Abbreviated Title: Anti-malaria MAb in Malian children
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Title: Safety and Efficacy of L9LS, a Human Monoclonal Antibody Against *Plasmodium falciparum*, in a Dose-Escalation Trial in Adults and Children and a Randomized, Double-Blind Trial of Children 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 (DCR), NIAID
Manufacturer	Vaccine Research Center (VRC), NIAID

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

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A. *Obtain information by intervening or interacting with living individuals for research purposes*
B. *Obtaining identifiable private information about living individuals*
C. *Obtaining the voluntary informed consent of individuals to be subjects*
D. *Makes decisions about subject eligibility*
E. *Studying, interpreting, or analyzing identifiable private information or data/specimens for research purposes*
F. *Studying, interpreting, or analyzing coded (linked) data or specimens for research purposes*
G. *Some/all research activities performed outside NIH*

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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:

- 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/FAPH Ethics Committee (EC) for review and approval. Approval of both the protocol and the consent forms 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 forms 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 form.

1 PROTOCOL SUMMARY

1.1 Synopsis

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

Study Description: A two-part, phase 2 trial evaluating the safety and tolerability of one-time subcutaneous (SC) or intravenous (IV) administration of monoclonal antibody (MAb) L9LS in healthy Malian adults and one-time SC administration of L9LS in healthy Malian children, as well as its protective efficacy against naturally occurring *Plasmodium falciparum* (Pf) infection over a 7-month malaria season in healthy Malian children 6-10 years of age. The primary study hypotheses are that L9LS will be safe and will produce protection against malaria infection when administered prior to the malaria season. Before study agent administration, all subjects will be given artemether-lumefantrine to clear any preexisting Pf blood-stage infection.

Age de-escalation and dose-escalation study: The first part of the study is an age de-escalation and dose-escalation study for safety and tolerability. Adult subjects (N=18) in the dose-escalation study will be assigned in open-label fashion to 1 of 3 L9LS dose arms (n=6 for each dose level). Dosing will begin in the lowest dose arm (300 mg SC). Once all subjects in that arm reach day 7 post-administration, if no safety concerns have arisen, dosing will begin at the next dose level (600 mg SC). Once all subjects in that arm reach day 7 post-administration, if no safety concerns have arisen, dosing will begin at the highest dose level (20 mg/kg IV). Once all adult subjects reach day 7 post-administration, if no safety concerns have arisen, 18 subjects aged 6-10 years will be randomized 1:1 in double-blind fashion to 150 mg of L9LS SC (n=9) versus placebo SC (n=9). Once all 18 subjects reach day 7 post-administration, if no safety concerns have arisen, an additional 18 subjects aged 6-10 years will be randomized 1:1 to 300 mg of L9LS SC (n=9) versus placebo SC (n=9). Randomization of subjects aged 6-10 years in each L9LS dose arm will be weight-stratified (26-30 kg, n=6; 20-25 kg, n=6; 15-19 kg, n=6) and enrollment will be weight de-escalated starting with

subjects weighing 26-30 kg. Adult subjects will be followed for safety to assess adverse events (AEs) at study visits 1, 3, 7, 14, 21, and 28 days after administration, and once every month thereafter through 28 weeks. Subjects aged 6-10 years will be followed at study visits 1, 3, 7, 14, 21, and 28 days after administration, and once every 2 weeks thereafter through 28 weeks. Primary study assessments include physical examination and blood collection for identification of Pf infection and other research laboratory evaluations. After the last subject in the pediatric 300-mg L9LS dose arm reaches day 7 safety follow-up, an interim safety evaluation will be performed before enrollment begins for the efficacy part of the study. Data from the 36 subjects aged 6-10 years enrolled in the dose-escalation study will be included in a secondary analysis to determine the relationship between L9LS concentration and the risk of Pf infection. For these 36 subjects, blinding will be maintained for treatment assignment (L9LS versus placebo), but not for possible L9LS dose group.

Efficacy study: The second part of the study is a weight-stratified, randomized, double-blind, placebo-controlled trial (N=225 total, n=75 for each of 2 treatment arms, n=75 for placebo arm) to assess safety and protective efficacy of L9LS and placebo administered SC in children 6-10 years of age. In this part of the study, 225 subjects will be randomized 1:1:1 to 150 mg of L9LS (n=75), 300 mg of L9LS (n=75), or placebo (n=75). Randomization of subjects in each arm will be weight-stratified (26-30 kg, n=75; 20-25 kg, n=75; 15-19 kg, n=75). Subjects in the efficacy study will receive the study agent prior to the malaria season 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.

Year 2 extension: Prior to the last study visit of the original protocol described above (January 2023 - February 2023), study participants who remain enrolled in the dose-escalation and efficacy studies will be invited to participate in a 12-month extension study. After the safety and efficacy results are unblinded (approximately March/April 2023), participants who agree to continue with the extension will be grouped into one of 3 arms based on their original study arm assignment:

Arm 1: Up to 84 subjects who received 150 mg of L9LS in year 1 (9 from the dose-escalation study, plus 75 from the efficacy study).

Arm 2: Up to 84 subjects who received 300 mg of L9LS in year 1 (9 from the dose-escalation study, plus 75 from the efficacy study).

Arm 3: Up to 93 subjects who received placebo in year 1 (18 from the dose-escalation study, plus 75 from the efficacy study).

The protocol extension employs a pre-specified, adaptive design based on the time-to-event efficacy of 150 mg and 300 mg of L9LS against *P. falciparum* infection as detected by blood smear observed after the first malaria season in the original protocol. Specifically, if 150 mg and 300 mg of L9LS both show ≥60% efficacy during the first malaria season (based on the upper bound of the two-sided 95% confidence interval [CI]), participants will be re-randomized 1:1 in a double-blind fashion within each arm to receive a single dose of either L9LS (150 or 300 mg depending on study arm) or placebo administered SC before the 2023 malaria season.

The same randomization scheme will be followed if in the first malaria season the 300-mg dose of L9LS shows ≥60% efficacy (based on the upper bound of the two-sided 95% CI) and the 150-mg dose of L9LS shows <60% efficacy (based on the upper bound of the two-sided 95% CI) but the difference between their respective point estimates of efficacy is ≤10%, with the exception that children who received placebo in year 1 will receive 300 mg of L9LS (or placebo) in year 2.

If 300 mg of L9LS shows ≥60% efficacy (based on the upper bound of the two-sided 95% CI) and 150 mg of L9LS shows <60% efficacy (based on the upper bound of the two-sided 95% CI) but the difference between their respective point estimates of efficacy is >10% during the first malaria season, participants will be re-randomized 1:1 in a double-blind fashion within each arm to receive a single dose of either 300 mg of L9LS or placebo administered SC before the 2023 malaria season.

If 150 mg and 300 mg of L9LS both show <60% efficacy (based on the upper bound of the two-sided 95% CI) after the first malaria season, the protocol extension will be abandoned.

Before study agent administration, all subjects will be given artemether-lumefantrine to clear any preexisting Pf blood-stage infection.

Subjects will be followed at study visits 1, 3, 7, 14, 21, and 28 days after administration, and once every 2 weeks thereafter through 28 weeks, with a final visit occurring on study day 252 (36 weeks) to collect a final PK sample. Primary study assessments include medical history, physical examination, and blood collection for pharmacokinetics (PK), anti-drug antibody (ADA) assessments, identification of Pf infection by microscopic examination of thick blood smears and RT-PCR, and other research laboratory evaluations.

Objectives:

Primary Objectives:

1. Dose escalation: To evaluate the safety and tolerability of L9LS administered at 300 mg and 600 mg SC and 20 mg/kg IV in healthy Malian adults, and at 150 mg and 300 mg SC in healthy Malian children.
2. Efficacy: To evaluate the safety and tolerability of L9LS administered at 150 mg and 300 mg SC in healthy Malian children.
3. Efficacy: To determine if SC administration of L9LS at 150 mg and 300 mg (compared to placebo) mediates protection against naturally occurring Pf infection in healthy Malian children during a single malaria season as detected from microscopic examination of thick blood smear.

Secondary Objectives:

Data from both parts of this study will be used to address the following objectives:

1. To evaluate the PK of L9LS throughout the study at dose levels of 300 mg and 600 mg SC and 20 mg/kg IV in healthy Malian adults, and at 150 mg and 300 mg SC in healthy Malian children, and to correlate L9LS serum concentration with Pf infection risk.

2. To determine if SC administration of L9LS at 150 mg and 300 mg (compared to placebo) mediates protection against naturally occurring Pf infection in healthy Malian children during a single malaria season as detected by reverse transcription polymerase chain reaction (RT-PCR).
3. To determine if SC administration of L9LS at 150 mg and 300 mg (compared to placebo) mediates protection against clinical malaria in healthy Malian children during a single malaria season as defined by an illness accompanied by measured axillary fever ≥37.5°C, or history of fever (subjective or objective) in the previous 24 hours, and Pf asexual parasitemia >5,000 parasites/µL as detected from microscopic examination of thick blood smear.
4. To determine if SC administration of L9LS at 150 mg and 300 mg (compared to placebo) mediates protection against clinical malaria in healthy Malian children during a single malaria season as defined by an illness accompanied by any level of Pf asexual parasitemia as detected from microscopic examination of thick blood smear that results in the administration of anti-malarial treatment.
5. To evaluate the PK of L9LS throughout the study at the dose of 150 mg and 300 mg and to correlate L9LS serum concentration with Pf infection risk.
6. To evaluate the PK of L9LS throughout the study at the dose of 150 mg and 300 mg and to correlate L9LS serum concentration with clinical malaria risk.

Extension Phase Primary Objectives:

1. To evaluate the safety and tolerability of a second SC dose of L9LS administered at 150 mg and 300 mg (compared to placebo) in healthy Malian children.
2. To evaluate the PK of L9LS throughout the study at dose levels of 150 mg and 300 mg SC in healthy Malian children.
3. To determine if ADAs to L9LS can be detected in sera of recipients at specific timepoints throughout the study and to correlate the occurrence of ADAs with L9LS PK.

Extension Phase Exploratory Objectives (see Figure 3 for study arm definitions):

1. To assess the efficacy of a second SC dose of L9LS at 150 mg and 300 mg (compared to placebo) in mediating protection against Pf infection and clinical malaria in healthy Malian children during a second malaria season (arms 1a vs. 1b; arms 2a vs. 2b), and to assess if this efficacy is influenced by whether or not a prior dose of L9LS was administered in year 1 (arms 1a vs. 3b; arms 2a vs. 3b).
2. To assess the efficacy of a first SC dose of L9LS at 150 mg or 300 mg (compared to placebo) in mediating protection against Pf infection and clinical malaria in healthy Malian children during the second malaria season (arms 3a vs. 3b).
3. To assess the risk of clinical malaria in children who received L9LS in year 1 but not year 2 (compared to children who received placebo both years; arms 1b vs. 3b; arms 2b vs. 3b).
4. To evaluate the PK of L9LS throughout the study at the dose of 150 mg and 300 mg and to correlate L9LS serum concentration with Pf infection risk.
5. To evaluate the PK of L9LS throughout the study at the dose of 150 mg and 300 mg and to correlate L9LS serum concentration with clinical malaria risk.

Endpoints:

Primary Endpoints:

1. Dose escalation and efficacy: Incidence and severity of local and systemic AEs occurring within 7 days after the administration of L9LS.
2. Efficacy: Pf blood-stage infection as detected by microscopic examination of thick blood smears obtained between 1 week and 24 weeks after administration of L9LS or placebo.

Secondary Endpoints:

Data from both parts of this study will be used to assess the following endpoints:

1. Measurement of L9LS in sera of recipients.
2. Pf blood-stage infection as detected by RT-PCR for 28 weeks after administration of L9LS or placebo for dose escalation subjects or 24 weeks after administration of L9LS or placebo for efficacy subjects.

3. Incidence of clinical malaria (definition 1, see section 2.2.2.3) for 28 weeks after administration of L9LS or placebo for dose escalation subjects or 24 weeks after administration of L9LS or placebo for efficacy subjects.
4. Incidence of clinical malaria (definition 2, see section 2.2.2.3) for 28 weeks after administration of L9LS or placebo for dose escalation subjects or 24 weeks after administration of L9LS or placebo for efficacy subjects.
5. PK analysis of L9LS and the association of L9LS concentration with Pf infection risk.
6. PK analysis of L9LS and the association of L9LS concentration with clinical malaria risk.

Extension Phase Primary Endpoints:

1. Incidence and severity of local and systemic AEs occurring within 7 days after the administration of L9LS.
2. Measurement of L9LS in sera of recipients.
3. Measurement of ADA in sera of recipients.

Extension Phase Exploratory Endpoints:

1. Pf blood-stage infection as detected by microscopic examination of thick blood smears obtained between 1 week and 28 weeks after administration of L9LS or placebo.
2. Pf blood-stage infection as detected by RT-PCR from dried blood spots obtained between 1 week and 28 weeks after administration of L9LS or placebo.
3. Incidence of clinical malaria (definition 1, see section 2.2.2.3) between 1 week and 28 weeks after administration of L9LS or placebo.
4. PK analysis of L9LS and the association of L9LS concentration with Pf infection risk.
5. PK analysis of L9LS and the association of L9LS concentration with clinical malaria risk.

Study Population: Healthy Malian adults (aged ≥18 to 55 years) and children (aged 6 to 10 years) residing in Kalifabougou and Torodo who are not receiving seasonal malaria chemoprevention (SMC).

The year 2 extension phase will include only participants who are currently enrolled in the study and agree to continue in the extension.

Phase:	2
Description of Sites/Facilities Enrolling Participants:	The MRTC clinics in Kalifabougou and Torodo. All screening, day 0 (study agent administration), and day 14 visits will be performed at Kalifabougou only. Both sites will conduct recruitment, enrollment, follow-up visits (other than day 14 as noted above), and unscheduled study visits. For the year 2 extension phase, all day 0 (study agent administration) will be performed at Kalifabougou only. Both sites (Kalifabougou and Torodo) will conduct recruitment, enrollment, follow-up visits, and unscheduled study visits.
Description of Study Intervention:	The study agent will be administered as a one-time SC injection or IV infusion as follows: L9LS at a dose of 150 mg SC (children only), 300 mg SC (children and adults), 600 mg SC (adults only), or 20 mg/kg IV (adults only), or matching placebo (children only). In the year 2 extension phase, the study agent will be administered as a one-time SC injection as follows: L9LS at a dose of 150 mg or 300 mg, or matching placebo.
Study Duration:	36 months.
Participant Duration:	7 months for dose escalation subjects (plus 2 months for those in the pediatric dose-escalation cohort who agree to additional follow-up visits, see Appendix E); 6 months for efficacy subjects. Year 2 extension phase: 12 months from the time of enrollment in the extension.

1.2 Schema

Figure 1a. Adult cohort.

