Abbreviated Title: L9LS MAb in Malian Adults
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Title: Safety and Efficacy of L9LS, a Human Monoclonal Antibody Against *Plasmodium falciparum*, in a Randomized, Double-Blind, Placebo-Controlled Trial of Adults in Mali

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Investigational Agent:

Drug Name	L9LS
Investigational New Drug (IND) Number	160213
Sponsor	Office of Clinical Research Policy and Regulatory Operations (OCRPRO), Division of Clinical Research, NIAID
Manufacturer	Vaccine Research Center (VRC), NIAID

Data and Safety Monitoring Board (DSMB): NIAID Intramural DSMB

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STATEMENT OF COMPLIANCE

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

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

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

The protocol and informed consent forms will be submitted to the FMOS Ethics Committee (EC) for review and approval. Approval of both the protocol and the consent form must be obtained before any subject is enrolled. Any amendment to the protocol will require review and approval by the EC before the changes are implemented to the study. In addition, all changes to the consent form will be EC approved; a determination will be made regarding whether a new consent needs to be obtained from subjects who provided consent using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title: Safety and Efficacy of L9LS, a Human Monoclonal Antibody Against *Plasmodium falciparum*, in a Randomized, Double-Blind, Placebo-Controlled Trial of Adults in Mali

Study Description: A phase 2 trial evaluating the safety and tolerability of a one-time subcutaneous (SC) administration of L9LS, as well its protective efficacy against naturally occurring *Plasmodium falciparum* (Pf) infection over a 6-month malaria season. The primary study hypotheses is that L9LS will be safe and protective against malaria infection. As a secondary objective, the efficacy of L9LS within three body weight strata among female participants will each be compared to placebo. Before study agent administration, all subjects will be given artemether-lumefantrine to clear any preexisting Pf blood-stage infection.

The study is a randomized, double-blind, placebo-controlled, sex-stratified (2:1 female to male ratio) and weight-stratified trial (N=288 total) with 2 treatment arms: L9LS 900 mg SC (n=216) and placebo (n=72) to assess safety and protective efficacy of L9LS and placebo.

Subjects will receive the study agent and be followed at study visits 1, 3, 7, 14, 21, and 28 days later, and once every 2 weeks thereafter through 24 weeks. Primary study assessments include physical examination and blood collection for identification of Pf infection and other research laboratory evaluations.

Objectives: Primary Objectives:

1. To evaluate the safety and tolerability of L9LS administered SC at 900 mg in healthy Malian adults.
2. To determine if SC administration of L9LS at 900 mg (compared to placebo) mediates protection against naturally occurring Pf infection in healthy Malian adults during a single malaria season as detected from microscopic examination of thick blood smear. The primary efficacy analysis will be based on time to the first infection.

Secondary Objectives:

1. To determine if SC administration of L9LS at 900 mg (compared to placebo) mediates protection against naturally occurring Pf infection in healthy Malian adults during a single malaria season as detected by reverse transcription polymerase chain reaction (RT-PCR).
2. To determine if SC administration of L9LS at 900 mg (compared to placebo) mediates protection against naturally occurring Pf infection in healthy Malian adult females during a single malaria season as detected from microscopic examination of thick blood smears.
3. To determine if SC administration of L9LS at 900 mg (compared to placebo) mediates protection against naturally occurring Pf infection in healthy Malian adult females during a single malaria season as detected by RT-PCR.

4. To determine if SC administration of L9LS at 900 mg (compared to placebo) mediates protection against naturally occurring Pf infection in healthy Malian adult females stratified by weight during a single malaria season as detected from microscopic examination of thick blood smears.
5. To determine if SC administration of L9LS at 900 mg (compared to placebo) mediates protection against naturally occurring Pf infection in healthy Malian adult females stratified by weight during a single malaria season as detected by RT-PCR.
6. To evaluate the pharmacokinetics (PK) of L9LS throughout the study.
7. To evaluate the PK of L9LS throughout the study and to correlate L9LS serum concentrations with Pf infection risk.

Endpoints:

Primary Endpoints:
1. Incidence and severity of local and systemic adverse events (AEs) occurring within 7 days after the administration of study agent.
2. Pf blood-stage infection as detected by microscopic examination of thick blood smear for 24 weeks after administration of study agent.

Secondary Endpoints:
1. Pf blood-stage infection as detected by RT-PCR for 24 weeks after administration of study agent.
2. Measurement of L9LS in sera of recipients.

Study Population: Healthy Malian adult females (aged 18 to 49 years) and males (aged 18 to 55 years) residing in Kalifabougou, Faladje, and Torodo (N=288; accrual ceiling: 860).

Phase: 2

Description of Sites/Facilities Enrolling Subjects: The MRTC clinics in Kalifabougou, Faladje, and Torodo. All day 0 (study agent administration) activities will be performed at Kalifabougou only. All three sites will conduct screening, recruitment, enrollment, follow-up visits, and unscheduled study visits.

Description of Study Intervention: The study agent (L9LS or matching placebo) will be administered as a one-time SC injection.

Study subjects will be randomized 3:1 to receive 1 of the following:
- Arm 1: 900 mg of L9LS SC
- Arm 2: Placebo (normal saline) SC

Study Duration: 12 months

Subject Duration: 8 months

1.2 SCHEMA

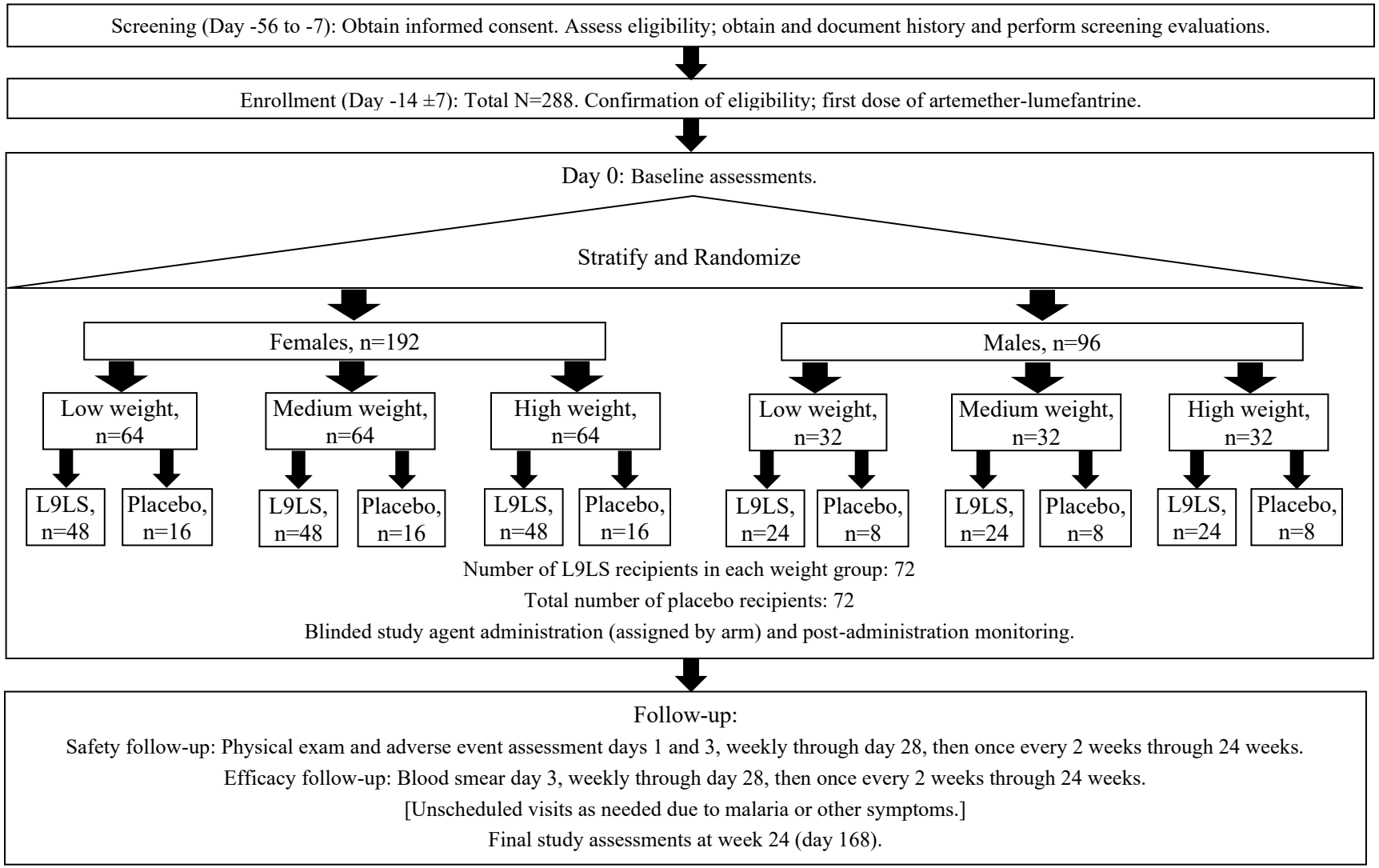

Figure 1. Study flow diagram.

1.3 SCHEDULE OF ACTIVITIES (SOA)

Study Day	Screen	Enroll [1]	0	1	3	7	14	21	28	42	56	70	84	98	112	126	140	154	168	Illness Visit	ET
Window (days)	-56 to -7	-14±7	-	+1	±1	±2	±3	±3	±3	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
Clinical Procedures/Evaluations																					
Study comprehension exam	X																				
Informed consent	X																				
Confirmation of identity, age, and residency	X																				
Physical exam [2]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X																				
Weight	X		X																		
Vital signs (temperature, blood pressure, and pulse)	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Medical history [2]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications [2]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Artemether-lumefantrine administration		X [3]																			
12-lead ECG	X																				
Randomization			X																		
Study agent administration			X [4]																		
Adverse events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Pregnancy prevention counseling	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Laboratory Evaluations																					
Test	Tube	(Blood volume in mL)																			
Urinalysis	-	X																			
Pregnancy test (urine/serum; for women)	-	X	X	X [5]					X		X		X		X		X		X		

Study Day		**Screen**	**Enroll** [1]	**0**	**1**	**3**	**7**	**14**	**21**	**28**	**42**	**56**	**70**	**84**	**98**	**112**	**126**	**140**	**154**	**168**	**Illness Visit**	**ET**
Window (days)		-56 to -7	-14±7	-	+1	±1	±2	±3	±3	±3	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		
HIV, HBV, HCV screen [6]	SST	3																				
CBC with differential	EDTA	3	3	3 [5]			3	3														
Hemoglobin type	EDTA	(X) [7]																				
ALT, Cr	SST	3	3	3 [5]			3	3														
Blood smear and dried blood spot for Pf RT-PCR [8]	-		X	X		0.5	X	X	0.5	X	0.5	X	0.5	X	0.5	X	0.5	X	0.5	X	X	
PK studies	SST			8 [9]	4		4	4		4		4		4		4		4		4		
Serum storage	SST			8			8			8				8						8		
ADA	SST			(X)			(X)			(X)				(X)						(X)		
PBMC storage	CPT			16				16												16		
Daily volume (mL)		**9**	**6**	**38**	**4**	**0.5**	**18**	**26**	**0.5**	**12**	**0.5**	**4**	**0.5**	**12**	**0.5**	**4**	**0.5**	**4**	**0.5**	**28**		
Cumulative volume (mL)		**9**	**15**	**53**	**57**	**57.5**	**75.5**	**101.5**	**102**	**114**	**114.5**	**118.5**	**119**	**131**	**131.5**	**135.5**	**136**	**140**	**140.5**	**168.5**	**-**	**-**

Abbreviations: ADA, anti-drug antibodies; ALT, alanine transaminase; CBC, complete blood count; CPT, cell preparation tube; Cr, creatinine; ECG, electrocardiogram; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; ET, early termination; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; PBMC, peripheral blood mononuclear cell; Pf, *Plasmodium falciparum*; PK, pharmacokinetics; SST, serum-separating tube; RDT, rapid diagnostic test; RT-PCR, reverse transcription polymerase chain reaction.

(X) indicates that no additional blood will be drawn; the test will be performed from the tube shown in the preceding row.

Notes:
- Day 0 visits will take place at Kalifabougou for all subjects, regardless of residence. All other visits can take place at any site.
- At any time during the study, the subject may have an unscheduled illness visit if experiencing malaria symptoms or other symptoms. The subject may be referred for standard care according to local guidelines. Unscheduled illness visits will take place at Kalifabougou, Faladje, or Torodo, depending on the subject's residence.

Footnotes:
[1] If enrollment is within 2 days of screening, duplicate procedures will not be repeated.
[2] Complete/comprehensive at screening; targeted/interim at other visits.
[3] Enrollment is defined as the time of first artemether-lumefantrine administration. (For women, negative pregnancy test must be confirmed prior to administration/enrollment.) The first dose will be directly observed in the clinic. The subsequent 5 doses given over 3 days will be observed by guides in the subject's home or at the clinic. All artemether-lumefantrine doses will be completed prior to day 0.

Study Day	**Screen**	**Enroll** [1]	**0**	**1**	**3**	**7**	**14**	**21**	**28**	**42**	**56**	**70**	**84**	**98**	**112**	**126**	**140**	**154**	**168**	**Illness Visit**	**ET**
Window (days)	-56 to -7	-14±7	-	+1	±1	±2	±3	±3	±3	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7		

[4] All other study procedures must be completed prior to study agent administration. Subject will be monitored during and for 60 minutes (±15 minutes) after each administration, and vital signs will be recorded directly and 1 hour (±15 minutes) after administration. Prior to discharge, subject will be assessed for local reactogenicity (including pain/tenderness, swelling, redness, bruising, and pruritus at the site of administration) and systemic reactogenicity (including fever, feeling unusually tired or unwell, muscle aches, headache, chills, nausea, and joint pain). If the subject is assessed as being unwell or has ongoing reactogenicity symptoms, he or she will be asked to remain in the clinic until evaluation and discharge by a study clinician. Clinicians will follow any solicited symptoms that are ongoing until they have resolved.
[5] Collected prior to study agent administration. For women, negative pregnancy test must be confirmed prior to administration.
[6] Viral screenings will be performed according to international guidelines. HIV testing will be 2 rapid diagnostic tests (RDTs), plus enzyme-linked immunosorbent assay (ELISA) if the RDTs are discordant; subject will be referred for medical care for 2 positive RDTs or a positive ELISA. Pre- and post-test HIV counseling will be provided. Hepatitis testing will be an HBV surface antigen test (ELISA) and HCV test (ELISA, PCR if indicated). A subject who is HBV and/or HCV positive will be referred for care regardless of the ALT result.
[7] EDTA tube will be stored at screening, and hemoglobin typing will be performed if the subject is enrolled; this test will not be used in eligibility assessments.
[8] If positive for malaria parasite infection, parasite genotyping may be performed. Sample can be obtained via finger prick or venipuncture.
[9] For PK, 4 mL serum will be collected prior to randomization and study agent administration, and an additional 4 mL serum will be collected about 1 hour (±30 minutes) after the administration.

2 INTRODUCTION

2.1 STUDY RATIONALE

Malaria is a mosquito-transmitted disease caused by *Plasmodium* parasites. Globally each year, there are 200-400 million cases of malaria resulting in over a half a million deaths, the majority of which occur in Africa among children and are caused by Pf.[1] Additionally, approximately 11 million pregnant women in Africa are exposed to malaria each year, resulting in significant maternal, fetal, and infant morbidity and mortality.[1, 2]

Currently available malaria-control measures include insecticide-treated bednets, early diagnosis and treatment with artemisinin-based combination therapies, and chemoprevention for high-risk groups including infants, children exposed to seasonal malaria, and pregnant women.[1] Despite these countermeasures, progress in reducing malaria cases and deaths has stalled in recent years[1] and is further threatened by the emergence of insecticide-resistant mosquitoes[3] and drug-resistant parasites.[4] In 2021, RTS,S/AS01 became the first malaria vaccine to be recommended by the World Health Organization (WHO). In a phase 3 trial, RTS,S/AS01 showed 36% efficacy against clinical malaria over a period of 4 years in children 5 to 17 months of age who had received four doses.[5] Until vaccines that induce high-level, durable protection against *P. falciparum* infection are developed, new tools are needed to complement existing countermeasures. Importantly, the primary endpoint for RTS,S/AS01 trials has been clinical (symptomatic) malaria,[5] as RTS,S/AS01 is not expected to reliably provide sterile protection from *P. falciparum* infection, particularly among adults residing in endemic areas.[6, 7] In this protocol, the primary endpoint is *P. falciparum* infection, since prevention of infection would be required to prevent the complications of malaria in pregnancy.

The VRC, NIAID, NIH, has developed monoclonal antibodies (MAbs) called CIS43LS and L9LS as possible preventive therapeutics against Pf infection. In a phase 1 trial of CIS43LS that evaluated doses of 20 and 40 mg/kg administered intravenously (IV), the 9 CIS43LS recipients never developed parasitemia, and 2 of these subjects had received CIS43LS (40 mg/kg IV) approximately 9 months prior to controlled human malaria infection (CHMI). The *in vivo* concentration of CIS43LS at the time of CHMI in the 9 protected subjects ranged from 50-500 µg/mL.[8] A phase 2 trial of healthy adults in Mali found that a single IV administration of CIS43LS was safe and provided 88.2% protective efficacy against Pf infection at a dose of 40 mg/kg, and 75.0% protective efficacy at a dose of 10 mg/kg over the 6-month malaria season.[9] The PK and pharmacodynamic analysis of the phase 2 CIS43LS trial in Mali is ongoing.

