AP 10-602 [GLA-LSQ] adjuvant. Group 3 participants will receive rCSP (diluted to the appropriate concentration) without adjuvant. Once diluted, the rCSP must be used within 4 hours. The rCSP plus AP 10-602 [GLA-LSQ] adjuvant admixture must be used within 4 hours of preparation. Both the diluted vaccine and vaccine plus adjuvant admixture will be held at 2° - 8° Celsius.

6.2.3 Administration

See the protocol-specific Manual of Procedures (MOP) for detailed information on the administration of study product for each group. Each dose of study vaccine will be administered as a single 0.5mL intramuscular (IM) injection in the deltoid muscle of the participant's arm.

Participants will be observed in the clinic for a minimum of 30 minutes following vaccination. After 30 minutes, the vaccination site will be examined, vital signs will be obtained, and the participant will be questioned about the presence of any localized or generalized reactogenicity symptoms. Any spontaneous AEs that occur will be assessed. Participants will be instructed to complete a symptom memory aid for 7 days post vaccination to record symptoms and to inform investigators immediately if they experience symptoms that may require medical attention. Participants will be instructed to return to the study center if they feel febrile on any individual day or if they develop any severe reactions following vaccination.

6.3 Modification of Study Intervention/Investigational Product for a Participant

The study product will be administered to enrolled participants according to the study schedule. There is no planned modification of a study product administration for participants, with the exception that vaccinated participants who become ineligible for further vaccinations or for CHMI will be precluded from these study procedures. Every effort will be made to follow vaccinated participants for safety evaluations, regardless of their eligibility for CHMI.

Subsequent vaccinations will not be given to participants who:

- Experience a related grade 3 clinical or laboratory AE or related SAE

- Become ineligible due to a change in status of exclusion criteria numbers 10 and/or 17.

6.4 Accountability Procedures for the Study Intervention/Investigational Product(s)

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

Used and unused study product will be retained until monitored and released for disposition, as applicable, and will be handled in accordance with the study MOP. Upon completion or termination of the study and after the final monitoring visit, any remaining unused study product will either be returned or destroyed appropriately at the clinical site as per Sponsor requirements and instructions. Disposition and accountability records will be maintained at the CVD and kept in the study file. Original accountability records will be transferred to the sponsor for the trial master file at the time of the study closeout visit with copies to remain in the CVD study file.

6.5 Assessment of Subject Compliance with Study Intervention/Investigational Product

The study product will be administered to enrolled subjects by study personnel at the clinical site and, as such, there will be no assessment of participant compliance.

6.6 Concomitant Medications/Treatments

At each study visit/contact, excluding telephone follow-up calls, from screening to study day 253 for Groups 1-5 and to study day 57 for group 6, the investigator will question the participant about any medication taken, including herbals, vitamins and holistic/naturopathic medications. Concomitant medication, including vaccines and any other medication, including any specifically contraindicated will be recorded in the CRF with trade name and/or generic name of the medication, medical indication, start and end dates of treatment. If a volunteer has initiated an antacid or kaolin, any administration of antimalarials will be timed to separate administration of antimalarial medication by more than 4 hours to prevent absorption interference.

6.7 Controlled Human Malaria Infection (CHMI)

Participants in groups 4, 5, and 6, will be challenged with sporozoite-infected mosquitoes to evaluate preliminary vaccine efficacy. Such challenges are mandated by the absence of any laboratory tests that will unequivocally predict protection. The challenge is scheduled to occur approximately 28 days (4 weeks) after the last vaccination visit for Groups 4 and 5. Participants in Groups 4 and 5 who receive the third vaccination outside of the prescribed window will be discussed on a case-by-case basis by the sponsor, investigators, and the DMID medical officer to determine if these participants should proceed to malaria challenge.

Participants in group 6 will not be immunized but will serve as infectivity controls to verify that mosquitoes used for challenge are able to transmit malaria and to calculate a preliminary efficacy estimate. The challenge of participants will be done at the University of Maryland CVD facility. Malaria-infected mosquitoes for challenge at the University of Maryland will be transported to the insectaries at the University of Maryland for challenge of human subjects.

The *A. stephensi* female mosquitoes infected with *P. falciparum* parasites of the NF54/3D7 strain used for challenge will be reared at Sanaria in their insectary facilities. These mosquitoes require carefully regulated environmental conditions such as temperature and humidity. At a designated time, Sanaria entomologic staff will accompany the mosquitoes, placed in secure transfer vessels, from Sanaria to the University of Maryland (a distance of approximately 40 miles from Rockville, MD). Upon transfer to the University of Maryland, CVD the containers of mosquitoes will be placed in the CVD insectary utilizing incubators that will maintain appropriate environmental conditions. The security of the insectary is critical and, as such, every effort is made to restrict access to the insectary and provide for a secure location. The University of Maryland has a protected suite devoted to an insectary, containment area, laboratory, and microscopy rooms, as well as incubation facilities for proper maintenance of temperature and humidity. The successful transfer of mosquitoes to and from Sanaria to the CVD has occurred on two previous occasions.

For each participant, exactly 5 mosquitoes will be placed in a container covered with mesh. The participant will place their forearm onto the mesh to facilitate mosquito feeding. Mosquitoes will be allowed to feed over 5-10 minutes, after which they will be euthanized and dissected to confirm the 5 mosquitoes both took a blood meal and were infected, and then the salivary glands scored. If required, additional mosquitoes will be allowed to feed until a total of 5, but not more than 5, infected mosquitoes with a minimum of +2 salivary gland score have fed. The salivary gland score rates the mosquito's salivary gland for the presence of sporozoites. The assessment is based on the following conditions:

0	No sporozoites observed
+1	1-10 sporozoites observed
+2	11-100 sporozoites observed
+3	101-1000 sporozoites observed
+4	1001-10,000 sporozoites observed
+5	>10,000 sporozoites observed

Participants will then be observed for at least 30 minutes following first exposure to mosquito bites in order to assess them for any evidence of acute allergic reactions related to mosquito exposure.

Volunteers undergoing malaria challenge will receive an emergency notification card that outlines their participation in the study with details on the exposure to malaria, as well as the appropriate investigator contact telephone numbers.

Subjects undergoing CHMI will be asked to avoid taking antibiotics starting 2 weeks before CHMI and for 28 days after CHMI unless prescribed by a physician. Subjects will be asked to notify the study team immediately if an antibiotic is prescribed for them or if they consider taking an antibiotic during this time.

7 STUDY SCHEDULE

Please refer also to the Schedule of Events in Appendix A.

7.1 Screening (Visit 00, days -60 to 1)

- Potential participants will be provided with a verbal description of the study (purpose and study procedures) and will be asked if they have any questions and to read/sign the consent form. The consent form will be signed and potential participants must score ≥70% on a comprehension quiz before the performance of any study procedures. Each potential participant will be allowed two attempts.
- The study staff will discuss with the participant his/her medical history, study eligibility criteria and concomitant medication use.
- For groups 4, 5 and 6, a 12-lead ECG will be done and analysis of 5-year cardiovascular event risk will be performed, based on the method published by Gaziano (29). The risk factors assessed will include sex, age, body mass index, blood pressure, history of diabetes mellitus, and history of smoking.
- Vital signs (height, weight, oral temperature, blood pressure, pulse) will be obtained.
- A physical exam will be performed by the investigator (lymph nodes, lungs, heart, liver, spleen).
- For females who are capable of bearing children, a serum pregnancy test will be performed at the clinical trial study site at the time of screening regardless of age unless written medical demonstration of sterility or amenorrhea (defined as one year with medical evaluation) can be provided.
- A 20mL blood sample will be collected from an arm vein to screen for health as follows:
    - Hematology: hemoglobin (Hgb), white blood cell count (WBC) with machine differential, and platelet count.
    - Chemistry: glucose (random), alanine aminotransferase (ALT), and creatinine.
    - Serology: HIV, HCV, and HBsAg.
- For groups 4, 5 and 6, an additional 3mL blood sample will be collected for hemoglobin electrophoresis testing (sickle cell screen)

- For group 6, an additional 2 mL blood sample will be collected for pre-CHMI humoral immune assays.
- A urine sample will be obtained for evaluation of the presence of urine blood and protein.

7.2 Enrollment/Baseline (Visit 01, Day 1) Groups 1-5

- Eligibility criteria will be reviewed with participants. Vital signs (oral temperature, blood pressure, pulse) and interim medical history will be obtained.
- A targeted physical exam will be performed if indicated by the medical history.
- Concomitant medications will be recorded.
- A urine pregnancy test will be performed, and negative results, within 24 hours before vaccination, will be confirmed on all females of childbearing potential.
- For groups 1-5, an 18mL venous blood sample for CBC, ALT, creatinine, and humoral immune assays will be collected before vaccination.
- (Groups 4 and 5) An additional 50mL venous blood sample for future use assessment of additional immune responses will be collected before vaccination.
- Group 1 consisting of 10 enrolled participants will receive the 10µg dose of rCSP/AP 10-602 [GLA-LSQ] vaccine via the IM route; Group 2 consisting of 10 enrolled participants will receive the 30µg dose of rCSP/AP 10-602 [GLA-LSQ] vaccine via the IM route; Group 3 consisting of 10 enrolled participants will receive the 30µg dose of rCSP vaccine without adjuvant via the IM route; Group 4 consisting of 10 enrolled participants will receive the 60µg dose of rCSP/AP 10-602 [GLA-LSQ] vaccine via the IM route; Group 5 consisting of 10 enrolled participants will receive a 10, 30 or 60 µg dose (depending on immunogenicity review) of rCSP/AP 10-602 [GLA-LSQ] vaccine via the IM route.
- For each group, two participants will be immunized first, if there are no safety concerns identified after 24 hours that trigger halting rules as per Section 9.5, then the other 8 volunteers will be immunized as per schedule.
- Participants will be observed in the clinic for a minimum of 30 minutes following vaccination. After 30 minutes, the vaccination site will be examined, vital signs (oral temperature, blood pressure, pulse) will be obtained, and the participant will be questioned about the presence of any localized or systemic reactogenicity symptoms.
- Any solicited or unsolicited AEs, AESIs, or SAEs that occur will be assessed.

- Participants will be instructed to complete a symptom memory aid for 7 days post vaccination to record symptoms and to inform investigators immediately if they experience symptoms that may require medical attention. For erythema and induration, participants will be provided rulers to measure size as explained by the memory aid.
- Participants will be instructed to return to the study center if they develop any severe reactions (grade 3) following vaccination.

7.3 Follow-up

7.3.1 Day 2 (+1), Telephone follow-up, Visit 2, Groups 1-5

- Participants will be contacted by telephone to ask about solicited and unsolicited symptoms following vaccination. During the 24 hour telephone call and the follow-up visits, the same set of specific symptoms which are listed on the memory aid will be actively elicited.
- Participants will be reminded about memory aid completion including recording oral temperatures and local and systemic reactions for the 7-day period after vaccination.