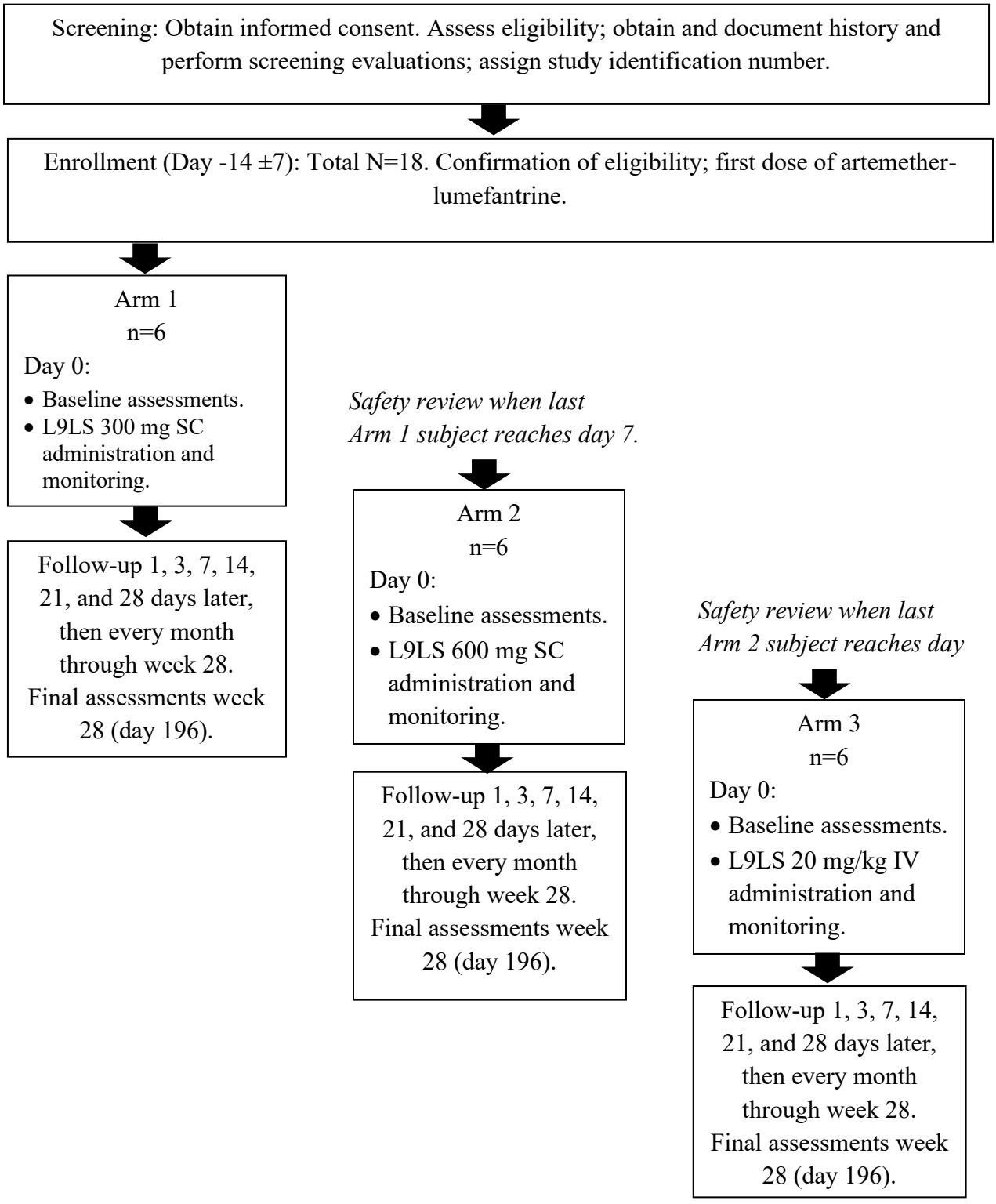
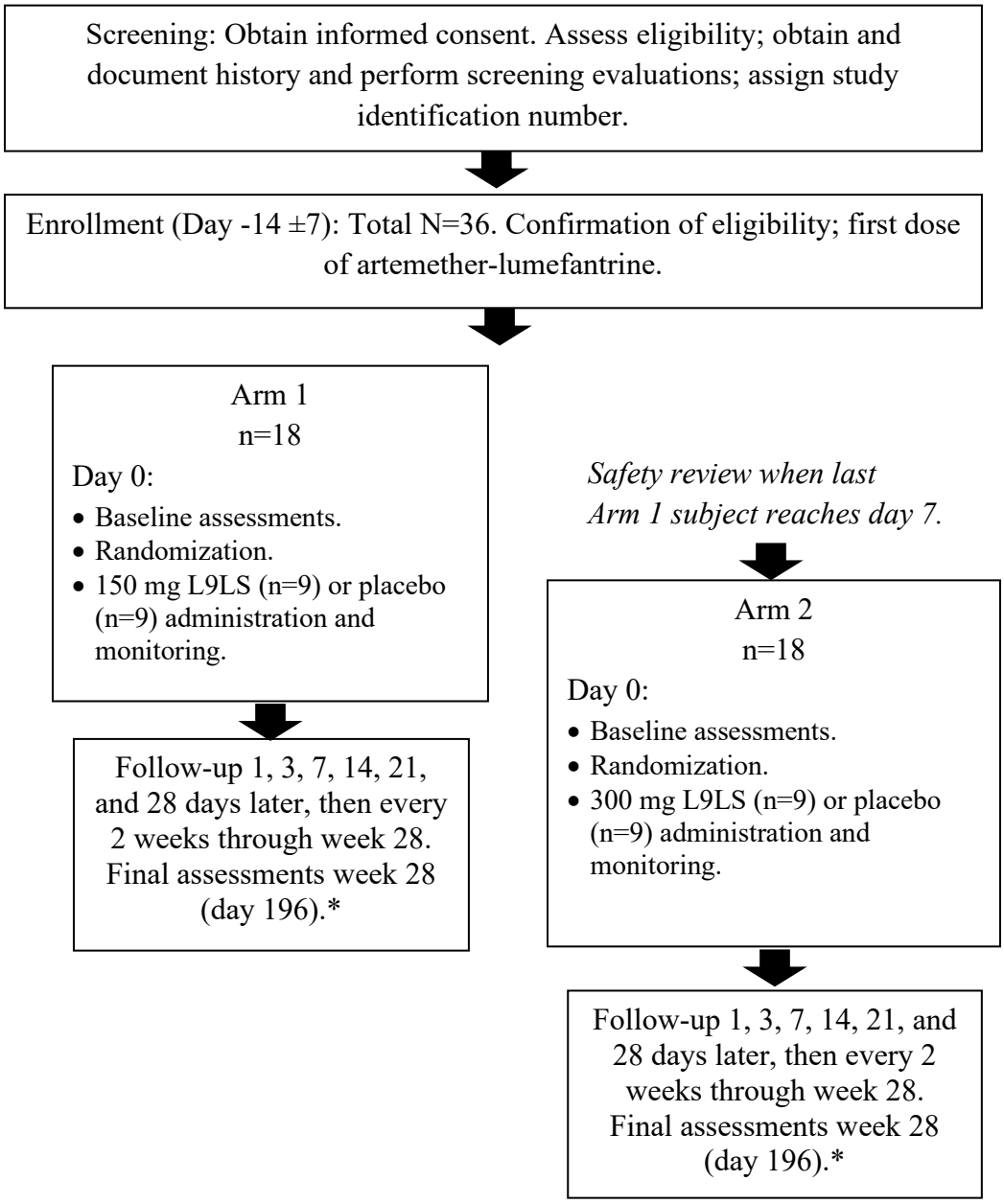


Figure 1b. 6-10 year old cohort.



Interim DSMB safety analysis after last Arm 2 subject reaches day 7, prior to first enrollment in efficacy study.

*Participants offered enrollment in extended follow-up for 2 additional months; see Appendix E.

Figure 1. Dose-escalation study flow diagrams.

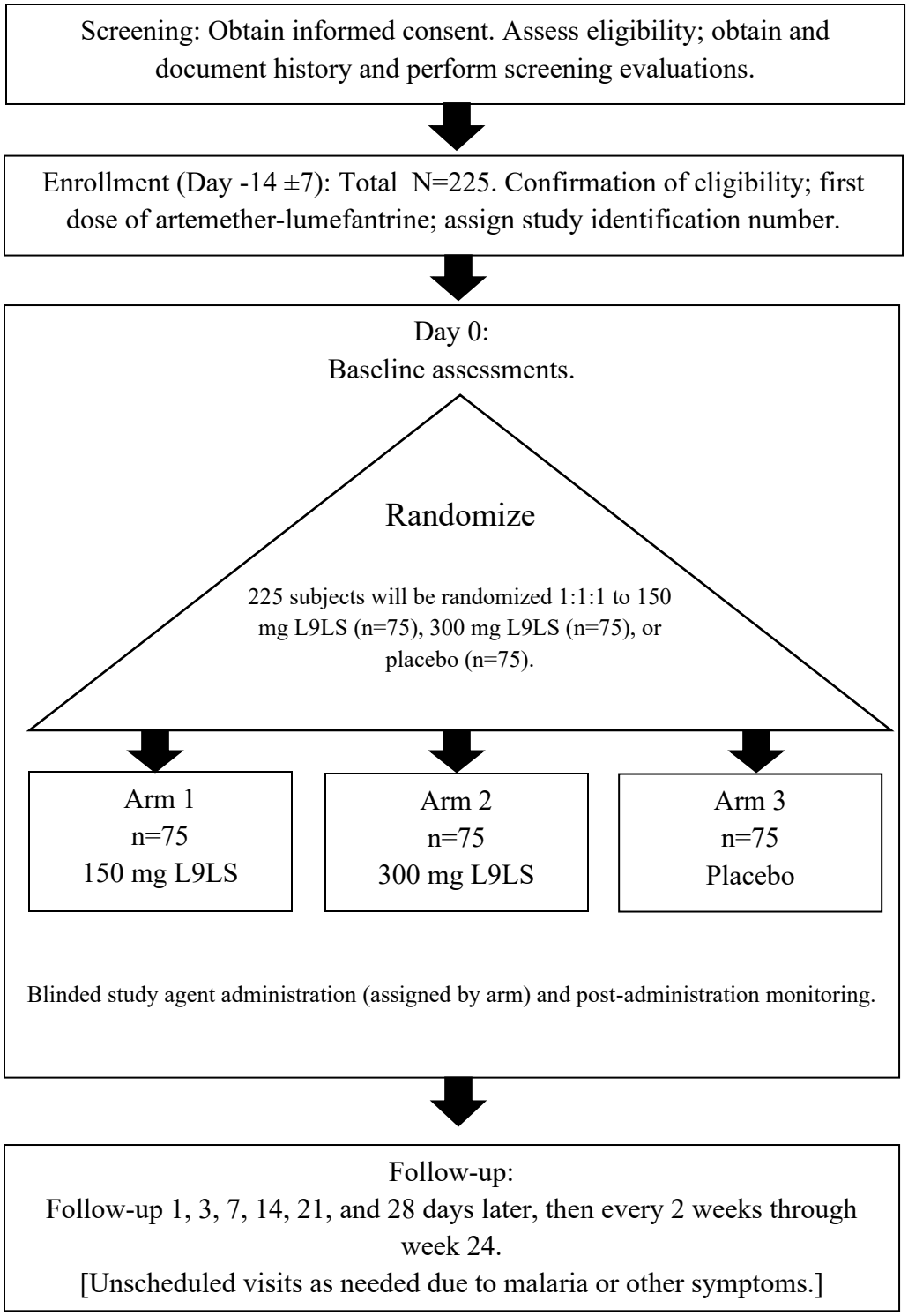


Figure 2. Efficacy study flow diagram.

Figure 3a. Extension study design if 150 mg and 300 mg of L9LS both show ≥60% efficacy by time-to-event analysis after the first malaria season (based on the upper bound of the two-sided 95% CI). Arm 3a or 3b may include 1 additional participant depending on randomization schema and total number that consent to the extension study.

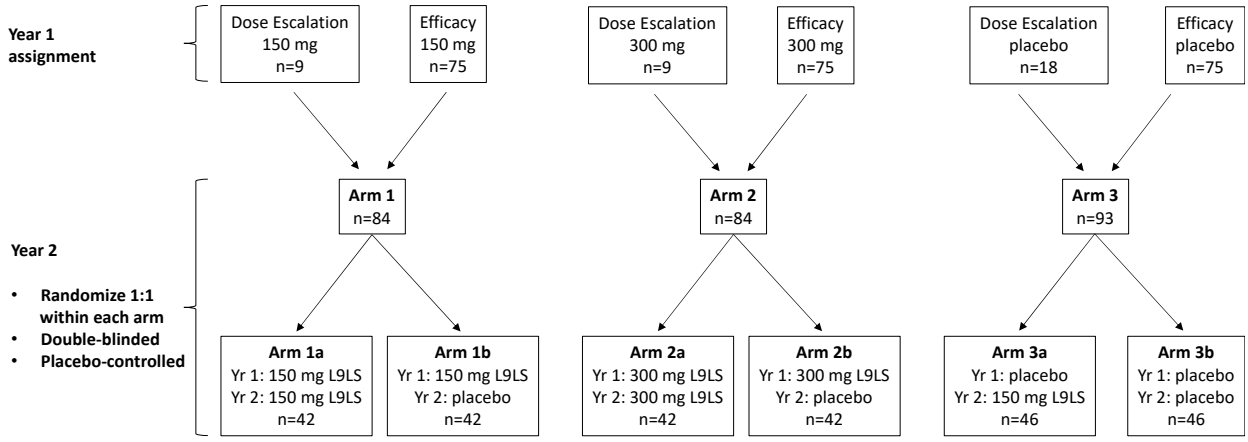


Figure 3b. Extension study design if 300 mg of L9LS shows ≥60% efficacy (based on the upper bound of the two-sided 95% CI) and 150 mg of L9LS shows <60% efficacy (based on the upper bound of the two-sided 95% CI) but the difference between their respective point estimates of efficacy is ≤10%. Arm 3a or 3b may include 1 additional participant depending on randomization schema and total number that consent to the extension study.