L9 was found to be more potent than CIS43 at mediating protection against mosquito bite challenge in mice (described in detail in Section 2.2.1.1).[10] The phase 1 trial of L9LS evaluated a dose range from 1 to 20 mg/kg delivered via IV administration, and 5 mg/kg delivered via SC administration. Of the 17 participants who received a single dose of L9LS, 15 (88%) were protected after CHMI. Parasitemia did not develop in any of the participants who received 5 or 20 mg/kg of IV L9LS. Parasitemia developed in 1 of 5 participants who received 1 mg/kg IV, and 1 of 5 participants who received 5 mg/kg SC. Protection conferred by L9LS was seen at serum concentrations as low as 9.2 µg per milliliter.[11] Based on the protective efficacy of low-dose SC L9LS in the phase 1 trial, the pre-clinical evidence that L9LS is more potent than

CIS43LS,[10] and the higher concentration of the L9LS formulation compared to CIS43LS formulation (150 mg/ml and 100 mg/ml, respectively), an ongoing phase 2 trial in Mali is evaluating the safety and efficacy of a single SC administration of L9LS (fixed doses of 150 mg or 300 mg in children weighing 15 – 30 kg, providing a dose range of 5 – 20 mg/kg) over a 6-month malaria season in a dose-escalation trial in adults (n=18) and children aged 6-10 years (n=36) and in a randomized, double-blind trial of children aged 6-10 years (n=225; ClinicalTrials.gov number, NCT05304611). In Kenya, where malaria transmission is perennial, an ongoing phase 2 trial is evaluating the safety and tolerability of a one-time SC administration of L9LS in children aged 5 months to 10 years (n=72), as well as the protective efficacy of 1 or 2 doses of L9LS against Pf infection over 1 year in a randomized, double-blind trial among children aged 5 months to 59 months (n=324; ClinicalTrials.gov number, NCT05400655). In the Kenya trial, dosing is based on 3 weight bands, and all doses are administered SC with fixed doses of 75 mg, 150 mg, or 225 mg of L9LS, resulting in a dose range of 10-19 mg/kg. In the ongoing phase 2 trials of L9LS in Mali and Kenya, there have been no safety concerns to date, and efficacy results are expected in early 2023 and 2024, respectively, which may inform a phase 3 trial of L9LS, potentially in 2024, that targets children aged 5 months to 59 months who are exposed to seasonal malaria—the highest priority age group that bears the highest burden of malaria morbidity and mortality.

In addition to the high-priority clinical-use case of prevention of malaria in infants and young children, prevention of malaria in pregnancy is a high-priority potential-use case for anti-malaria MAbs. As MAbs are safe and can prevent infection, they may be an ideal intervention for use in pregnancy. The present protocol is designed to assess the safety and efficacy of L9LS in healthy adults in Mali, with a particular focus on females of childbearing potential. Female participants in this trial would provide data on the safety, PK, and efficacy of SC administration of L9LS that would lay the foundation for clinical trials of L9LS in pregnant women. In addition, female and male participants in this trial would provide data that could lay the foundation for potential clinical trials targeting seasonal workers and malaria elimination.

2.2 BACKGROUND

2.2.1 Study Agent: L9LS

2.2.1.1 Development

The VRC, NIAID, NIH, has developed a MAb called L9LS as a possible preventive therapeutic against Pf infection. L9LS represents a second-generation anti-malaria MAb that follows the anti-malaria MAb CIS43LS, which was also developed by the VRC. L9, the wild-type parent of L9LS, was isolated by sorting the Pf circumsporozoite protein (CSP)-reactive memory B cells obtained from a subject immunized with a radiation-attenuated Pf whole-sporozoite malaria vaccine in the VRC 314 clinical trial (NCT02015091) using a junctional epitope mimic probe designed to select for “CIS43-like” MAbs. Epitope mapping showed L9 bound to NPNV motifs associated with NVDP minor repeats of PfCSP. When compared to a published panel of protective human PfCSP MAbs, L9 protected mice against IV and mosquito bite sporozoite challenge, and demonstrated the lowest ED80 and EC80 values (325.7 µg and 145.1 µg/mL, respectively) of any MAb evaluated, including CIS43LS (685.92 µg and 363.93 µg/mL, respectively).[10] The unique preference of L9 for NPNV motifs was further underscored by the

fact that all four NVDP tetrapeptide motifs had to be mutated to NANP to disrupt the recognition of L9 for the NPNV motifs found in recombinant PfCSP. As 100% of known Pf field isolates have one or more NVDP motifs, these data suggest that L9 should bind all circulating strains of Pf malaria.[10]

Half-life extension of L9 was accomplished by modifying the L9 Fc heavy chain to include an LS mutation (L9LS). Restriction enzymes were used to digest plasmids carrying the L9 heavy chain and the CIS43LS heavy chain; subsequently, the variable region of the L9 heavy chain was ligated to the Fc heavy chain bearing the LS mutation. The LS mutation is a methionine to leucine (L) and asparagine to serine (S) (M432L/N434S, collectively, LS) replacement, and changes have been reported to increase antibody Fc region binding affinity to FcRn, resulting in the extended recirculation of functional IgG and consequent longer antibody serum half-life. Other than these two amino acid sequences in the heavy chain Fc, the sequences of L9LS and L9 are identical. Immune protection and half-life data acquired with L9 and L9LS identify L9LS as a promising clinical candidate for passive malaria prophylaxis.

2.2.1.2 Preclinical Experience

To assess L9LS as a candidate for clinical trials, research-grade MAb was evaluated for in vitro functional activity including binding properties, auto-reactivity, and PK, and in mouse models of in vivo protection following challenge. In addition, PK studies in non-human primates (NHPs) were performed. In mice, L9 was more potent than CIS43 and a panel of several other human MAbs against PfCSP that were known to be highly protective. Both L9LS and L9 mediated the same potency following challenge. In NHP PK studies, L9LS exhibited significantly longer half-life in blood as compared to the parental L9 MAb without the LS mutation.

Two preclinical toxicology studies were conducted with a process-representative developmental batch of L9LS. An in vitro tissue cross-reactivity (TCR) assay to screen for potential cross-reactivity and an in vivo rat toxicity study to demonstrate safety both were conducted in compliance with 21 CFR 58 Good Laboratory Practice (GLP) for Nonclinical Laboratory Studies.

2.2.1.2.1 TCR Study

The TCR assay screened a standard panel of normal human tissues (3 donors per tissue) and Sprague Dawley rat tissues (2 rats per tissue). Mammalian cells transfected to express CSP were used as a tissue-positive control. Two concentrations of L9LS were tested: 1.15 µg/mL (selected as the concentration which saturated the positive control tissue), and 11.5 µg/mL (10-fold excess). A negative control IgG1κ antibody (GR338422-1, no mammalian target antigen) was tested at the same 2 concentrations. L9LS exhibited scattered specific membrane binding in 3/3 human salivary gland tissue samples and localized to the epithelial cells lining the ducts and acini. Binding was rare (1-5% of these epithelial cells) at 1.15 µg/mL, and rare to occasional (5-25% of these epithelial cells) at 11.5 µg/mL. The cause of the salivary epithelial cell membrane binding has not been identified. Specific membrane binding to salivary gland tissue was not observed for the negative control antibody. L9LS did not bind to rat salivary epithelium. No specific membrane binding to other human tissues was observed. The potential clinical significance of the epithelial findings is unclear as the antibody is not expected to distribute into healthy normal epithelium; any effect on the salivary gland from L9LS, if present, is expected to

be transient due to the clearance of the antibody over time and the rapid turnover of salivary gland epithelium. As a precaution, however, the initial phase 1 and 2 clinical protocols conducted with L9LS excluded subjects with potential salivary gland abnormalities based on clinical judgement; and included monitoring for potential salivary gland toxicity to assess whether this finding translates into clinical risk, specifically by asking study participants if they experienced unusual dry mouth symptoms at each study visit. No subjects who received L9LS in the VRC phase 1 trial or the Mali pediatric phase 2 trial have reported any symptoms consistent with potential salivary gland toxicity.

2.2.1.2.2 Rat Toxicity Study

Sprague Dawley rats were dosed with L9LS to evaluate toxicity and toxicokinetics. Rats received 0, 40, or 400 mg/kg by IV bolus injection twice (Day 1 and Day 11). Female rats were dosed with 10 mg/kg by SC injection once (D1); male rats were dosed with 10 mg/kg SC twice (D1 and D11); both male and female rats were dosed with 100 mg/kg SC twice (Day 1 and Day 11). For all dose levels, the main group was necropsied at Day 12 to evaluate potential immediate effects, and recovery animals were necropsied at Day 46, to evaluate the potential for delayed effects and recovery. Treated rats exhibited a transient increase in body temperature post-dose (up to + 0.5ºC, considered a non-adverse response). Serum clinical chemistry tests detected slightly increased globulin, consistent with the administration of L9LS, an IgG MAb. For IV dosing, the no observed adverse effect level (NOAEL) was the high dose, 400 mg/kg IV x2. For SC dosing, the NOAEL was the high-dose, 100 mg/kg SC x2. SC injection of L9LS did not cause reactogenicity (edema, erythema, eschar) in any rat. Histopathology evaluation of the skin at the SC injection site reported minimal to moderate SC mixed cell infiltration, considered treatment related and predictive for human volunteers. One treated rat (in the 100 mg/kg SC group) was found dead; this event was attributed to a procedural error the previous day and is not considered treatment related. One treated female (in the 100 mg/kg SC recovery group, n=30) had grossly visible heart enlargement (2-fold increase in both heart weight and heart-body weight compared to the control means), with normal heart histology. Spontaneous cardiomyopathies are occasionally observed for the Sprague Dawley strain of rat. Based on the singular incidence, this observation is not considered treatment related.

With reference to the US Food and Drug Administration (FDA) Center for Drug Evaluation and Research 2005 Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers, scaling based on body weight is appropriate for a MAb expected to distribute mainly in the vascular space. The rat IV NOAEL of 400 mg/kg x2 supports the IV clinical high dose with a 20-fold dose margin. The rat SC NOAEL of 100 mg/kg x2 supports the SC clinical dose with a 20-fold dose margin.

The FDA provides guidance on “the nonclinical safety studies recommended to support human clinical trials” in the Guidances for Industry ICH M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals and ICH S6 Addendum to Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals. Taken together, the L9LS TCR and rat toxicity study results meet the safety standard set in these guidances and supported proceeding with L9LS clinical trials.

2.2.1.3 Clinical Experience

The first generation anti-malaria MAb CIS43LS was shown to be safe and conferred complete protection against experimental CHMI in a phase 1 study in healthy adult volunteers at the VRC.[12] In the phase 1 study, subjects were protected against malaria infection when challenged in CHMI at about 4 weeks to 36 weeks post-product administration. A phase 2 trial of healthy adults in Mali (ClinicalTrials.gov Identifier: NCT04329104) found that a single IV administration of CIS43LS was safe and provided 88.2% protective efficacy against Pf infection at a dose of 40 mg/kg, and 75.0% protective efficacy at a dose of 10 mg/kg over the 6-month malaria season.[9] The PK and pharmacodynamic analysis of the phase 2 CIS43LS trial in Mali is ongoing.

L9LS was evaluated in a phase 1 trial that evaluated a dose range from 1 to 20 mg/kg delivered via IV administration, and 5 mg/kg delivered via SC administration. For SC administrations, the total dose was divided into 1 or 2 injections, not exceeding 2.0 mL each. Most SC injections were abdominal, but the upper arm was also used if preferred by the participant and clinician. L9LS was found to be safe and well tolerated by both the IV and SC routes. Of the 17 participants who received a single dose of L9LS, 15 (88%) were protected after CHMI. Parasitemia did not develop in any of the participants who received 5 or 20 mg/kg of IV L9LS. Parasitemia developed in 1 of 5 participants who received 1 mg/kg IV, and 1 of 5 participants who received 5 mg/kg SC. Protection conferred by L9LS was seen at serum concentrations as low as 9.2 μg per milliliter.[11]

An ongoing phase 2 trial in Mali is evaluating the safety and efficacy of a single SC administration of L9LS (fixed doses of 150 mg or 300 mg in children weighing 15 – 30 kg, providing a dose range of 5 – 20 mg/kg) over a 6-month malaria season in a dose-escalation trial in adults (n=18) and children aged 6-10 years (n=36) and in a randomized, double-blind trial of children aged 6-10 years (n=225; ClinicalTrials.gov number, NCT05304611). In the dose-escalation phase of the phase 2 trial in Mali, adults received up to 600 mg of L9LS SC, divided into 2 injections of 2.0 ml each, while children received up to 300 mg of L9LS SC, divided into 2 injections of 1.0 ml each. SC injections of L9LS were well tolerated in adults and children and there have been no safety concerns to date.

In Kenya, where malaria transmission is perennial, an ongoing phase 2 trial is evaluating the safety and tolerability of a one-time SC administration of L9LS in children aged 5 months to 10 years (n=72), as well as the protective efficacy of 1 or 2 doses of L9LS against Pf infection over 1 year in a randomized, double-blind trial among children aged 5 months to 59 months (n=324; ClinicalTrials.gov number, NCT05400655). In the Kenya trial, dosing is based on 3 weight bands and all doses are administered SC with fixed doses of 75 mg, 150 mg, or 225 mg of L9LS, resulting in a dose range of approximately 10-19 mg/kg. Participants are randomized at 6 months to receive the second dose. In the ongoing phase 2 trial of L9LS in Kenya, there have been no safety concerns to date, and SC administration of L9LS has been well tolerated up to 1.5 mL in children. Efficacy results from the Mali and Kenya phase 2 trials are expected in early 2023 and 2024, respectively. The protocol and consent will be updated if new information about risks and side effects becomes available from the ongoing phase 2 L9LS trials.

2.2.2 Laboratory Assessments of L9LS

Some laboratory assessments in this study are designed to characterize the investigational product. This includes PK analysis and evaluation for anti-drug antibody (ADA) development after product exposure. Other assays may also be completed from stored samples at a later date. The LIG International Center of Excellence in Research Lab at the USTTB in Bamako will process blood and store coded samples and will either perform sample testing or ship coded samples to designated research laboratories at LIG/NIAID and VRC/NIAID or other approved collaborators. The key to the code will remain at the USTTB. See section 1.3 for schedules, blood volumes, and tube types to be used for research sample collection. Research assays will be performed on samples from both study product recipients and placebo controls at baseline and throughout the study.

Tube types for clinical labs are according to institutional requirements and are shown in section 1.3 to estimate blood volumes. Different tubes for clinical evaluations may be used to meet site requirements. Research sample tube types and blood volumes must be used as shown or as otherwise instructed by the manufacturer. In some instances, coded samples may be transported directly by study staff to the laboratory of an approved collaborator.

2.2.2.1 PK Analysis

Concentrations of L9LS will be measured by a Meso Scale Discovery LLC–based automation platform and similar methodology as previously described for other VRC MAb products.[13]

2.2.2.2 Detection of Anti-Drug Antibodies

Assays for detection of ADA will be performed at specified timepoints following product administration and compared to baseline status using a similar methodology as previously described for other VRC MAb products.[13]

2.2.2.3 Measures of MAb-Mediated Protection Against Pf Infection

L9LS-mediated protection against naturally occurring Pf infection during a single 6-month malaria season will be assessed and compared to control subjects. The endpoint defining MAb-mediated protection from Pf infection is the absence of Pf parasites in blood samples obtained from L9LS recipients collected from day 7 through week 24 (day 168) after study agent administration just prior to the malaria season. The criteria for a case of Pf infection is based on blood smear analysis. Giemsa-stained thick blood films will be prepared and examined by trained personnel following the standard operating procedure (SOP) based on the standard WHO protocol. Thick blood smears will be prepared from the blood remaining in the collection device, or (at timepoints when no blood collection is planned) from a finger prick or venipuncture blood sample. The smears will be examined microscopically.

Additionally, another secondary endpoint, *Plasmodium* 18S ribosomal ribonucleic acid (rRNA) RT-PCR, will be performed by Dr. Sean Murphy, University of Washington. The assay will be applied to dried blood spots using methods substantially equivalent to those reviewed extensively by the FDA for Biomarker Qualification of the 18S rRNA by the University of Washington for

CHMI trials (Biomarker Qualification Letter DDTBMQ000044, Oct 12, 2018). The assay has been validated for use on dried blood spots.

Research blood samples may also be used for Pf malaria parasite genome analysis that will be conducted by Dr. Daniel Neafsey, Harvard School of Public Health.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 Known Potential Risks

Risks of L9LS: L9LS was evaluated as safe and well tolerated in the VRC 614 study.[11] Local reactions at the injection site included mild to moderate pain, redness, bruising, swelling, and pruritus. Similarly, no significant safety concerns have arisen to date in the ongoing phase 2 trials of L9LS in Mali and Kenya, for which there have been no serious adverse events (SAEs) and no safety pauses.

In a preclinical TCR study, L9LS produced membrane and cytoplasmic binding in infrequent ductal and acinar epithelial cells in the human salivary gland which is of unclear clinical significance. Binding occurred in only 1-5% of cells and was scattered without evidence of clustering. Histopathology findings suggests the human salivary tissues were sourced from surgical excisions with evidence of pre-existing inflammation, which may have contributed to the observed binding. We suspect any effect on the salivary glands from L9LS, if present, would be transient due to the clearance of the passively transferred antibody and the rapid turnover of salivary gland epithelium. To address these findings, the trial will exclude any population at an increased risk for salivary gland dysfunction and will prospectively monitor for signs and symptoms that may be indicative of salivary gland hypofunction. Subject matter experts in salivary gland disorders at the National Institute of Dental and Craniofacial Research who are board-certified by the American Board of Oral and Maxillofacial Pathology have agreed to assist the protocol team by being available for consultation throughout the trial. In addition, safety assessment in the phase 1 trial of L9LS[11] and the 2 ongoing trials of L9LS in Mali and Kenya included prospective monitoring for signs and symptoms that may be indicative of salivary gland hypofunction by asking study participants if they experienced unusual dry mouth symptoms at each study visit. No subjects who received L9LS in the VRC phase 1 trial or the Mali and Kenya phase 2 trials have reported any symptoms consistent with potential salivary gland toxicity. The protocol and consent will be updated if new information about risks and side effects becomes available from the ongoing phase 2 trials in Mali and Kenya.