7.3.2 Day 8 (±2), Visit 3, Groups 1-5

- Participants will be evaluated 7 days after vaccination. Vital signs (oral temperature, blood pressure, pulse) will be taken, and information regarding systemic and local reactions will be solicited and recorded. An examination of the vaccination site will be performed.
- Any solicited or unsolicited AEs, AESIs, or SAEs that have occurred since the last visit will be assessed.
- Interim medical history will be obtained.
- A targeted physical exam will be performed as indicated.
- Concomitant medications will be reviewed and updated, if applicable.
- Obtain 16 mL blood sample for safety laboratory and antibody testing (complete blood count, ALT, creatinine, and humoral immune assays).
- Participants will be reminded to notify study personnel if they develop any severe reactions during the study.

7.3.3 Day 29 (±3), Visit 4, 2nd vaccination, Groups 1-5

- Exclusion criteria numbers 10 and 17 will be reviewed with participants. Vital signs (oral temperature, blood pressure, pulse) will be assessed and recorded. Interim medical history will be obtained.
- Any solicited or unsolicited AEs, AESIs, or SAEs that have occurred since the last visit will be assessed.
- A targeted physical exam will be performed as indicated.
- Concomitant medications will be reviewed and updated, if applicable.
- A urine pregnancy test will be performed, and negative results, within 24 hours before vaccination, will be confirmed on all females of childbearing potential
- A 16 mL venous blood sample for CBC, ALT, creatinine, and humoral immune assays will be collected before vaccination.
- (Groups 4 and 5) An additional 50mL venous blood sample for future use assessment of additional immune responses will be collected before vaccination.
- Group 1 consisting of 10 enrolled participants will receive the 10µg dose of rCSP/AP 10-602 [GLA-LSQ] vaccine via the IM route; Group 2 consisting of 10 enrolled participants will receive the 30µg dose of rCSP/AP 10-602 [GLA-LSQ] vaccine via the IM route; Group 3 consisting of 10 enrolled participants will receive the 30µg dose of rCSP without adjuvant via the IM route; Group 4 consisting of 10 enrolled participants will receive the 60µg dose of rCSP/AP 10-602 [GLA-LSQ] vaccine via the IM route; Group 5 consisting of 10 enrolled participants will receive a 10, 30 or 60 µg dose (depending on immunogenicity review) of rCSP/ AP 10-602 [GLA-LSQ] vaccine via the IM route.
- Participants will be observed in the clinic for a minimum of 30 minutes following vaccination. After 30 minutes, the vaccination site will be examined, vital signs will be obtained, and the participant will be questioned about the presence of any localized or generalized reactogenicity symptoms.
- Participants will be instructed to complete a symptom memory aid for 7 days post vaccination to record symptoms and to inform investigators immediately if they experience symptoms that may require medical attention. For erythema and induration, participants will be provided rulers to measure size as explained by the memory aid.
- Participants will be instructed to come back to the study center if they feel febrile on any individual day or if they develop any severe (grade 3) reactions following vaccination.

7.3.4 Day 30 (+1), Telephone follow-up, Visit 5, Groups 1-5

- Participants will be contacted by telephone to ask about solicited and unsolicited symptoms following vaccination. During the 24 hour telephone call and the follow-up visits, the same set of specific symptoms which are listed on the memory aid will be actively elicited.
- Participants will be reminded about memory aid completion including recording oral temperatures and local and systemic reactions for the 7-day period after vaccination.

7.3.5 Day 36 (±3), Visit 6, Groups 1-5

- Participants will be evaluated 7 days after vaccination. Vital signs (oral temperature, blood pressure, pulse) will be taken, and information regarding systemic and local reactions will be solicited and recorded. An examination of the vaccination site will be performed.
- Any solicited or unsolicited AEs, AESIs, or SAEs that have occurred since the last visit will be assessed.
- A targeted physical exam will be performed as indicated.
- Concomitant medications will be reviewed and updated, if applicable.
- Obtain 16 mL blood sample for safety laboratory and antibody testing (complete blood count, ALT, creatinine, and humoral immune assays).
- Participants will be reminded to notify study personnel if they develop any severe reactions (grade 3) during the study.

7.3.6 Day 57 (±3), Visit 7, Groups 1-5

- Subjects will be evaluated. Vital signs (oral temperature, blood pressure, pulse) will be assessed and recorded. Interim medical history will be obtained.
- Any solicited or unsolicited AEs, AESIs, or SAEs AEs that have occurred since the last visit will be assessed.
- A targeted physical exam will be performed as indicated.
- Concomitant medications will be reviewed and updated, if applicable.
- Obtain 16 mL blood sample for safety laboratory and antibody testing (complete blood count, ALT, creatinine, and humoral immune assays).

7.3.7 Day 85 (±3), Visit 8, 3rd vaccination, Groups 1-5

- Exclusion criteria numbers 10 and 17 will be reviewed with participants. Vital signs (oral temperature, blood pressure, pulse) and interim medical history will be obtained.
- Any solicited or unsolicited AEs, AESIs, or SAEs that have occurred since the last visit will be assessed.
- A targeted physical exam will be performed as indicated.
- Concomitant medications will be reviewed and updated, if applicable.
- A urine pregnancy test will be performed, and negative results, within 24 hours before vaccination, will be confirmed on all females of childbearing potential
- A 16 mL venous blood sample for CBC, ALT, creatinine and humoral immune assays will be collected before vaccination.
- (Groups 4 and 5) An additional 50mL venous blood sample for future use assessment of additional immune responses will be collected before vaccination.
- Group 1 consisting of 10 enrolled participants will receive the 10µg dose of rCSP/AP 10-602 [GLA-LSQ] vaccine via the IM route; Group 2 consisting of 10 enrolled participants will receive the 30µg dose of rCSP/AP 10-602 [GLA-LSQ] vaccine via the IM route; Group 3 consisting of 10 enrolled participants will receive the 30µg dose of rCSP without adjuvant via the IM route; Group 4 consisting of 10 enrolled participants will receive the 60µg dose of rCSP/AP 10-602 [GLA-LSQ] vaccine via the IM route; Group 5 consisting of 10 enrolled participants will receive a 10, 30 or 60 µg dose (depending on immunogenicity review) of rCSP/AP 10-602 [GLA-LSQ] vaccine via the IM route.
- Participants will be observed in the clinic for a minimum of 30 minutes following vaccination. After 30 minutes, the vaccination site will be examined, vital signs will be obtained, and the participant will be questioned about the presence of any localized or generalized reactogenicity symptoms.
- Participants will be instructed to complete a symptom memory aid for 7 days post vaccination to record symptoms and to inform investigators immediately if they experience symptoms that may require medical attention. For erythema and induration, participants will be provided rulers to measure size as explained by the memory aid.
- Participants will be instructed to come back to the study center if they feel febrile on any individual day or if they develop any severe (grade 3) reactions following vaccination.

7.3.8 Day 86 (+1), Telephone follow-up, Visit 9, Groups 1-5

- Participants will be contacted by telephone to ask about solicited and unsolicited symptoms following vaccination. During the 24 hour telephone call and the follow-up visits, the same set of specific symptoms which are listed on the memory aid will be actively elicited.
- Participants will be reminded about memory aid completion including recording oral temperatures and local and systemic reactions for the 7-day period after vaccination.

7.3.9 Day 92 (±3), Visit 10, Groups 1-5

- Participants will be evaluated 7 days after vaccination. Vital signs (oral temperature, blood pressure, pulse) will be taken, and information regarding systemic and local reactions will be solicited and recorded. An examination of the vaccination site will be performed.
- Any solicited or unsolicited AEs, AESIs, or SAEs that have occurred since the last visit will be assessed.
- A targeted physical exam will be performed as indicated.
- Concomitant medications will be reviewed and updated, if applicable.
- Obtain 16 mL blood sample for safety laboratory and antibody testing (complete blood count, ALT, creatinine, and humoral immune assays).
- Participants will be reminded to notify study personnel if they develop any severe reactions (grade 3) during the study.

7.3.10 Day 113 (±3), Visit 11, All Groups (Group 6 Day 1)

All Groups Pre-Challenge:

- Subjects will be evaluated.
- Vital signs (oral temperature, blood pressure, pulse) will be assessed and recorded.
- Interim medical history will be obtained.
- Any solicited or unsolicited AEs, AESIs (Groups 4 and 5), or SAEs that have occurred since the last visit will be assessed.
- A targeted physical exam will be performed as indicated.
- Concomitant medications will be reviewed and updated, if applicable.

- (Groups 1-5) Obtain 18 mL blood sample for safety laboratory and antibody testing (complete blood count, ALT, creatinine, and humoral immune assays.).
- (Groups 4 and 5) An additional 50mL venous blood sample for future use assessment of additional immune responses will be collected.

Groups 4-6, Malaria Challenge:

Before the malaria challenge:

- All screening laboratories will be reviewed for group 6 only
- Exclusion criteria numbers 10 and 17 will be reviewed
- A urine pregnancy test will be performed, and negative results, within 24 hours before challenge, will be confirmed on all females of childbearing potential
- Participants will be issued study participation cards identifying them as having been exposed to malaria and listing contact numbers for study physicians. If they do not own a cell phone, a beeper or cell phone will be issued to facilitate contacting study personnel during the post-challenge period.

- The malaria challenge will be administered.
- After challenge, the participants will be observed for a minimum of 30 minutes.
- Post-challenge vital signs (oral temperature, blood pressure, and pulse) will be recorded.
- Participants will be instructed to contact study personnel immediately should they manifest any signs or symptoms they perceive as serious.
- Participants will be counseled on mosquito avoidance behavior (handout).

7.3.11 Days 118-120, Visits 12-14, Outpatient Post-malaria Challenge Surveillance, Groups 4, 5 and 6 (Group 6 Days 6-8)

- Participants will follow-up in the clinic to be interviewed.
- A review of all current medications (focusing on antibiotics) will be conducted
- Vital signs (oral temperature, blood pressure, pulse) will be recorded
- Any solicited or unsolicited AEs, AESIs (groups 4 and 5), or SAEs that have occurred since the last visit will be assessed
- A targeted physical exam will be performed as indicated

- 2 mL of venous blood will be collected for malaria diagnostics (qPCR with thick blood smear as backup if needed).
- Participants will be counseled on mosquito avoidance behavior.