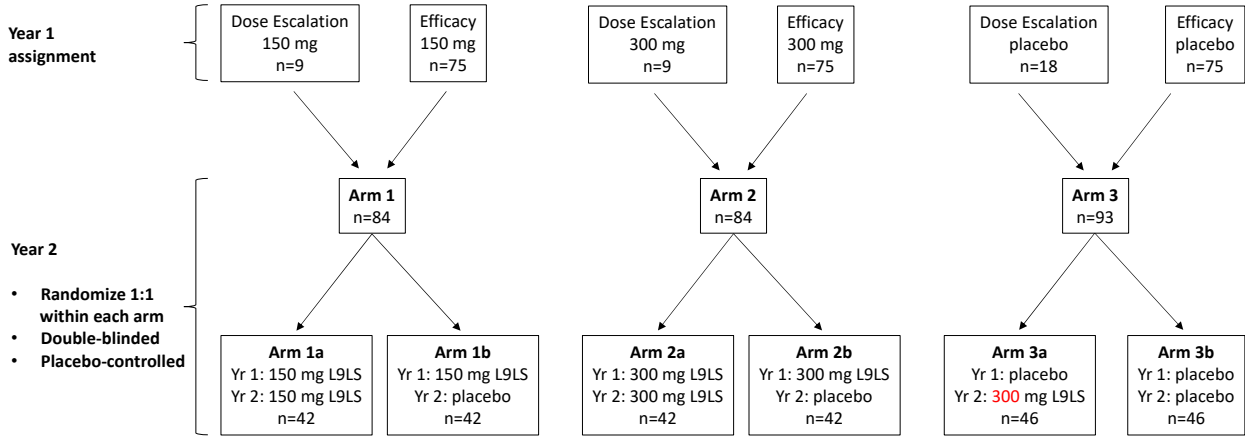
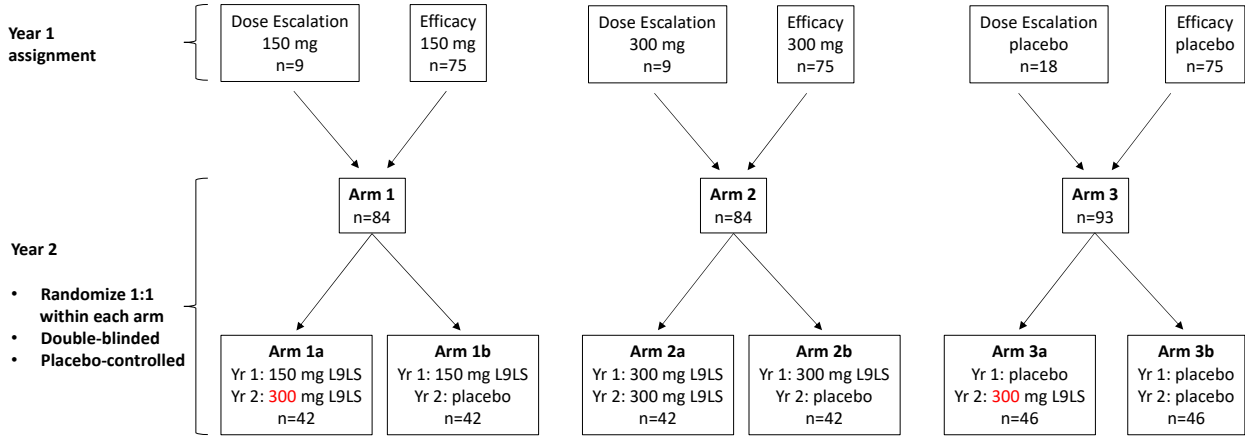


Figure 3c. Extension study design if 300 mg of L9LS shows ≥60% efficacy (based on the upper bound of the two-sided 95% CI) and 150 mg of L9LS shows <60% efficacy (based on the upper bound of the two-sided 95% CI) but the difference between their respective point estimates of efficacy is >10%. Arm 3a or 3b may include 1 additional participant depending on randomization schema and total number that consent to the extension study.



Note: If 150 mg and 300 mg of L9LS both show <60% efficacy (based on the upper bound of their two-sided 95% CIs) by time-to-event analysis after the first malaria season, the protocol extension will be abandoned.

Figure 3. Year 2 extension study design.

1.3 Schedule of Activities (SOA)

1.3.1 Adult cohort dose escalation

Study Day		Screen	Enroll [1]	0	1	3	7	14	21	28	56	84	112	140	168	196	Illness Visit	ET Visit
Window (days)		-56 to -7	-14±7	-	+1	±1	±2	±3	±3	±3	±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
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
Medical history [2]		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
Artemether-lumefantrine administration			X [3]															
ECG		X																
Study agent administration				X [4]														
Adverse events			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
Laboratory Evaluations																		
Test	Tube	(Blood volume in mL)																
Urinalysis	-	X																

Study Day		**Screen**	**Enroll** [1]	**0**	**1**	**3**	**7**	**14**	**21**	**28**	**56**	**84**	**112**	**140**	**168**	**196**	**Illness Visit**	**ET Visit**
Window (days)		-56 to -7	-14±7	-	+1	±1	±2	±3	±3	±3	±7	±7	±7	±7	±7	±7		
Pregnancy test (urine/serum; for women)	-	X	X	X [5]						X	X	X	X	X	X	X		
HIV, HBV, HCV screen [6]	SST	3																
CBC with differential	EDTA	3	3	3 [5]		3	3	3										
Hemoglobin type	EDTA	(X) [7]																
ALT, Cr	SST	3	3	3 [5]		3	3	3										
Blood smear and dried blood spot for Pf RT-PCR [8]	-		X	X				X	0.5	X	X	X	X	X	X	X	X	
PK studies	SST			8 [9]	4		4	4		4	4	4	4	4	4	4		
Serum storage	SST			8			8			8		8			8	8		
ADA	SST			(X)			(X)			(X)		(X)			(X)	(X)		
PBMC storage	CPT			8			8			8						8		
RNA-seq	PaxGene		2.5	2.5 [5]			2.5			2.5		2.5						
Daily volume (mL)		**9**	**8.5**	**32.5**	**4**	**6**	**28.5**	**10**	**0.5**	**22.5**	**4**	**14.5**	**4**	**4**	**12**	**20**		
Cumulative volume (mL)		**9**	**17.5**	**50**	**54**	**60**	**88.5**	**98.5**	**99**	**121.5**	**125.5**	**140**	**144**	**148**	**160**	**180**	**-**	**-**

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; RDT, rapid diagnostic test; RT-PCR, reverse transcription polymerase chain reaction; SST, serum-separating tube.

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

Notes:

- Screening, day 0, and day 7 visits will take place at Kalifabougou for all subjects, regardless of residence. All other visits can take place at either 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 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. The study investigators will provide a fat-containing drink (e.g. milk) to study participants immediately prior to administering artemether-lumefantrine to enhance its absorption. All artemether-lumefantrine doses will be completed prior to day 0.
[4] All other study procedures must be completed prior to study agent administration. Subject will be monitored after each administration, and vital signs will be recorded directly after administration and hourly during post-administration monitoring. The first subject in a dose arm will be monitored for at least 4 hours after administration; all other subjects will be monitored for at least 2 hours 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. Other indicated tests may be performed after 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 will be obtained by venipuncture.
[9] For PK, 4 mL serum will be collected prior to study agent administration, and an additional 4 mL serum will be collected about 1 hour (±30 minutes) after administration.

1.3.2 6-10 year old cohort dose escalation

Study Day	**Screen**	**Enroll [1]**	**0**	**1**	**3**	**7**	**14**	**21**	**28**	**42**	**56**	**70**	**84**	**98**	**112**	**126**	**140**	**154**	**168**	**182**	**196**	**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	±7	±7		
Clinical Procedures/Evaluations																							
Study comprehension exam	X																						
Informed consent	X																						
Confirmation of identity, age, and residency	X																						

Study Day		**Screen**	**Enroll** [1]	**0**	**1**	**3**	**7**	**14**	**21**	**28**	**42**	**56**	**70**	**84**	**98**	**112**	**126**	**140**	**154**	**168**	**182**	**196**	**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	±7	±7		
Physical exam [2]		X	X	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								X			X			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	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	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	X	X
Artemether-lumefantrine administration			X [3]																					
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	X		X
Laboratory Evaluations																								
Test	Tube	(Blood volume in mL)																						
Urinalysis	-	X																						
HIV, HBV, HCV screen [6]	SST	(X)																						
CBC with differential	EDTA	1	1	1 [5]		1	1	1																
Hemoglobin type	EDTA	(X) [7]																						
ALT, Cr	SST	3	3	3 [5]		3	3	3																
Blood smear and dried blood spot for Pf RT-PCR [8]	-		X	X		X	X	X	0.5	X	0.5	X	0.5	X	0.5	X	0.5	X	0.5	X	0.5	X	X	
PK studies	SST		4	4 [9]			4			4		4		4		4		4		4		4		
Serum storage	SST			(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**	**182**	**196**	**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	±7	±7		
ADA	SST			(X)			(X)			(X)				(X)						(X)		(X)		
PBMC storage	CPT			4			4			4												4		
RNA-seq	PaxGene		(X)	(X)[5]			(X)			0.5				0.5										
Daily volume (mL)		**4**	**8**	**12**	**-**	**4**	**12**	**4**	**0.5**	**8.5**	**0.5**	**4**	**0.5**	**4.5**	**0.5**	**4**	**0.5**	**4**	**0.5**	**4**	**0.5**	**8**		
Cumulative volume (mL)		**4**	**12**	**24**	**24**	**28**	**40**	**44**	**44.5**	**53**	**53.5**	**57.5**	**58**	**62.5**	**63**	**67**	**67.5**	**71.5**	**72**	**76**	**76.5**	**84.5**	**-**	**-**

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 a tube collected for another indicated evaluation.

Notes:

- Screening, day 0, and day 14 visits will take place at Kalifabougou for all subjects, regardless of residence. All other visits can take place at either 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 or Torodo, depending on the subject's residence.
- Participants will be offered extended follow-up visits for an additional 2 months after Day 196, see Appendix E.

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. 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. The study investigators will provide a fat-containing drink (e.g. milk) to study participants immediately prior to administering artemether-lumefantrine to enhance its absorption. All artemether-lumefantrine doses will be completed prior to day 0.

[4] All other study procedures must be completed prior to study agent administration. For the dose-escalation study, the first subject in a dose arm will be monitored for at least 4 hours after study agent administration and vital signs will be recorded hourly; all other subjects will be monitored for at least 2 hours after study agent administration and vital signs will be recorded hourly. Prior to discharge, subject 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). 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. Other indicated tests may be performed after study agent 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] The blood smear will be read in real time only if medical history and/or physical exam are suggestive of clinical malaria. If positive for malaria parasite infection, parasite genotyping may be performed.
[9] For PK, 4 mL serum will be collected about 1 hour (±30 minutes) after administration.

1.3.3 6-10 year old cohort efficacy

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								X			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	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]																			
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

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		
Laboratory Evaluations																						
Test	Tube	(Blood volume in mL)																				
Urinalysis	-	X																				
HIV, HBV, HCV screen [6]	SST	(X)																				
CBC with differential	EDTA	1	1	1 [5]		1	1	1														
Hemoglobin type	EDTA	(X) [7]																				
ALT, Cr	SST	3	3	3 [5]		3	3	3														
Blood smear and dried blood spot for Pf RT-PCR [8]	-		X	X		X	X	X	0.5	X	0.5	X	0.5	X	0.5	X	0.5	X	0.5	X	X	
PK studies	SST		4	4 [9]			4			4		4		4		4		4		4		
Serum storage	SST			(X)			(X)			(X)				(X)						(X)		
ADA	SST			(X)			(X)			(X)				(X)						(X)		
PBMC storage	CPT			4			4			4										4		
RNA-seq	PaxGene		(X)	(X)[5]			(X)			0.5				0.5								
Daily volume (mL)		**4**	**8**	**12**	**-**	**4**	**12**	**4**	**0.5**	**8.5**	**0.5**	**4**	**0.5**	**4.5**	**0.5**	**4**	**0.5**	**4**	**0.5**	**4**		
Cumulative volume (mL)		**4**	**12**	**24**	**24**	**28**	**40**	**44**	**44.5**	**53**	**53.5**	**57.5**	**58**	**62.5**	**63**	**67**	**67.5**	**71.5**	**72**	**76**	**-**	**-**

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 a tube collected for another indicated evaluation.

Notes:

- Screening, day 0, and day 14 visits will take place at Kalifabougou for all subjects, regardless of residence. All other visits can take place at either 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 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. 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. The study investigators will provide a fat-containing drink (e.g. milk) to study participants immediately prior to administering artemether-lumefantrine to enhance its absorption. All artemether-lumefantrine doses will be completed prior to day 0.
[4] All other study procedures must be completed prior to study agent administration. For the efficacy study, each subject will be monitored for at least 60 minutes after study agent administration and vital signs will be recorded 1 hour (± 15 min) after study agent administration. Prior to discharge, subject 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). 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. Other indicated tests may be performed after study agent 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] The blood smear will be read in real time only if medical history and/or physical exam are suggestive of clinical malaria. If positive for malaria parasite infection, parasite genotyping may be performed.
[9] For PK, 4 mL serum will be collected about 1 hour (±30 minutes) after administration.

1.3.4 Year 2 Extension

Study Day	Screen	Enroll [1]	0	1	3	7	14	21	28	42	56	70	84	98	112	126	140	154	168	182	196	224	252	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	±7	±7	±7	±7		
Clinical Procedures/Evaluations																									
Study comprehension exam	X																								
Verify informed consent for extension	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	X	X	X	X
Height	X																								
Weight	X	X	X								X			X			X				X	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	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	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	X	X	X	X
Artemether-lumefantrine administration		X [3]																							
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	X	X	X		X
Laboratory Evaluations																									
Test	Tube	(Blood volume in mL)																							
Urinalysis	-	X																							
HIV, HBV, HCV screen [6]	SST	(X)																							

Study Day		**Screen**	**Enroll** [1]	**0**	**1**	**3**	**7**	**14**	**21**	**28**	**42**	**56**	**70**	**84**	**98**	**112**	**126**	**140**	**154**	**168**	**182**	**196**	**224**	**252**	**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	±7	±7	±7	±7		
CBC with differential	EDTA	1	1	1 [5]		1	1	1																		
ALT, Cr	SST	3	3	3 [5]		3	3	3																		
Blood smear and dried blood spot for Pf RT-PCR [7]	-		X	X		X	X	X	0.5	X	0.5	0.5	0.5	X	0.5	0.5	0.5	X	0.5	0.5	0.5	X	0.5	X	0.5	
PK studies	SST		4	4 [8]			4			4				4				4				4		4		
Serum storage	SST			(X)			(X)			(X)				(X)				(X)				(X)		(X)		
ADA	SST			(X)			(X)			(X)				(X)				(X)				(X)		(X)		
PBMC storage	CPT			4			4			4												4		4		
RNA-seq	PaxGene		(X)	(X)[5]			(X)			0.5				0.5												
Daily volume (mL)		**4**	**8**	**12**	**-**	**4**	**12**	**4**	**0.5**	**8.5**	**0.5**	**0.5**	**0.5**	**4.5**	**0.5**	**0.5**	**0.5**	**4**	**0.5**	**0.5**	**0.5**	**8**	**0.5**	**8**	**0.5**	
Cumulative volume (mL)		**4**	**12**	**24**	**24**	**28**	**40**	**44**	**44.5**	**53**	**53.5**	**54**	**54.5**	**59**	**59.5**	**60**	**60.5**	**64.5**	**65**	**65.5**	**66**	**74**	**74.5**	**82.5**	**-**	**-**

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 a tube collected for another indicated evaluation.

Notes:

- All day 0 visits will take place at Kalifabougou for all subjects, regardless of residence. All other visits can take place at either 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 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. 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. The study investigators will provide a fat-containing drink (e.g. milk) to study participants immediately prior to administering artemether-lumefantrine to enhance its absorption. All artemether-lumefantrine doses will be completed prior to day 0.
[4] All other study procedures must be completed prior to study agent administration. The first 30 subjects will be monitored for at least 2 hours (+/- 15 minutes) after study agent administration and vital signs will be recorded for 1 hour (+/- 15 minutes) after study agent administration (based on randomization this will be approximately 5 subjects in each sub-arm). Thereafter, all subjects will be monitored for at least 60 minutes (+/- 15 minutes) after study agent administration and vital signs will be recorded 1 hour (+/- 15 minutes) after study agent administration. Prior to discharge, subject 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). 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. Other indicated tests may be performed after study agent 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] The blood smear will be read in real time only if medical history and/or physical exam are suggestive of clinical malaria. If positive for malaria parasite infection, parasite genotyping may be performed.
[8] For PK, 4 mL serum will be collected about 1 hour (±30 minutes) after administration.