Risks of MAb Administration: Administration of MAbs may cause immune reactions such as acute anaphylaxis, serum sickness, and the generation of ADA. However, these reactions are rare and more often associated with MAbs targeted to human proteins or with the use of mouse MAbs that would have a risk of human anti-mouse antibodies.[14] In this regard, because L9LS is targeted to a parasite antigen and is a human MAb, it is expected to have a low risk of such side effects.

Typically, the side effects of MAbs are mild to moderate and may include local reactions at the injection site (including pain, redness, bruising, swelling, pruritis) and systemic reactions such as fever, chills, rigors, nausea, vomiting, pain, headache, myalgia, arthralgia, dizziness, fatigue,

shortness of breath, bronchospasm, hypotension, hypertension, pruritus, rash, urticaria, angioedema, diarrhea, tachycardia, or chest pain. Healthcare staff will be appropriately trained and necessary medical equipment will be readily available at the clinic where the study agent is administered. Clinical use of MAbs that are targeted to cytokines or antigens associated with human cells may be associated with an increased risk of infections;[14] however, this is not expected to be a risk for a MAb targeted to a parasite antigen.

Severe reactions, such as anaphylaxis, angioedema, bronchospasm, hypotension, and hypoxia, are infrequent and more often associated with MAbs targeted to human proteins or with non-human MAbs, such as a mouse MAb.[14] Most administration-related events occur within the first 24 hours after initiation of MAb administration.

Published experience with human MAbs directed against cell surface targets on lymphocytes shows that administration of a MAb may be associated with cytokine release, causing a reaction known as cytokine release syndrome (CRS).[15] CRS reactions commonly occur within the first few hours of administration and with the first MAb administration received. This is because the cytokine release is associated with lysis of the cells targeted by the MAb and the burden of target cells is greatest at the time of the first MAb treatment. With licensed therapeutic MAbs, CRS is managed by administering histamine blockers.[16] Supportive treatment may also be indicated for some signs and symptoms.

Delayed allergic reactions that include a serum sickness type of reaction characterized by urticaria, fever, lymph node enlargement, and joint pains, typically occur several days after MAb exposure and are more commonly associated with chimeric types of MAbs.[14] In general, and with due consideration of the needs dictated by individual subject symptoms and treating clinician discretion, immediate and delayed reactions to study product will be managed according to the principles of the American Academy of Allergy, Asthma, and Immunology guidelines.[17]

Participation in this study may limit a subject's eligibility for future MAb studies.

Risks of Placebo Administration: There are no risks of the placebo (normal saline) other than injection-related events such as transient headache, dizziness, hypertension, and vasovagal-mediated hypotension.

Risks of Blood Collection: Collecting blood by venipuncture or finger prick may cause pain, bruising, and a feeling of lightheadedness or fainting. Rarely, an infection may develop at the site where blood is taken.

Risks of ECG: The ECG is not painful, but the electrodes may cause discomfort or a self-limiting rash. Participants may be asked to remove clothing for electrode placement, which may be uncomfortable or embarrassing. This will be done in a private setting.

Risks of Artemether-Lumefantrine Administration: Before study agent administration, all subjects will be given artemether-lumefantrine to clear any preexisting Pf blood-stage infection. Clearance of preexisting Pf blood-stage infection is necessary to accurately assess the primary

endpoint of Pf blood-stage infection as detected by microscopic examination of thick blood smear after administration of MAb or placebo, since preexisting Pf blood-stage parasites cannot be reliably distinguished from newly transmitted Pf blood-stage parasites. The historical prevalence of asymptomatic Pf blood-stage infection among adults at the end of the dry season (time of anticipated enrollment into the efficacy study) at the Kalifabougou study site is as high as 60%, as detected by PCR analysis of dried blood spots (Figure 2).[18] In areas of high Pf transmission such as Mali, the true prevalence of asymptomatic Pf blood-stage infection is generally considered to be higher than that detected by PCR of dried blood spots, which has a limit of detection of approximately 1 parasite/µL of blood.[19] Therefore, for this protocol it is assumed that all subjects are infected with Pf blood-stage parasites at the time of enrollment.

An observational study in Kalifabougou found that treating asymptomatic Pf infection in children with artemether-lumefantrine at the end of the dry season does not increase the risk of clinical malaria upon reinfection, suggesting that asymptomatic Pf infection does not provide benefit by maintaining immunity to clinical malaria.[18] A recent observational study conducted in Kenya found that asymptomatic Pf infections were associated with an increased 1-month likelihood of symptomatic malaria in both children and adults, suggesting that treatment of asymptomatic infection may be beneficial[20], although this could vary by Pf transmission setting.

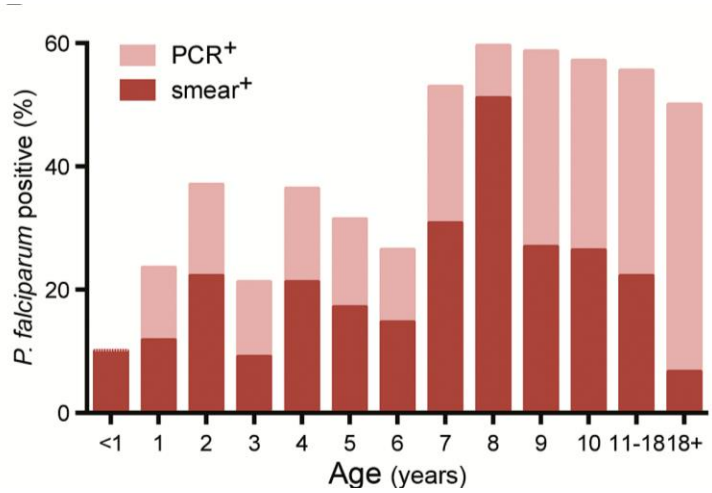

Figure 2. Age-stratified point prevalence of asymptomatic *P. falciparum* infection detected by polymerase chain reaction (PCR) or blood smear at the end of the dry season in Kalifabougou, Mali (ClinicalTrials.gov Identifier: NCT01322581).

Although there is some evidence to suggest that exposure to Pf blood-stage infection may interfere with the human immune response to malaria vaccine candidates, clearance of preexisting Pf blood-stage infection is not considered necessary for CIS43LS or L9LS efficacy since they directly neutralize sporozoites independently of the host immune system.[10]

Adverse reactions (ARs) to artemether-lumefantrine occurring in more than 30% of adults are headache, anorexia, dizziness, asthenia, arthralgia, and myalgia. Reactions typically do not

require stopping treatment. Individuals who may have any contraindication for the use of this drug (e.g., prolonged corrected QT interval [QTc]) will be excluded at screening. In postmarketing experience, serious hypersensitivity reactions including anaphylaxis and serious skin reactions (bullous eruption) have been reported. Individuals with known sensitivity or contraindications to the antimalarials administered in this study are excluded from participation. A complete list of side effects and contraindications is provided in the package insert.[21]

Of note, artemether-lumefantrine is the first-line antimalarial drug for uncomplicated malaria in an ongoing observational cohort study that has been conducted in Kalifabougou, Mali since 2011 (ClinicalTrials.gov Identifier: NCT01322581; same study site as current protocol). To date, no serious reactions to artemether-lumefantrine have been noted in the Kalifabougou cohort study.

2.3.2 Known Potential Benefits

In this study, subjects may not receive direct health benefit from study participation. Depending on whether L9LS confers protective efficacy at the dose tested, subjects receiving a sufficient dose of L9LS may experience some protection against Pf infection and clinical malaria during part or all of the malaria transmission season.

In the future, others may benefit from knowledge gained in this study that may aid in the development of malaria prevention.

2.3.3 Assessment of Potential Risks and Benefits

The study population lives in an area where malaria is endemic and so is at significant risk of malarial infection and disease. As described above, it is possible that some subjects may benefit from study participation by receiving some protection during the malaria season. Therefore, the value of the information that will be gained from this study for developing malaria prevention strategies justifies the potential risks of study participation described above. Additionally, potential risks are minimized by careful design of subject eligibility criteria and monitoring after study product administration.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
1. To evaluate the safety and tolerability of L9LS administered SC at 900 mg in healthy Malian adults. 2. To determine if SC administration of L9LS at 900 mg (compared to placebo) mediates protection against naturally occurring Pf infection in healthy Malian adults during a single malaria season as detected from microscopic	1. Incidence and severity of local and systemic AEs occurring within 7 days after the administration of study agent. 2. Pf blood-stage infection as detected by microscopic examination of thick blood smear for 24 weeks after administration of study agent.	1. Assessment of AEs is a standard measure of study agent safety and tolerability. 2. Blood smear is the gold standard for diagnosis of blood-stage Pf infection.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
examination of thick blood smear. The primary efficacy analysis will be based on time to the first infection.		
Secondary		
1. To determine if SC administration of L9LS at 900 mg (compared to placebo) mediates protection against naturally occurring Pf infection in healthy Malian adults during a single malaria season as detected by RT-PCR. 2. To determine if SC administration of L9LS at 900 mg (compared to placebo) mediates protection against naturally occurring Pf infection in healthy Malian adult females during a single malaria season as detected from microscopic examination of thick blood smears. 3. To determine if SC administration of L9LS at 900 mg (compared to placebo) mediates protection against naturally occurring Pf infection in healthy Malian adult females during a single malaria season as detected by RT-PCR. 4. To determine if SC administration of L9LS at 900 mg (compared to placebo) mediates protection against naturally occurring Pf infection in healthy Malian adult females stratified by weight during a single malaria season as detected from microscopic examination of thick blood smears. 5. To determine if SC administration of L9LS at 900 mg (compared to placebo) mediates protection against naturally occurring Pf infection in healthy Malian adult females	1. Pf blood-stage infection as detected by RT-PCR for 24 weeks after administration of study agent. 2. Measurement of L9LS in sera of recipients.	1. RT-PCR is more sensitive than blood smear for detecting Pf blood-stage infection. 2. Concentrations of L9LS in blood will help assess durability of L9LS and will allow for correlation with Pf infection risk.

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
stratified by weight during a single malaria season as detected by RT-PCR. 6. To evaluate the PK of L9LS throughout the study. 7. To evaluate the PK of L9LS throughout the study and to correlate L9LS serum concentrations with Pf infection risk.		
Tertiary/Exploratory		
1. To evaluate the relationship between weight-based dosing of L9LS at 900 mg administered SC and protection against naturally occurring Pf infection in healthy Malian adults during a single malaria season as detected by blood smears and RT-PCR. 2. To determine whether ADA to L9LS can be detected in sera of recipients at specific timepoints throughout the study. 3. To assess for IgG1 allotypes and allotype-specific effects on L9LS PK. 4. To explore and characterize the cellular immune response to L9LS. 5. To determine if the efficacy of L9LS is specific to certain Pf parasite genotypes at the CSP locus. 6. To explore the impact of pre-existing parasitemia on the protective efficacy and PK of L9LS. 7. To explore the impact of pre-existing CSP antibodies on the protective efficacy and PK of L9LS.	1. Pf blood-stage infection as detected by microscopic examination of thick blood smear and RT-PCR for 24 weeks after administration of study agent 2. Measurement of ADA to L9LS in sera of recipients. 3. Assessment of IgG1 allotypes and allotype-specific effects on L9LS PK. 4. Characterization of the cellular immune response to L9LS. 5. CSP genotyping of parasites isolated from study subjects. 6. Pre-existing parasitemia detected by microscopic examination of thick blood smears or RT-PCR before L9LS administration. 7. Pre-existing CSP-specific antibodies measured in sera collected before L9LS administration.	1. Blood smear is the gold standard for diagnosis of blood-stage Pf infection and RT-PCR is more sensitive than blood smear for detecting Pf blood-stage infection. 2. ADA to L9LS may impact the PK and activity of L9LS. 3. Subject IgG1 allotype may impact the PK and activity of L9LS. 4. L9LS-induced cellular immune responses may be associated with Pf infection risk. 5. L9LS efficacy may be specific to certain Pf parasite genotypes at the CSP locus. 6. Pre-existing parasitemia may impact the protective efficacy and PK of L9LS. 7. Pre-existing CSP-specific antibodies may impact the protective efficacy and PK of L9LS.

4 STUDY DESIGN

4.1 OVERALL DESIGN

This is a phase 2 trial evaluating the safety and tolerability of one-time SC administration of L9LS in healthy adults in Mali, as well as its protective efficacy against naturally occurring Pf infection over a 6-month malaria season. A secondary objective is to determine if SC administration of L9LS at 900 mg (compared to placebo) mediates protection against naturally occurring Pf infection in healthy Malian adult females stratified by weight during a single malaria season as detected from microscopic examination of thick blood smears and RT-PCR.

The primary study hypotheses are that L9LS will be safe and will confer protection against Pf infection. The study will recruit from 3 MRTC clinics, 1 in Torodo, 1 in Faladje, and 1 in Kalifabougou. All day 0 visits will take place at Kalifabougou for all subjects, regardless of residence.

The rural village of Kalifabougou is situated 46 km from the MRTC laboratory in Bamako where biological samples collected for this protocol will be processed and stored. Torodo is located 12 km north of Kalifabougou, and Faladje is located 25 km north of Kalifabougou. The economy is based on subsistence farming. Kalifabougou, Faladje, and Torodo are similar in terms of geographic, demographic, and epidemiological characteristics, and all three typically experience intense seasonal Pf transmission from July through December each year.[22] Based on data collected in Kalifabougou from 2011 through 2018, 68%-90% of adults who are uninfected (PCR negative) before the malaria season become infected with Pf during the ensuing 6-month malaria season.[22] Accordingly, in the phase 2 trial of CIS43LS at the same study site, 78.2% of participants who received placebo became infected over the 6-month malaria season.[9]

This is a randomized, double-blind, placebo-controlled, sex- and weight-stratified trial (N=288 total) to assess safety and protective efficacy of L9LS and a placebo arm. Study enrollment will be staged to allow early safety to be assessed in an initial group of participants prior to proceeding with full study enrollment, as described in section 6.1.2.1. Adult subjects will receive 1 dose of study agent and be followed at regular study visits for about 6 months. Primary study assessments include physical examination and blood collection for research laboratory evaluations, including assessment of protection against naturally occurring Pf infection over 6 months (a single malaria season). The primary efficacy endpoint is the absence of Pf parasites in blood smears obtained between 2 weeks and 6 months after study agent administration in recipients of L9LS and placebo. The primary efficacy analysis will be based on time to the first infection. A secondary endpoint is the absence of Pf parasites as assessed by RT-PCR between 2 weeks and 6 months after study agent administration in recipients of L9LS and placebo. The adult phase 2 CIS43LS trial conducted in Mali assessed protective efficacy between 1 week and 6 months after study agent administration.[9] Since CIS43LS was administered intravenously in the adult phase 2 trial in Mali, the maximum serum concentration of CIS43LS (C_{max}) occurred during the immediate post-infusion period. In contrast, the phase 1 trial of L9LS showed that C_{max} after subcutaneous administration occurred at 5.9 (±2.2) days,[11] therefore the current trial will assess protective efficacy between 2 weeks and 6 months after study agent administration to ensure that C_{max} has been reached in study participants.

Subjects will be followed for safety via AE assessment at each study visit.

4.2 Scientific Rationale for Study Design

This study was designed to test L9LS in the setting of naturally occurring Pf infection and in a population that could potentially benefit from a novel therapeutic for malaria prevention. It will use both randomization and a double blind to minimize bias in subject selection and study assessments. The placebo will be inactive (normal saline) rather than a comparator MAb, as currently there are no licensed anti-malaria MAbs available.

4.3 Justification for Dose

The L9LS dose used in this trial was selected based on data generated in the phase 1 VRC trial of L9LS, which evaluated a dose range from 1 to 20 mg/kg delivered via IV administration and 5 mg/kg delivered via SC administration. Of the 17 participants who received a single dose of L9LS, 15 (88%) were protected after CHMI that occurred within 2 – 6 weeks. Parasitemia did not develop in any of the participants who received 5 or 20 mg/kg of IV L9LS. Parasitemia developed in 1 of 5 participants who received 1 mg/kg IV, and 1 of 5 participants who received 5 mg/kg SC. Protection conferred by L9LS after CHMI was observed with serum concentrations of L9LS as low as 9.2 µg/ml.[11] PK modeling indicated that a concentration of L9LS in the range of 5 to 11 µg/mL would remain in circulation at 6 months in participants who received 5 mg/kg SC. As described below, the dose range of L9LS being tested in this trial is approximately 10 – 20 mg/kg for females and approximately 9 – 18 mg/kg for males. To be conservative, a minimum dose of 9 – 10 mg/kg of L9LS SC was selected for this trial since the phase 1 trial of L9LS involved only a single challenge of 5 infective mosquito bites, whereas in this trial in Mali participants are expected to be exposed to repetitive infections over 6 months. In addition, a dose of 10 mg/kg SC aligns with the minimum dose used in the phase 2 trial of L9LS in Kenya, and with the WHO's preferred product characteristics for MAbs for malaria prevention.[23] The weight range of adult females and males in Kalifablugou and Torodo Mali is shown below (Figure 3). For females, the weight range encompassing the 2.5 – 97.5% quantiles is approximately 45 – 90 kg. For males, the weight range encompassing the 2.5 – 97.5% quantiles is approximately 50 -100 kg. Participants above or below these weight ranges will be excluded from participation in the trial, and enrollment will be stratified by weight to approximate an even distribution of weight-based (mg/kg) dosing. Therefore, a single 900-mg dose of L9LS will provide a weight-based dose range of approximately 10 – 20 mg/kg for females and approximately 9 – 18 mg/kg for males.