7.3.12 Days 121-131, Visits 15-25, Daily Post-malaria Challenge Surveillance, Groups 4, 5 and 6 (Group 6 Days 9-19)

- Participants will be checked daily for the presence of falciparum malaria in their blood (qPCR with thick blood smear as backup, if needed) during the period of time they are most at risk for the development of falciparum malaria.
- A review of all current medications (focusing on antibiotics) will be conducted.
- Vital signs (oral temperature, blood pressure, pulse) will be recorded.
- Any solicited or unsolicited AEs, AESIs (groups 4 and 5), or SAEs that have occurred since the last visit will be assessed
- A targeted physical exam will be performed as indicated.
- A small quantity of blood (2 mL) will be obtained daily for malaria diagnostics (qPCR with thick blood smear as backup if needed). Febrile or otherwise symptomatic individuals will have malaria diagnostics performed every 8-12 hours at the discretion of the investigator for the purposes of malaria diagnosis and treatment until they are declared malaria positive.
- For those with two qPCR positive tests, as defined by assay validation and study SOPs, or a thick blood smear positive for malaria, they will be considered malaria-positive and will receive artemether/lumefantrine tablets (20/120 mg) orally according to the following schedule: 1) Day 1- four tablets initially, and four tablets again after eight hours, 2) Days 2&3- four tablets twice daily; representing a total daily treatment dose of 40/240 mg, as first line therapy. In case of artemether/lumefantrine intolerance, second line therapy will be atovaquone/proguanil at 4 tablets (250/100 mg) orally for three days, representing a total daily treatment dose of 1 gram/400 mg.
- 7 mL of venous blood will be collected on the day a participant is considered malaria-positive. The sample will be used to evaluate hemoglobin, WBC, platelets, ALT, and serum creatinine.
- Study participants who have been diagnosed with malaria may discontinue the scheduled daily follow-up visits after completion of oral antimalarial therapy. Next follow-up visit will be on day 141 ($\pm$1).

- Urine will be collected on all females of childbearing potential to perform a urine β-HCG pregnancy test on the day a participant is considered malaria-positive.
- If by Day 131 for groups 4-5 (Day 19 for group 6), the study volunteer has not contracted malaria and is free of signs and symptoms of malaria, they will continue with scheduled clinical evaluations and malaria diagnostics.

7.3.13 Days 133 (±1), 135 (±1), 137 (±1) and 139 (±1), Visits 26-29, Outpatient Post-malaria Challenge Surveillance for Participants who Remain Malaria Free, Groups 4, 5 and 6 (Group 6 Days 21, 23, 25,27)

- Participants who have not had a positive malaria test to date will be seen in clinic for continued malaria surveillance and interviewed.
- A review of all current medications (focusing on antibiotics) will be conducted.
- Vital signs (oral temperature, blood pressure, pulse) will be recorded.
- Any solicited or unsolicited AEs, AESIs (groups 4 and 5), or SAEs that have occurred since the last visit will be assessed and a targeted physical exam will be performed as indicated.
- A small quantity of blood (2 mL) will be obtained for malaria diagnostics.
- Participants will be counseled on mosquito avoidance behavior.

7.3.14 Day 141 (±1), Visit 30, Outpatient Post-malaria Challenge Surveillance, Groups 4, 5 and 6 (Group 6 Day 29)

- A review of all current medications (focusing on antibiotics) will be conducted.
- Vital signs (oral temperature, blood pressure, pulse) will be recorded.
- Any solicited or unsolicited AEs, AESIs (groups 4 and 5), or SAEs that have occurred since the last visit will be assessed and a targeted physical exam will be performed as indicated.
- For those who remain malaria negative, a small quantity of blood (2 mL) will be obtained for malaria diagnostics.
- For those who were previously diagnosed with malaria, a small quantity of blood (2 mL) will be obtained for malaria diagnostics to confirm malaria cure.
- For group 6, an additional 2 mL blood sample will be collected for post-CHMI humoral immune assays.
- Participants will be counseled on mosquito avoidance behavior.

7.3.15 Day 169 (±7), Visit 31, Groups 1-5 and 6 (Group 6 Day 57)

- Subjects will be evaluated. Vital signs (oral temperature, blood pressure, pulse) will be assessed and recorded. Interim medical history will be obtained.
- Any solicited or unsolicited AEs, AESIs (groups 4 and 5), or SAEs that have occurred since the last visit will be assessed.
- A targeted physical exam will be performed as indicated.
- Concomitant medications will be reviewed and updated, if applicable.
- For groups 1-5, obtain 16 mL blood sample for safety laboratory and antibody testing (complete blood count, ALT, creatinine, and humoral immune assays)

7.3.16 Day 253 (±14), Visit 32, Groups 1-5

- Subjects will be evaluated. Vital signs (oral temperature, blood pressure, pulse) will be assessed and recorded. Interim medical history will be obtained.
- Any solicited or unsolicited AEs, AESIs, or SAEs that have occurred since the last visit will be assessed.
- A targeted physical exam will be performed as indicated.
- Concomitant medications will be reviewed and updated, if applicable.
- A 9 mL venous blood sample for humoral immune assays will be collected.

7.3.17 Day 450 (±14), Visit 33, Telephone follow-up, Groups 1-5

- Participants will be contacted by telephone to ask about occurrence of serious AEs since the last study vaccination was administered

7.4 Final Study Visit

For participants in groups 1-5, the telephone follow-up at day 450 (±14), visit 33 is considered the final visit.
For participants in group 6, the final visit occurs at day 57 (±7), visit 31.

7.5 Early Termination Visit

Participants may be discontinued from follow-up if any of the following criteria are met:

- Death
- Serious illness or disability making it impossible to maintain follow-up
- Lost to follow-up
- Participant choice or withdrawal of consent
- Termination of the study
- Any circumstance in the opinion of the investigator that would prevent the participant from completing follow-up or that would put the participant at risk.

If a participant is terminated from the study early, when applicable, every effort should be made to perform the following procedures:

- Review current health status and note any changes since the last visit.
- Record all concomitant medications.
- Obtain 7 mL blood sample for safety laboratory testing (CBC, ALT, creatinine).
- Obtain 11 mL blood sample for the humoral immune assays.
- For Groups 4 & 5, obtain 50 mL blood sample for future additional immune assays.
- Perform a targeted physical examination, as indicated.
- Treatment of possible incubating malaria infection (if terminated after CHMI and before antimalarial treatment administration)
- Solicit information regarding AEs. Any ongoing related AEs will be followed to resolution or until a stable chronic condition has been established.
- Participants will be encouraged to permit continued follow-up of AEs if possible.

7.6 Unscheduled Visit

Participants may be asked to come in for additional clinic visits if the need arises for follow-up of local or systemic AE such as neurologic or febrile illnesses, or for requests for repeat blood draws for scheduled laboratory testing from the clinical laboratory due to sample hemolysis or clotting. Neurological symptoms will be evaluated with a full neurological exam and neurological consultation if needed. Any febrile illness will be evaluated as necessary to identify the etiology following standard procedures at the site.

A supplemental visit source document will be filled and signed by the appropriate personnel. All unscheduled visits will be entered into the Emmes Corporation Advantage eClinical® internet data entry system on the appropriate eCRFs. Please see the Manual of Procedures for details.

8 STUDY PROCEDURES/EVALUATIONS

8.1 Clinical Evaluations

Clinical evaluations are detailed in the previous "**Section 7.3 Follow-up**".

Safety and reactogenicity will be assessed by determining rates of solicited and unsolicited adverse events.

12-lead Electrocardiogram
A 12-lead ECG will be performed at screening prior to enrollment for the purposes of evaluating for occult cardiovascular disease and to serve as a baseline comparator in case a repeat ECG is clinically indicated during participation in the study. All ECGs will be evaluated by a cardiologist. An abnormal ECG will be defined as specified previously in the exclusion criteria.

8.2 Laboratory Evaluations

The total amount of blood collected at each visit and over the course of the entire study for each group is detailed in Appendix A: Schedule of Events.

8.2.1 Clinical Laboratory Evaluations

Screening:
The following laboratory tests will be performed at the time of participant screening and will be reviewed before enrollment into the protocol. Each laboratory result will be compared to upper and lower limit norms for the CVD's contracted laboratory system (Garcia Laboratories). Results will be categorized as normal, meaning that the values were within standard limits determined based on population results for the laboratories, or abnormal/positive based on outlying values beyond the upper or lower limit of normality. The Garcia Laboratories, Jackson, MI (or University of Maryland Reference Facility as a back-up) will perform these assays. It is estimated that 7 mL will be sufficient to complete the biochemical and hematological analysis. Additionally, 16 mL will be sufficient to complete viral serologies and sickle cell trait analysis.

- Hematology: hemoglobin, white blood cells (WBC) and, platelet count [3 mL]
- Biochemistry: glucose (random), renal function test (creatinine), and liver enzyme (ALT [Alanine aminotransferase]) [4 mL]

- Viral serologies: Anti-Hepatitis C virus (HCV), Hepatitis B surface antigen (HBsAg) and Human Immunodeficiency Virus (HIV) will be recorded at baseline [13 mL]
- Sickle Cell Trait (Groups 4, 5 and 6 only): Hemoglobin electrophoresis screening for sickle cell trait will be recorded at baseline [3 mL]
- Urinalysis: A dipstick test will be performed examining urine for the presence of protein or blood. Trace values will be accepted but values quantified as "moderate" and/or > 1+ will be deemed abnormal. Hematuria deemed related to menstruation will be followed up to verify clearance of blood. Abnormal values will be sent to Garcia Laboratories for full analysis.

Enrollment:
In addition to hematology and biochemistry that will be repeated at scheduled intervals; the following assay will be performed and repeated according to the study schedule.

- Pregnancy Test: A serum pregnancy test will be performed at the clinical trial study site at the time of screening on all female participants regardless of age unless written medical demonstration of sterility or amenorrhea (defined as one year with medical evaluation) can be provided. A Food and Drug Administration (FDA)-approved testing kit for urine β-hCG pregnancy testing will be performed at the clinical trial study site on all female participants before each vaccination and, for CHMI participants within 24 hours of the malaria challenge event. In addition, testing will be performed on the first day of a positive malaria test.

8.2.2 Special Assays or Procedures

8.2.2.1 Humoral Immune Assays

A total of 11mL of venous blood will be drawn at enrollment and at regular intervals for Groups 1-5 to quantify the humoral immune response. A CSP repeat region (NANP)6-specific or a full length CSP-specific ELISA has been well developed by the community and will be used to quantify the anti-CSP antibody response generated by the rCSP/AP 10-602 [GLA-LSQ] vaccine. Serum will also be probed with a diversity-reflecting peptide microarray per substudy described in 4.1.

8.2.2.2 Additional Immune Assays

A total of 50 mL of venous blood will be drawn on study days 1, 29, 85, and 113 in Groups 4 and 5 to allow for future use assessment of additional immune responses against *P. falciparum* CSP and related targets, including potentially whole sporozoites. These tests may include number and types of PBMC collected, cytokine responses after stimulation, and antibody types and functions,

among others. Samples will be collected from Groups 4 and 5 only as these groups are planned for CHMI, and their immune responses can potentially be correlated to protection against CHMI if that is observed.

8.2.2.3 PCR analysis

A sensitive real-time polymerase chain reaction (PCR) will be used for the detection of *P. falciparum* parasites. qPCR will be run off venous blood collected in EDTA tubes. Optimization experiments have established a high degree of sensitivity. PCR primers will be based on the published sequence of the highly conserved (32), stage specific (33) *P. falciparum* 18S ribosomal RNA gene. Primer sequences are identical to the corresponding sequence of the NF54 strain. Samples will be blinded to treatment group, participant ID, and visit number, and assays run daily. Each sample will be run in triplicate along with a water control. The data will be analyzed using the Applied Biosystem 7300 Absolute Quantification Software.