2 INTRODUCTION

2.1 Study Rationale

Malaria is a mosquito-borne protozoan disease belonging to the genus *Plasmodium* that affects approximately 219 million people and kills approximately 435,000 individuals annually, with an enormous economic impact in the developing world, especially sub-Saharan Africa.[1] The 5 recognized species of *Plasmodium* that cause human malaria infection are Pf, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*. Among these, Pf causes more deaths in children worldwide than any other single infectious agent. An estimated 30,000 travelers from North America, Europe, and Japan contract malaria per year. Although malaria is preventable with chemoprophylaxis and completely curable with early intervention, drug treatment is not readily accessible in many parts of the world. Additionally, the use of antimalarial drugs over time has been associated with the emergence of drug-resistant strains. Lack of compliance with preventive drug treatment by individuals travelling to endemic areas may also result in fatal malaria infection. The world's first malaria vaccine, RTS,S/AS01 (Mosquirix™), a recombinant protein-based vaccine targeting Pf, was approved for use by European regulatory authorities in 2015. RTS,S/AS01 is currently being evaluated in an ongoing pilot immunization program in sub-Saharan Africa despite having been found to provide only partial protection against malaria to children and infants.[2,3] Based on results from the ongoing pilot program, the World Health Organization (WHO) recently recommended widespread use of RTS,S/AS01 in a schedule of 4 doses in infants from 5 months of age in sub-Saharan Africa and in other regions with moderate to high Pf malaria transmission.[4] Therefore, the development of safe and effective malaria vaccines or protective antibodies for complete prevention and ultimate elimination of malaria remains an urgent unmet medical need with the potential to have a major impact on improving public health worldwide.

2.1.1 Year 2 Extension

The primary objectives of the protocol extension are to assess the safety and PK of a second dose of L9LS in children exposed to seasonal malaria transmission in Mali. There are several potential clinical use cases for anti-malarial MAbs that may involve repeated administrations of the same MAb to the same individual. For example, the WHO currently recommends repeated, intermittent (e.g., monthly) courses of chemoprevention for high-risk groups such as children under 5 years of age exposed to seasonal malaria, and pregnant women.[5] Although chemoprevention is a critically important tool, its effectiveness may be limited by the challenge of delivering frequent treatment courses,[6,7] and the emergence of drug resistance.[8,9] A single dose of a monoclonal antibody that prevents infection for up to 6 months could be administered before each malaria season for at-risk children and in early pregnancy, complementing chemoprevention and other malaria control measures. Therefore, it is important to assess the safety and PK of repeated dosing of L9LS in target populations such as children exposed to seasonal malaria who could receive annual dosing of an anti-malaria MAb before each malaria season. In addition, the study extension offers the opportunity to explore the efficacy of a second

dose of L9LS (first exploratory objective), to explore the risk of 'rebound' malaria in children who received L9LS in the first year of the study but not in the second year (third exploratory objective), and to explore the relationship between the PK of L9LS and the risk of Pf infection and clinical malaria (fourth and fifth exploratory objectives, respectively).

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. CIS43LS was shown to be safe and conferred complete protection against controlled Pf infection in a phase 1 study in healthy volunteers at the VRC.[10] In a recent phase 2 trial involving healthy adults in Mali, the safety and efficacy of a single IV infusion of CIS43LS at doses of 10 mg/kg or 40 mg/kg were assessed. There were no evident safety concerns. Over the 6-month malaria season, 86 (78.2%) participants in the placebo group, 39 (35.5%) in the CIS43LS 10 mg/kg group, and 20 (18.2%) in the CIS43LS 40 mg/kg group had *P. falciparum* infections detected by blood smear. By time-to-event analysis, compared to placebo at 6 months, CIS43LS efficacy was 88.2% (adjusted 95% CI, 79.3 to 93.3; P<0.0001) for 40 mg/kg, and 75.0% (adjusted 95% CI, 61.0 to 84.0; P<0.0001) for 10 mg/kg (Kayentao et al. *N Engl J Med, In press*; ClinicalTrials.gov Identifier: NCT04329104; FMOS/FAPH Protocol Number: 2020/32/CE/FMOS/FAPH).

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).[11] 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.[11]

Half-life extension of L9 was accomplished by modifying the L9 Fc heavy chain to include a 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 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 (three donors per tissue) and Sprague Dawley rat tissues (two 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 two 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.

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 support 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 controlled human malaria infections (CHMI) in a phase 1 study in healthy adult volunteers at the VRC.[10] 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. As noted above, a phase 2 trial involving healthy adults in Mali demonstrated the safety and protective efficacy of a single IV infusion of CIS43LS at doses of 10 mg/kg or 40 mg/kg. By time-to-event analysis, compared to placebo at 6 months, CIS43LS efficacy was 88.2% (adjusted 95% CI, 79.3 to 93.3; P<0.0001) for 40 mg/kg, and 75.0% (adjusted 95% CI, 61.0 to 84.0; P<0.0001) for 10 mg/kg (Kayentao et al. *N Engl J Med, In press*; ClinicalTrials.gov

Identifier: NCT04329104; FMOS/FAPH Protocol Number: 2020/32/CE/FMOS/FAPH). Together, the phase 1 and 2 trials of CIS43LS provide proof of concept that a MAb with an extended half-life can safely confer protection against Pf infection.

L9LS was recently evaluated in a phase 1, dose-escalation, open-label clinical trial with CHMI to evaluate safety and protective efficacy in healthy, malaria-naive adults at the VRC (ClinicalTrials.gov Identifier: NCT05019729). The trial was a two-part, dose-escalation, adaptive-design study evaluating the safety, tolerability, PK, and protective efficacy of L9LS. Doses evaluated ranged from 1 to 20 mg/kg delivered via IV administration, and 5 mg/kg delivered via SC administration. No safety concerns were identified. A total of 17 L9LS recipients and 6 control participants underwent CHMI. 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, 1 of 5 participants who received 5 mg/kg SC, and all 6 control participants through 21 days after CHMI. Protection conferred by L9LS was seen at serum concentrations as low as 9.2 μg/mL. Thus, in this small phase 1 trial, L9LS administered IV or SC protected recipients against malaria after controlled infection, without evident safety concerns, and all IV and SC administrations were well tolerated.[12]

Unlike CIS43LS, which was administered IV in the Mali phase 2 trial, L9LS can be administered SC in children given its higher potency. As a follow-up to the VRC phase 1 trial of L9LS, this protocol is designed to evaluate the safety and efficacy of L9LS administered SC in healthy children aged 6-10 years in Mali, where Pf malaria infection is endemic. Unlike children aged 1-5 years in Mali, who receive SMC as standard of care, SMC is not currently recommended in children aged 6-10 years in Mali.

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 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 and Clinical Malaria

L9LS-mediated protection against naturally occurring Pf infection during a single 7-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 28 (day 196) after study agent administration just prior to the malaria season for the dose escalation subjects. The efficacy subjects will have samples collected through week 24 (day 168). 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 venipuncture blood sample. The smears will be examined microscopically.

As secondary endpoints, L9LS-mediated protection against clinical malaria during a single 7-month malaria season will be assessed and compared to control subjects from day 7 through week 28 (day 196) after study agent administration for the dose escalation subjects. The efficacy subjects will be assessed through week 24 (day 168). The two definitions of clinical malaria in this protocol are 1) an illness accompanied by measured fever ≥37.5°C, or history of fever (subjective or objective) in the previous 24 hours, and Pf asexual parasitemia >5,000 parasites/µL as detected from microscopic examination of thick blood smear, and 2) an illness accompanied by any level of Pf asexual parasitemia as detected from microscopic examination of thick blood smear that results in the administration of anti-malarial treatment. These definitions of clinical malaria are consistent with definitions used in recent trials of SMC and the RTS,S vaccine conducted in the Sahel region of sub-Saharan Africa.[14,15]

Clinical malaria will be assessed by both passive surveillance (unscheduled sick visits) and active surveillance (medical history, physical exam, and blood smear at all scheduled visits), but the blood smear will be read in real time only if the medical history and/or physical exam are suggestive of clinical malaria. Thick blood smears will be prepared from the blood remaining in

the collection device, or from a venipuncture sample at timepoints when no other blood collection is planned. Giemsa-stained thick blood films will be prepared and examined microscopically by trained personnel following the SOP based on the standard WHO protocol. Thick blood smears will be used for diagnosis throughout the study.

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.

2.2.3 Repeat Dosing: ADA, PK

All MAbs are potentially immunogenic and can result in the formation of ADA, particularly with repeated dosing.[16] An ADA response can result in diminished efficacy by altering the PK properties of the MAb, and can also promote adverse reactions to the MAb, including infusion reactions and local and systemic immune reactions.[16,17] In the case of the widely used tumor necrosis factor (TNF)-alpha inhibitor MAbs, rates of ADA formation are generally higher in patients treated with chimeric anti-TNF constructs compared with fully human MAbs.[18] Formation of ADA to anti-TNF MAbs has also been linked to subtherapeutic serum MAb levels that may occur during intermittent dosing.[19] Moreover, some studies suggest that intramuscular and subcutaneous administration of MAbs is more immunogenic than intravenous administration.[19]

As described above, L9 is a fully human IgG1 MAb that was modified with the Fc region LS mutation (L9LS) to increase neonatal Fc receptor (FcRn) binding and consequent antibody half-life. Other human IgG1 MAbs with LS mutations produced by the VRC that target HIV and Ebola have been tested in over 30 clinical trials, and ADA has not been observed to date, including in individuals who received up to five administrations of the same MAb. For example, VRC01 was found to be safe and well tolerated at doses ranging from 5-40 mg/kg administered IV and at 5 mg/kg SC in clinical trials that involved HIV-infected and non-infected adults.[13,20-22] In these studies, VRC01 retained its expected neutralizing activity in participants' serum and no ADA responses were detected over the course of multiple administrations. ADA responses against the anti-malarial MAbs CIS43LS and L9LS have not yet been assessed in serum samples collected during the phase 1 US trials[10,12] or the ongoing phase 2 trials in Mali.

Regulatory authorities in the US and Europe request premarketing evaluations for immunogenicity (ADA testing) for all novel biologics.[17,23] FDA recommends the following regarding the implementation of ADA testing in clinical trials:

*"FDA recommends that sponsors obtain **pre-treatment samples** from all subjects. Because there is the potential for pre-existing antibodies or confounding components in the matrix, understanding the degree of reactivity before treatment is essential. The sponsor should obtain subsequent samples, with the timing depending on the frequency of dosing. Optimally, samples taken **7 to 14 days after the first exposure** can help elucidate an early IgM response. Samples taken at **3 to 6 weeks after the first exposure** are generally optimal for determining IgG responses. IgA responses may peak earlier than IgG responses, at around 2 to 3 weeks after antigen exposure (Schütz et al. 2013; Macpherson et al. 2008). For individuals receiving a single dose of a therapeutic protein product, these time frames may be adequate. However, for subjects receiving a therapeutic protein product at multiple times during the trial, the sponsor should obtain samples at appropriate intervals throughout the trial and obtain a sample approximately 30 days after the last exposure. For products with long half-lives, samples should be obtained **approximately five half-lives after last exposure**."*

"Samples to determine serum concentrations of the therapeutic protein product should be obtained at the same time as immunogenicity samples. Testing such samples can provide information on whether the therapeutic protein product in the samples is interfering with ADA testing and whether ADA is altering the therapeutic protein product's pharmacokinetics."

In addition to measuring ADA in the pre-MAb administration samples (already collected in the first year of the study), the protocol extension will follow FDA recommendations by collecting serum samples for PK and ADA testing before re-dosing of L9LS/placebo and periodically after re-dosing. The final assessment time point (Day 252) represents approximately 5 half-lives after the last exposure since the estimated half-life of L9LS was 56 days in the phase 1 US trial.

2.2.4 Rebound/Delayed Malaria

Repeated exposures to *P. falciparum* leads to the acquisition of clinical immunity that decreases the incidence and severity of malaria symptoms as children age (see Figure 6 below), although the acquisition of immunity that prevents *P. falciparum* infection altogether appears rare (see Figure 5 below).[24,25] Therefore, when a highly effective malaria prevention measure is introduced into a population for a limited period of time and then withdrawn, there is a risk that in the subsequent period the population which received the intervention may be at greater risk from clinical malaria than if they had not received the intervention. This phenomenon has been commonly termed "rebound" malaria, and more recently has been termed "delayed" malaria when in reference to young children.[26] The risk of rebound malaria will be explored in year 2 of the study by comparing the risk of clinical malaria in children who receive L9LS in year 1 and placebo in year 2 to those who receive placebo in both years.

2.3 Risk/Benefit Assessment

2.3.1 Known Potential Risks

Risks of L9LS: As noted above, a similar malaria antibody, CIS43LS, was evaluated as safe and well tolerated, both in the VRC phase 1 study involving US adults,[10] and in the phase 2 trial involving adults in Mali (Kayentao et al. *N Engl J Med, In press*). Similarly, no significant safety concerns were evident following administration of L9LS in a dose range of 1 mg/kg to 20 mg/kg IV and 5 mg/kg SC in the VRC phase 1 study involving US adults.[12]

In this ongoing phase 2 trial of L9LS in Mali, all participants have been enrolled and there have been no significant safety concerns or serious adverse events (SAEs) to date. Specifically, in the open-label adult dose-escalation arm, 18 participants were enrolled and received L9LS at 300 mg SC (n=6), 600 mg SC (n=6), and 20 mg/kg IV (n=6). In the pediatric dose-escalation arm which remains blinded, 36 participants were enrolled and randomized 1:1 in a double-blind fashion and received L9LS 150 mg SC (n=9) vs. placebo SC (n=9), or L9LS 300 mg SC (n=9) vs. placebo SC (n=9). In the pediatric efficacy study which remains blinded, 225 participants were enrolled and randomized 1:1:1 in a double-blind fashion and received L9LS 150 mg SC (n=75), L9LS 300 mg SC (n=75), or placebo SC (n=75). All IV and SC administrations of study agent were well tolerated. Among adults in the dose-escalation study (N=18), there have been a total of 42 AEs reported in 14 participants (77.8% of participants), all of which were mild to moderate in severity and resolved without sequelae. The most common AEs were upper respiratory illness and headache, accounting for 38% of all AEs. There have been no clear trends or groupings among AEs by dose group in the adult dose-escalation study. Among children in the dose-escalation study (N=36), there have been a total of 73 AEs reported in 30 participants (83.3% of participants). Except for 1 AE (describe in detail below), all AEs were mild to moderate in severity and resolved without sequelae. One AE involved a participant who had a grade 4 white blood cell count (WBC) increase that resolved after several weeks with no intervention. That participant never experienced any symptoms or manifested physical examination findings related to the WBC increase and the etiology remains unknown after detailed clinical, laboratory, and radiographic evaluations. The study investigators remain blinded to this participant's randomization assignment. In the pediatric efficacy study (n=225), which remains blinded, there have been a total of 383 AEs reported for 174 participants (77.3%), all of which have been mild to moderate in severity and resolved without sequelae.