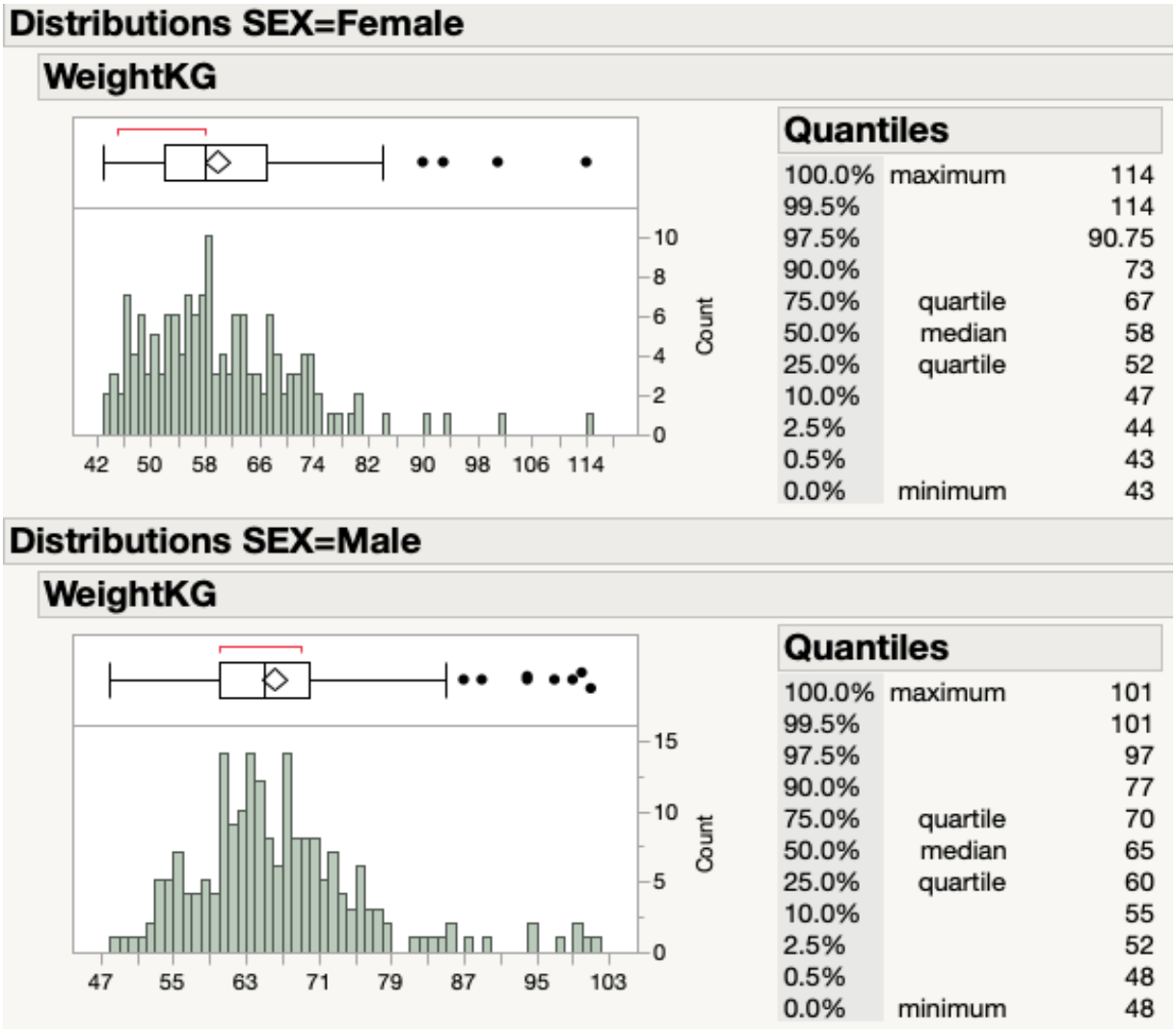

Figure 3. Sex-stratified distribution of body weight (kg) among adults in Kalifabougou and Torodo, Mali.

4.4 Justification for Sex and Weight Stratification

Weight stratification is employed in this protocol to approximate an even distribution of weight-based (mg/kg) dosing of L9LS, which will be administered at a single fixed dose of 900 mg. Sex stratification at a female to male ratio of 2:1 is employed in this protocol to obtain robust data on L9LS safety, PK, and efficacy among women of child-bearing potential in preparation for a phase 1 trial of L9LS in pregnant women—a high-priority clinical-use case for anti-malarial MAbs. Data on adult males could inform future studies of migrant workers who are intermittently exposed to malaria, as well as elimination trials that target all sexes and age groups.

For weight stratification, the female weight distribution was divided into 3 strata based on the 33.3% and 66.6% quantiles: 45.0 – 54.9 kg (low), 55.0 – 63.9 kg (medium), and 64.0 – 90.0 kg (high), thus yielding weight-based dosing strata of 16.7 – 20 mg/kg, 14.3 – 16.4 mg/kg, and 10 – 14.1 mg/kg, respectively. Similarly, the male weight distribution was divided into 3 strata based

on the 33.3% and 66.6% quantiles: 50.0 – 61.9 kg (low), 62.0 – 72.9 kg (medium), and 73.0 – 100.0 kg (high), thus yielding weight-based dosing strata of 14.8 – 18 mg/kg, 12.5 – 14.5 mg/kg, and 9 – 12.3 mg/kg, respectively. For females, there will be 64 (i.e., 192/3) participants in each of the 3 weight strata; however, to minimize barriers to eligibility and enrollment, the required minimum number of participants in each weight strata is 40, beyond which any participant within the eligible weight range (45.0 – 90.0 kg) can be enrolled. For males, there will be 32 (i.e., 96/3) participants in each of the 3 weight strata; however, to minimize barriers to eligibility and enrollment, the required minimum number of participants in each weight strata is 20, beyond which any participant within the eligible weight range (50.0 – 100.0 kg) can be enrolled.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

Individuals must meet all of the following criteria to be eligible for study participation:

1. Females aged ≥18 and ≤49 years and weighing ≥ 45.0 and ≤ 90.0 kg.
2. Males aged ≥18 and ≤55 years and weighing ≥ 50.0 and ≤ 100.0 kg.
3. Able to provide proof of identity to the satisfaction of the study clinician completing the enrollment process.
4. In good general health and without clinically significant medical history.
5. Able to provide informed consent.
6. Willing to have blood samples and data stored for future research.
7. Resides in or near Kalifabougou, Faladje, or Torodo, Mali, and available for the duration of the study.
8. Females of childbearing potential must be willing to use reliable contraception from 21 days prior to study day 0 through the final study visit as described below.
    a. Reliable methods of birth control include 1 of the following: confirmed pharmacologic contraceptives via parenteral delivery or intrauterine or implantable device.
    b. Nonchildbearing women will be required to report date of last menstrual period, history of surgical sterility (i.e., tubal ligation, hysterectomy) or premature ovarian insufficiency, and will have urine or serum pregnancy test performed per protocol.

5.2 EXCLUSION CRITERIA

Individuals meeting any of the following criteria will be excluded from study participation:

1. Pregnancy, as determined by a positive urine or serum beta-human choriogonadotropin (β-hCG) test (if female).
2. Currently breastfeeding.
3. Behavioral, cognitive, or psychiatric disease that in the opinion of the investigator affects the ability of the subject to understand and comply with the study protocol.
4. Study comprehension examination score of <80% correct or per investigator discretion.

5. Hemoglobin, white blood cell, absolute neutrophil, or platelet count outside the local laboratory-defined limits of normal. (Subjects may be included at the investigator's discretion for "not clinically significant" values.)
6. Alanine transaminase (ALT) or creatinine (Cr) level above the local laboratory-defined upper limit of normal. (Subjects may be included at the investigator's discretion for "not clinically significant" values.)
7. Infected with human immunodeficiency virus (HIV), hepatitis C virus (HCV), or hepatitis B virus (HBV).
8. Known or documented sickle cell disease by history. (Note: Known sickle cell trait is NOT exclusionary.)
9. Clinically significant abnormal electrocardiogram (ECG; QTc >460 or other significant abnormal findings, including unexplained tachycardia or bradycardia).
10. Evidence of clinically significant neurologic, cardiac, pulmonary, hepatic, endocrine, rheumatologic, autoimmune, hematological, oncologic, or renal disease by history, physical examination, and/or laboratory studies including urinalysis.
11. Receipt of any investigational product within the past 30 days.
12. Participation or planned participation in an interventional trial with an investigational product until the last required protocol visit. [Note: Past, current, or planned participation in observational studies is NOT exclusionary; participation in the placebo arm of the Mali adult CIS43LS MAb trial (ClinicalTrials.gov Identifier: NCT04329104) is NOT exclusionary.]
13. Medical, occupational, or family problems as a result of alcohol or illicit drug use during the past 12 months.
14. History of a severe allergic reaction or anaphylaxis.
15. Severe asthma (defined as asthma that is unstable or required emergent care, urgent care, hospitalization, or intubation during the past 2 years, or that has required the use of oral or parenteral corticosteroids at any time during the past 2 years).
16. Pre-existing autoimmune or antibody-mediated diseases including but not limited to: systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, Sjögren's syndrome, or autoimmune thrombocytopenia.
17. Salivary gland disorder diagnosed by a doctor (e.g., parotitis, sialadenitis, sialolithiasis, salivary gland tumors).
18. Known immunodeficiency syndrome.
19. Known asplenia or functional asplenia.
20. Use of chronic (≥14 days) oral or IV corticosteroids (excluding topical or nasal) at immunosuppressive doses (i.e., prednisone >10 mg/day) or immunosuppressive drugs within 30 days of day 0.
21. Receipt of a live vaccine within the past 4 weeks or a killed vaccine within the past 2 weeks prior to study agent administration.
22. Receipt of immunoglobulins and/or blood products within the past 6 months.
23. Previous receipt of an investigational malaria vaccine or monoclonal antibody in the last 5 years.

24. Known allergies or contraindication against artemether-lumefantrine.
25. Other condition(s) that, in the opinion of the investigator, would jeopardize the safety or rights of a subject participating in the trial, interfere with the evaluation of the study objectives, or render the subject unable to comply with the protocol.

5.3 Inclusion or Exclusion of Vulnerable Participants

- **Children:** Children are not eligible to participate in this clinical trial. There are ongoing phase 2 trials of L9LS in Mali and Kenya that involve children.
- **Pregnant Women:** Pregnant women are excluded from this study because the effects of L9LS on pregnant women or the developing human fetus are unknown with the potential for teratogenic or abortifacient effects.
- **Decisionally Impaired Adults:** Individuals must be able to provide consent in order to be eligible for participation in this study. Enrolled subjects who lose decision-making capability during study participation will be withdrawn (see sections 8.4.3 and 10.1.2).
- **Illiterate Individuals:** We anticipate that many individuals eligible for this study will be illiterate in French, so the study team will translate the consent orally into local languages when appropriate, as described in section 10.1.

5.4 Lifestyle Considerations

In addition to prohibited treatments and procedures listed in section 6.5, subjects must refrain from donating blood for at least 1 year after study drug administration.

Women must not breastfeed during the study because there could be unknown risks to the child secondary to the mother's receipt of the study drug.

5.5 Screen Failures

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

Individuals who initially do not meet the criteria for participation in this trial (screen failure) because of an acute illness or a transient lab or other screening procedure findings (e.g., abnormal ECG, ALT, or other transient lab evaluations) may be rescreened or may repeat individual screening procedures at the investigator's discretion. All data (screening and re-screening) will be collected under the same subject number.

5.6 Strategies for Recruitment and Retention

Healthy adult volunteers will be selected based on the eligibility criteria described in sections 5.1 and 5.2. The total target sample size across all three study sites is 288 subjects (accrual ceiling 860). We expect to enroll all subjects within the first 6 months of the study. The study includes a

higher proportion of women given the importance of obtaining safety, PK, and efficacy data in women of child-bearing potential to lay the foundation for future studies in pregnant women. Subject selection will not be limited based on sex, race, or ethnicity.

The study team will hold community meetings in Kalifabougou, Faladje, and Torodo to explain and discuss the study and obtain community permission from the village elders, heads of families, and other community members in each village where the study will take place (section 10.1.1.1). Afterward, an announcement via local radio or another traditional channel of communication may be made to invite households to come to participating clinics to learn about the study.

5.6.1 Costs

There are no costs associated with participation in this trial.

5.6.2 Compensation

Subjects will be compensated for the time and inconvenience of participating in the study as follows:

- 5,000 Communauté Financière Africaine (CFA) Franc for each of the following: screening visit, enrollment visit, and day 0 visit (study drug administration).
- 3,000 CFA Franc for all other visits.

The total compensation amount will be 63,000 CFA Franc (valued at approximately USD $115). Payment will be provided in cash after the completion of each visit.

Subjects will be provided with transportation to and from study visits.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION ADMINISTRATION

6.1.1 Study Intervention Description

The study intervention involves a single administration of L9LS or placebo to each subject. All study agent administration will take place at the Kalifabougou study site.

6.1.2 Dosing and Administration

Study agent and dosing will be dependent on study arm assignment (Table 1). Procedures for SC administration are described in section 6.1.2.4.

Table 1. Study agent assignment and dosing by arm.

Arm	Subjects	Study Agent and Dose
1	216	900 mg L9LS SC
2	72	Placebo (normal saline) SC

6.1.2.1 Staged Enrollment and Study Agent Administration

L9LS was vialed at a concentration of 150 mg/mL; therefore, the 900-mg dose will be administered as two separate 3-mL SC injections. To maintain blinding across study arms, the placebo (normal saline) will also be administered as two separate 3-mL SC injections. In the ongoing phase 2 trial of L9LS in Mali, SC injections of 600 mg of L9LS (two separate 2-mL injections) were well tolerated in adults during the dose escalation, age de-escalation phase of the study. In the VRC phase 1 study of L9LS, SC injections up to 2 mL were well tolerated.[11] In an ongoing extension of the phase 1 trial of CIS43LS, multiple SC administrations of CIS43LS were given with a maximum volume of 2.5 mL per injection site and were found to be safe and well tolerated.

In this protocol, enrollment and dosing will start with 12 participants (approximately 9 receiving L9LS at a dose of 900 mg, and approximately 3 receiving placebo) to assess the safety and tolerability of two 3-mL SC injections at two separate sites. Once all 12 subjects complete day 7 post-administration, if no safety concerns have arisen, then enrollment will continue for the remaining subjects.

6.1.2.2 Dose Limiting Toxicity

Pausing and halting rules are provided in sections 8.4.5 and 8.4.6.

6.1.2.3 Dose Modifications

This study involves administering a single fixed dose to each subject, so there will be no dose modifications.

6.1.2.4 Drug Administration

Prior to study agent administration on day 0, subjects will undergo vital signs measurement, a targeted physical examination (as needed based on signs, reported symptoms, or interim medical history), and, for women, a urine pregnancy test (confirmed negative prior to administration).

For all subjects, 6 mL of assigned study agent will be divided into 2 syringes. The study agent will be administered in each upper outer triceps area as a SC injection (1 injection per arm), using proper technique to ensure administration into SC fatty layer and a slow push to minimize discomfort or the excessive distention of overlying skin. The abdomen may be used as an alternate injection site if the outer triceps area is not suitable. SC administration sites in the abdomen should be at least 2 inches apart from each other and at least 2 inches away from the umbilicus. Up to 3 SC injection sites may be used if deemed necessary by the clinician. Because L9LS has a slight yellow tint, and the placebo (normal saline) is colorless, all syringes will be covered with transparent yellow tape by the study pharmacist prior to administration to maintain blinding. L9LS is more viscous than normal saline, so the study agents will only be administered to subjects by designated individuals who remain separate from the team of blinded investigators who conduct all subsequent follow-up study assessments.

All subjects will be observed for at least 60 minutes (+/- 15 minutes) following completion of product administration. Prior to discharge from the clinic, vital signs will be recorded and

subjects will be assessed for local reactogenicity (including pain/tenderness, swelling, redness, bruising, and pruritus at the site of injection) and systemic reactogenicity (including fever, feeling unusually tired or unwell, muscle aches, headache, chills, nausea, and joint pain). Any subject who is assessed as being unwell or has ongoing reactogenicity symptoms will be asked to remain in the clinic until evaluation and discharge by a study clinician. If necessary, the subject will be referred to the district hospital to evaluate for safety and possible treatment. Clinicians will follow any solicited symptoms that are ongoing until they have resolved.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 Acquisition and Accountability

Acquisition: L9LS will be shipped from the US to the study site where administration will take place, in compliance with all FDA, US Department of Transportation, and United Nations transport guidelines for shipping biohazardous materials. The placebo product will either be purchased in the US and shipped to Mali at ambient temperature or purchased in Mali.

Accountability: The study pharmacist will be responsible for maintaining an accurate record of the study arm codes, inventory, and an accountability record of study agent supplies. Electronic documentation as well as paper copies may be used.

The empty vials and the unused portion of a vial will be discarded in a biohazard containment bag and incinerated or autoclaved in accordance with the institutional or pharmacy policy. Any unopened vials that remain at the end of the study will be returned to the production facility or discarded at the discretion of the manufacturer in accordance with policies that apply to investigational agents. Partially used vials will not be administered to other subjects or used for *in vitro* experimental studies. These vials will be disposed of in accordance with institutional or pharmacy policy.

6.2.2 Formulation, Appearance, Packaging, and Labeling

L9LS was manufactured under current Good Manufacturing Practice by the Vaccine Clinical Materials Program operated under contract by Leidos Biomedical Research, Inc., Frederick, MD. L9LS is a sterile, aqueous, buffered solution that is filled into single-dose vials at 150 + 15 mg/mL to a target fill volume of 2.25 mL in a 3-mL vial. The formulation buffer is the same as the drug substance. The drug product container closure system consists of Type I glass vials, chlorobutyl rubber stoppers, and seals purchased from approved manufacturers. Any diluent composition will be described in the IND. The placebo product will be sterile isotonic (0.9%) normal saline. The products will be prepared by an unblinded pharmacist and placed in a sterile syringe for SC administration. All syringes will be covered to maintain blinding as described above (section 6.1.2.4).

Vials of L9LS will be individually labeled with the name of the material, volume, lot number, concentration, storage instructions, Investigational Use Statement (“Limited by Federal Law to Investigational Use”), and manufacturer information.