8.2.2.4 Blood malaria smear

A small aliquot of blood (10 µL) will be placed upon microscope slides for the creation of thick malaria smears. The thick smear will be allowed to dry, lysed with distilled water and stained with Giemsa for analysis of intra-erythrocytic ring forms consistent with malaria. Trained investigators blinded to randomization results, examine five separate passes along the 1 cm axis of a blood smear using the 100x oil immersion lens of calibrated microscopes. This will be doubled to ten passes for symptomatic individuals. Ten passes performed by microscopists examines a total of 0.9-1.1 µL of blood (refer to site standard operating procedures). The peripheral blood smear provides comprehensive information on the stages, and the density of parasitemia with a sensitivity of 5 to 20 parasites/µL of blood for an experienced laboratory professional although this level can be much higher taking into account reader variability. These assays will be performed on-site in the Malaria Laboratory and Insectary of the CVD and the collective experience of the CVD staff is a sensitivity of 2 parasites/µL. The minimum acceptance criteria is NO false positive reads on thick smears and a positive smear will be defined as two unquestionable parasites present on smear. The above technique has proven to be highly effective and accurate with ~45% of individuals being diagnosed before malaria symptoms (personal experience, UMD). The slides will be stored for future review as necessary.

8.2.3 Specimen Preparation, Handling, and Shipping

Blood will be collected from study participants by venipuncture up to 30 times during the study, including screening. The maximum amount of blood requested from any participant for standard

collection during the study for research purposes will not exceed 550 mL over an eight-week period. However, additional blood may be obtained as deemed necessary by the investigators or clinicians to evaluate any illness or condition. Research samples will be prepared, handled, and shipped according to the study manual of procedures (MOP).

8.2.3.1 Instructions for Specimen Preparation, Handling, and Storage

Blood will be obtained by research staff as part of the clinical evaluation of the participant. Assays will be sent to the University of Maryland central laboratory processing, to Fisher repository or on to Garcia diagnostics depending on the nature of the testing. Research samples will be prepared, handled and stored according to the study MOP. qPCR assays will be run at the Center for Vaccine Development.

8.2.3.2 Specimen Shipment

Specimens collected during the course of the study will be shipped according to the study MOP. Specimen labeling requirements will also be included in the study MOP.

9 ASSESSMENT OF SAFETY

9.1 Specification of Safety Parameters

The primary safety measurements of this trial are:

1. Occurrence of solicited local reactions within 7 days following vaccination (day of vaccination and 7 subsequent days)

2. Occurrence of solicited systemic reactions within 7 days following vaccination (day of vaccination and 7 subsequent days)

3. Occurrence of unsolicited AEs considered related to vaccination and that are severe (Grade 3) within 28 days following vaccination (day of vaccination and 28 subsequent days), according to the Medical Dictionary for Regulatory Activities (MedDRA classification)

4. Occurrence of serious adverse events (SAEs) considered related to vaccination within 28 days following vaccination (day of vaccination and 28 subsequent days), according to the MedDRA classification

5. Occurrence of SAEs at any point during the planned participant follow-up period, according to the MedDRA classification

9.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

9.2.1 Adverse Events

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

Adverse events will be documented in terms of a medical diagnosis. When this is not possible, the adverse event will be documented in terms of signs and/or symptoms observed by the investigator or reported by the subject at each study visit. All AEs occurring while on study will be documented appropriately regardless of relationship. Pre-existing conditions or signs and/or symptoms (including any which are not recognized at study entry but are recognized during the study period) present in a participant before the start of the study will be recorded on the participant's Medical History CRF and will be recorded as an AE if deterioration or exacerbation in the condition occurs during the study. Any hospitalization will be considered a serious adverse event. Information to be collected include event description, date of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis, which would include an MD, PA, Nurse Practitioner, DO or DDS), and date of resolution/stabilization of the event. All AEs will be followed to adequate resolution or stabilization. Solicited adverse events to be recorded as endpoints are described below in Tables 9-1, 9-2, 9-3 and 9-4. Systemic and local solicited symptoms are recorded on subject memory aids and reviewed at scheduled clinic visits through day 8. All AEs must be graded for severity and relationship to study product.

Severity of Event: All AEs will be assessed by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or appropriate sub-investigator using a protocol defined grading system (see Sections 9.2.2 and 9.2.3). For events not included in the protocol defined grading system, than the following guidelines will be used to quantify severity.

- Mild (Grade 1): events require minimal or no treatment and do not interfere with the subject's daily activities.
- Moderate (Grade 2): events result in a low level of inconvenience or concern with therapeutic measures. Moderate events may cause some interference with functioning and daily activities.
- Severe (Grade 3): events that prevent the subject's usual daily activities and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

The severity of systemic and clinical laboratory adverse events will be graded according to Section 9.2.2 and the toxicity tables included in the Appendices to this protocol.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of intensity. When AEs are intermittent, the onset and duration of each episode will be documented.

Relationship to Study Products: The study physician's assessment of an AE's relationship to study product is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. The relationship to study product must be assessed for AEs using the terms: related or not related. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used:

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- Not Related – There is **not** a reasonable possibility that the administration of the study product caused the event.

9.2.2 Reactogenicity (for Vaccine Studies and Some Therapeutic Trials)

Reactogenicity events are AEs that are known to occur with this type of study vaccine. The following Toxicity Grading Scales will be used to grade solicited local (injection site) and systemic (subjective and quantitative) reactions:

Table 9-1: Injection Site Reactogenicity Grading

Local (Injection Site) Reaction	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Pain – experienced without touching the injection site (spontaneous discomfort)	Subject is aware of pain but it does not interfere with daily activity **and** no pain medication is taken	Subject is aware of pain; there is interference with daily activity **or** it requires use of pain medication	Subject is aware of pain **and** it prevents daily activity (incapacitating)
Tenderness – hurts only when injection site is touched or the arm is moved	The area immediately surrounding the injection site hurts only when touched or with arm motion, **and** it does **not** interfere with daily activity	The area immediately surrounding the injection site hurts when touched or with arm motion, **and** it interferes with daily activity	The area immediately surrounding the injection site hurts when touched or with arm motion, **and** it prevents daily activity (incapacitating)
Swelling*	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity (incapacitating)

* Will be also measured in cm but size will not be used as halting criteria.

Erythema and swelling as analyzed by measurement will be graded as follows:

Table 9-2: Injection Site Reactogenicity Measurements

Local (Injection Site) Reaction	Small (Grade 1)	Medium (Grade 2)	Large (Grade 3)
Erythema (Redness)*	2.5 – 5 cm	5.1 – 10 cm	> 10 cm
Induration (Hardness)/Swelling*	2.5 – 5 cm	5.1 – 10 cm	> 10 cm

* Will not be used as halting criteria.

Table 9-3: Subjective Systemic Reactogenicity Grading

Systemic (Subjective)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)**
Chills	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Myalgia (Body Aches/Muscular Pain)*	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Arthralgia (Joint Pain)*	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Nausea	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Vomiting	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Headache	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Dizziness	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Malaise	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Fatigue	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity

* Not at injection site; ** incapacitating

Oral temperature[#] will be graded as follows:

Table 9-4: Quantitative Systemic Reactogenicity Grading

Systemic (Quantitative)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Fever* - oral[†]	38.0°C – 38.4°C	38.5°C – 38.9°C	>=39.0°C

[#] Oral temperature assessed on Day 1 (Visit 01) before the first study vaccination will be considered as baseline.

*Note: A fever can be considered not related to the study product if an alternative etiology can be documented.

† Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes before taking oral temperature.

9.2.3 Serious Adverse Events

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

- Death,
- a life-threatening adverse event*,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

* Life-threatening adverse event. An adverse event is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk

of death. It does not include an adverse event that, had it occurred in a more severe form, might have caused death.

All SAEs will be:

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

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

The site Principal Investigator or appropriate co-investigator is responsible for reporting all AEs/ SAEs that are observed or reported during the study, regardless of the relationship to study product. AEs/SAEs, abnormal clinical laboratory test values, or abnormal clinical findings will be documented, reported and followed appropriately.

9.3 Reporting Procedures

9.3.1 Serious Adverse Events

AEs will be followed to resolution even if this extends beyond the study reporting period. Resolution of an AE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

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

DMID Pharmacovigilance Group

Clinical Research Operations and Management Support (CROMS)

6500 Rock Spring Dr. Suite 650

Bethesda, MD 20817, USA

SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)

SAE FAX Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)

SAE Email Address: PVG@dmidcroms.com

In addition to the SAE form, selected SAE data fields must also be entered into Advantage eClinical®. Please see the protocol-specific MOP for details regarding this procedure.

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

The site will notify the ISM when an SAE is provided to the DMID Pharmacovigilance Group. The DMID medical monitor will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct.

At any time after completion of the study, if the site principal investigator or appropriate co-investigator becomes aware of an SAE that is suspected to be related to study product, the investigator will report the event to the DMID Pharmacovigilance Group.

9.3.2 Regulatory Reporting for Studies Conducted Under DMID-Sponsored IND

Following notification from the investigator, DMID, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected. DMID will report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event. DMID will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting as specified in 21 CFR 312.32. DMID will also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information. Relevant follow-up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, DMID will submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

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

9.3.3 Adverse Events of Special Interest

Adverse Events of Special Interest (AESI):
Clinical trials of vaccines with novel adjuvants are required to include collection of a specific list of AESI. These unsolicited AESI will be recorded and reported on the AE/SAE CRF within 24 hours of site awareness, regardless of their attributed relationship to study injections. For this protocol, the AESI list includes the following diagnoses made in study vaccine recipients during the course of the study follow-up period:

- **Neuroinflammatory Disorders**: Optic Neuritis, Uveitis, Multiple Sclerosis, Demyelinating Disease, ADEM, Myasthenia Gravis, Myelitis/Transverse Myelitis, Guillain-Barré Syndrome.
- **Musculoskeletal and Connective Tissue Diseases**: Rheumatoid Arthritis, Juvenile Rheumatoid Arthritis, Systemic Lupus Erythematosus, Cutaneous Lupus, Dermatomyositis, Polymyositis, Polymyalgia Rheumatica, Psoriasis/Psoriasis Arthritis, Ankylosing Spondylitis, Scleroderma, Sjögren's Syndrome, Temporal Arteritis, Wegener's Granulomatosis, Sarcoidosis, Mixed Connective Tissue Disease, Behçet's Syndrome.

- **Gastrointestinal Disorders**: Crohn's disease, Ulcerative Colitis, Celiac Disease, Ulcerative Proctitis, Autoimmune Hepatitis, Inflammatory Bowel Syndrome (non-specific).
- **Hematologic Disorders:** ITP, Anti-Phospholipid Syndrome, Autoimmune Hemolytic Anemia.
- **Thyroid Disorders:** Grave's Disease, Thyroiditis, Basedow's Disease
- **Renal and Urinary Disorders:** Nephritis, Glomerulonephritis
- **Vascular Disorders:** Vasculitis, Anti-neutrophilic Cytoplasmic Antibodies (ANCA)-Associated Vasculitis
- **Others:** Carditis, Myocarditis, Pericarditis, Insulin-Dependent Diabetes Mellitus (IDDM)

All AESI will be:

- Reviewed and evaluated by a study physician at the site, the DMID Medical Monitor, and the SMC.
- Recorded on the AE/SAE CRF.