As noted above, 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. Of note, the phase 1 trial of L9LS in healthy adult volunteers at the VRC included prospective monitoring for signs and symptoms that may be indicative of salivary gland hypofunction, and none were noted.[12] In addition, this ongoing phase 2 trial of L9LS in Malian adults and children has included prospective monitoring for signs and symptoms that may be indicative of salivary gland hypofunction, and none have been noted to date.

Risks of MAb Administration: Administration of MAbs may cause immune reactions such as acute anaphylaxis, serum sickness, and the generation of anti-drug antibodies. 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.[27] 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;[27] 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.[27] 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).[28] 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.[29] 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.[27] 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.[30]

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 Drawing and IV insertion: Drawing blood by venipuncture or inserting an IV line may cause pain, bruising, and a feeling of lightheadedness or fainting. Rarely, an infection may develop at the site where blood is taken or the IV line is placed.

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 L9LS 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 6-10 year olds at the end of the dry season (time of anticipated enrollment into the efficacy study) at the Kalifabougou study site ranges from 20 – 60%, as detected by PCR analysis of dried blood spots (Figure 4).[31] 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.[32] 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.[31] 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,[33] although this could vary by Pf transmission setting.

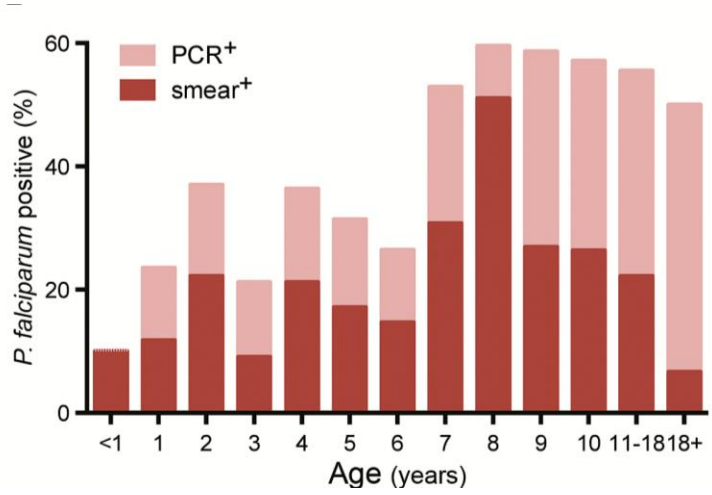


Figure 4. Age-stratified point prevalence of asymptomatic *P. falciparum* infection detected by polymerase chain reaction (PCR) or blood smear at the end of the dry season in Kalifabougou, Mali (ClinicalTrials.gov Identifier: NCT01322581).

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 L9LS efficacy since it directly kills sporozoites independently of the host immune system.[11]

Adverse reactions (ARs) to artemether-lumefantrine occurring in more than 12% of children are pyrexia, cough, vomiting, anorexia, and headache. 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.

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.

A complete list of side effects and contraindications is provided in the package insert.[34]

2.3.2 Known Potential Benefits

In the dose-escalation study and efficacy study, subjects may not receive direct health benefit from study participation. Depending on whether L9LS confers protective efficacy, 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. Dose escalation: To evaluate the safety and tolerability of L9LS administered at 300 mg and 600 mg SC and 20 mg/kg IV in healthy Malian adults, and at 150 mg and 300 mg SC in healthy Malian children. 2. Efficacy: To evaluate the safety and tolerability of L9LS administered at 150 mg and 300 mg SC in healthy Malian children. 3. Efficacy: To determine if SC administration of L9LS at 150 mg and 300 mg (compared to placebo) mediates protection against naturally occurring Pf infection in healthy Malian	1. Dose escalation and efficacy: Incidence and severity of local and systemic AEs occurring within 7 days after the administration of L9LS. 2. Efficacy: Pf blood-stage infection as detected by microscopic examination of thick blood smears obtained between 1 week and 24 weeks after administration of L9LS or placebo.	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.

children during a single malaria season as detected from microscopic examination of thick blood smear.		
Secondary (to be addressed using data from both parts of the study)		
1. To evaluate the PK of L9LS throughout the study at dose levels of 300 mg and 600 mg SC and 20 mg/kg IV in healthy Malian adults, and at 150 mg and 300 mg SC in healthy Malian children, and to correlate L9LS serum concentration with Pf infection risk. 2. To determine if SC administration of L9LS at 150 mg and 300 mg (compared to placebo) mediates protection against naturally occurring Pf infection in healthy Malian children during a single malaria season as detected by RT-PCR. 3. To determine if SC administration of L9LS at 150 mg and 300 mg (compared to placebo) mediates protection against clinical malaria in healthy Malian children during a single malaria season as defined by an illness accompanied by measured axillary fever ≥37.5°C, or history of fever (subjective or objective) in the previous 24 hours, and Pf asexual parasitemia >5,000 parasites/μL as detected from microscopic examination of thick blood smear. 4. To determine if SC administration of L9LS at 150 mg and 300 mg (compared to placebo) mediates protection against clinical malaria in healthy Malian children during a single malaria season as defined	1. Measurement of L9LS in sera of recipients. 2. Pf blood-stage infection as detected by RT-PCR for 28 weeks after administration of L9LS or placebo in the dose escalation study and for 24 weeks after administration of L9LS or placebo in the efficacy study. 3. Incidence of clinical malaria (definition 1, see section 2.2.2.3) for 28 weeks after administration of L9LS or placebo in the dose escalation study and for 24 weeks after administration of L9LS or placebo in the efficacy study. 4. Incidence of clinical malaria (definition 2, see section 2.2.2.3) for 28 weeks after administration of L9LS or placebo in the dose escalation study and for 24 weeks after administration of L9LS or placebo in the efficacy study. 5. PK analysis of L9LS and the association of L9LS concentration with Pf infection risk. 6. PK analysis of L9LS and the association of L9LS concentration with clinical malaria risk.	1. Concentrations of L9LS in blood will help assess durability of L9LS at each dose level. 2. RT-PCR is more sensitive than blood smear for detecting Pf blood-stage infection. 3. 6–10-year-old children in Mali are at high risk for clinical malaria. 4. 6–10-year-old children in Mali are at high risk for clinical malaria. 5. Concentrations of L9LS in blood will help assess durability of L9LS and will allow for correlation with Pf infection risk. 6. Concentrations of L9LS in blood will help assess durability of L9LS and will allow for correlation with clinical malaria risk.

by an illness accompanied by any level of Pf asexual parasitemia as detected from microscopic examination of thick blood smear that results in the administration of anti-malarial treatment. 5. To evaluate the PK of L9LS throughout the study at the dose of 150 mg and 300 mg and to correlate L9LS serum concentration with Pf infection risk. 6. To evaluate the PK of L9LS throughout the study at the dose of 150 mg and 300 mg and to correlate L9LS serum concentration with clinical malaria risk.		
Tertiary/Exploratory		
1. To determine whether ADA to L9LS can be detected in sera of recipients at specific timepoints throughout the study. 2. To assess for IgG1 allotypes and allotype-specific effects on L9LS PK. 3. To explore and characterize the cellular immune response to L9LS. 4. To determine if the efficacy of L9LS is specific to certain Pf parasite genotypes at the CSP locus. 5. To explore the impact of pre-existing parasitemia on the protective efficacy and PK of L9LS. 6. To explore the impact of pre-existing CSP antibodies on the protective efficacy and PK of L9LS.	1. Measurement of ADA to L9LS in sera of recipients. 2. Assessment of IgG1 allotypes and allotype-specific effects on L9LS PK. 3. Characterization of the cellular immune response to L9LS. 4. CSP genotyping of parasites isolated from study subjects. 5. Pre-existing parasitemia detected by microscopic examination of thick blood smears or RT-PCR before L9LS administration. 6. Pre-existing CSP-specific antibodies measured in sera collected before L9LS administration.	1. ADA to L9LS may impact the PK and activity of L9LS. 2. Subject IgG1 allotype may impact the PK and activity of L9LS. 3. L9LS-induced cellular immune responses may be associated with Pf infection risk. 4. L9LS efficacy may be specific to certain Pf parasite genotypes at the CSP locus. 5. Pre-existing parasitemia may impact the protective efficacy and PK of L9LS. 6. Pre-existing CSP-specific antibodies may impact the protective efficacy and PK of L9LS.
Extension Phase Primary		

1. To evaluate the safety and tolerability of a second SC dose of L9LS administered at 150 mg and 300 mg (compared to placebo) in healthy Malian children. 2. To evaluate the PK of L9LS throughout the study at dose levels of 150 mg and 300 mg SC in healthy Malian children. 3. To determine if ADAs to L9LS can be detected in sera of recipients at specific timepoints throughout the study and to correlate the occurrence of ADAs with L9LS PK.	1. Incidence and severity of local and systemic AEs occurring within 7 days after the administration of L9LS. 2. Measurement of L9LS in sera of recipients. 3. Measurement of ADA in sera of recipients.	1. Assessment of AEs is a standard measure of study agent safety and tolerability. 2. Concentrations of L9LS in blood will help assess durability of L9LS at each dose level. 3. ADA to L9LS may impact the PK and activity of L9LS.
Extension Phase Exploratory (see Figure 3 for study arm definitions)		
1. To assess the efficacy of a second SC dose of L9LS at 150 mg and 300 mg (compared to placebo) in mediating protection against Pf infection and clinical malaria in healthy Malian children during a second malaria season (arms 1a vs. 1b; arms 2a vs. 2b), and to assess if this efficacy is influenced by whether or not a prior dose of L9LS was administered in year 1 (arms 1a vs. 3b; arms 2a vs. 3b). 2. To assess the efficacy of a first SC dose of L9LS at 150 mg or 300 mg (compared to placebo) in mediating protection against Pf infection and clinical malaria in healthy Malian children during the second malaria season (arms 3a vs. 3b). 3. To assess the risk of clinical malaria in children who received L9LS in year 1 but not year 2 (compared to children who received placebo both years; arms 1b vs. 3b; arms 2b vs. 3b).	1. Pf blood-stage infection as detected by microscopic examination of thick blood smears obtained between 1 week and 28 weeks after administration of L9LS or placebo. 2. Pf blood-stage infection as detected by RT-PCR from dried blood spots obtained between 1 week and 28 weeks after administration of L9LS or placebo. 3. Incidence of clinical malaria (definition 1, see section 2.2.2.3) between 1 week and 28 weeks after administration of L9LS or placebo. 4. PK analysis of L9LS and the association of L9LS concentration with Pf infection risk. 5. PK analysis of L9LS and the association of L9LS concentration with clinical malaria risk.	1. Blood smear is the gold standard for diagnosis of blood-stage Pf infection. 2. RT-PCR is more sensitive than blood smear for detecting Pf blood-stage infection. 3. 6–10-year-old children in Mali are at high risk for clinical malaria. 4. Concentrations of L9LS in blood will help assess durability of L9LS and will allow for correlation with Pf infection risk. 5. Concentrations of L9LS in blood will help assess durability of L9LS and will allow for correlation with clinical malaria risk.

4. To evaluate the PK of L9LS throughout the study at the dose of 150 mg and 300 mg and to correlate L9LS serum concentration with Pf infection risk. 5. To evaluate the PK of L9LS throughout the study at the dose of 150 mg and 300 mg and to correlate L9LS serum concentration with clinical malaria risk.		

4 STUDY DESIGN

4.1 Overall Design

This is a two-part, phase 2 trial evaluating the safety and tolerability of one-time SC or IV administration of L9LS in healthy Malian adults and one-time SC administration of L9LS in healthy Malian children, as well as its protective efficacy against naturally occurring Pf infection over a 7-month malaria season in healthy Malian children 6-10 years of age. The primary study hypotheses are that L9LS administered prior to the malaria season will be safe and will produce protection against malaria infection. Before study agent administration, all subjects will be given artemether-lumefantrine to clear any preexisting Pf blood-stage infection. Interim safety data from the ongoing VRC phase 1 trial of L9LS will be regularly reviewed, and if needed, the protocol and relevant study documents will be amended to reflect any new information.

The study will recruit from 2 MRTC clinics, 1 in Torodo and 1 in Kalifabougou. All of the screening, day 0, and day 14 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. The economy is based on subsistence farming. Kalifabougou and Torodo are similar in terms of geographic, demographic, and epidemiological characteristics, and both typically experience intense seasonal Pf transmission from June through December each year.[25] Based on data collected in Kalifabougou, approximately 85% of children aged 6-10 years who are uninfected (PCR negative) before the malaria season become infected with Pf during the ensuing malaria season (Figure 5).[25]

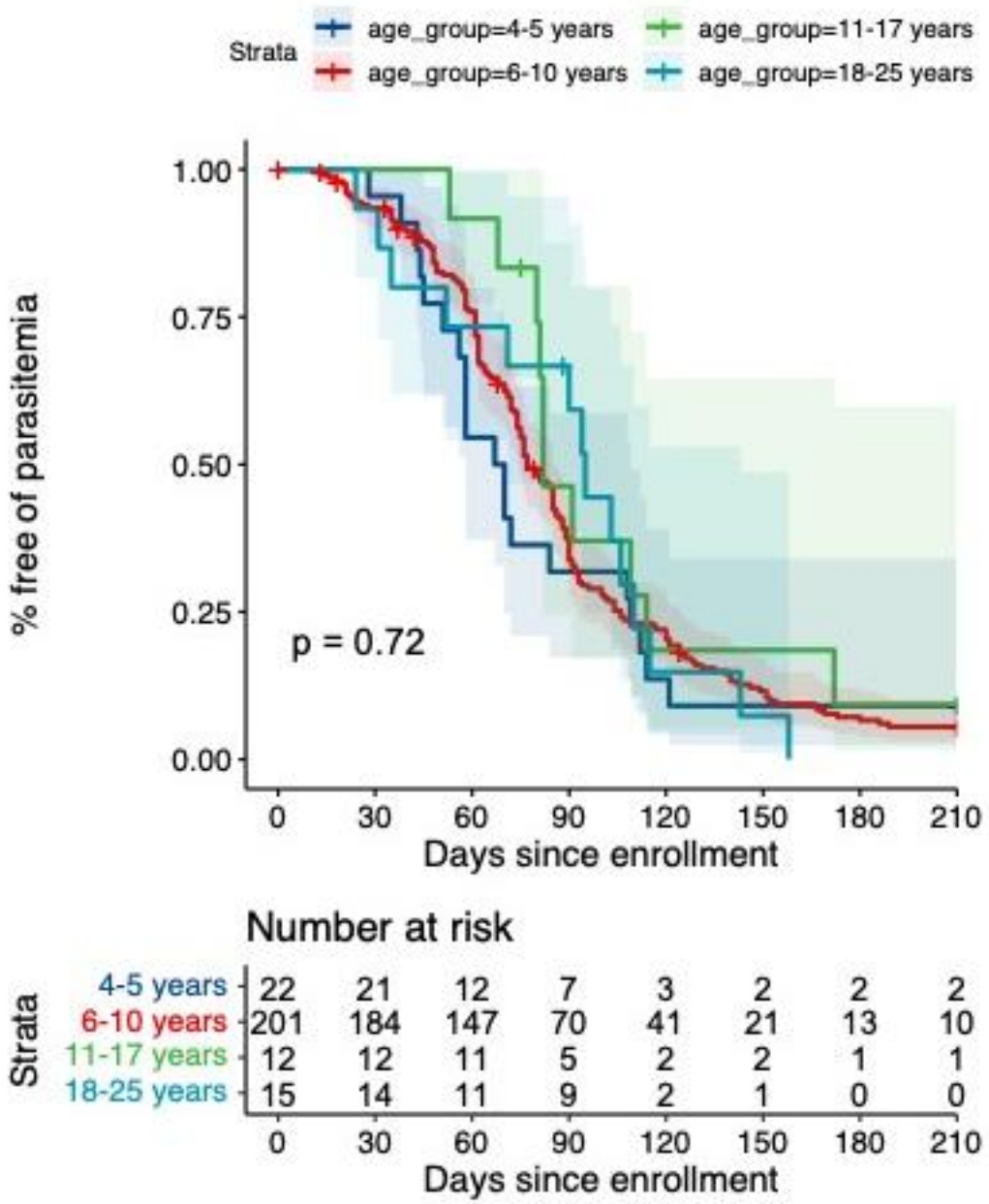


Figure 5. Kaplan-Meier plots, stratified by age for time to first *Pf* infection by PCR during the malaria season in Kalifabougou, Mali (ClinicalTrials.gov Identifier: NCT01322581).