6.2.3 Product Storage and Stability

L9LS: L9LS vials should be stored frozen at −35°C to −15°C in a qualified, continuously-monitored, temperature-controlled freezer. The site pharmacist must promptly report any storage temperature excursions outside of the normal allowance for the storage device to the IND sponsor. The affected product must be quarantined in a separate area under protocol-specific temperature ranges until further notice from the sponsor. If the excursion results in thawed material, the material should not be refrozen; the thawed, vialed material should be stored at 2°C to 8°C.

When a storage/shipping/handling excursion occurs, the IND sponsor designee must send a notification of the occurrence of an excursion to VRCProductinquiries@nih.gov. An automatic email reply will be sent to the notifier, including (as an attachment) the Clinical Excursion Reporting Form, which can be filled electronically (or manually and scanned, if needed). The completed form and relevant attachments (e.g., temperature charts) must be emailed to the VRC via the same email address (VRCProductinquiries@nih.gov) using the "reply" function. The IND sponsor will notify the site pharmacist if continued clinical use of the product is acceptable or will provide further instructions.

Prior to preparation for SC administration, vials should be thawed and equilibrated for a minimum of 90 minutes at ambient temperature (15°C to 32°C). If thawed vials are removed from 2°C to 8°C, they should be equilibrated at ambient temperature for a minimum of 30 minutes.[24]

After product preparation in a syringe, the prepared L9LS may be stored at 2°C to 8°C for a maximum of 24 hours and/or at ambient temperature (15°C to 32°C) for a maximum of 4 hours. Product may not be stored in direct sunlight.[24]

Placebo: Normal saline will be stored at room temperature in a controlled room per product standards.

6.2.4 Preparation

Study product will be prepared by an unblinded pharmacist. Two syringes will be prepared for each subject for a total of two 3-mL SC injections for each subject. The pharmacist will cover all syringes with transparent yellow tape to maintain blinding. Up to 3 syringes may be prepared if deemed necessary by the clinician to use 3 injection sites.

For each SC injection order, the dose level and study arm code will be included in the pharmacy order. To prepare a SC injection, the pharmacist will do the following:

- For subjects assigned to L9LS:
    1) Retrieve the minimum number of thawed vials required to prepare the full dose. After vials are thawed, vials should be gently swirled for 30 seconds while avoiding foaming. DO NOT SHAKE THE VIAL.

2) Withdraw the total amount of L9LS from the vial into the syringe(s) using a 5-micron filter needle. A new filter needle must be used for each syringe. The filter needle must be discarded prior to dispensing and replaced with a needle suitable for SC injection at the time of administration.

- For subjects assigned to placebo:
    1) Withdraw 3 mL of normal saline placebo into the syringe(s) using a 5-micron filter needle. A new filter needle must be used for each syringe. The filter needle must be discarded prior to dispensing and replaced with a needle suitable for SC injection at the time of administration.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

6.3.1 Randomization

Subjects will be randomized to 1 of 2 treatment arms: the L9LS arm or the placebo arm. Randomization lists will be generated by the unblinded study statistician, and the randomization code lists will be maintained by a designated pharmacist at the study site where the study intervention will take place.

Randomization is further described in section 9.4.1.

6.3.2 Blinding

The study will be conducted with a double blind. Because L9LS has a slight yellow tint, and the placebo (normal saline) is colorless, the pharmacy team will cover all syringes with transparent yellow tape to maintain blinding. The subjects, the clinical staff, and the study team will be blinded to study treatment allocation, with the exception of designated individuals who administer the study agents and remain separate from the team of blinded investigators who conduct all subsequent follow-up study assessments. The pharmacy team at the study site where administration is taking place will be unblinded, and they are responsible for maintaining security of study treatment assignments.

Data will remain blinded until the last subject completes the final study visit. Subjects will then be informed about their study treatment assignment.

Unscheduled unblinding, either intentional (e.g., in the case of a medical emergency in a subject) or unintentional, will be handled according to SOPs. Intentional and unintentional unscheduled unblinding will be documented in the appropriate source and/or research record and will include the reason for the unscheduled unblinding, the date it occurred, who approved the unblinding, who was unblinded, who was notified of the unblinding, and the plan for the subject.

The principal investigator will report all cases of intentional and unintentional unscheduled unblinding to the DSMB in writing within 1 business day after site awareness via email to the DSMB mailbox (niaiddsmbia@niaid.nih.gov) outlining the reason for the unblinding and the date it occurred. The report will also be submitted to the EC and the SMM.

If an SAE or other event merits unblinded review for safety (i.e., per the principal investigator, regulatory authority, or the SMM), the unblinding occurrence and a summary of how unblinded review and assessment was conducted for safety (preferably by the DSMB), will be summarized in the SAE or regulatory safety report. However, the actual treatment assignment will not be disclosed unless the particular participant is being globally unblinded per study leadership and sponsor.

6.4 Study Intervention Compliance

Study intervention administration will be documented by study staff.

6.5 Concomitant Therapy

All concomitant prescription and nonprescription (including over-the-counter, herbal, or traditional) medications taken during study participation will be recorded. For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician.

Treatment with the following drugs and procedures will not be permitted unless discussed with and approved by the investigator:

- Live vaccines within 4 weeks of study agent administration.
- Killed vaccine or any COVID-19 vaccine within 2 weeks of study agent administration. (In Mali there are no standard vaccines for adults).
- Immunoglobulins and/or blood products for the duration of the study.
- Receipt of any investigational product or co-enrollment in other clinical studies of investigational products.
- Oral or IV corticosteroids at immunosuppressive doses (i.e., prednisone >10 mg/day) or immunosuppressive drugs for the duration of the study.
- Antimalarials and antibiotics with known antimalarial activity (with the exception of artemether-lumefantrine given at enrollment).

Emergent use of any drugs or blood products to treat life-threatening conditions does not require approval by the investigator and will be recorded.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Intervention

Study intervention may be discontinued for a protocol-defined group or arm (i.e., pausing), or it may be discontinued for all subjects and enrollment suspended (i.e., halting). Pausing and halting are described in sections 8.4.5 and 8.4.6. Subjects who have already received the study agent at the time of a pause or halt will continue planned follow-up under the protocol.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request. Plans for managing the involuntary withdrawal of a subject are provided in section 8.4.3. The reason for subject discontinuation or withdrawal from the study will be recorded on the case report form (CRF).

Subjects who withdraw after receiving study agent but prior to study completion will be encouraged to attend an early termination visit, where they will complete as many of the procedures and evaluations indicated in the schedule of activities (section 1.3) as possible.

7.3 MISSED VISITS AND LOST TO FOLLOW-UP

If a participant misses 1 visit after D28 or 2 visits after D28 that are not sequential, these will not be considered protocol deviations. However, 2 missed visits in a row or a total of 3 or more missed visits (regardless of whether or not they are sequential) over the duration of the study will be considered protocol deviations.

A subject will be considered lost to follow-up if he or she fails to return for 3 consecutive scheduled visits and is unable to be contacted by the study site staff.

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

- The site will attempt to contact the subject and reschedule the missed visit within 7 days, counsel the subject on the importance of maintaining the assigned visit schedule, and ascertain if the subject wishes to and/or should continue in the study. If a missed visit cannot be rescheduled, the participant may continue with the next scheduled visit.
- Before a subject is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the subject (where possible, 3 telephone calls or home visits). These contact attempts should be documented in the subject's medical record or study file.
- Should the subject continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 SCREENING PROCEDURES

Screening will be performed at all study sites. The study staff will explain the study to the prospective subject, complete the study comprehension examination and obtain consent (section 10.1), and assess eligibility. Consent will be obtained before any study-related procedures are performed.

The following screening procedures and evaluations must be performed within -56 to -7 days of study intervention. Screening may take place over multiple visits if necessary.

- Confirmation of identity, age, and residency.

- Complete review of medical history and medication use.
- Complete physical examination, including height and weight.
- Vital signs (temperature, blood pressure, and pulse).
- 12-lead ECG.
- Urine collection for urinalysis (urine dipstick or formal urinalysis; acceptable laboratory parameters defined in Appendix A).
- For women, serum β-hCG pregnancy test.
- Blood collection via venipuncture for screening evaluations:
    - HIV tests: 2 rapid diagnostic tests (RDTs), plus ELISA if the RDTs are discordant. A subject will be referred for medical care for 2 positive RDTs or a positive ELISA. If the ELISA is negative, no further work-up will be done. Pre- and post-test HIV counseling will be provided.
    - HBV ELISA and HCV ELISA (PCR, if indicated) tests. If either test is positive, the subject will be referred for care regardless of the ALT result.
    - Hemoglobin typing sample will be stored at screening, and hemoglobin typing will be performed if the subject is enrolled; this test will not be used in eligibility assessments.
    - Complete blood count (CBC) with differential.
    - ALT.
    - Cr.
- Pregnancy prevention confirmation and counseling.

A prospective subject who has any clinically significant abnormal finding and/or is diagnosed with a medical condition at screening or during the conduct of the study will be notified and referred for medical care. Per national requirements for reporting communicable diseases, confirmed positive test results for HIV, HBV, and HCV will be reported to the local health department according to applicable laws and appropriate medical referrals initiated. The cost of initial and long-term treatment and care of medical conditions diagnosed during the screening process will not be reimbursed by the study but referrals to relevant specialist will be provided.

Screening evaluations may be repeated as described in section 5.5, at the discretion of the investigator. If screening is completed outside the specified window, all screening procedures and evaluations must be repeated.

Enrollment: If the individual is eligible and agrees to participate, he or she will be scheduled to come for an enrollment visit, as described in section 1.3. Enrollment is defined as the time of artemether-lumefantrine administration. A clinician will discuss the target dates and timing of the study agent administration and sample collections before completing an enrollment to help ensure that the subject can comply with the projected schedule.

8.2 EFFICACY ASSESSMENTS

8.2.1 Clinical Evaluations

The following clinical evaluations will be performed as efficacy assessments.

Medical History and Medication Review: A complete review of all medical history and medications will be conducted at screening. Subsequent visits will include a targeted review of changes in medical history or medications since the last study visit.

Physical Examination: A complete physical examination (including height and weight) will be done at screening. A targeted physical examination based on signs, reported symptoms, and medical history will be conducted at subsequent study visits. Weight will also be recorded on day 0 prior to study agent administration.

Artemether-Lumefantrine: At enrollment, all subjects will be orally administered standard artemether-lumefantrine treatment (4 tablets twice daily for 3 days) to clear any possible Pf blood-stage infection prior to study agent administration. Study investigators will provide a fat-containing drink (e.g., milk) to study participants immediately prior to administering artemether-lumefantrine to enhance its absorption, as per 2021 WHO Guidelines for Malaria.[25]

Randomization Procedures: Randomization for the efficacy study is described in sections 6.3.1 and 9.4.1.

Study Agent Administration and Monitoring: Study agent administration and monitoring will be performed according to the assigned arm, as described in section 6.1.

Illness Visit: A subject will be instructed to come in for an unscheduled visit if he or she has symptoms of malaria or other symptoms. The subject will be evaluated by the study team and referred for standard care according to local guidelines. At an illness visit, the subject may undergo review of medical history and concomitant medications, a focused physical exam for symptoms of malaria or other diseases, vital sign measurement, and a fingerprick or venipuncture blood collection for blood smear for malaria diagnosis as well as a dried blood spot for Pf RT-PCR for research purposes.

Malaria Diagnosis and Management: If a subject has a malarial infection, we will share these results with the subject and provide standard treatment in accordance with the recommendations of the Mali National Malaria Control Program. According to the national guidelines, asymptomatic malarial infections in adults are not treated. Malaria treatment is given only when symptoms are present along with positive blood smear results. RT-PCR is not commonly used for routine malaria diagnosis.

8.2.2 Biospecimen Evaluations

Blood will be collected under this protocol by the following methods:

- Venipuncture will be performed with single-use needles. Venous blood samples will be used as follows:

  - Safety evaluations described in section 8.3.
  - Shipment to the research laboratory in Bamako and NIAID for evaluation (including assays described in section 8.2.3) and storage.
  - Blood smear and dried blood spot for Pf RT-PCR if unable to obtain ample sample from finger prick.
- Finger prick will be performed using single-use disposable lancets. Finger prick blood samples will be used as follows:
  - Blood smear and dried blood spot for Pf RT-PCR.

The amount of blood drawn for research purposes will be within the limits allowed for adult research subjects by the NIH Clinical Center: no more than 10.5 mL/kg or 550 mL (whichever is smaller) over an 8-week period.

The collection schedule, volumes, and test tubes are presented in section 1.3.

8.2.3 Correlative Studies for Research/Pharmacokinetic Studies

The following evaluations will be performed according to the schedule presented in section 1.3.

Blood smear: Thick blood smears will be prepared and analyzed by the standard WHO method (section 2.2.2.3) to identify Pf infection for the primary endpoint. This evaluation will be performed centrally in the laboratory in Bamako, unless the subject is displaying signs or symptoms of clinical malaria, in which case the blood smear will be analyzed at the time of the scheduled or unscheduled visit.

Pf RT-PCR: RT-PCR will be performed to identify Pf infection as a secondary endpoint. This evaluation will be performed at the University of Washington on coded dried blood spots.

PK studies: Blood L9LS concentrations will be measured by Meso Scale Discovery LLC–based automation platform. The concentration at the visit prior to the first Pf infection will be used to assess L9LS-mediated protection. This evaluation will be performed at the NIAID.

ADA detection: Assays for detection of ADA will be performed at specified timepoints (section 1.3) following product administration and compared to baseline status. This evaluation will be performed at the NIAID.

Parasite genotyping: For subjects who become infected during the study, coded blood samples collected around the time of the first infection will be used to perform a genotypic sieve analysis to analyze sequences of breakthrough parasites (section 9.4.11). This evaluation will be performed by Dr. Daniel Neafsey at the Harvard School of Public Health.

8.2.4 Samples for Genetic/Genomic Analysis

8.2.4.1 Description of the scope of genetic/genomic analysis

Genetic testing performed in this protocol will be limited to analyzing the genetic material of infection-inducing parasites in blood samples. No human genetic analyses will be performed.

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

Privacy and confidentiality of medical information and biological samples is described in section 10.3.

8.2.4.3 Management of Results

As no human genetic analyses will be performed in this protocol, no genetic results will be returned to subjects.

8.2.4.4 Genetic counseling

Not applicable.

8.3 Safety and Other Assessments

The following study procedures and evaluations will be done according to the schedule in section 1.3 to monitor safety and support the understanding of the study intervention's safety. The assessment and collection of safety events such as AEs are described in section 8.4.2.

Physical Examination: As described in section 8.2.1, physical examination will also be performed for assessment of safety.

Vital Signs: Vital signs (temperature, blood pressure, and heart rate) will be collected at visits, including before and after study agent infusion, as described in section 8.2.1.

Safety Blood Laboratory Evaluations: The following safety laboratory evaluations will be performed at a frequency presented in section 1.3:

- CBC with differential.
- ALT, Cr.

Pregnancy Testing: Women will have a serum pregnancy test at screening and urine tests at enrollment, day 0 (confirmed negative prior to administration of the study agent), and monthly through the final study visit.

Pregnancy Prevention Counseling: Female subjects will be counseled on the importance of not becoming pregnant during study participation and on acceptable methods of contraception.

8.4 Safety Definitions, Management, and Sponsor Reporting

8.4.1 Definitions

Adverse Event: An AE is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the research.

Adverse Reaction: An AR means any AE caused (see "Causality" below) by a study agent. ARs are a subset of all suspected adverse reactions (SARs; defined below) where there is reason to conclude that the study agent caused the event.

Suspected Adverse Reaction: SAR means any AE for which there is a reasonable possibility that the study agent caused the AE.

Per US FDA guidance:
For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal (see "Causality" below) relationship between the study agent and the AE. A SAR implies a lesser degree of certainty about causality than an AR, which means any AE caused by a study agent.

SARs are the subset of all AEs for which there is a reasonable possibility that the study agent caused (see "Causality" below) the event. Inherent in this definition, and in the requirement to report SARs, is the need for the sponsor to evaluate the available evidence and make a judgment about the likelihood that the study agent actually caused the AE.

The sponsor is responsible for making the causality judgment.

Serious Adverse Event: An SAE:

- is an AE that results in death.
- is an AE that is a life-threatening event (places the subject at immediate risk of death from the event as it occurred).
- is an AE that requires inpatient hospitalization or prolongs an existing hospitalization.
  NOTE:
  - Hospitalization is considered required if outpatient treatment would generally be considered inappropriate.
  - Same-day surgical procedures that are required to address an AE are considered hospitalizations, even if they do not involve an overnight admission.
  - Hospitalization due to a condition that has not worsened and that pre-dates study participation (e.g., elective correction of an unchanged baseline skin lesion), or due to social circumstance (e.g., prolonged stay to arrange aftercare), or that is planned/required "per protocol" AND that proceeds without prolongation or complication, is NOT considered an SAE by this criterion.
- is, or results in, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- is a congenital anomaly/birth defect/miscarriage/stillbirth.
  NOTE: This definition is more inclusive than some commonly published definitions. It includes an affected conceptus/neonate whose:
  - biological mother was exposed to a study agent at any point from conception through the end of the pregnancy, AND/OR, if breastfeeding, the 30-day neonatal period; or

- biological father was exposed to a study agent at any point during the 90 days prior to conception.

This is separate from, and in addition to, general reporting of pregnancy in a study participant or female partner of a male participant (see section 8.4.2.3.4 below).

- is a medically important event.

  NOTE: Medical and scientific judgment should be exercised. Events that significantly jeopardize the subject and/or require intervention to prevent one of the SAE outcomes listed above are generally considered medically important, and are thus SAEs.

Unexpected Adverse Event: An AE is unexpected if it is not listed in the investigator's brochure or package insert (for marketed products) at the frequency, AND specificity, AND severity that has been observed.

NOTE:

- Such events should also be evaluated for possible reporting as unanticipated problems (UPs) (see section 8.4.2.3.3 below).
- Unexpected, as used in this definition, also refers to AEs or SARs that are mentioned in the investigator's brochure as occurring with a class of drugs/biologics, or as anticipated from the pharmacological properties of the study agent but are not specifically mentioned as occurring with the particular study agent under investigation.