9.3.4 Reporting of Pregnancy

All pregnancies that develop in participants within 30 days of the last vaccination or within the 30-day window after the CHMI event (Day of challenge plus 30 days) will be reported by the Investigator to the Sponsor and IRB within 24 hours of learning of its occurrence. The pregnancy will be documented on the Pregnancy Reporting From provided by Emmes, and this form will be used for the pregnancy event data entry into Advantage eClinical®. All study-mandated blood samples will be obtained and the participant will continue in follow-up for safety events. Pregnancies will be followed until 30 days after delivery to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. No subsequent vaccinations or CHMI procedures will be administered to pregnant participants.

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

Participants who experience AEs that are related to the study product will be followed to resolution or stabilization.

9.5 Halting Rules

Vaccination Phase

Individual Halting Rules

The following Individual halting rules will apply to each subject. The Principal Investigator (PI) will monitor the individual halting rules and will make recommendations regarding the continuation of vaccinations in a subject. If the individual halting rules are met, no further vaccination will be administered to that subject until the investigators have conferred with the Medical Monitor (MM) and Medical Officer (MO) and a written report is submitted to the SMC. After a thorough review by the PI, MM, MO and sponsor, vaccination of the subject may resume only if all parties agree it is safe to resume vaccination.

- Any solicited local injection site adverse event (AE) at the discretion of the PI.
- Any study vaccine-related injection site ulceration, abscess, or necrosis.
- Any study vaccine-related solicited systemic Grade 3 AE persisting at Grade 3 for greater than 2 days (subjective AE corroborated by study personnel).
- Any study vaccine-related unsolicited Grade 3 AE persisting at Grade 3 for greater than 2 days.
- Any study vaccine-related Grade 3 laboratory AE persisting at Grade 3 for greater than 2 days.
- Any acute allergic reaction or anaphylactic shock following the administration of study vaccine.
- Any study vaccine-related serious adverse event (SAE).

Study Group Halting Rules

Two sentinel subjects from each study group will be vaccinated one day prior to the remainder of the group for the first study vaccination. If the sentinel subject halting rules are met within 1 day of study vaccination, vaccinations will be held for the eight remaining subjects within the affected study group and for the subjects in other study groups. The study site member first aware of the event meeting the sentinel subject halting rules will immediately notify the PI. The PI will notify the MM and MO immediately (within 24 hours). An ad hoc SMC review will be performed. Vaccinations may resume only if the MM, MO, the PI, the sponsor and the SMC agree it is safe to resume vaccinations.

- Any subject experiences immediate hypersensitivity reaction (in the 30 to 60 minutes following vaccination)
- Any subject experiences cytokine storm
- Any subject experiences a study vaccine-related Grade 3 solicited systemic AE (subjective AE corroborated by study personnel).
- Any subject experiences a study vaccine-related Grade 3 unsolicited AE.
- Any subject experiences a study vaccine-related SAE.

Study Group Advancement Halting Rules

Advancement from enrollment of Group 1 to enrollment of Groups 2 and 3 will be based on review of all available safety data collected on Group 1 from the day of first study vaccination through at least study day 7. The Protocol PI will review this data, and study group advancement will proceed if none of the study group advancement halting rules specified below are met:

- Two or more subjects experience the same study vaccine-related Grade 3 solicited injection site AE (except measured erythema) related to the study product and persisting at Grade 3 for greater than 2 days.
- Two or more subjects experience the same study vaccine-related Grade 3 solicited systemic AE and persisting at Grade 3 for greater than 2 days (subjective AE corroborated by study personnel).
- Two or more subjects experience the same (same organ system at the MedDRA PT level) study vaccine-related Grade 3 unsolicited systemic AE and persisting at Grade 3 for greater than 2 days.
- Two or more subjects experience generalized urticaria within 3 days of study vaccination.
- Three or more subjects experience a study vaccine-related Grade 3 laboratory AE and persisting at Grade 3 for greater than 2 days.
- Any subject experiences a study vaccine-related SAE.

If one or more study group advancement halting rules are met no further vaccination will be administered in any study group until the PI has conferred with the MM, MO, and a full written report has been submitted to the SMC. After a study group advancement halting rule is activated, a thorough review by the PI, MM, MO, and an ad hoc SMC review will be performed. Vaccination

of the subjects within the affected group and the other study groups may resume only if the MM, MO, the PI, the SMC, and the sponsor agree it is safe to resume vaccinations and the following considerations are discussed.

- Relationship of the AE or SAE to the vaccine.
- Relationship of the AE or SAE to the vaccine dose (i.e, only associated with high dose group).
- If appropriate, additional screening or laboratory testing is provided to other subjects to identify subjects who may develop similar symptoms.

Study Halting Rules

Study enrollment and vaccination will be halted and an ad hoc SMC review will be performed if any of the following occur across all study groups:

- Two or more subjects die after study vaccination regardless of relatedness to the study vaccine.
- Two or more subjects experience study vaccine-related ulceration, abscess, or necrosis at the site of injection.
- Any subject experiences study vaccine-related anaphylaxis within 1 day following study vaccination.
- Any subject experiences a study vaccine-related SAE.
- Any subject experiences a study vaccine-related AESI.
- Two or more subjects experience generalized urticaria within 3 days of study vaccination.
- Two or more subjects experience the same (same organ system at the MedDRA PT level) study vaccine-related Grade 3 unsolicited AE and persisting at Grade 3 for greater than 2 days.
- The study will also be halted for SMC review/recommendation if, during the 7 days after each vaccination, any of the following occurs across all study groups:
    - Two or more subjects who received at least one dose of study vaccine to date experience the same study vaccine-related, Grade 3 solicited local injection site AE (except measured erythema) and persisting at Grade 3 for greater than 2 days.

- Two or more subjects who received at least one dose of study vaccine to date experience the same study vaccine-related, Grade 3 solicited systemic AE and persisting at Grade 3 for greater than 2 days (subjective AE corroborated by study personnel).
- Three or more subjects who receive at least one dose of study vaccine to date experience a study vaccine-related Grade 3 laboratory AE and persisting at Grade 3 for greater than 2 days.

CHMI Phase

Administration of CHMI study product will be halted if the study PI makes the determination that it will be unsafe for the subjects to proceed with the CHMI challenge.
DMID retains the authority to suspend additional enrollment and study interventions/administrations of study product during the entire study period, as applicable.

9.6 Safety Oversight (ISM plus SMC)

9.6.1 Independent Safety Monitor (ISM)

The ISM is a physician with relevant expertise whose primary responsibility is to provide independent safety monitoring in a timely fashion. The ISM will review SAEs and other AEs as needed and provide an independent assessment to DMID. The independent monitoring of this DMID-sponsored study will be performed by an ISM only.

A qualified and experienced physician not otherwise associated with this protocol will serve as the Independent Safety Monitor (ISM) at the site for this study as per the specifications set forth in the DMID standard operating procedures. The ISM's *curriculum vitae* will be maintained on record. If safety concerns are identified, the ISM may request a meeting of the Safety Monitoring Committee (SMC) to review safety data. The ISM will also review all serious adverse events and provide an unbiased written report of the event within 10 calendar days of the initial report. At a minimum, the ISM will comment on the outcomes of the SAE and relationship of the SAE to the study product. The ISM will also indicate whether he/she concurs with the details of the report provided by the study investigator.

Furthermore, the ISM, a licensed physician, with relevant expertise will have the primary responsibility to provide independent safety monitoring in a timely fashion. The ISM will review

SAEs and other adverse events as needed and provide an independent assessment to DMID. The ISM may attend the open session of the SMC meetings.

9.6.2 Safety Monitoring Committee (SMC)

This clinical trial will utilize an SMC, which is an independent group of experts that advises DMID and the study investigators for many Phase 1 and smaller Phase 2 trials. The primary responsibility of the SMC is to monitor subject safety. The committee is external to DMID, composed of at least three voting members. Its activities will be described in an SMC charter that will delineate membership, responsibilities, and the scope and frequency of data reviews. DMID or the SMC may convene ad hoc meetings of the SMC according to protocol criteria or if there are concerns that arise during the study. The SMC will have access to unblinded data during its closed session, if applicable. During the closed session of the meeting the committee may request unblinding of the treatment assignments for individual subjects or by group if necessary to adequately assess the safety data presented. After its assessment, the SMC will recommend continuation, modification, or termination of the clinical trial.
The SMC will review aggregate safety data (primary endpoint) for increased rate of occurrence of serious adverse reactions. The SMC meets the specifications set forth in the DMID standard operating procedures. The safety data will be compiled by The Emmes Corporation.

SMC reviews will occur:

1) The SMC will electronically review all available safety data for enrolled study Groups 1-3 including laboratory monitoring done 7 days after the second vaccination and make recommendation to proceed with the enrollment of Groups 4-6.
2) Ad hoc meeting is convened:
    a. If any of the study group halting rules including the sentinel subject halting rules, the study group advancement halting rules, and the study halting rules are met during the vaccination phase.
    b. to discuss any issue of safety raised by an investigator, the sponsor, the ISM, or a member of the SMC.

Additional details of study safety oversight, including scheduled meetings and roles and responsibilities of SMC are described in the study charter.

10 CLINICAL MONITORING

In general, site monitoring is conducted to ensure that:

- human subjects' rights and well-being are protected;
- data are accurate, complete, and verifiable from source documents;
- the study complies with the protocol/amendment(s), ICH Good Clinical Practice (GCP) guidelines, and applicable regulatory requirements.

In order to ensure protocol compliance, monitoring visits by a sponsor-designated professional or monitor will occur at scheduled intervals before, during, and at study completion. The visit frequency will be defined in a monitoring plan (refer to Section 10.1 below) and communicated before study start to the principal investigator and all other appropriate study personnel.

10.1 Site Monitoring Plan

Site monitoring will be conducted to ensure that human participant protection, study procedures, laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, GCP/ICH, and regulatory guidelines, and that the study is conducted in accordance with the protocol and sponsor standard operating procedures. DMID, the sponsoring agency, or its designee will conduct site-monitoring visits as detailed in the monitoring plan or in the Manual of Procedures.

Site visits will be made at standard intervals as defined by DMID and may be made more frequently as directed by DMID. Site visits will be conducted by study monitors from the DMID contractor for clinical monitoring (CROMS). Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, CRFs, informed consent forms, medical and laboratory reports, and protocol compliance. Study monitors will meet with investigators to discuss any problems and actions to be taken and document visit findings and discussions. The principal investigator will be responsible for review, follow-up and resolution of monitoring findings.

11 STATISTICAL CONSIDERATIONS

11.1 Study Hypotheses

Hypothesis 1: The rCSP/AP 10-602 [GLA-LSQ] candidate malaria vaccine will induce an immune response in a dose-dependent manner as measured by anti-CSP antibody titer via ELISA.

Hypothesis 2: The rCSP/AP 10-602 [GLA-LSQ] candidate malaria vaccine will provide a minimum of 50% efficacy in vaccinees compared to unvaccinated infectivity controls.