The first part of the study is an age de-escalation and dose-escalation study for safety and tolerability. Adult subjects (N=18) in the dose-escalation study will be assigned in open-label fashion to 1 of 3 L9LS dose arms (n=6 for each dose level). Dosing will begin in the lowest dose arm (300 mg SC). Once all subjects in that arm reach day 7 post-administration, if no safety concerns have arisen, dosing will begin at the next dose level (600 mg SC). Once all subjects in that arm reach day 7 post-administration, if no safety concerns have arisen, dosing will begin at the highest dose level (20 mg/kg IV). Once all adult subjects reach day 7 post-administration, if

no safety concerns have arisen, 18 subjects aged 6-10 years will be randomized 1:1 in double-blind fashion to 150 mg of L9LS versus placebo administered SC. Once all 18 subjects reach day 7 post-administration, if no safety concerns have arisen, an additional 18 subjects aged 6-10 years will be randomized 1:1 to 300 mg of L9LS versus placebo administered SC. Randomization of subjects aged 6-10 years in each L9LS dose arm will be weight-stratified (26-30 kg, n=6; 20-25 kg, n=6; 15-19 kg, n=6) and enrollment will be weight de-escalated starting with subjects weighing 26-30 kg. All subjects will be followed for safety to assess AEs at study visits over 28 weeks. Primary study assessments include medical history, physical examination and blood collection for identification of Pf infection and other research laboratory evaluations. After the last 6-year-old subject in the 300-mg L9LS arm reaches day 7 safety follow-up, an interim safety evaluation will be performed before enrollment begins for the efficacy part of the study. Data from the 36 subjects aged 6-10 years enrolled in the dose-escalation study will be included in a secondary analysis to determine the relationship between L9LS concentrations and the risk of Pf infection. For these 36 subjects, blinding will be maintained for treatment assignment (L9LS versus placebo), but not for possible L9LS dose group.

The second part of the study is a weight-stratified, randomized, double-blind, placebo-controlled trial to assess safety and protective efficacy of L9LS and placebo in children 6-10 years of age. In this part of the study, 225 subjects will be randomized 1:1:1 to SC administration of 150 mg of L9LS (n=75), 300 mg of L9LS (n=75), or placebo (n=75). Randomization of subjects in each arm will be weight-stratified (26-30 kg, n=75; 20-25 kg, n=75; 15-19 kg, n=75). Subjects in the efficacy study will receive the study agent and be followed at study visits over 24 weeks. Primary study assessments include medical history, physical examination, and blood collection for identification of Pf infection and other research laboratory evaluations.

4.1.1 Year 2 Extension

In the extension phase, all participants who are currently enrolled in the dose escalation or efficacy study will be invited to extend their participation for approximately 1 year from the time of re-consenting. After the results of the first year of the study are unblinded (approximately March 2023), children will be re-randomized 1:1 in a double-blind fashion within their original study arm assignment to receive either L9LS or placebo administered SC before the 2023 malaria season (Figure 3).

The protocol extension employs a pre-specified, adaptive design based on the time-to-event efficacy of 150 mg and 300 mg of L9LS observed after the first malaria season in the original protocol. Specifically, if 150 mg and 300 mg of L9LS both show ≥60% time-to-event efficacy against *P. falciparum* infection as detected by blood smear during the first malaria season (based on the upper bound of the two-sided 95% CI), children who received 150 mg of L9LS in year one will be randomized 1:1 in year 2 to receive either a second 150-mg dose of L9LS (arm 1a) or placebo (arm 1b); children who received 300 mg of L9LS in year one will be randomized in year 2 to receive either a second 300-mg dose of L9LS (arm 2a) or placebo (arm 2b); and children

who received placebo in year 1 will be randomized in year 2 to receive either a first 150-mg dose of L9LS (arm 3a) or placebo (arm 3b) (Figure 3a).

If the 300-mg dose of L9LS shows ≥60% efficacy (based on the upper bound of the two-sided 95% CI) and the 150-mg dose of L9LS shows <60% efficacy (based on the upper bound of the two-sided 95% CI) but the difference between their respective point estimates of efficacy is ≤10%, children who received 150 mg of L9LS in year one will be randomized 1:1 in year 2 to receive either a second 150-mg dose of L9LS (arm 1a) or placebo (arm 1b); children who received 300 mg of L9LS in year one will be randomized in year 2 to receive either a second 300-mg dose of L9LS (arm 2a) or placebo (arm 2b); and children who received placebo in year 1 will be randomized in year 2 to receive either a first 300-mg dose of L9LS (arm 3a) or placebo (arm 3b) (Figure 3b). In the unlikely event that the 150-mg dose of L9LS shows ≥60% efficacy (based on the upper bound of the two-sided 95% CI) and the 300-mg dose of L9LS shows <60% efficacy (based on the upper bound of the two-sided 95% CI) but the difference between their respective point estimates of efficacy is ≤10%, the same design (Figure 3b) will be employed with the following exception: children who received placebo in year 1 will be randomized in year 2 to receive either a first 150-mg dose of L9LS (arm 3a) or placebo (arm 3b).

If the 300-mg dose of L9LS shows ≥60% efficacy (based on the upper bound of the two-sided 95% CI) and the 150-mg dose of L9LS shows <60% efficacy (based on the upper bound of the two-sided 95% CI) but the difference between their respective point estimates of efficacy is >10%, the 150 mg dose will be abandoned and children who received 150 mg of L9LS in year one will be randomized 1:1 in year 2 to receive either a 300-mg dose of L9LS (arm 1a) or placebo (arm 1b); children who received 300 mg of L9LS in year one will be randomized in year 2 to receive either a second 300-mg dose of L9LS (arm 2a) or placebo (arm 2b); and children who received placebo in year 1 will be randomized in year 2 to receive either a first 300-mg dose of L9LS (arm 3a) or placebo (arm 3b) (Figure 3c).

If 150 mg and 300 mg of L9LS both show <60% efficacy (based on the upper bound of the two-sided 95% CI) by time-to-event analysis after the first malaria season, the protocol extension will be abandoned.

Before study agent administration, all subjects will be given artemether-lumefantrine to clear any preexisting Pf blood-stage infection.

Subjects will be followed at study visits 1, 3, 7, 14, 21, and 28 days after administration, and once every 2 weeks thereafter through 28 weeks, with a final visit occurring on study day 252 (36 weeks) to collect a final PK sample. Primary study assessments include medical history, physical examination and blood collection for PK, ADA assessments, identification of Pf infection by microscopic examination of thick blood smears and RT-PCR, and other research laboratory evaluations.

If 150 mg and 300 mg of L9LS both show ≥60% efficacy during the first malaria season in the original protocol, the schema depicted in Figure 3a will be followed, and safety outcomes will be evaluated within arms to assess the safety of a second 150-mg dose of L9LS vs. placebo (1a vs. 1b); the safety of a second 300-mg dose of L9LS vs. placebo (2a vs. 2b), and the safety of a first 150-mg dose of L9LS vs. placebo (3a vs. 3b). In addition, safety outcomes will be evaluated between arms to assess the safety of a second 150-mg dose of L9LS vs. a first 150-mg dose of L9LS (1a vs. 3a). Finally, safety outcomes of L9LS vs. placebo will be evaluated in aggregate (1a + 2a + 3a vs. 1b + 2b + 3b).

PK of L9LS will be evaluated throughout the study at dose levels of 150 mg and 300 mg SC. The presence of ADA to L9LS will be assessed in sera of recipients at specific timepoints throughout the study and will be correlated with L9LS PK.

In exploratory analysis, L9LS serum concentration will be correlated with the risk of Pf infection (assessed by blood smear and RT-PCR) and clinical malaria. The protective efficacy of L9LS against *P. falciparum* infection (by blood smear and RT-PCR) and clinical malaria will be assessed by comparing the following arms:

1. Children who receive a second 150 mg dose of L9LS versus children who receive placebo (arms 1a vs. 1b).
2. Children who receive a second 300 mg dose of L9LS versus children who receive placebo (arm 2a vs. 2b).
3. Children who receive a second 150 mg dose of L9LS versus children who received placebo in years 1 and 2 (arms 1a vs. 3b).
4. Children who receive a second 300 mg dose of L9LS versus children who received placebo in years 1 and 2 (arms 2a vs. 3b).
5. Children who receive a first 150 mg dose of L9LS versus children who received placebo in years 1 and 2 (arms 3a vs. 3b).

The risk of ‘rebound’ malaria will be assessed in year two of the study by comparing the risk of clinical malaria in the following arms:

1. Children who received a 150 mg dose of L9LS in year 1 and placebo in year 2 versus children who received placebo in years 1 and 2 (arms 1b vs. 3b).
2. Children who received a 300 mg dose of L9LS in year 1 and placebo in year 2 versus children who received placebo in years 1 and 2 (arms 2b vs. 3b)

If 300 mg of L9LS shows ≥60% efficacy (based on the upper bound of the two-sided 95% CI) and 150 mg of L9LS shows <60% efficacy (based on the upper bound of the two-sided 95% CI) but the difference between their respective point estimates of efficacy is ≤10% during the first malaria season, the schema depicted in Figure 3b will be followed, and safety, PK, and efficacy

outcomes will be conducted as described above, with the exception of Arm 3a that will evaluate children who received placebo in year 1 followed by 300 mg of L9LS in year 2.

If 300 mg of L9LS shows ≥60% efficacy (based on the upper bound of the two-sided 95% CI) and 150 mg of L9LS shows <60% efficacy (based on the upper bound of the two-sided 95% CI) but the difference between their respective point estimates of efficacy is >10% during the first malaria season, the schema depicted in Figure 3c will be followed, and safety, PK, and efficacy outcomes will be conducted as described above, with the exception of Arm 1a that will evaluate children who received 150 mg of L9LS in year 1 followed by 300 mg of L9LS in year 2.

4.2 Scientific Rationale for Study Design

This two-part 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, with initial safety data collected from adults prior to proceeding with the enrollment of children.

Both parts of the study will use randomization and a double-blind design in the cohorts of children 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.

4.2.1 Year 2 Extension

The protocol extension was designed to evaluate the safety and PK of a second dose of L9LS in the setting of naturally occurring Pf infection and in a population that could potentially benefit from a novel therapeutic for malaria prevention.

As with the original protocol, the protocol extension will use randomization and a double-blind design in the cohorts of children 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 doses used in this study were selected based on preclinical data and data generated in the ongoing phase 1 VRC study, which tested the dose range included in this protocol. This study will test fixed doses of L9LS in children in a defined range of subject weights to approximate the weight-based dosing used in the phase 1 VRC study. Compared to weight-based dosing, fixed dosing will provide a greater range of antibody concentrations in vivo for determining a protective L9LS titer. Fixed dosing is also simpler to administer, reduces the risk of medication errors, and is generally more cost effective—factors that are relevant to the potential use of L9LS in malaria-endemic regions.

In the safety study, subjects aged ≥18 years weighing 60 kg or less will receive a SC dose of 300 mg (2 mL) or 600 mg (4 mL), approximating the weight-based dosing of 5 mg/kg and 10 mg/kg, respectively, in a 60-kg adult. An additional group of subjects aged ≥18 years weighing 60 kg or less in the safety study will receive an IV dose of 20 mg/kg. This dose will be administered IV rather than SC because 4 mL is the maximum volume of L9LS that will be administered SC in this study. In Kalifabougou, the average weight of adult females and males is 55.6 kg and 61.7 kg, respectively. In the safety and efficacy studies, subjects aged 6-10 years weighing ≥15 kg and ≤30 kg will receive a dose of 150 mg or 300 mg SC, approximating the weight-based dosing of 5 mg/kg and 10 mg/kg, respectively, in a 30-kg child, and 10 mg/kg and 20 mg/kg, respectively, in a 15-kg child. Based on recent data from the study site, approximately 85% of children aged 6-10 years in Kalifabougou weigh between 15 kg and 30 kg. The doses used in the efficacy study will complement the data generated in the ongoing phase 1 VRC study. The doses used in the phase 1 VRC study were derived from 1) efficacy data from the challenge studies performed in mice showing that the protective concentration of antibody *in vivo* is between 5-100 μg/mL in 2 different mouse models of malaria infection;[11] and 2) PK data from nonhuman primate studies with L9LS and prior clinical experience in healthy adults with human MAbs targeting HIV (i.e., VRC01, VRC01LS, and VRC07-523LS) and ebolavirus (MAb114) at the same dose.

5 STUDY POPULATION

5.1 Inclusion Criteria

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

1. Is within the appropriate age range for the respective cohort:
    a. Children: Aged ≥6 years and <11 years.
    b. Adults: Aged ≥18 years.
2. Able to provide proof of identity to the satisfaction of the study clinician completing the enrollment process.
3. In good general health and without clinically significant medical history.
4. Adult participants or parent and/or guardian of minor participants able to provide informed consent.
5. Willing to have blood samples and data stored for future research.
6. Resides in or near Kalifabougou or Torodo, Mali, and available for the duration of the study.
7. For the adult cohort, 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.
    - Reliable methods of birth control include 1 of the following: confirmed pharmacologic contraceptives via parenteral delivery or intrauterine or implantable device.

- 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.

5.1.1 Year 2 Extension Inclusion Criteria

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

1. Participated in the first year of the protocol.
2. Able to provide proof of identity to the satisfaction of the study clinician completing the enrollment process.
3. In good general health and without clinically significant medical history.
4. Parent and/or guardian able to provide informed consent.
5. Willing to have blood samples and data stored for future research.
6. Resides in or near Kalifabougou or Torodo, Mali, and available for the duration of the study.