Serious and Unexpected Suspected Adverse Reaction (SUSAR): A SUSAR is an SAR (defined above) that is both serious and unexpected.

Unanticipated Problem: A UP is any incident, experience, or outcome that meets all the following criteria:

1. Unexpected (in terms of nature, severity, or frequency) given
   a. the research procedures that are described in the protocol-related documents, such as the EC-approved research protocol and informed consent document; and
   b. the characteristics of the subject population being studied; and
2. Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research), and
3. Suggests the research places subjects or others (which may include research staff, family members or other individuals not directly participating in the research) at a greater risk of harm (including physical, psychological, economic, or social harm) related to the research than was previously known or expected.

   NOTE:
   - Per the sponsor, an SAE always meets this "greater risk" criterion.
   - An incident, experience, or outcome that meets the definition of a UP generally will warrant consideration of changes to the protocol or informed consent form, or to study procedures (e.g., the manual of procedures for the study), in order to protect the safety, welfare, or rights of participants or others. Some UPs may

warrant a corrective and preventive action plan at the discretion of the sponsor or other oversight entities.

Unanticipated Problem that is not an Adverse Event (UPnonAE): A UPnonAE belongs to a subset of UPs that:

- meets the definition of a UP, AND
- does NOT fit the definition of an AE or an SAE.

  NOTE: Examples of UPnonAEs include, but are not limited to:
  - a breach of confidentiality
  - prolonged shedding of a vaccine virus beyond the anticipated timeline
  - unexpectedly large number of pregnancies on a study
  - subject departure from an isolation unit prior to meeting all discharge criteria
  - accidental destruction of study records
  - unaccounted-for study agent
  - overdosage, underdosage, or other significant error in administration or use of study agent or intervention, even if there is no AE/SAE
  - development of an actual or possible concern for study agent purity, sterility, potency, dosage, etc.

    NOTE: A decision to temporarily quarantine, or to permanently not use all or part of study agent supply due to an unexpected finding or event (e.g., particulate, cloudiness, temperature excursion), even if there is no known or proven issue (i.e., out of an "abundance of caution"), is considered a UPnonAE.

Protocol Deviation: Any change, divergence, or departure from the EC-approved research protocol (except for some missed visits as specified in section 7.3).

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

Noncompliance: Failure of investigator(s) to follow the applicable laws, regulations, or institutional policies governing the protection of human subjects in research, or the requirements or determinations of the EC, whether intentional or not.

1. **Serious noncompliance:** Noncompliance, whether intentional or not, that results in harm or otherwise materially compromises the rights, welfare and/or safety of the subject. Noncompliance that materially affects the scientific integrity or validity of the research may be considered serious non-compliance, even if it does not result in direct harm to research subjects.
2. **Continuing noncompliance:** A pattern of recurring noncompliance that either has resulted, or, if continued, may result in harm to subjects or otherwise materially

compromise the rights, welfare and/or safety of subjects, affect the scientific integrity of the study or validity of the results. The pattern may comprise repetition of the same non-compliant action(s), or different noncompliant events. Such non-compliance may be unintentional (e.g., due to lack of understanding, knowledge, or commitment), or intentional (e.g., due to deliberate choice to ignore or compromise the requirements of any applicable regulation, organizational policy, or determination of the EC).

8.4.2 Documenting, Assessing, Recording, and Reporting Events

ALL AEs, including those that may appear to have a non-study cause (see "Causality" below), will be documented (e.g., on the clinical chart/progress notes/clinical laboratory record), recorded (e.g., in the study-specified CRF/research database), and reported (e.g., cumulatively from the research database, or according to protocol-specified expedited reporting mechanism) to the sponsor from the time informed consent is obtained through the timeframe specified below. At each contact with the subject, information regarding AEs will be elicited by open-ended questioning and examinations.

AEs and SAEs will generally be recorded, assessed, and reported according to the timeframes outlined in Table 2.

Table 2. Standard event recording, assessment, and reporting timeframes.

Event type	Record, assess, and report through
Related SAEs	End of subject participation in study, or if study personnel become aware thereafter
Unrelated SAEs	End of subject participation in study
Related non-serious AEs of grade 1 to 3	End of subject participation in study
All other related non-serious AEs	End of subject participation in study
Unrelated non-serious AEs	End of subject participation in study

8.4.2.1 Investigator Assessment of Adverse Events

The investigator will initially assess all AEs with respect to **seriousness** (according to SAE definition above), **severity** (intensity or grade, see below), and **causality** (relationship to study agent and relationship to participation in the research, see below). The Clinical Safety Office (CSO)/SMM is responsible for final/regulatory determinations of expectedness and causality.

8.4.2.1.1 Severity Grading

The investigator will grade the severity of fever (by non-oral temperature reading) and each blood laboratory testing AE according to the "Mali Adverse Event Grading Scale" provided in APPENDIX B: MALI ADVERSE EVENT GRADING SCALE. Events that are not gradable

using this table (e.g., urinalysis abnormalities) will be graded according to the "Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" which can be found at: https://www.fda.gov/media/73679/download.

Events that are NOT gradable using either of the above specified tables will be graded as follows:

- Mild = grade 1
- Moderate = grade 2
- Severe = grade 3
- Potentially life threatening = grade 4
- Death = grade 5

NOTE: A subject death should always be reported as grade 5.

8.4.2.1.1.1 Laboratory Value Assessment and Clinical Significance Criteria

Except as specified below, ALL abnormal lab values of grade 1 or above are REPORTABLE.

Grade 1 and 2 abnormal laboratory values are considered CLINICALLY SIGNIFICANT, and are to be recorded in the research database, and reported, if they meet ONE or more of the following criteria:

- result in a study agent dosage adjustment, interruption, or discontinuation
- are accompanied by clinically abnormal signs or symptoms that are likely related to the laboratory abnormality (e.g., clinical jaundice)
- indicate a possible organ toxicity (e.g., elevated serum creatinine)
- result in additional/repeat testing or medical intervention (procedures/treatments) (e.g., ECG to evaluate arrhythmia potential with a high serum potassium; one or more ECGs to assess an elevated troponin level; potassium supplementation for hypokalemia)
- indicates possible over-dosage
- are considered clinically significant by the investigator or SMM

8.4.2.1.2 Causality

Causality (likelihood that the event is caused by the study agents) will be assessed by the principal investigator or designee considering the factors listed under the following categories:

Definitely Related

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

Probably Related

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

Possibly Related

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

Unlikely Related

- does not have a reasonable temporal relationship

AND/OR

- there is good evidence for a more likely alternative etiology

Not Related

- does not have a temporal relationship

AND/OR

- definitely due to an alternative etiology

Note: Other factors (e.g., dechallenge, rechallenge, if applicable) should also be considered for each causality category when appropriate. Causality assessment is based on available information at the time of the assessment of the AE. The investigator may revise the causality assessment as additional information becomes available.

Causality assessment will be reviewed by the sponsor. The sponsor may make a separate and final determination on the "reasonable possibility" that the event was "related" (comprising definitely, probably, and possibly related) or "unrelated" (comprising unlikely and not related) to the study agent, in keeping with applicable (US FDA) guidance on sponsor IND safety reporting.

8.4.2.2 Recording of Events

AEs will be promptly recorded in the research database, regardless of possible relationship to study interventions. If a diagnosis is clinically evident (or subsequently determined), the diagnosis rather than the individual signs and symptoms or laboratory abnormalities will be recorded as the AE. The investigator will review events regularly to ensure they have been captured correctly and to perform assessment of events individually and cumulatively to assess possible safety trends.

8.4.2.3 Investigator Reporting Responsibilities

The principal investigators and/or equally qualified designees will check daily for events that may require expedited reporting.

The principal investigators and/or equally qualified designees will also monitor all accumulating data no less than weekly, or according to superseding NIH or NIAID policy, whichever is more frequent.

Data will be reviewed by the principal investigators/designees on a regular basis for accuracy and completeness.

Data will be submitted to the sponsor in keeping with all applicable agreements and when requested, such as for periodic safety assessments, review of IND annual reports, review of IND safety reports, and preparation of final study reports.

The principal investigators and/or other study designee will ensure prompt reporting to safety oversight bodies (e.g., CSO, DSMB), regulatory entities, and stakeholders as specified below, and according to any additional requirements or agreements.

8.4.2.3.1 Adverse Events

Unless otherwise specified above, AE data will be entered into the research database no less than every other week and will include all data through 1 week prior to database entry.

8.4.2.3.2 Serious Adverse Events (Expedited Reporting)

Unless otherwise specified above, all SAEs (regardless of relationship and whether or not they are also UPs) must be reported to the CSO as specified by the CSO (e.g., Research Electronic Data Capture [REDCap] system; use the Safety Expedited Report Form [SERF]/email if REDCap is not available). If the preferred/indicated mechanism for reporting is not available, the CSO/SMM should be contacted by telephone, fax, or other reasonable mechanism to avoid delays in reporting.

CSO CONTACT INFORMATION:
Clinical Safety Office
5705 Industry Lane
Frederick, MD 21704
Phone: 301-846-5301
Fax: 301-846-6224
Email: rchspsafety@mail.nih.gov
REDcap: https://crimsonredcap.cc.nih.gov/redcap/index.php

Unless otherwise specified above, deaths and immediately life-threatening SAEs must be reported to the CSO promptly, and no later than the **first business day** following the day of study personnel awareness.

All other SAEs must be reported to the CSO no later than the **third business day** following the day of study personnel awareness.

If an individual subject experiences multiple SAEs in a closely timed/overlapping “cause-and-effect” (cascade) sequence, the principal investigators, after careful evaluation, will report ONLY primary/precipitating event(s) individually. SAEs that are determined to be definitely secondary to other SAEs will be detailed in the narrative portion of the report of the relevant primary/precipitating SAE. A clinical rationale and findings to support such reporting should be part of the narrative.

For each SAE report, the research database entry MUST match the corresponding entries on the SAE report (e.g., start and stop dates, event type, relationship, and grade), and **must be updated if necessary** (e.g., if the SAE report was generated after the corresponding AE was entered in the research database).

Unless otherwise specified above, SAEs that have not resolved by the end of the per-protocol follow-up period for the subject are to be followed until final outcome is known (to the degree permitted by the EC-approved informed consent form). If it is not possible to obtain a final outcome for an SAE (e.g., the subject is lost to follow-up), and to update the CSO, the last known status and the reason a final outcome could not be obtained will be recorded by the investigator on an SAE report update and the CRF.

8.4.2.3.3 Unanticipated Problems

Unless otherwise specified above, UPs (as defined in this protocol, or as defined by the EC of record, whichever definition is more conservative) that are also AEs or SAEs, must be reported to the CSO (by REDCap, or by email and SERF if REDCap is not available) no later than when they are due to be reported to the EC.

UPnonAEs are NOT reported to the CSO but must be reported to the Clinical Trials Management (CTM) group according to their requirements and preferred methods. If the UPnonAE raises a significant potential subject safety concern, the SMM should be consulted by email or phone no later than when reports are made to the CTM.

8.4.2.3.4 Pregnancy

Unless otherwise specified above, all pregnancies will be reported (by REDCap, or by email and SERF if REDCap is not available) to the CSO no later than the first business day following the day of study personnel awareness.

Pregnancy outcome data (e.g., delivery outcome, spontaneous or elective termination of the pregnancy) will be reported to the CSO no later than the third business day following the day of study personnel awareness (by REDCap, or by email and SERF if REDCap is not available).

Pregnancy itself is not an AE. Events that meet AE or SAE criteria in relation to pregnancy, delivery, or the conceptus/neonate (see section 8.4.1) are reportable (by REDCap, or by email and SERF if REDCap is not available).

In the event of pregnancy in a study subject exposed to study agent, the following actions will be taken, with the goal of ensuring maternal and fetal well-being, in consultation with the SMM, independent safety monitor (ISM), and DSMB:

- Sample collection will continue per guidelines below:
    - Only proceed with blood draw if subject's hemoglobin value is ≥ 8.0 gm/dL (assessed via finger prick).
    - PK samples (4 mL) will be collected according to the regular study schedule (Day 1, Day 7, Day 14, Day 28, Day 56, Day 84, Day 112, Day 140 and Day 168).

  - The finger prick used to measure hemoglobin at the visits specified above will also be used to collect blood smears for detection of Pf infection by microscopic examination, and dried blood spots for Pf RT-PCR. A venipuncture (up to 0.5 mL) may also be performed if the investigators believe that a finger prick would not provide a sufficient volume of blood to prepare dried blood spots for the Pf RT-PCR assay.
  - Serum storage samples will not be drawn if a subject becomes pregnant.
- Continue to follow for safety for the duration of the pregnancy and for a period of up to 12 months (per investigator discretion) following delivery for assessment of the neonate (see Appendix C).
- Request to unblind the subject, if applicable, AND if doing so would offer a benefit to the subject.
- Report, no later than the first business day after study personnel awareness, to the ISM and DSMB.
- Advise subject to notify the obstetrician of study participation and study agent exposure, providing contact information for the obstetrician to contact the study principal investigator, should this be required, and with the subject's consent.

8.4.2.4 Sponsor's Reporting Responsibilities

Events reported to the sponsor will be promptly evaluated and will be reported as required according to FDA IND safety reporting guidance and regulations. IND safety reports will be sent to other investigators conducting research under the same IND and will be shared with other stakeholders according to applicable agreements.

The sponsor will also submit an IND annual report of the progress of the investigation to the FDA as defined in 21 CFR 312.33.

All UPs will be evaluated by the sponsor, and a summary of the event, and any necessary (corrective/preventative) actions, will be distributed to investigators conducting research under the same IND as may be relevant and appropriate.

8.4.3 Withdrawal Criteria for an Individual Subject

An individual subject will be withdrawn from the study for any of the following:

- An individual subject's decision. (The investigator should attempt to determine the reason for the subject's decision.)
- Non-compliance with study procedures to the extent that it is potentially harmful to the subject or to the integrity of the study data.
- A change in the subject's condition as follows:
  - Loss of the ability to provide informed consent.
  - Withdraws permission to have data stored for future research.
- The investigator determines that continued participation in the study would not be in the best interest of the subject.

8.4.3.1 Re-enrollment and Unplanned Procedure Repetition

Unless otherwise specified within this protocol (e.g., rescreening as described in section 5.5), each person who is a subject in this study may be enrolled and may pass through each step and process outlined in the protocol, only **ONCE** (i.e., subjects may not "go back" and repeat a protocol step already completed).

8.4.3.2 Replacement of Withdrawn Subjects or Subjects Who Discontinue Study Agent

Subjects withdrawn prior to study agent administration will be replaced.

All subjects exposed to study agents MUST be included in the safety dataset.

8.4.4 Additional Safety Oversight

8.4.4.1 Safety Review and Communications Plan

A safety review and communication plan (SRCP) is required for this protocol. The SRCP is an internal communications document between the principal investigators and the CSO, as sponsor representative, which delineates key safety oversight responsibilities of the principal investigators, the CSO, and other stakeholders. The SRCP includes a plan for conducting periodic safety surveillance assessments by the CSO.

8.4.4.2 Sponsor Medical Monitor

A SMM, representing the sponsor, has been appointed for oversight of safety in this clinical study. The SMM will be responsible for performing safety assessments as outlined in the SRCP.

8.4.4.3 Oversight Committees

8.4.4.3.1 Independent Safety Monitor in Mali

The ISM is an expert who does not have direct involvement in the conduct of the study and has no significant conflicts of interest as defined by NIAID policy. An ISM in Mali will review the study prior to initiation and will be available to advise the investigators on study-related medical issues and act as a representative for the welfare of the subjects. The ISM will conduct independent safety monitoring. The ISM is an expert in the field of oversight of clinical trials conducted in Mali and internal medicine, specifically in the population under study in Mali.

All deaths, SAEs, UPs, pregnancies, and FDA IND safety reports will be reported by the principal investigators to the ISM prior to or at the same time they are submitted to the EC or CSO unless otherwise specified herein. The ISM will be notified immediately if any pausing rule is met. The principal investigators will also notify the ISM if intentional or unintentional unblinding occurs. The ISM will have access to blinded data only. If the ISM is unblinded to the study agent given to an individual subject during medical management, the ISM will report that unblinding to the DSMB Executive Secretary.

8.4.4.3.2 Data and Safety Monitoring Board

The NIAID intramural DSMB includes independent experts that do not have direct involvement in the conduct of the study and have no significant conflicts of interest as defined by NIAID policy. The DSMB may include an *ad hoc* member from the host country, as needed. The DSMB will review the study protocol, consent documents, and investigator brochure prior to initiation and twice a year thereafter, or as may be determined by the DSMB.

The DSMB may convene additional reviews as necessary. The DSMB will review the study data as needed to evaluate the safety, efficacy, study progress, and conduct of the study.

All deaths, SAEs, UPs, pregnancies, and IND safety reports will be reported to the DSMB at the same time they are submitted to the EC and CSO unless otherwise specified herein.

All cases of intentional or unintentional unblinding will be reported to the DSMB not later than 1 business day from the time of study personnel awareness.

The principal investigators will notify the DSMB at the time pausing or halting criteria are met and obtain a recommendation concerning continuation, modification, or termination of the study.

8.4.5 Pausing Rules

"Pausing" is discontinuation of study intervention/treatment/dosing (agent/placebo/procedure, etc.) in a protocol-defined group or "arm," until a decision is made to either resume or permanently discontinue such activity. Subjects continue to be followed for safety during a pause.

The pausing criteria for this study include any one or more of the following:

- A subject experiences an SAE that is unexpected (per the investigator's brochure or product label) and possibly, probably, or definitely related to a study agent;
- A subject experiences 2 grade 3 or greater AEs that are unexpected (per the investigator's brochure or product label) and possibly, probably, or definitely related to a study agent.