11.2 Sample Size Considerations

The primary objective of this Phase 1 trial is to provide preliminary safety evaluation in a small group of closely observed subjects. As such, the trial is not powered for statistical comparisons. Descriptive numeric and graphical summaries will be used in the assessment of safety. Numeric summaries and visual displays of data will be used to characterize immune responses.

The study has limited power to detect efficacy and is intended to provide a preliminary estimate for confirmatory testing in larger studies. Assuming that the 6 infectivity controls have a 0.99 chance of becoming infected, if there are 10 vaccinees with a 0.22 chance of becoming infected (which corresponds to an efficacy of 78%) the study will have at least 80% power to detect a difference in the proportions infected between groups. If groups 4 and 5 receive the same dose and are combined into a group of 20, then the study will have more than 97% power assuming the true efficacy is at least 78%. The combined sample size of 20 would provide power to detect differences if the true efficacy is lower as well. For example, if the chance of a vaccinee becoming infected is 36% (corresponding to efficacy of 64%), then this combined sample size would provide at least 80% power.

11.3 Planned Interim Analyses

11.3.1 Interim Safety Review

The SMC will electronically review when all available safety data for enrolled study Groups 1-3 including laboratory monitoring done 7 days after the second vaccination and make recommendation to proceed with the enrollment of groups 4-6.

Additional interim safety review may include enrollment and demographic information, medical history, concomitant medications, physical assessments, clinical laboratory values, dosing, and solicited and unsolicited AE/SAEs. Additional data may be requested by the PI and/or SMC, and interim statistical reports may be generated as deemed necessary and appropriate by DMID. The SMC may receive data in aggregate and presented by group. The SMC may also be provided with expected and observed rates of the expected AEs in an unblinded fashion. The SMC will meet and review this data at scheduled time points or ad hoc as needed during the study as defined in the SMC charter. As an outcome of each review/meeting, the SMC will make a recommendation as to the advisability of proceeding with study vaccinations (as applicable), and to continue, modify, or terminate the study. Additionally, the study will be monitored to determine if any of the halting rules described in Section 9.5 are met.

11.3.2 Interim Immunogenicity Review

The multiplicative change from baseline (fold-rise) will be calculated for day 57 for Groups 1, 2 and 3. The mean, median, and geometric mean titer for day 57 will be calculated. If at least fourfold increase in geometric mean anti-CSP antibody or geometric mean anti-CSP titer of 20 is found, then the 10 volunteers in Group 5 will receive the lowest rCSP dose that gives this predefined immunogenic response ((either 10 or 30µg rCSP) + AP 10-602 [GLA-LSQ]), otherwise, Group 5 will receive 60µg rCSP + AP 10-602 [GLA-LSQ].
Data for the safety and immunogenicity interim analysis for groups 1-3 collected up to day 57 will be cleaned but not locked for the planned safety and immunogenicity analysis for review. Due to the importance of this data to the vaccine community, these results may be shared with the greater scientific community via meeting abstracts, professional presentations, and manuscript submissions to scientific journals. The data to be presented and the authorship will be discussed between investigators and sponsors, and approved by the sponsors, before any official communication.

11.3.3 Handling of Missing Data

All attempts will be made to collect all data per protocol. No imputation will be performed for missing values. Any data point that appears to be erroneous or inexplicable based on clinical judgment will be investigated as a possible outlier. If data points are identified as outliers, sensitivity analyses may be performed to examine the impact of including or excluding the outliers. Any substantive differences in these analyses may be reported.

11.4 Final Analysis Plan

The primary analysis will be conducted on data and samples collected on all groups until study day 141 on clean but unlocked data. This analysis will include all available data on the primary and secondary outcome measures listed in Section 3.2 and will be presented in a 'Primary Analysis Report' which will be shared with investigators before the clinical study report is generated and will be available for public presentation and publication. Subsequent data collected will be analyzed and included in the Clinical Study Report following completion of all protocol defined follow-up visits.

This study, like other Phase I studies, is exploratory rather than confirmatory; its purpose is to estimate event rates and patterns of immune responses rather than to test formal statistical hypotheses. Estimates will be presented with their 95% confidence intervals for safety and efficacy endpoints. Descriptive approaches will be used to meet the protocol objectives as stated in this protocol. Results will be presented in tabular format, as well as graphically when appropriate.

It is anticipated that the results of this primary analysis and Clinical Study Report will be presented separately and/or together to the scientific community via oral presentations at meetings and written publications in scientific journals. The data to be presented and the authorship will be discussed between investigators and sponsors, and approved by the sponsors, before any official communication.

The official report of the primary analysis and Clinical Study Report will be drafted by Emmes and finalized by the PI and DMID. These reports will contain detailed information about the participants, their tolerance of the rCSP/AP 10-602 [GLA-LSQ] malaria vaccine, their side effects and laboratory abnormalities, their immunologic response to vaccination measured by anti-CSP antibody ELISA, as well as their response to CHMI (for groups 4 through 6).

The Clinical Study Report will be conducted on cleaned and locked data and samples collected until for all groups through the end of protocol defined follow-up using the same format as described for the primary analysis. The official report of the final analysis will be drafted by Emmes and finalized by the PI and DMID. This report will contain detailed information about the participants, their tolerance of the rCSP/AP 10-602 [GLA-LSQ] malaria vaccine, their side effects and laboratory abnormalities, their immunologic response to vaccination, as well as their response to CHMI.

11.4.1 Final Analysis Plan

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

11.4.2 Analysis of Safety

The number of subjects experiencing unsolicited adverse events and the number of spontaneous events, classified using MedDRA® System Organ Classes and Preferred Terms, reported after vaccination through the end of protocol-defined follow-up will be tabulated per group. The intensity and relationship to study product of the unsolicited adverse events reported will also be assessed. Serious adverse events will be described. A complete listing of adverse experiences for each subject will provide details including severity, relationship to vaccine, onset, duration, and outcome.

The number of subjects experiencing clinical safety laboratory adverse events and the number of events, following vaccination will be tabulated per group.

Solicited local and systemic reactogenicity will be analyzed similarly to spontaneously reported adverse events but will be listed by specific symptom documented as well as categorized as local or systemic. Additional assessments of product safety will include clinical observation and monitoring of hematological, chemical, and immunologic parameters.

11.4.3 Analysis of Efficacy

Proportions of participants infected after CHMI will be compared between vaccinees and infectivity controls. Efficacy will be calculated as 1 – RR, where RR is the risk ratio, and will be presented with its 95% confidence interval. To assess whether the vaccine reduces time to infection, Kaplan Meier curves will be presented, and a log rank test will compare survival curves between vaccinees and infectivity controls.

11.4.4 Immunology Analyses

Antibody titers against the malaria circumsporozoite antigen at days 1, 8, 29, 36, 57, 85, 92, 113, 169 and 253 post-enrollment, using ELISA will be presented at baseline and by study day using

descriptive statistics. Changes from baseline to each visit will be presented. A graph of immunological response data over time for each dose will be presented with confidence limits. In addition, geometric mean and fold-rise in ELISA level will be calculated.

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

In compliance with ICH E6, Section 4.9, complete source documentation (which include but are not limited to outpatient and inpatient records, laboratory test reports, and malaria diagnostics results) will be maintained on every subject throughout the duration of the study. Source documents derived from the electronic CRFs will be the central record for recording data on volunteers enrolled in the study. All clinical surveillance reports will be recorded into the CRF and results from source documentation will be recorded into the electronic CRF. The accuracy of this transfer of data will be the responsibility of the investigator. Only authorized study personnel will have direct access to these documents and the management of these documents will be the joint responsibility of the principal investigator and nurse study coordinator. As part of participating in a DMID-sponsored, DMID-affiliated, or manufacturer-sponsored study, authorized representatives of the sponsor(s), DMID, and regulatory agencies will be allowed to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress

13 QUALITY CONTROL AND QUALITY ASSURANCE

The Quality Management Plan will comply with DMID Clinical Quality Management (CQMP) policy; and the implementation of that plan benefits the internal site audits by:

- Supporting substantive performance measurements/findings/corrective actions, as required, and
- Providing data to support reporting requirements, as applicable.

The University of Maryland Center for Vaccine Development core Quality Management (QM) Plan is accepted by DMID and is in place onsite, and available upon request. As defined in the core QM plan, a separate protocol-specific clinical research QM plan outlining the sample size, priority of protocol review, frequency of quality assurance audits, communication of findings, and annual review will be prepared for DMID QMP reviewers.

DMID-designated clinical monitors will verify that the clinical trial data are generated, documented (recorded), and reported in compliance with the protocol, GCP standards, and the applicable regulatory requirements.

The PI will provide direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The PI will ensure all study personnel are appropriately trained and applicable documentations are maintained on site.

The Statistical and Data Coordinating Center (SDCC) will implement quality control procedures beginning with the data entry system; database quality control checks will be implemented. Any missing data or data anomalies will be communicated to the site(s) for clarification and resolution.

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Ethical Standard

The investigators will ensure that this study is conducted in full conformity with the Declaration of Helsinki, or with the ICH GCP regulations and guidelines, whichever affords the greater protection to the participant.

The investigators will also ensure that this study is conducted in full conformity with the principles of the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR 50 and 56. The Institution will hold a current FWA issued by OHRP for federally funded research.

14.2 Institutional Review Board

Before enrollment of participants into this trial, the protocol and protocol-related documents including the informed consent form will be reviewed and approved by the University of Maryland IRB.

The responsible official for the IRB will sign the IRB letter of approval of the protocol before the start of this trial and a copy will be provided to DMID. Notification of the IRB's composition and the institutions Federal Wide Assurance number will be provided to DMID. The University of Maryland IRB currently holds and will maintain a U.S. FWA issued by OHRP for the entirety of this study.

Should amendments to the protocol be required, the amendments will be written by the sponsor and provided to the investigator for submission to the IRB.

Participants will be compensated for their participation in this study. Compensation will be in accordance with the local IRB's policies and procedures and requires IRB approval.

The local IRB will be notified if any of the study group advancement halting rules and the study halting rules are activated or released.

14.3 Informed Consent Process

The investigator will choose participants in accordance with the eligibility criteria detailed previously. The investigator will not exercise selectivity so that bias is prevented. All participants must sign an informed consent form that complies with the requirements of both 21 CFR Part 50 and Health Insurance Portability and Accountability Act (HIPAA) before entering the trial. A consent form that complies with the requirements of 21 CFR Part 50 will be used.

Before the trial, participants will receive a comprehensive explanation of the proposed vaccine product (and/or CHMI as appropriate), including the nature and risks of the trial, any known adverse events associated with the trial product, the investigational status of the components, the procedure and risks of malaria challenge, and the other elements that are part of obtaining proper informed consent. Participants will also receive a detailed explanation of the proposed use and disclosure of their protected health information, including specifically their biological specimens. Participants will be allowed sufficient time to consider participation in the trial, after having the nature and risks of the trial explained to them. The consent form must not include any exculpatory statements.