5.2 Exclusion Criteria

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

1. Body weight <15 kg or >30 kg for children, or >60 kg for adults.
2. Currently receiving or planning to receive SMC.
3. Any history of menses (for 6-10 year old cohort) or positive pregnancy test at screening (for adult cohort).
4. 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.
5. Subject (for adult subjects) or parental (for minor subjects) study comprehension examination score of <80% correct or per investigator discretion.
6. 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.)
7. 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.)
8. Infected with HIV, hepatitis C virus (HCV), or hepatitis B virus (HBV).
9. Known or documented sickle cell disease by history. (Note: Known sickle cell trait is NOT exclusionary.)
10. Clinically significant abnormal electrocardiogram (ECG; QTc >460 or other significant abnormal findings, including unexplained tachycardia or bradycardia).

11. 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.
12. Receipt of any investigational product within the past 30 days.
13. 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.)
14. Medical, occupational, or family problems as a result of alcohol or illicit drug use during the past 12 months.
15. History of a severe allergic reaction or anaphylaxis.
16. 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).
17. Salivary gland disorder diagnosed by a doctor (e.g., parotitis, sialadenitis, sialolithiasis, salivary gland tumors).
18. Pre-existing autoimmune or antibody-mediated diseases including but not limited to: systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, Sjogren's syndrome, or autoimmune thrombocytopenia.
19. Known immunodeficiency syndrome.
20. Known asplenia or functional asplenia.
21. 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.
22. Receipt of a live vaccine within the past 4 weeks or a killed vaccine or COVID-19 vaccine within the past 2 weeks prior to study agent administration.
23. Receipt of immunoglobulins and/or blood products within the past 6 months.
24. Previous receipt of an investigational malaria vaccine or monoclonal antibody in the last 5 years.
25. Known allergies or contraindication against artemether-lumefantrine.
26. Clinical signs of malnutrition.
27. 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.

5.2.1 Year 2 Extension Exclusion Criteria

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

1. Currently receiving or planning to receive SMC.

2. Any history of menses.
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. Parental 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 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 another interventional trial with an investigational product other than L9LS until the last required protocol visit. (Note: Past, current, or planned participation in observational studies 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. Currently active salivary gland disorder diagnosed by a doctor (e.g., parotitis, sialadenitis, sialolithiasis, salivary gland tumors).
17. Pre-existing autoimmune or antibody-mediated diseases including but not limited to: systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, Sjogren's syndrome, or autoimmune thrombocytopenia.
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 or COVID-19 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 any other monoclonal antibody other than L9LS in the last 5 years.
24. Known allergies or contraindication against artemether-lumefantrine.
25. Clinical signs of malnutrition.
26. 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 of Vulnerable Participants

- **Children:** L9LS is currently being evaluated in an ongoing phase 1 clinical trial to evaluate safety and protective efficacy of L9LS in healthy, malaria-naive adults. Interim safety results available from that trial as of December 26, 2021 (see section 2.3.1) indicate that L9LS is safe and well tolerated upon IV and SC administration. Therefore, this protocol will proceed with administration of L9LS in healthy adults followed by children aged 6-10 years in Mali, an age group that is particularly vulnerable to symptomatic malaria infection (Figure 6). Unlike children aged 1-5 years in Mali, who receive SMC as standard of care, SMC is not currently recommended in children aged 6-10 years in Mali. Children and adolescents aged 11-17 years have acquired some degree of clinical immunity to malaria and are less likely to experience symptomatic malaria (Figure 6). Irrespective of the decreased risk of clinical malaria risk with increased age, children, adolescents, and adults in Mali appear to be equally susceptible to Pf infection as detected by PCR (Figure 5).

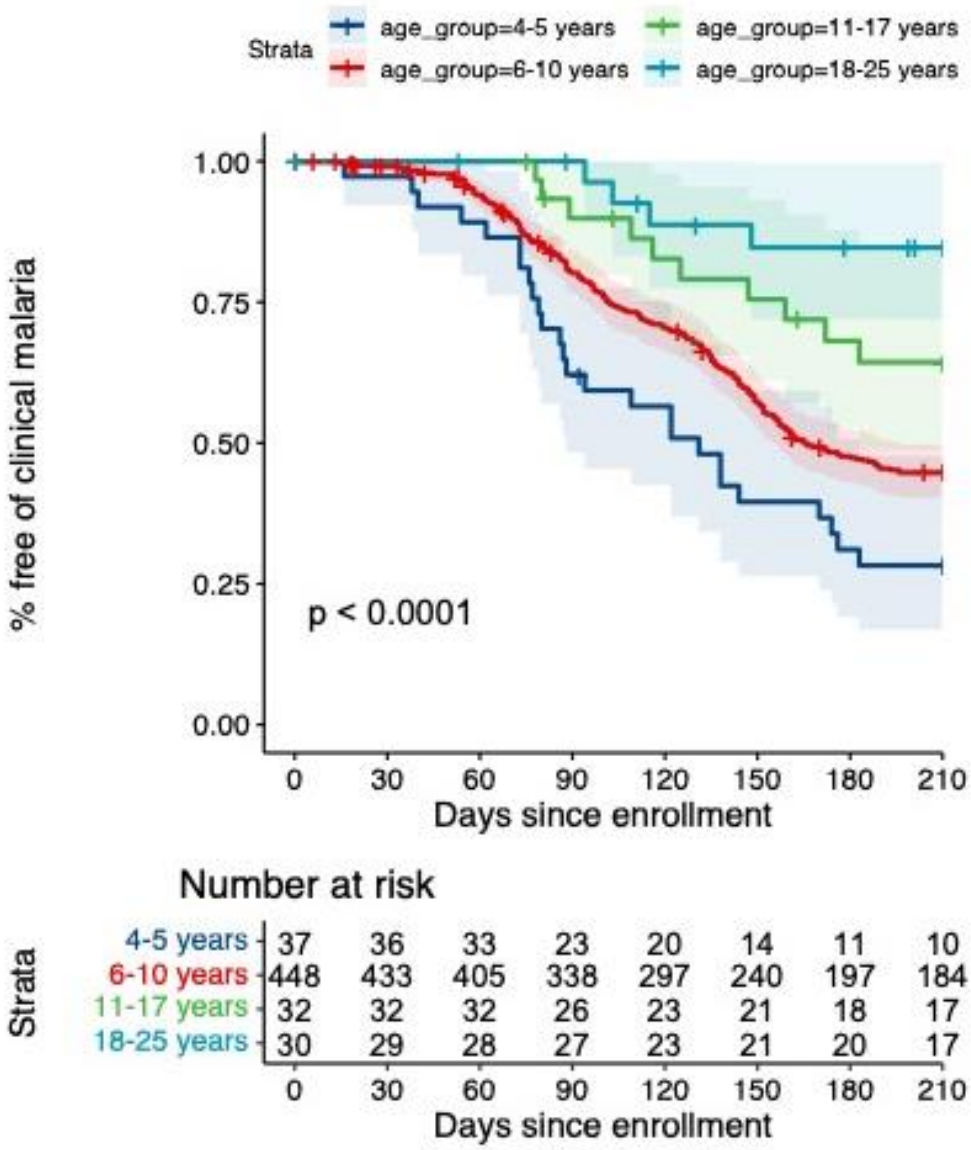


Figure 6. Kaplan-Meier plot stratified by age for time to first clinical malaria episode during the malaria season in Kalifabougou, Mali. Enrollment occurred just prior to the malaria season. Clinical malaria is defined by an axillary temperature of ≥37.5°C and ≥2,500 asexual Pf parasites/µL of blood (ClinicalTrials.gov Identifier: NCT01322581).

Children aged 6-10 years who weigh <15 kg are excluded because they are below the 15th percentile in weight-for-age according to WHO growth standards. Children who weigh >30 kg are excluded because the 150 mg or 300 mg doses administered to children in this study would fall below the minimum weight-based dosing target of 5 mg/kg and 10 mg/kg, respectively. The maximum volume of study agent that will be administered to children in this study is 2 mL (300 mg of L9LS). Based on recent data from the study site, approximately 85% of children aged 6-10 years in Kalifabougou weigh between 15 kg and 30 kg.

- **Illiterate Individuals:** We anticipate that many individuals eligible for this study and their parents/guardians 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 Inclusion of Pregnant Women, Fetuses or Neonates

Not applicable.

5.5 Lifestyle Considerations

Beyond the prohibited treatments and procedures listed in section 6.5, this study would not impact the lifestyle of the subjects.

5.6 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.

5.7 Strategies for Recruitment and Retention

Study subjects will be selected based on the eligibility criteria described in sections 5.1 and 5.1.1. The total target sample size across both study sites is 54 subjects in the dose-escalation study (accrual ceiling 90). The total target sample size across both study sites is 225 subjects in the efficacy study (accrual ceiling 380). We expect to enroll all subjects for both parts of the study within the first 6 months of the study from March through August. Subject selection will not be limited based on sex, race, or ethnicity.

The study team will hold community meetings in Kalifabougou 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.

For the year 2 extension, study participants who remain enrolled in the dose-escalation and efficacy studies will be invited to participate in the extension study before completing their last visit on the original study.

5.7.1 Costs

There are no costs associated with participation in this trial.

5.7.2 Compensation

Adult subjects and parents or guardians of minor 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 (injection).
- 3,000 CFA Franc for all other visits.

The total compensation amount for adults subjects will be 51,000 CFA Franc (valued at approximately USD $88) and for the 6-10 year old subjects will be 69,000 CFA Franc (valued at approximately USD $119) in the dose escalation study. Total compensation amount for the 6-10 year old subjects will be 63,000 CFA Franc (valued at approximately USD $109) in the efficacy study. Total compensation for the year 2 extension study will be 75,000 CFA Franc (valued at approximately USD $112). Payment will be provided in cash after the completion of each visit.

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

6 STUDY INTERVENTION

6.1 Study Interventions(s) Administration

6.1.1 Study Intervention Description

The study intervention involves a single administration of L9LS SC or IV or placebo SC to each subject. An additional single SC administration of L9LS or placebo will be delivered to participants who enroll in the year 2 extension study. 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 the progress of the study and study arm assignment (Table 1). The dose-escalation study dosing plan is described in section 0. Procedures for administration are described in section 6.1.2.4.

Table 1. Study agent assignment and dosing by arm.

Arm	Total Subjects	Study Agent and Dose
Dose-escalation study – adult cohort		
1	6	300 mg L9LS SC
2	6	600 mg L9LS SC
3	6	20 mg/kg L9LS IV
Dose-escalation study – 6-10 year old cohort		
1	18	150 mg L9LS (n=9) or placebo SC (normal saline; n=9)
2	18	300 mg L9LS (n=9) or placebo SC (normal saline; n=9)
Efficacy study – 6-10 year old cohort		
1	75	150 mg L9LS SC
2	75	300 mg L9LS SC
3	75	Placebo (normal saline) SC
Year 2 extension study		
1a	42	150 mg L9LS SC
1b	42	Placebo (normal saline) SC
2a	42	300 mg L9LS SC
2b	42	Placebo (normal saline) SC
3a	46	150 mg L9LS SC [a]
3b	46	Placebo (normal saline) SC

[a] This dose will be 300 mg if schema b or c are implemented as shown in Figure 3.

6.1.2.1 Dose Escalation

In the dose-escalation study, dose escalation will proceed in 3 study arms for the adult cohort and 2 study arms for the 6-10 year old cohort. The study will begin with enrollment into the adult cohort. When the last subject in arm 3 reaches day 7, if there are no safety concerns, enrollment into the 6-10 year old cohort will begin. For each cohort, enrollment will begin with arm 1. Subjects enrolled in this arm will be scheduled for study intervention, with at least 1 hour of observation between study agent administration in each subject, and follow-up procedures. Once all subjects in arm 1 complete day 7 post-injection, if no safety concerns have arisen, then enrollment will begin for arm 2. In the adult cohort, once all subjects in arm 2 complete day 7 post-injection, if no safety concerns have arisen, then enrollment will begin for arm 3.

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 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 and a targeted physical examination (as needed based on signs, reported symptoms, or interim medical history). For subjects receiving study agent SC, the total volume of assigned study agent will be divided into 2 syringes, except for the 300 mg adult cohort which will receive 1 syringe only. The study agent will be administered in each upper outer triceps area as a SC injection (1 injection per arm, except for the 300 mg adult cohort which will receive 1 injection), 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. 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. For subjects receiving study agent IV, each subject will have IV access placed in an arm vein in an aseptic manner. A different site may be used for collection of PK blood samples; however, the same site may be used after flushing the line if another site is not available. The study agent will be administered with approximately 100 mL of normal saline IV over about 30-60 minutes, with a target of 30 minutes. Infusions lasting longer than 30 minutes are allowed. If the subject experiences side effects during the infusion, the rate of infusion may be slowed or stopped to alleviate the symptoms. At the end of product administration, the IV administration set must be flushed with about 30 mL (or appropriate volume) of normal saline. The IV will remain in for safety through the end of post-administration observation. Only adult subjects in Arm 3 of the dose-escalation study will receive study agent IV, and this will be done in open-label fashion.

The first subject in each arm of the dose-escalation study will be observed for at least 4 hours following completion of product administration, and subsequent subjects will be observed for at least 2 hours following completion of product administration. All subjects in the efficacy study will be observed for at least 60 minutes following completion of product administration. In the year 2 extension study, the first 30 subjects will be monitored for at least 2 hours (+/-15 minutes) after study agent administration and vital signs will be recorded for 1 hour (+/-15 minutes) after study agent administration (based on randomization this will be approximately 5 subjects in each sub-arm). Thereafter, all subjects will be monitored for at least 60 minutes (+/-15 minutes) after study agent administration and vital signs will be recorded 1 hour (+/-15 minutes) after study agent 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 would 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 and the normal saline placebo 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.

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 (cGMP) by the Vaccine Clinical Materials Program (VCMP) operated under contract by Leidos Biomedical Research, Inc., Frederick, MD. It 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 (DS). 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 when preparing for SC administration. Because L9LS has a slight yellow tint, and the placebo (normal saline) is colorless, the pharmacist will cover all syringes with transparent yellow tape to maintain blinding. L9LS is more viscous than normal saline, so the study agents will only be administered to subjects SC by designated individuals who remain separate from the team of blinded investigators who conduct all subsequent follow-up study assessments.

Vials of L9LS and placebo 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, DO NOT REFREEZE; store the thawed, vialed material 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 and IV administration, thaw and equilibrate vials 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, equilibrate at ambient temperature for a minimum of 30 minutes.[35]

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.[35]

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

6.2.4 Preparation

For children: Study product will be prepared by an unblinded pharmacist. For the dose-escalation study, one syringe will be prepared for each subject in the 150-mg L9LS/placebo arm. For the 300-mg L9LS/placebo arm of the dose-escalation study, all arms of the efficacy study, and all arms of the year 2 extension study, two syringes will be prepared for each subject for a total of two 1-mL SC injections for each subject. Because L9LS has a slight yellow tint, and the placebo (normal saline) is colorless, the pharmacist will cover all syringes with transparent yellow tape to maintain blinding.