The principal investigators or the CSO may also pause dosing/study interventions for one or more subjects for any safety issue. The study safety oversight bodies (e.g., DSMB, ISM) may recommend a pause to the CSO.

8.4.5.1 Reporting a Pause

If a pausing criterion is met, a description of the AE(s) or safety issue must be reported by the principal investigators within 1 business day to the CSO and the EC according to their requirements. The principal investigators will also notify the DSMB and ISM. In addition, the CSO or designee will notify all other site investigators by email or through the specified pathway.

8.4.5.2 Resumption Following a Pause

The CSO, in collaboration with the principal investigators and DSMB and ISM, will determine if study activities, including agent administration and/or other study interventions may be resumed, and any additional modifications or requirements that may apply, for the impacted subject(s), or whether the events that triggered the pause require expansion to a study halt (see below).

The CSO or sponsor designee will notify the principal investigators of the decision. The principal investigators will notify the EC of the decision according to the EC's process.

8.4.5.3 Discontinuation of Study Agent

A subject who does not resume study agent/intervention/treatment will continue to be followed for protocol-specified safety assessments or as clinically indicated, whichever is more conservative.

8.4.6 Halting Rules for the Protocol

"Halting" is discontinuation of study intervention/treatment/dosing (agent/placebo/procedure, etc.) for all subjects in a study and suspension of enrollment until a decision is made to either resume or permanently discontinue such activity. Subjects continue to be followed for safety during a halt.

The halting rules are:

- Two or more subjects experience the same or similar grade 3 or greater AEs that are unexpected and possibly, probably, or definitely related to a study agent;

  OR

- Any safety issue that the principal investigators or the CSO determines should halt the study. The study safety oversight bodies (e.g., DSMB) may recommend a halt to the CSO.

In addition, the FDA, Malian Ministry of Health, FMOS EC, or any regulatory body having oversight authority may halt the study at any time. The DSMB or ISM may recommend a study halt.

8.4.6.1 Reporting a Study Halt

If a halting criterion is met, a description of the AE(s) or safety issue must be reported by the principal investigators, within 1 business day to the CSO and the EC according to their requirements. The principal investigators will also notify the DSMB and ISM. In addition, the CSO or designee will notify all other site investigators by email or through the specified pathway.

8.4.6.2 Resumption of a Halted Study

The CSO, in collaboration with the principal investigators and DSMB and ISM will determine if study activities, including enrollment, study agent administration, and/or other study interventions, may be resumed and any additional modifications or requirements that may apply.

The CSO or sponsor designee will notify the principal investigators of the decision. The principal investigators will notify the EC of the decision according to the EC's process.

8.4.6.3 Discontinuation of Study Agent

Subjects who do not resume study agent/study intervention will continue to be followed for protocol-specified safety assessments or as clinically indicated, whichever is more conservative.

8.5 UNANTICIPATED PROBLEMS

8.5.1 Definition of an Unanticipated Problem

The definition of a UP is provided in section 8.4.1.

8.5.2 Unanticipated Problem Reporting

The investigator will report UPs to the FMOS EC according to NIH Human Research Protection Program (HRPP) Policy 801, as described in section 8.6.1.

8.6 ADDITIONAL REPORTING REQUIREMENTS

8.6.1 Reporting to the FMOS EC

Non-compliance and other reportable events will be reported to the FMOS EC according to NIH HRPP Policy 801, which requires reporting as described below.

The following will be reported within 7 calendar days of any investigator or individual associated with the protocol first becoming aware:

- Actual or suspected noncompliance.
- Actual or suspected major deviation.
- Actual or suspected UPs.
- New information that might affect the willingness of a subject to enroll or remain in the study.
- Suspension or termination of research activities, including holds on new enrollment, placed upon the research by the sponsor, NIH or NIAID leadership, or any regulatory agency.

Any death of a research subject that is possibly, probably, or definitely related to the research must be reported within 24 hours of the investigator becoming aware of the death.

Additionally, investigators must provide the following information to the EC in summary format at the time of continuing review, or when otherwise specifically requested by the EC or the Office of Human Subjects Research Protections (OHSRP) Office of Compliance and Training:

- Major and minor protocol deviations.
- Noncompliance reported to the EC that is not related to a protocol deviation.
- AEs and SAEs that do not meet the definition of an UP.

- UPs reported to the EC.

8.6.2 Reporting to the NIAID Clinical Director

The principal investigator will report UPs, major protocol deviations, and deaths to the NIAID clinical director according to institutional timelines.

8.6.3 Reporting Protocol Deviations that Result from the COVID-19 Pandemic

The following addresses the reporting requirements to the FMOS EC with regard to protocol deviations that result from disruption of study visits from the COVID-19 pandemic. These requirements follow the direction of the FMOS EC as well as the NIH reporting requirements. Investigators may modify the protocol without prospective FMOS EC approval when necessary to prevent an immediate apparent harm to a study subject. Typically, when this occurs the event must be reported to the EC via a Reportable Event Form (REF) within 7 days of the deviation. Given the potential need for this to occur on a much larger than usual scale, it is not required that all planned deviations be reported to the FMOS EC in an expedited timeframe. Only those deviations which meet the definition of a major deviation will require reporting, as defined in section 8.4.1.

If a subject cannot complete a protocol-specified study visit or intervention, the principal investigator should assess the impact of the missed visit on the safety of the subject and the scientific validity of the trial. If in the principal investigator's determination neither of these are meaningfully impacted by the deviation, these do not need to be reported to the FMOS EC in an expedited manner. The event should be included in the summary of events reported at the time of continuing review.

If in the opinion of the principal investigator the missed visit or intervention poses a risk to the safety of the subject, the investigator should develop a plan to minimize the impact of the deviation. For example, if the subject is scheduled to return to the study site for safety lab work, the investigator may arrange for labs to be drawn at a location closer to the subject's home. In cases such as this, if the change is necessary to assure the safety of the subject, the investigator may implement the change without prospective FMOS EC approval. If the change meets the definition of a major deviation, it must be reported via a REF within 7 days.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESIS

The hypotheses are that L9LS will be safe and will confer protection against Pf infection. The primary objective is to evaluate the safety, tolerability, and efficacy of L9LS compared to placebo.

Primary Endpoints:

- Incidence and severity of local and systemic AEs occurring within 7 days after the administration of study agent

- Pf blood-stage infection as detected by microscopic examination of thick blood smear for 24 weeks after administration of study agent. The primary efficacy endpoint will be the time to first infection during the 24 week follow-up.

Secondary Endpoints:

- Pf blood-stage infection as detected by RT-PCR for 24 weeks after administration of study agent
- Measurement of L9LS in sera of recipients

9.2 SAMPLE SIZE DETERMINATION

9.2.1 Sample Size Considerations for Safety Evaluation

The ability of the study to identify safety events can be expressed in terms of the probability of observing 1 or more event of interest (e.g., AEs) within each arm. Within a group of 72 participants, there is over a 90% chance to observe at least 1 AE if the true rate is at least 0.032 and over a 90% chance to observe no AE if the true rate is no more than 0.001. Probabilities of observing 0 or more than 1 AE in a group of size 72 and 48, the number of participants in each weight group and the number of female participants in each weight group, respectively, are presented in Table 3 for a range of possible true event rates.

Table 3. Probability (Pr) of events for different safety scenarios within a group (n=72 or 48).

True event rate	n=72		n=48	
	Pr(0)	Pr(>1)	Pr(0)	Pr(>1)
0.005	0.697	0.051	0.786	0.024
0.01	0.485	0.162	0.617	0.083
0.02	0.233	0.423	0.379	0.249
0.035	0.077	0.722	0.181	0.504
0.05	0.025	0.881	0.085	0.699
0.1	0.001	0.995	0.006	0.960
0.15	<0.001	>0.999	<0.001	0.996

9.2.2 Sample Size Considerations for Efficacy Evaluation

The study is designed to evaluate protective efficacy of L9LS at 900 mg by testing the null hypothesis,

H0: protective efficacy = 0%,
versus the alternative hypothesis,
H1: protective efficacy ≠ 0%.

The sample size consideration is primarily based on the power of the comparison of L9LS recipients in each weight group with the placebo recipients. Assuming a dropout rate of 10%, Table 4 presents the power assuming an exponential proportional hazards model with a 2-sided significance level of 0.05 over a range of possible protective efficacy and 24-week infection rates under placebo, where efficacy is 1 minus the ratio of the infection rate under L9LS over the

infection rate under placebo. With 72 L9LS recipients in a weight group and 72 placebo recipients, the trial has over 90% power to claim protective efficacy of L9LS if the underlying efficacy is greater than or equal to 0.6 and the infection rate under placebo is no less than 0.5. When restricted to female participants, with 48 female recipients of L9LS in each weight group, the trial has over 80% power to claim protective efficacy of L9LS if the underlying efficacy is greater than or equal to 0.6 and the infection rate under placebo is no less than 0.5. Of note, the infection rate under placebo in the phase 2 trial of CIS43LS at the same study sites was 78.2%.[9]

Table 4. Power for efficacy evaluation under a 2-sided type I error rate of 0.05 assuming a dropout rate of 10%.

Sample size per arm	Infection rate under placebo	Protective efficacy	Power (%)
72	0.5	0.6	93
	0.5	0.7	98
	0.5	0.8	100
	0.6	0.6	98
	0.6	0.7	100
	0.6	0.8	100
	0.7	0.6	100
	0.7	0.7	100
	0.7	0.8	100
48	0.5	0.6	81
	0.5	0.7	91
	0.5	0.8	96
	0.6	0.6	90
	0.6	0.7	96
	0.6	0.8	99
	0.7	0.6	96
	0.7	0.7	99
	0.7	0.8	100
40	0.5	0.6	73
	0.5	0.7	85
	0.5	0.8	93
	0.6	0.6	84
	0.6	0.7	93
	0.6	0.8	97
	0.7	0.6	92
	0.7	0.7	97
	0.7	0.8	99

Table 5 presents the minimum detectable difference between two arms assuming an exponential proportional hazards model, with 2-sided type I error rate of 0.05 under a range of infection rates in the high infection arm and a 10% dropout rate.

Table 5. Minimum detectable difference in the infection rate between two arms under a 2-sided type I error rate of 0.05 assuming 10% dropout rate.

Sample size within each arm (#)	Infection rate (in high infection arm)	Detectable with 80% power		Detectable with 90% power	
		Difference	Infection rate (in low infection arm)	Difference	Infection rate (in low infection arm)
72	0.2	0.164	0.036	0.185	0.015
	0.3	0.203	0.097	0.231	0.069
	0.4	0.228	0.172	0.262	0.138
	0.5	0.244	0.256	0.282	0.218
48	0.2	0.193	0.007	N/A	N/A
	0.3	0.242	0.058	0.275	0.025
	0.4	0.277	0.123	0.317	0.083
	0.5	0.298	0.202	0.346	0.154

9.3 POPULATIONS FOR ANALYSES

The following datasets will be considered in study analyses:

- Intention-to-treat analysis dataset will include all subjects that receive assignment and will be analyzed according to the initial randomization assignment.
- Modified intention-to-treat (MITT) analysis dataset will include all randomized subjects that receive the study intervention and will be analyzed according to the initial randomization assignment.
- Per-protocol analysis dataset will include all randomized subjects that receive the study intervention consistent with the initial randomization assignment and complete the scheduled visits and will be analyzed according to the initial randomization assignment. In cases where subjects receive an intervention other than the one randomly assigned, an as-treated analysis will additionally be performed according to the actual intervention received.

9.3.1 Evaluable for Toxicity

All subjects will be evaluable for toxicity from the time of their study agent administration.

9.3.2 Evaluable for Objective Response

Not applicable.

9.3.3 Evaluable Non-Target Disease Response

Not applicable.

9.4 STATISTICAL ANALYSES

9.4.1 General Approach

In general, descriptive statistics will be tabulated by treatment arm for endpoints of interest. This will include point estimates (mean, geometric mean, median, or proportions) and their respective 95% confidence intervals. Formal comparisons will use standard methods, contingency tables for categorical variables, t-tests for comparing means if data (or log-transformed data) follow a normal distribution (log transformation or not depends on which method is closer to normality), or nonparametric analogs for comparing medians if otherwise. Unless specified in the subsequent sections, comparisons will be two-sided with type I error rate of 0.05.

Missing data will be considered as "missing completely at random" provided missing data is modest (e.g., <10%). We will examine the "missing completely at random" assumption if missing data is more than 10%. If the assumption does not hold, missing data will be handled under the "missing at random" assumption (that is, missingness depends only on observed variables) via methods such as multiple imputation and inverse propensity weighting. To handle the possibility of "missing not at random," a sensitivity analysis will be performed by imputing missing binary observations with the observed proportion in the opposite arm. A secondary sensitivity analysis will be considered by imputing missing binary observations as failures.

Randomization: Randomization will be 3:1 allocation to L9LS 900 mg and placebo arm stratified by sex (2:1 female to male ratio) and weight (1:1:1 to three weight groups). To limit the number of dropouts before administration of study agent, randomization and administration will occur as close in time as possible.

9.4.2 Analysis of the Primary Endpoints

Analysis for the primary endpoint for safety and tolerability is described in section 9.4.5.

Analysis for the primary efficacy endpoint, protective efficacy with Pf infection determined by blood smear, is described in section 9.4.4.

9.4.3 Analysis of the Secondary Endpoints

Analysis of the secondary efficacy endpoint, with Pf infection determined by RT-PCR, is described in section 9.4.4.

Analyses of the secondary endpoints related to PK and the association of monoclonal antibody concentration with Pf infection risk are described in section 9.4.6.

9.4.4 Efficacy Analyses

Incidence of malaria infection is primarily defined as blood smear–positive Pf infection through 24 weeks after administration, and secondarily as determined by RT-PCR. The efficacy analyses will be MITT.

The primary efficacy analysis will be based on time to the first infection. The survival patterns will be described by Kaplan-Meier curves for each arm. The protective efficacy of the study product will be assessed from the Cox proportional hazards model with study arm as the only regressor and estimated by one minus the hazard ratio. This will be achieved via R package "straweib" that accounts for interval censoring. To address the heterogeneity of the study population, as a sensitivity analysis, a Cox regression with regressors other than the study arm will be additionally performed to account for potential differences among participants. The regressors will include time of enrollment and possibly those covariates that are significantly different between the study product arm and the placebo arm in spite of randomization.

The secondary efficacy analysis will be based on the proportion of subjects who become infected during the 24-week follow-up period. The proportion of subjects who become infected during the 24-week follow-up period will be estimated for each arm and compared across arms based on Kaplan-Meier estimates along with 95% confidence intervals via R package "bpcp." This estimation accounts for right censoring and the comparison should be equivalent to Fisher's exact test in case of no censoring. Though not accounting for interval censoring, the Kaplan-Meier estimates are appropriate as the interest is on infection at 24 weeks only.

Protective efficacy of L9LS will be primarily assessed over all participants by comparing all L9LS recipients with placebo recipients. As a secondary objective, the protective efficacy of L9LS will also be assessed among female participants by comparing female recipients of L9LS with female placebo recipients; and a stratified analysis by weight will also be performed. An exploratory analysis will be conducted to assess how the weight-based dosage, in terms of mg of L9LS per kg of subject body weight, affects the protective efficacy. No multiplicity adjustment will be applied to the secondary and exploratory analyses.

9.4.5 Safety Analyses

Safety analysis will be primarily MITT where individuals who receive assignment but do not receive any product are excluded. Because of blinding and the brief length of time between assignment and administration, such cases will be very few.

Safety data will be presented by line listing and tables at the individual level to provide details on safety events such as severity, duration, and relationship to study product. The number and percentage of subjects with 1 or more AEs will be summarized by dose arm along with the exact 95% confidence intervals of the AE rate. For subjects experiencing more than 1 AE, the subjects will be counted once under the event of highest severity.

In the efficacy study, comparisons between the dose arms and the control arm will be additionally performed in terms of the proportions of solicited AEs, related AEs, and SAEs.

In the rare case of subjects receiving a regimen different from assignment, a per-protocol analysis will be performed as a secondary analysis, which will include subjects according to the product they actually receive in the study.

9.4.6 Pharmacokinetics Analysis

PK analysis will be carried out for subjects with blood samples collected at defined timepoints as listed in section 1.3. The following PK analysis will be performed in each monoclonal antibody (mAb) arm.

Individual Subject PK Analysis: A non-compartmental (NC) PK analysis will be performed on mAb concentration data generated from each subject. Individual subject and dosing arm concentration-versus-time profiles will be constructed in linear and semi-log scales. In the NC analysis, the maximum concentration and time of maximal concentration will be taken directly from the observed data. The area under the concentrations vs. time curve (AUC) will be calculated using the trapezoidal method and determined out to the final concentration collected. If a subject's mAb concentration falls below the quantitative limit (QL) of the assay, the sample with concentration below the QL will be assigned a mAb concentration value of "0" for AUC calculations. In addition to the total AUC, partial AUCs will also be determined over certain time intervals. The time-weighted average concentrations (Cave) during these intervals will be calculated as the AUC divided by the AUC collection interval (e.g., $Cave_{0\text{-}16WK}$ = ($AUC_{0\text{-}16WK}$) / 16 weeks). The terminal slope, λ_z, will be determined by regression of the terminal, log-linear portion of the concentration-versus-time profile. If the final PK sample has measurable mAb concentrations greater than the assay QL, the AUC post-final PK collection ($AUC_{last\text{-}infinity}$) will be estimated as C_{last} / λ_z and $AUC_{0\text{-}infinity}$ will be calculated as the sum of $AUC_{0\text{-}last}$ + $AUC_{last\text{-}infinity}$.

Population PK Analyses: Based on preclinical PK results for mAb and known PK behavior studies of mAbs, the two-compartment model will be used for population PK analysis. The population analysis will estimate compartmental PK parameters such as the clearance (CL), central and peripheral volumes of distribution (Vd1 and Vd2), and intercompartmental clearance (Q). Total volume of distribution at steady-state will be calculated as the sum of Vd1 + Vd2. Alpha and beta half-lives will be calculated from CL, Q, Vd1, and Vd2 using standard equations.[26]

To assess the association of mAb concentration with protection, we will perform a Cox proportional hazards regression for the time to the first infection with mAb concentration as a time-varying covariate. A logistic regression analysis will be additionally performed to model the infection rate as a function of mAb concentration.