DMID will provide the investigator, in writing, any new information that bears significantly on the participants' risk to receive the investigational product. This new information will be communicated by the investigator to participants who consent to participate in the trial in accordance with IRB requirements. The informed consent document will be updated and participants will be re-consented, if necessary.

Site staff may employ recruitment efforts before the participant consenting; however, before any protocol-specific procedures are performed to determine protocol eligibility, an informed consent form must be signed. Participants will be given a copy of all consent forms that they sign.

The informed consent process will be initiated before a volunteer agrees to participate in the study and should continue throughout the individual's study participation. The subject will sign the informed consent document before any procedures are undertaken for the study. A copy of the signed informed consent/assent document will be given to the subject or the legal guardian for their records. The consent will explain that subjects may withdraw consent at any time throughout the course of the trial. Extensive explanation and discussion of risks and possible benefits of this investigation will be provided to the subjects in understandable language. Adequate time will be provided to ensure that the subject has time to consider and discuss participation in the protocol.

The consent form will describe in detail the study interventions/products/procedures and risks/benefits associated with participation in the study. By signing the informed consent form, the participant agrees to complete all evaluations required by the trial, unless the participant withdraws voluntarily or is terminated from the trial for any reason.

14.3.1 Informed Consent/Assent Process (in Case of a Minor)

Not applicable.

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

All healthy adults between the ages of 18 to 45 years of age, who meet the inclusion/exclusion criteria, regardless of religion, sex, or ethnic background, will be included in the study. Due to the nature of the study, and the fact that no benefit exists to the U.S. malaria-naïve volunteer, children will not be enrolled at this time. Malaria infection during pregnancy can have adverse effects on both mother and fetus, including maternal anemia, fetal loss, premature delivery, intrauterine growth retardation and delivery of low birth-weight infants. As such, women who are pregnant or plan to become pregnant up to 30 days after the last vaccination (Groups 1-3) or up to 30 days after CHMI and persons <18 years of age are excluded from the study.

14.5 Subject Confidentiality

Subject confidentiality is held strictly in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participating subjects. Participants will be assigned a unique study number. All results will be keyed to this number. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

Study records will only be available to staff members and will be kept locked at the study site conforming to the investigators' SOPs. All computer entry will be done by coded number only, and all local databases will be secured with password-protected access systems. The study monitor or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

14.6 Study Discontinuation

In the event that the study is discontinued before completion, all volunteers who received a malaria challenge and did not develop clinical symptoms of malaria, will be offered Malarone® malaria treatment doses to prevent erythrocytic-stage *P. falciparum* parasite development. Treatment doses are as follows: 4 tablets (250/100 mg) orally for three days, representing a total daily treatment dose of 1 gram/400 mg. The participants will be asked to follow up weekly (x 4) with the study team and complete surveillance visits to assure that malaria therapy is taken reliably and that malaria does not develop. In the event that the study volunteer has developed malaria with eradication of the parasite after protocol-specified treatment with artemether/lumefantrine or atovaquone/proguanil, the participant will be asked to follow up with the study team for debriefing. In the event that the study volunteer refuses drug treatment, attempts will be made to convince them to return to the CVD for daily malaria testing. If they refuse, daily contact with maintenance of solicited and unsolicited symptoms will be recorded. If a volunteer refuses therapy and symptoms of malaria develop, he/she will be asked to notify study personnel immediately and treatment will be offered through the University of Maryland Medical System. Study team personnel will be available for questions or follow-up should evaluation be needed. Study team personnel will also be available the entire time period of passive follow-up should symptoms arise relating to malaria. Participants will be telephoned at 12 months after the last vaccination.

14.7 Costs, Subject Compensation, and Research Related Injuries

There is no cost to subjects for the research tests, procedures, and study product while taking part in this trial. Procedures and treatment for clinical care may be billed to the subject, subject's insurance or third party. Subjects may be compensated for their participation in this trial. Compensation will be in accordance with the local IRB's policies and procedures, and subject to IRB approval.

If it is determined by the site principal investigator that an injury occurred to a subject as a direct result of the tests or treatments that are done for this trial, then referrals to appropriate health care facilities will be provided to the subject. Study personnel will try to reduce, control, and treat any complications from this trial. Immediate medical treatment may be provided by the participating site. No financial compensation will be provided to the subject by the NIAID, NIH to the subject, or by the participating site for any injury suffered due to participation in this trial.

14.8 Future Use of Stored Specimens and Data

If residual sera are available following the assays described in this protocol, additional immunological or microbiological assays may be performed on those samples for which permission was expressly granted for preserving samples for future studies at the time of informed consent at study enrollment. Allowing future use of samples for additional immunology testing is a stipulation of the informed consent for Groups 4 and 5. Samples from participants in Groups 1-3 who did not grant permission to preserve samples will be discarded after the analyses described in this protocol have been completed.

The residual specimens can be shared with other investigators listed on the protocol and will be linked to the participant using their study identification number only if the participant grants permission for samples to be linked to their study information. If a participant requests that samples be de-identified before further studies are conducted, then these samples will be de-identified for future studies. Residual specimens may be maintained at Fisher BioServices. Future studies utilizing these samples must first be approved by the University of Maryland IRB and DMID. No increased risk to the study participants is expected in association with the storage of residual specimens. Subjects must agree to the storage and use of their residual specimens and will state their preference on the informed consent form (refer to study informed consent document). Subjects will not be re-contacted for additional information or to receive the results of future studies.

15 DATA HANDLING AND RECORD KEEPING

15.1 Data Management Responsibilities

The principal investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported.

Forms for use as source documents will be derived from the eCRFs and provided by the Emmes Corporation to record and maintain data for each participant enrolled in the study. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue ink is required to ensure clarity of reproduced copies. When making a change or correction, cross out the original entry with a single line and initial and date the change. Do not erase, overwrite, or use correction fluid or tape on the original.

Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained.

The sponsor will provide guidance to investigators on making corrections to the source documents and eCRFs.

All source documents and laboratory reports must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. Adverse events must be graded, assessed for seriousness, severity and causal relationship, and reviewed by the site principal investigator or designee. Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site principal investigator. During the study, the investigator must maintain complete and accurate documentation for the study.

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

A detailed data management plan for the study will be included in the study Manual of Procedures. This data management plan includes information pertaining to:

- Reference to source documentation,
- CRFs, including how they are derived,
- Instructions for completing forms,
- Data handling, security, and monitoring,
- Maintaining subject confidentiality,

- Record retention per the sponsor's requirements, and
- Disaster recovery plans.

15.2 Data Capture Methods

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

15.3 Types of Data

Data for this study will include safety, laboratory (clinical safety and immunologic), and efficacy outcome measures (e.g. reactogenicity, immunogenicity, efficacy, and PCR quantification of malaria parasitemia).

15.4 Timing/Reports

The SMC will be provided with an abbreviated focused adverse event summary to evaluate safety data up to Day 57 for groups 1-3. Immunogenicity data up to Day 57 for groups 1-3 will be provided to the PI for evaluation. Safety and immunogenicity data will be compiled by the Emmes Corporation and the interim report provided within the agreed upon time frame for distribution to the SMC and study team members.

Adverse experiences (spontaneous reports) will be coded into MedDRA preferred terms by Emmes in real-time as data is uploaded to the IDES. Solicited local and systemic reactogenicities will be analyzed similarly to spontaneously reported Adverse Events but will be listed by specific symptom documented as well as categorized as local or systemic. Additional assessments of product safety will include clinical observation and monitoring of hematologic, chemical, and immunologic parameters.

Data clarification will take place in real-time with routine reports sent to the site from the Emmes Corporation for query resolution; a final data clarification query set will be sent from Emmes to the site for resolution before freezing the database for analysis. Data analysis will occur at the end of the study (primary at day 141 and final at the end of protocol defined follow-up) to prepare the Clinical Study Report. For safety reasons, additional interim data analyses may be requested by

the SMC and must be agreed upon by the sponsor DMID and investigators before database freeze and analysis.

15.5 Study Records Retention

Study documents will be retained for a minimum of 2 years after the last approval of a marketing application and until there are no pending or contemplated marketing applications, or when at least 2 years have elapsed since the formal discontinuation of clinical development of an investigational product. These documents will be retained for a longer period, however, if required by local regulations. No record will be destroyed without the written consent of the sponsor.

15.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, Good Clinical Practice (GCP), or Manual of Procedures requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.
These practices are consistent with ICH E6:

4.5 Compliance with Protocol, Sections 4.5.1, 4.5.2, and 4.5.3
5.1 Quality Assurance and Quality Control, Section 5.1.1
5.20 Noncompliance, Sections 5.20.1, and 5.20.2.

It is the responsibility of the site principal investigator and personnel to use continuous vigilance to identify and report deviations within five working days of identification of the protocol deviation, or within five working days of the scheduled protocol-required activity. All deviations must be promptly reported to DMID per the DCC protocol deviation reporting procedures.

All protocol deviations, as defined above, must be addressed in study subject data collection forms. A completed copy of the DMID Protocol Deviation Form must be maintained in the Regulatory File, as well as in the subject's chart. Protocol deviations must be sent to the local IRB/IEC per their guidelines. The site principal investigator and personnel are responsible for knowing and adhering to their IRB requirements.

16 PUBLICATION POLICY

Following completion of the study, the investigator may publish the results of this research in a scientific journal. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine (NLM). Other biomedical journals are considering adopting similar policies. It is the responsibility of DMID to register this trial in an acceptable registry. In compliance with Public Law 110-85, the Food and Drug Administration Amendments Act of 2007 (FDAAA), DMID will also post the results of the trial in accordance to the legal requirements.

The ICMJE defines a clinical trial as any research project that prospectively assigns human participants to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (*e.g.*, Phase 1 trials), would be exempt from this policy. This study is of great public health interest. As a result, this study will be registered in the NLM registry, ClinicalTrials.gov.

All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. The NIH Public Access Policy ensures the public has access to the published results of NIH funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication.