9.4.7 Baseline Descriptive Statistics

Treatment arms will be compared for baseline subject characteristics using descriptive statistics. For continuous variables, the mean or median will be calculated for each treatment arm. For categorial variables, the proportion under each category will be calculated for each arm.

9.4.8 Planned Interim Analyses

No interim safety analysis will be performed.

9.4.9 Sub-group Analyses

Above-described analyses will also be performed over the subpopulation of female participants.

9.4.10 Tabulation of Individual Participant Data

Safety data will be presented by line listing and tables at the individual level, as described in section 9.4.5.

9.4.11 Exploratory Analyses

To explore the impact of mAb on the genotype of infection-inducing parasites at the CSP locus, a genotypic sieve analysis will be performed to analyze CSP sequences of breakthrough parasites in the blood samples of infected subjects. The sieve analysis will differentiate protective efficacy against different genotypes of infection-inducing parasites with genotype defined by, for example, number of mismatches to the mAb footprint.

10 REGULATORY AND OPERATIONAL CONSIDERATIONS

10.1 Informed Consent Process

10.1.1 Consent Procedures and Documentation

The informed consent process for this study will involve obtaining initial community permission followed by individual informed consent.

10.1.1.1 Community Permission for the Conduct of the Study

Prior to the start of this study, community permission will be obtained as described in section 5.6. Following the process of Diallo and colleagues,[27] the community permission process will involve the following:

1. Study investigators/personnel explain the study to village leaders, including the village chief, family heads, women association, and elders.
2. The village leaders discuss the study with family heads and community members and relay any additional questions or concerns to the study personnel.
3. The study and the informed consent process are explained in detail to heads of families by study investigators/personnel.

Discussions during the community permission process will address the need for both a husband and wife to agree to avoid pregnancy for the specified period if a wife chooses to volunteer for the study.

10.1.1.2 Individual Informed Consent

The study informed consent form will be written in French. The study team will review the consent form word-for-word and will translate it orally into local languages, since most potential study subjects do not read or speak French. An independent witness who is not a member of the study team will verify that oral translations are accurate and that potential subjects understand the contents of the consent form.

Local households and families will be invited to come to the study clinic for review of the informed consent. At the consenting visit, the subject will read the consent form or have it explained to them (in cases of illiteracy). Individuals in each family will be separately consented, and not all individuals from a household need to participate. Individuals who agree to participate will sign or fingerprint (if illiterate) the consent form.

Also, a study comprehension examination will be conducted to make sure that the study is understood by the potential subjects prior to signing consent. The exam will be written in French and translated orally into local languages. All incorrect responses will be reviewed, and individuals must orally confirm their understanding of all incorrect responses. A score of at least 80% correct responses is mandatory to enroll. For individuals scoring below 80%, study staff may choose to review study details again and reassess comprehension by repeating the examination. At the discretion of the investigator, any individual whose comprehension is questionable, regardless of score, may be excluded from enrollment.

10.1.2 Participation of Subjects who are/become Decisionally Impaired

Adults unable to give consent are excluded from enrolling in the protocol. However, reconsent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because subjects might not benefit from research participation (section 2.3.3), subjects who lose the ability to consent during participation will be withdrawn.

10.2 Study Discontinuation and Closure

The study may be temporarily suspended or permanently terminated as described in the halting rules (section 8.4.6). In addition to the reporting described in that section, the principal investigator(s) will promptly contact the study subjects, provide the reason(s) for the termination or suspension, and, if applicable, inform them of changes to study visit schedule.

The principal investigators will consult with the EC prior to resuming the study following a halt.

10.3 Confidentiality and Privacy

All records will be kept confidential to the extent provided by federal, state, and local law. The study monitors and other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records. Records will be kept locked and data will be coded. Any personally identifiable information maintained for this study will be kept on restricted-access computers and networks. Personally identifiable information will only be shared with individuals authorized to receive it

under this protocol. Individuals not authorized to receive personally identifiable information will be provided with coded information only, as needed. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the FMOS/FAPH EC, FDA, NIAID, Office for Human Research Protections (OHRP), the VRC, or the sponsor's designee.

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

Samples and data will be collected and stored under this protocol. All of the stored study research samples are labeled by a code that only the investigators can link to the subject. Samples are stored in secure research laboratories in locked freezers with limited access at the USTTB, Bamako, and the NIH. Data will be kept in password-protected computers. Only investigators or their designees will have access to the samples and data.

Samples and data acquired under this protocol will be tracked using BSI Systems software. Any loss or unanticipated destruction of samples (for example, due to freezer malfunction) or data (for example, misplacing a printout of data with identifiers) that meets the definition of a reportable event will be reported to the FMOS/FAPH EC.

Additionally, subjects may decide at any point not to have their samples stored. In this case, the principal investigator will destroy all known remaining samples and report what was done to both the subject and to the EC. This decision will not affect the individual's participation in this protocol or any other protocols at NIH.

10.4 Future Use of Stored Specimens and Data

Subjects are consented at enrollment for permission to indefinite storage and future use of specimens and data. Samples, specimens, and data collected under this protocol may be used to study malaria and the immune system. Genetic testing may be performed.

Storage and Tracking: Access to and tracking of stored samples and data will be secured and limited as described above (section 10.3).

Disposition: In the future, other investigators (both at NIH and outside) may wish to use these samples and/or data for research purposes. If the planned research falls within the category of "human subjects research" on the part of the researchers, EC review and approval will be obtained. This includes the researchers sending out coded and linked samples or data and getting results that they can link back to their subjects.

10.5 Safety Oversight

Safety oversight is described in section 8.4.4.

10.6 Clinical Monitoring

According to the ICH E6(R2) GCP guidelines, section 5.18, and FDA 21 CFR 312.50, clinical protocols are required to be adequately monitored by the study sponsor. This study monitoring will be conducted according to the "NIAID Intramural Clinical Monitoring Guidelines." Monitors under contract to the NIAID/OCRPRO will visit the clinical research site to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent documents and documentation of the consent process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points in DFdiscover and prompt reporting of all SAEs; 3) to compare abstracted information entered into DFdiscover with individual subjects' records and source documents (subjects' charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (OHRP, FDA) and applicable guidelines (ICH GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make study documents (e.g., consent forms, DFdiscover abstracts) and pertinent hospital or clinical records readily available for inspection by the local EC, FDA, the site monitors, and the NIAID staff for confirmation of the study data.

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

10.7 Quality Assurance and Quality Control

To help ensure that NIH Office of Research Support and Compliance procedures and GCP are being carried out, a Clinical Trials Management designee within the Office of Clinical Research Policy and Regulatory Operations, Regulatory Compliance and Human Subjects Protection Program will conduct a study initiation visit before study enrollment begins. The purpose of this meeting is to review with the principal investigator and study team designees the roles and responsibilities concerning their commitment to adhere to the requirements of the protocol, especially in terms of NIH OHSRP reporting requirements for reportable events. In addition, the quality management and data management plan for the study will be reviewed.

10.8 Data Handling and Record Keeping

10.8.1 Data Collection and Management Responsibilities

Study data will be maintained in electronic CRFs and collected directly from subjects during study visits and telephone calls or will be abstracted from subjects' medical records. Source

documents include all recordings of observations or notations of clinical activities, including CRFs, and all reports and records necessary to confirm the data abstracted for this study. Data entry into CRFs will be performed by authorized individuals. The investigator is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner. Study data, including cumulative subject accrual numbers, should be generated via the chosen data capture method and submitted to study oversight bodies as needed.

10.8.2 Study Records Retention

The investigator is responsible for retaining all essential documents listed in the ICH GCP guidelines. Study records will be maintained by the principal investigator according to the timelines specified in 21 CFR 312.62 or a minimum of 7 years, and in compliance with institutional, EC, state, and federal medical records retention requirements, whichever is longest. No records will be destroyed without the written consent of the principal investigator and sponsor, as applicable.

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

10.9 Protocol Deviations

The definition of a protocol deviation is provided in section 8.4.1. It is the responsibility of the investigator to use continuous vigilance to identify and report deviations to the FMOS EC according to NIH HRPP Policy 801 (as described in section 8.6.1). All deviations must be addressed in study source documents and reported as specified in the protocol quality management plan and/or monitoring plan. The investigator is responsible for knowing and adhering to the reviewing EC requirements.

10.10 Publication and Data Sharing Policy

10.10.1 Human Data Sharing Plan

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

- NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.
- This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-

reviewed journals. Data from this study may be requested from other researchers indefinitely after the completion of the primary endpoint by contacting Peter Crompton or LIG.

10.10.2 Genomic Data Sharing Plan

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

10.11 COLLABORATIVE AGREEMENTS

10.11.1 Agreement Type

Not applicable.

10.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership will follow policies and procedures for all study group members to disclose and manage all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

11 ABBREVIATIONS

ADA	Anti-drug antibody
AE	Adverse event
ALT	Alanine transaminase
AR	Adverse reaction
AUC	Area under the curve
β-hCG	Beta-human choriogonadotropin
Cave	Time-weighted average concentrations
CBC	Complete blood count
CFA	Communauté Financière Africaine
CFR	Code of Federal Regulations
CHMI	Controlled human malaria infection
CL	Clearance
CONSORT	Consolidated Standards of Reporting Trials
COVID-19	Coronavirus disease 2019
CPT	Cell preparation tube
Cr	Creatinine
CRF	Case report form

CRS	Cytokine release syndrome
CSO	Clinical Safety Office
CSP	Circumsporozoite protein
CTM	Clinical Trials Monitoring
DSMB	Data and safety monitoring board
EC	Ethics committee
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
FMOS	Faculté de Médecine Pharmacie d'Odontostomatologie
GCP	Good clinical practice
GLP	Good laboratory practices
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HPF	High power field
HRPP	Human Research Protection Program
ICH	International Council on Harmonisation
Ig	Immunoglobulin
IND	Investigational new drug
ISM	Independent safety monitor
IV	Intravenous(ly)
LIG	Laboratory of Immunogenetics
MAb	Monoclonal antibody
MITT	Modified intention to treat
MRTC	Malaria Research and Training Center
NC	Non-compartmental
NHP	Non-human primate
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NOAEL	No observed adverse effect level
OCRPRO	Office of Clinical Research Policy and Regulatory Operations
OHRP	Office for Human Research Protections
OHSRP	Office of Human Subjects Research Protections
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
Pf	*Plasmodium falciparum*
PK	Pharmacokinetics
Pr	Probability
Q	Intercompartmental clearance
QL	Quantitative limit
QTc	QT interval, corrected
RBC	Red blood cell
RDT	Rapid diagnostic test

REDCap	Research Electronic Data Capture
REF	Reportable Event Form
rRNA	Ribosomal ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction
SAE	Serious adverse event
SAR	Suspected adverse reaction
SC	Subcutaneous(ly)
SERF	Safety Expedited Report Form
SMM	Sponsor medical monitor
SOA	Schedule of Activities
SOP	Standard operating procedure
SRCP	Safety review and communications plan
SST	Serum-separating tube
SUSAR	Serious and unexpected suspected adverse reaction
TCR	Tissue cross-reactivity
UP	Unanticipated problem
UPnonAE	Unanticipated problem that is not an adverse event
US	United States
USTTB	University of Science, Techniques and Technologies of Bamako
Vd1, Vd2	Volumes of distribution
VRC	Vaccine Research Center
WBC	White blood cell
WHO	World Health Organization

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APPENDIX A: MRTC URINE LABORATORY NORMAL VALUES

Urine Dip/Urinalysis

Urine[1]	Reference Ranges
Protein	None or Trace
Blood (Microscopic) – RBC/HPF	None or Trace < 5

Abbreviations: HPF, high power field; RBC, red blood cell.

[1] The laboratory values provided in the table are based on Bancoumana, Malian adults (age 18-45 years).

APPENDIX B: MALI ADVERSE EVENT GRADING SCALE

Evaluation	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-threatening (Grade 4)
Hematology and Biochemistry Values[1, 2]				
Hemoglobin (Female) – gm/dL	8.0 – 9.0	7.0 – 7.9	6.0 – 6.9	< 6 and/or requiring transfusion
Hemoglobin (Male) – gm/dL	9.5 – 10.3	8.0 – 9.4	6.5 – 7.9	< 6.5 and/or requiring transfusion
WBC Increase – 10^3/µL	11.5 – 15.0	15.1 – 20.0	20.1 – 25.0	> 25.0
WBC Decrease – 10^3/µL	2.5 – 3.3	1.5 – 2.4	1.0 – 1.4	< 1.0 with fever
Neutrophil/Granulocyte Decrease[3] – 10^3/µL	0.80 – 1.00	0.50 – 0.79	< 0.50	< 0.50 with fever
Platelet Decrease – 10^3/µL	100 – 110	70 – 99	25 – 69	< 25
Creatinine (Male) – µmol/L	124.00 – 150.99	151.00 – 176.99	177.00 – 221.00	> 221.00 and requires dialysis
Creatinine (Female) – µmol/L	107.00 – 132.99	133.00 – 159.99	160.00 – 215.99	> 216.00 and requires dialysis
Liver Function Tests/ALT – U/L	75.0 – 150.9	151.0 – 300.9	301.0 – 600.0	> 600.0
Other Values				
Fever[4] – °C	37.5 – 37.9	38.0 – 38.4	38.5 – 39.5	> 39.5

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

[1] The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

[2] The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as potentially life-threatening (grade 4). For example, a low sodium value that falls within a grade 3 parameter should be recorded as a grade 4 hyponatremia event if the subject had a new seizure associated with the low sodium value.

[3] Note: Neutropenias are graded and followed, but based on previous experience in African populations, should be interpreted with caution since lower values are more frequently observed in people of African descent. [28, 29]

[4] Values presented are for non-oral temperature reading (i.e., axillary or no-touch), which is the preferred method at the study site. If oral temperature is taken, the US Food and Drug Administration toxicity grading scale will be used to grade fever.

APPENDIX C: PREGNANCY FOLLOW-UP

Pregnancy Follow-up Rationale

If a woman becomes pregnant during study participation, we will follow her for safety for the duration of the pregnancy and for 12 months following delivery for assessment of the neonate. In addition to the procedures described in section 8.4.2.3.4, we will perform additional research procedures to collect information regarding the delivery outcome and the health of the neonate/infant and mother over the months following delivery. This will allow an initial assessment of PK values after delivery as well as the effects of the investigational agent on pregnancy course and outcome, and infant health.

Pregnancy Follow-up Procedures

Participants who become pregnant during the study will be offered participation in the pregnancy follow-up phase and undergo an informed consent process (see section 10.1). Women who agree to participate will sign the pregnancy follow-up consent and will undergo the following assessments at the time of delivery:

- Hemoglobin measure using HemoCue
- Peripheral malaria test (RDT, blood smear, filter paper)
- Placental malaria using blood smear, filter paper
- Placental malaria histology
- PK blood draw
- AE assessment
- Breast milk to measure L9LS concentrations

After delivery, we will collect a breast milk sample at each of the scheduled infant visits described below.

At the infant follow-up visits during months 6, 9, and 12, 4 mL of blood will also be collected from the mother for detection of malaria and for PK analysis.

The neonate/infant and mother will undergo research assessments according to Table 6.

Table 6. Schedule of assessments for mother and neonate/infant participants.

Study Month	**Month 0/ Delivery**	3	6	9	12	**Illness Visit**	**ET Visit**
Window (days)	+7	±7	±7	±7	±7		
Clinical Procedures/Evaluations							
Heel/finger stick (infant)	X	X	X	X	X	X	X
Cord blood collection	X						
Weight (infant)	X	X	X	X	X	X	X
Ballard assessment (infant)	X						

Study Month		**Month 0/ Delivery**	3	6	9	12	**Illness Visit**	**ET Visit**
Window (days)		+7	±7	±7	±7	±7		
Apgar (infant)		X						
Length (infant)		X	X	X	X	X	X	X
Head circumference (infant)		X	X	X	X	X	X	X
Neurological assessment (infant)			X	X	X	X	X	X
Vaccination/EPI review [a] (infant)			X	X	X	X	X	X
AE assessment (mother and infant)		X	X	X	X	X	X	X
Breast milk sample (collected from mother)		X	X	X	X	X		X
Venous blood (collected from mother)				4 mL	4 mL	4 mL	4 mL	4 mL
Laboratory Evaluations								
Test	Tube	(infant blood volume)						
PK (heel/ finger stick)	SST	50 uL	50 uL	50 uL	50 uL	50 uL		50 uL
PK (cord blood)	SST	X						
Malaria blood smear/RDT [b]	N/A	(X)	(X)	(X)	(X)	(X)	3 to 4 drops	(X)
Hemoglobin	N/A		(X)	(X)	(X)	(X)	(X)	(X)

Abbreviations: AE, adverse event; ET, early termination; EPI, Expanded Programme of Immunization; PK, pharmacokinetics; RDT, rapid diagnostic test.

(X) indicates that no additional blood will be drawn; the test will be performed from blood collected for another evaluation listed.

[a] Record vaccination information from EPI card.

[b] Only performed if enough blood is available.

The month 12 visit will be the final study visit for participants; participation will be complete after this timepoint.

For both women and children, any AEs related to the blood collection procedures and all SAEs will be followed through resolution.

Compensation

Subjects will be compensated 3,000 CFA Franc for each study visit for the time and inconvenience of participation. Mothers and neonates/newborns will each receive compensation for study visits. Payment will be provided in cash after the completion of each visit. The parent/guardian will receive the payments for the neonate/infant participants.

Subjects will be provided with transportation to and from study visits but will not receive additional reimbursement for travel.

Additional Procedures and Processes

Refer to main protocol section 2.3.1, section 8, and section 10 for additional information related to risks, safety definitions and reporting, and human subject protection procedures.