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18 SUPPLEMENTS/APPENDICES

APPENDIX A: SCHEDULE OF STUDY PROCEDURES & EVALUATIONS

Table 18-1: Schedule of events for Groups 1, 2 and 3[1]

Visit Number	00	01	02	03	04	05	06	07	08	09	10	11	31	32	33
Day of Study	-60 to 1	1	2	8	29	30	36	57	85	86	92	113	169	253	450
Window Period			(+1)	(±2)	(±3)	(+1)	(±3)	(±3)	(±3)	(+1)	(±3)	(±3)	(±7)	(±14)	(±14)
Clinical Evaluations	Screen	Enroll													
Informed Consent	x														
Comprehension quiz	x														
Eligibility Review	x	x			x				x						
Physical exam(only targeted, if indicated, for all visits other than visit 00), vitals	x	x		x	x		x	x	x		x	x	x	x	
Medical history	x	x		x	x			x	x			x	x	x	
Post-vaccination vitals		x			x				x						
Concomitant medications	x	x		x	x		x	x	x		x	x	x	x	
Vaccination and post-vaccination assessment		#1	phone		#2	phone			#3	phone					phone
Clinical reactogenicity		x	x	x	x	x	x		x	x	x				
Review of unsolicited AE		x	x	x	x	x	x	x	x	x	x	x	x	x	
Review of SAEs and AESIs		x	x	x	x	x	x	x	x	x	x	x	x	x	x
Serum pregnancy test	x														
Urine pregnancy test		x			x				x						
CBC	x	x		x	x		x	x	x		x	x	x		
ALT and creatinine	x	x		x	x		x	x	x		x	x	x		
Glucose (random)	x														
Urinalysis (protein and blood)	x														
HIV	x														
HBsAg	x														
Hepatitis C antibody	x														
Humoral immune assays		x		x	x		x	x	x		x	x	x	x	
Visit blood volume(s) (mL)	20	18		16	16		16	16	16		16	18	16	9	
Cumulative blood volume (mL)	20	38		54	70		86	102	118		134	152	168	177	

[1]If a participant is terminated from the study early, when applicable, every effort should be made to perform the following procedures: Review current health status and note any changes since the last visit, record all concomitant medications, obtain 7 mL blood sample for safety laboratory testing (CBC, ALT, creatinine) and 11 mL blood sample for the humoral immune assays, perform a targeted physical examination, as indicated, solicit information regarding AEs, and encourage permission for continued follow-up of AEs, if possible. Any ongoing related AEs will be followed to resolution or until a stable chronic condition has been established. For more details, see Section 7.5.

Table 18-2: Schedule of Events for Groups 4 and 5[1]

Visit Number	00	01	02	03	04	05	06	07	08	09	10	11	12-14	15-25	26-29	30	31	32	33
Day of Study	-60 to 1	1	2	8	29	30	36	57	85	86	92	113	118 119 120	121 - 131	133 135 137 139	141	169	253	450
Window Period			(+1)	(±2)	(±3)	(+1)	(±3)	(±3)	(±3)	(+1)	(±3)	(±3)			(±1)	(±1)	(±7)	(±14)	(±14)
Clinical Evaluations	Screen	Enroll										CHMI							
Informed Consent	x																		
Comprehension quiz	x																		
Eligibility Review	x	x			x				x			x							
Physical exam(only targeted, if indicated, for all visits other than visit 00), vitals	x	x		x	x		x	x	x		x	x	x	x	x	x	x	x	
Post-vaccination or post-challenge vitals		x			x				x			x							
Concomitant medications	x	x		x	x		x	x	x		x	x	x	x	x	x	x	x	
Medical history	x	x		x	x			x	x			x					x	x	
Vaccination and post-vaccination assessment		#1	☎		#2	☎			#3	☎									☎
Clinical reactogenicity		x	x	x	x	x	x		x	x	x								

Visit Number	00	01	02	03	04	05	06	07	08	09	10	11	12-14	15-25	26-29	30	31	32	33
Day of Study	-60 to 1	1	2	8	29	30	36	57	85	86	92	113	118 119 120	121 - 131	133 135 137 139	141	169	253	450
Window Period			(+1)	(±2)	(±3)	(+1)	(±3)	(±3)	(±3)	(+1)	(±3)	(±3)			(±1)	(±1)	(±7)	(±14)	(±14)
Review of unsolicited AE		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Review of SAEs and AESIs		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
CHMI participation card												x							
Counseling on mosquito avoidance												x	x	x	x	x			
Serum pregnancy	x																		
Urine pregnancy		x			x				x			x		x	x				
CBC	x	x		x	x		x	x	x		x	x		x			x		
ALT, creatinine	x	x		x	x		x	x	x		x	x		x			x		
Glucose (random)	x																		
Urinalysis	x																		
HIV	x																		
HBsAg	x																		
Hepatitis C	x																		
Hemoglobin electrophoresis	x																		
ECG	x																		
Humoral immune assays		x		x	x		x	x	x		x	x					x	x	
Additional immune assays		50			50				50			50							
Malaria diagnostics													x[2]	x[2]	x[2]	x[2]			
Visit blood volume(s) (mL)	23	68		16	66		16	16	66		16	68	6	29	8	2	16	9	

Visit Number	00	01	02	03	04	05	06	07	08	09	10	11	12-14	15-25	26-29	30	31	32	33
Day of Study	-60 to 1	1	2	8	29	30	36	57	85	86	92	113	118 119 120	121 - 131	133 135 137 139	141	169	253	450
Window Period			(+1)	(±2)	(±3)	(+1)	(±3)	(±3)	(±3)	(+1)	(±3)	(±3)			(±1)	(±1)	(±7)	(±14)	(±14)
Cumulative blood volume (mL)	23	91		107	173		189	205	271		287	355	361	390	398	400	416	425	

[1]If a participant is terminated from the study early, when applicable, every effort should be made to perform the following procedures: Review current health status and note any changes since the last visit, record all concomitant medications, obtain 7 mL blood sample for safety laboratory testing (CBC, ALT, creatinine), 11 mL blood sample for the humoral immune assays, and 50 mL for future additional immune assays, perform a targeted physical examination, as indicated, solicit information regarding AEs, and encourage permission for continued follow-up of AEs, if possible. Any ongoing related AEs will be followed to resolution or until a stable chronic condition has been established. For more details, see Section 7.5. Also, if the participant has undergone CHMI but not received treatment, they should receive treatment for any possible incubating malaria infection as outlined in 7.3.12.

[2]Per section 7.3.12, those with two qPCR positive tests or a thick blood smear positive for malaria will be considered malaria-positive and will receive artemether/lumefantrine or atovaquone/proguanil (dosage and schedule per section 7.3.12). After completing treatment, next follow-up visit will be on day 141 (+1).

Table 18-3: Schedule of Events for Group 6[1]

Visit Number	00	11	12-14	15-25	26-29	30	31
Day of Study	-60 to 1	1	6-8	9-19	21 23 25 27	29	57
Clinical Evaluations	Screen	CHMI			(±1)	(±1)	(±7)
Informed Consent	x						
Comprehension quiz	x						
Eligibility Review	x	x					
Physical exam (only targeted, if indicated, for all visits other than visit 00), vitals	x	x	x	x	x	x	x
Post-challenge vitals		x					
Concomitant Medications	x	x	x	x	x	x	x
Medical history	x	x					x
Review of unsolicited AEs/SAEs		x	x	x	x	x	x
CHMI participation card		x					
Counseling on mosquito avoidance		x	x	x	x	x	
Serum pregnancy	x						
Urine pregnancy		x			x		
CBC	x			x			
ALT, creatinine	x			x			
Glucose (random)	x						
Urinalysis	x						
HIV	x						
HBsAg	x						
Hepatitis C	x						
Hemoglobin electrophoresis	x						
ECG	x						
Malaria Diagnostics			x[2]	x[2]	x[2]	x[2]	
Humoral immune assays	x					x	
Visit blood volume(s) (mL)	25	0	6	29	8	4	
Cumulative blood volume (mL)	25	25	31	60	68	72	72

[1]If a participant is terminated from the study early, when applicable, every effort should be made to perform the following procedures: Review current health status and note any changes since the last visit, record all concomitant medications, obtain 7 mL blood sample for safety laboratory testing (CBC, ALT, creatinine) and 11 mL blood sample for the humoral immune assays, perform a targeted physical examination, as indicated, solicit information regarding AEs, and encourage permission for continued follow-up of AEs, if possible. For more details, see Section 7.5. Any ongoing related AEs will be followed to resolution or until a stable chronic condition has been established.

[2]Per section 7.3.12, those with two qPCR positive tests or a thick blood smear positive for malaria will be considered malaria-positive and will receive artemether/lumefantrine or atovaquone/proguanil (dosage and schedule per section 7.3.12). After completing treatment, next follow-up visit will be on day 29 (±1).

APPENDIX B: TOXICITY TABLES

Table 18-4: Vital Signs

Vital Signs			
Vital Signs	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Fever (°C) *	38.0-38.4	38.5-38.9	≥ 39.0
Hypertension (systolic) mm Hg **	141-150	151-160	>160
Hypertension (diastolic) mm Hg	91-95	96-100	>100
Hypotension (systolic) mm Hg	85-89	80-84	<80
Bradycardia – beats per minute	50-54 or 45-50 if baseline <60	45-49 or 40-44 if baseline <60	<45 or <40 if baseline <60
Tachycardia – beats per minute	101-115	116-130	>130 or ventricular dysrhythmia

* Oral temperature; no recent hot or cold beverages or smoking

** Assuming supine position, 10 minutes at rest conditions, not sleeping subjects, measurements on the same arm and several concordant results

Table 18-5: Hematology and Chemistry

Hematology	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
WBC Decrease –cell/mm^3	2,500 – 3,500	1,500 – 2,499	<1500
WBC Increase –cell/mm^3	11,001 – 15,000	15,001 – 20,000	>20,000
Hgb g/dL (Female)	11.0 – 11.5	9.5 – 10.9	<9.5
Hgb g/dL (Male)	12.0 – 12.5	10.0 – 11.9	<10.0
Platelets Decrease –cell/mm^3	120,000 – 130,000	100,000 – 119,999	<100,000

Chemistry	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
ALT Increase IU/L	46 – 105	106 – 175	>175
Creatinine mg/dL (Increase) (Female)	>ULN – 1.7	1.8 – 2.0	>2.0
Creatinine mg/dL (Increase) (Male)	>ULN – 1.7	1.8 – 2.0	>2.0
Glucose-hypoglycemia mg/dL	65-67	55-64	<55
Glucose-hyperglycemia random-mg/dL	140-159	160-200	>200

Table 18-6: Urinalysis

Urinalysis	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Protein*	1+	2+	>2+
Blood*	1+	2+	>2+

*Trace protein or blood at screening (Visit 00) is acceptable for inclusion into the study.

APPENDIX C: LABORATORY REFERENCE RANGES

Table 18-7: Reference ranges from Garcia Laboratories (2016)

Test	Gender	Age	Low	High	Units
WBC	Both	>13 yrs	4.0	10.0	$x10^3$/uL
Neutrophils	Both	>13 yrs	1.56	8.10	$x10^3$/uL
Lymphocytes	Both	>13 yrs	1.40	5.10	$x10^3$/uL
Monocytes	Both	>13 yrs	0	1.33	$x10^3$/uL
Eosinophils	Both	>13 yrs	0	0.80	$x10^3$/uL
Basophils	Both	>13 yrs	0	0.2	$x10^3$/uL
Hemoglobin	Male	>13 yrs	13.5	17.5	g/dL
	Female	>13 yrs	12.0	16.0	g/dL
Platelets	Both	>13 yrs	150	400	$x10^3$/uL
Alt	Male	> 18 yrs	7	45	IU/L
	Female	> 18 yrs	7	45	IU/L
Creatinine	Male	> 15 yrs	0.7	1.3	mg/dL
	Female	> 15 yrs	0.6	1.2	mg/dL
Glucose, fasting	Both	>18 yrs	70	105	mg/dL
HCV Antibody	Both	All	Negative	Negative	
HBsAg	Both	All	Negative	Negative	
HIV 1/0/2	Both	All	Non-Reactive		
Hemoglobin electrophoresis	Both	All	Absence of Hgb S