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 in the case of multiple syringes. The filter needle must be discarded prior to dispensing and replaced with a needle suitable for SC injection at the time of administration.
    3) For subjects assigned to the 150-mg L9LS arm in the efficacy study and the year two extension study ONLY: Withdraw half of the total amount of L9LS from the vial into each of 2 syringes using a 5-micron filter needle, and then fill each syringe with normal saline as needed for a total volume of 1 mL in each syringe. 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 1 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.

For adult SC administration: Study product will be prepared by an unblinded pharmacist. For subjects receiving the 300-mg dose, one syringe will be prepared which will contain 2 mL of L9LS, as described below. For subjects receiving the 600-mg dose, two syringes will be prepared and each syringe will contain 2 mL of L9LS (for a total of two 2-mL SC injections for each subject), as described below. The adult safety study is open-label without a placebo arm.

For each SC injection order, the dose level and study arm code will be included in the pharmacy order. To prepare a SC L9LS injection, the pharmacist will do the following:

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 in the case of multiple syringes. The filter needle must be discarded prior to dispensing and replaced with a needle suitable for SC injection at the time of administration.

For adult IV administration: Study product will be prepared by an unblinded pharmacist. For each IV infusion order, the subject's weight, dose level, and study arm code will be included in the pharmacy order. The adult safety study is open-label without a placebo arm. To prepare an IV infusion, the pharmacist will do the following:

1) Calculate the total milligrams of L9LS needed.
2) Retrieve the minimum number of thawed vials required to prepare the full dose. After vials are thawed, prior to preparation for administration in the IV bag, vials should be gently swirled for 30 seconds while avoiding foaming. DO NOT SHAKE THE VIAL.
3) Withdraw all air from the 100-mL bag of normal saline.
4) Withdraw the necessary amount of L9LS.
5) Add this volume to a 150-mL capacity, partial fill, 100-mL bag of normal saline using sterile compounding techniques to maintain sterility.

An in-line filter infusion set must be used for IV product administrations and MUST comply with the following specifications: 1.2-micron polyethersulfone filter membrane, diethylhexylphthalate-free, latex-free (equivalent to Braun #473994 filter extension set). When the in-line filter is added to the tubing, the administration set must then be primed.

6.3 Measures to Minimize Bias: Randomization and Blinding

6.3.1 Randomization

Randomization lists for each randomized phase of the trial will be generated by the blinded 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 9.5.1.

6.3.2 Blinding

This trial will be conducted with a double blind for subjects aged 6-10 years and for the year 2 extension study; dose assignments of adult subjects will be open label. 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 for subjects aged 6-10 years and those in the year 2 extension study, 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. For the 36 subjects

aged 6-10 years enrolled in the dose-escalation study, blinding will be maintained for treatment assignment (L9LS versus placebo), but not for possible L9LS dose group.

Data will remain blinded for the original protocol until the last subject completes the final study visit of the original protocol. Data will remain blinded for the protocol extension until the last subject completes the final study visit of the protocol extension. Blinded 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. If an SAE has resulted in unblinding, this information will be included in the SAE Report.

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 6-10 year old children or 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.

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

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/parent/guardian and reschedule the missed visit within 7 days, counsel them on the importance of maintaining the assigned visit schedule, and ascertain if they wish 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/parent/guardian (where possible, 3 telephone calls). These contact attempts should be documented in the subject's medical record or study file.
- Should the subject/parent/guardian 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 the Kalifabougou and Torodo study sites. The study staff will explain the study to the prospective subject/parent/guardian, complete the study comprehension examination (completed by adult subject or parent/guardian of minor subject), obtain consent (section 10.1), and assess eligibility. Consent will be obtained before any study-related procedures are performed. At this time, the subject will be assigned a unique study identification number in the clinical database. The identification number will link subject samples and data collected throughout the study.

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, including solicited information related to recurrent or persistent dry mouth or swelling of salivary glands.
- Complete review of 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).
- Blood collection via venipuncture for screening evaluations:
    - HIV tests: 2 rapid diagnostic tests (RDTs), plus enzyme-linked immunosorbent assay (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 and HCV tests. If either test is positive, the subject will be referred for care regardless of the ALT result.
    - Hemoglobin typing (will not be repeated for the protocol extension).
    - Complete blood count (CBC) with differential.
    - ALT.
    - Cr.
- Pre- and post-test HIV counseling.
- Pregnancy test (urine or serum; females in adult cohort only).

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 5.6, at the discretion of the investigator. If screening is completed outside the specified window, all screening procedures and evaluations must be repeated. If an individual screens and is enrolled into the dose-escalation study but for any reason does not receive study agent, they may later consent to be screened and enrolled in the efficacy study.

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 to clear any possible Pf blood-stage infection prior to study agent administration. Dosing in adults will be 4 tablets twice daily for 3 days. Dosing in children will follow standard treatment dosing. 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.[36]

Randomization: At randomization, a randomization code will be assigned. Randomization procedures are 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 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 children 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. In adults assigned to IV drug administration, blood may be drawn from the IV line if an adequate venipuncture site is not available. 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.

The amount of blood drawn for research purposes will be within the limits allowed for research subjects by the NIH Clinical Center:

- Adult subjects: no more than 10.5 mL/kg or 550 mL, whichever is smaller, over any 8-week period.
- Pediatric subjects: no more than 5 mL/kg in a single day, and no more than 9.5 mL/kg over any 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 and to identify clinical malaria for a secondary 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 9.5.11). This evaluation will be performed by Dr. Daniel Neafsey at the Harvard School of Public Health.

Whole blood RNA-seq: Whole blood will be collected into PaxGene tubes to preserve RNA for human transcriptome analysis, to correlate transcriptomic signatures with Pf infection risk.

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 include performing human RNA-seq/transcriptomic analysis of whole blood as well as analyzing the genetic material of infection-inducing parasites in blood samples.

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 the human transcriptomic analyses performed in this protocol are research tests that do not have clinical implications for participants, 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 injection, 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: Urine or serum pregnancy tests will be conducted for female subjects in the adult cohort only. Results must be confirmed negative prior to study agent administration.

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 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 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).

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 (separate tables provided for adults and children). 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/regulatory-information/search-fda-guidance-documents/toxicity-grading-scale-healthy-adult-and-adolescent-volunteers-enrolled-preventive-vaccine-clinical

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 Cr)
- 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., Clinical Safety Office [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
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, ISM, and DSMB:

- Sample collection will continue per guidelines below:
    - Only proceed with PK blood draw if subject's hemoglobin value is ≥8.0 gm/dL (assessed via venipuncture collection of 0.5 mL of blood).
    - PK samples (4-8 mL) will be collected according to the regular study schedule (see sections 1.3.1 and 1.3.2).
    - The venipuncture 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.
    - Serum storage samples will not be drawn if a subject becomes pregnant.
- Continue to follow for safety on a monthly basis for the duration of the pregnancy and for a period of up to 6 months (per investigator discretion) following delivery for assessment of the neonate.
- 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/parent's/guardian's decision. (The investigator should attempt to determine the reason for the 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 of the subject/parent/guardian to provide informed consent.
    - Withdraws permission to have blood samples or data stored for future research.
    - Positive pregnancy test prior to study agent administration.
- 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 5.6), 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). On a case-by-case basis, a request for re-enrollment, or for repetition of a protocol step or procedure already completed, may be submitted to, reviewed by, and approved by the SMM in writing. The SMM may also recommend or require consultation of the EC and/or DSMB and ISM.

8.4.3.2 Replacement of Withdrawn Subjects or Subjects Who Discontinue Study Agent

In the dose-escalation study, subjects withdrawn prior to the day 7 safety evaluation will be replaced. In the efficacy study, subjects withdrawn prior to study agent administration will be replaced. Subjects who are withdrawn in the year 2 extension study will not 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. 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. The ISM will have access to unblinded data, by group, only as the entire DSMB does. The ISM may also serve as an *ad hoc* member of the DSMB.

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 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. Additionally, the DSMB will conduct 1 interim analysis when safety data are available from the dose-escalation study subjects (section 9.5.8). They will also review available data from Year 1 of the study prior to starting participant visits for Year 2.

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 a group or arm (e.g., a specific dosing group) in 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

If a decision is made to permanently discontinue study agent administration, any subject who has received the study agent or undergone any other study 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/FAPH 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

If a decision is made to permanently discontinue study agent administration, any subjects who have received study agent or undergone any other 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 Unanticipated Problems

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/FAPH 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/FAPH EC

Non-compliance and other reportable events will be reported to the FMOS/FAPH 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. Determinations by the FMOS/FAPH EC of serious and/or continuing non-compliance by an NIH investigator must also be reported to the NIH within 7 calendar days.
- 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 NIH Office of Human Subjects Research Protections 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/FAPH 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/FAPH EC as well as the NIH reporting requirements.

Investigators may modify the protocol without prospective FMOS/FAPH 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/FAPH 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/FAPH 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/FAPH 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 primary study hypotheses are that L9LS will be safe and will confer protection against Pf infection. In the dose-escalation study, the primary objective is to evaluate the safety and tolerability. In the efficacy study, the primary objective is to evaluate the efficacy of L9LS compared to placebo.

Primary Endpoints:

- Dose escalation and efficacy: Incidence and severity of local and systemic AEs occurring within 7 days after the administration of L9LS.
- Efficacy: Pf blood-stage infection as detected by microscopic examination of thick blood smears obtained between 1 week and 24 weeks after administration of L9LS or placebo.

Secondary Endpoints:

Data from both parts of the study will be used to assess the following endpoints:

- Measurement of L9LS in sera of recipients.
- Pf blood-stage infection as detected by RT-PCR for 28 weeks after administration of L9LS or placebo for the dose escalation study and for 24 weeks after administration of L9LS or placebo for the efficacy study.
- Incidence of clinical malaria (definition 1, see section 2.2.2.3) for 28 weeks after administration of L9LS or placebo for the dose escalation study and for 24 weeks after administration of L9LS or placebo for the efficacy study.
- Incidence of clinical malaria (definition 2, see section 2.2.2.3) for 28 weeks after administration of L9LS or placebo for the dose escalation study and for 24 weeks after administration of L9LS or placebo for the efficacy study.
- PK analysis of L9LS and the association of L9LS concentration with Pf infection risk.
- PK analysis of L9LS and the association of L9LS concentration with clinical malaria risk.

9.2 Sample Size Determination

9.2.1 Sample Size Considerations for the Dose-Escalation Study

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. With sample size n=6 in each arm, there is over 90% chance to observe at least 1 AE if the true rate is at least 0.319 and over a 90% chance to observe no AE if the true rate is no more than 0.017. With sample size n=9 in each arm, there is over a 90% chance to observe at least 1 AE if the true rate is at least 0.226 and over a 90% chance to observe no AE if the true rate is no more than 0.011. Probabilities of observing 0 or more than 1 AE within a group are presented in Table 3 for a range of possible true event rates.

Table 3. Probability of events for different safety scenarios within an arm (n=6 or n=9).

True event rate	n=6		n=9	
	Pr(0)	Pr(>1)	Pr(0)	Pr(>1)
0.005	0.97	<0.001	0.956	0.001
0.01	0.941	0.001	0.914	0.003
0.02	0.886	0.006	0.834	0.013

True event rate	n=6		n=9	
	Pr(0)	Pr(>1)	Pr(0)	Pr(>1)
0.035	0.808	0.017	0.726	0.037
0.05	0.735	0.033	0.63	0.071
0.1	0.531	0.114	0.387	0.225
0.15	0.377	0.224	0.232	0.401
0.2	0.262	0.345	0.134	0.564
0.3	0.118	0.58	0.04	0.804

Abbreviations: Pr, probability.

9.2.2 Sample Size Considerations for the Efficacy Study

The efficacy study is designed to evaluate protective efficacy (PE) of L9LS at each dose by testing the null hypothesis,

H0: PE = 0%,

versus the alternative hypothesis,

H1: PE ≠ 0%,

where PE is 1 minus the relative risk of infection under L9LS versus under placebo. To account for the two comparisons, each L9LS dose arm with the placebo arm, two-sided tests each with significance level of 0.025 will be adopted to ensure the overall type I error rate is no more than 0.05. The sample size considerations are based on the power of rejecting H0 over a range of possible protective efficacy and infection rates under placebo (Figure 5). Assuming a drop-out rate of 10%, Table 4 presents the power under a 2-sided log-rank test with significance level of 0.025. If the infection rate under placebo is 0.4, there will be 90% power to detect the protective efficacy of 0.7 with 75 participants enrolled into each arm of the efficacy study. Based on historical data collected in Kalifabougou, approximately 85% of children aged 6-10 years who are uninfected (PCR negative) before the malaria season become infected with Pf during the ensuing malaria season (Figure 5). Therefore, the conservative estimate of infection rate under placebo of 0.4 will ensure that the study has adequate power if the malaria season is unexpectedly delayed or truncated.

Table 4. Power for efficacy evaluation from a 2-sided log-rank test with type I error rate of 0.025 assuming 10% dropout.

Sample size (per arm)	Infection rate under placebo	Protective efficacy	Power (%)
60	0.4	0.6	68
	0.4	0.7	83
	0.4	0.8	92
	0.5	0.6	82

	0.5	0.7	92
	0.5	0.8	97
	0.6	0.6	92
	0.6	0.7	97
	0.6	0.8	99
75	0.4	0.6	78
	0.4	**0.7**	**91**
	0.4	0.8	97
	0.5	0.6	90
	0.5	0.7	97
	0.5	0.8	99
	0.6	0.6	97
	0.6	0.7	99
	0.6	0.8	100

Table 5 presents the minimum detectable difference between an L9LS arm and placebo arm in terms of the event rate, for example the proportion of infection, based on a two-sided proportion test with type I error rate of 0.025.

Table 5. Minimum detectable difference in the event rate in arms receiving L9LS or placebo under a 2-sided type I error rate of 0.025.

Sample size within each arm (#)	**Event rate (under placebo)**	**Detectable with 80% power**		**Detectable with 90% power**	
		Difference	**Event rate (under L9LS)**	**Difference**	**Event rate (under L9LS)**
75 under L9LS and 75 under placebo	0.4	0.226	0.174	0.253	0.147
	0.5	0.243	0.257	0.273	0.227
	0.6	0.25	0.35	0.283	0.317
	0.7	0.247	0.453	0.282	0.418

9.3 Sample Size Considerations for the Year 2 Extension Study

For safety assessment, Table 6 shows the precision for estimating the AE rate within each arm of 42 participants for a range of underlying event rates.

Table 6. Exact 95% CI of AE rate estimate within each arm of size 42.

Observed number of events	Observed event rate	95% confidence interval	
		Lower bound	Upper bound
0	0	0	0.084

1	0.024	0.001	0.126
2	0.048	0.006	0.162
3	0.071	0.015	0.195
4	0.095	0.027	0.226
5	0.119	0.04	0.256
6	0.143	0.054	0.285
7	0.167	0.07	0.314
8	0.19	0.086	0.341
9	0.214	0.103	0.368
10	0.238	0.121	0.395
11	0.262	0.139	0.42
12	0.286	0.157	0.446
13	0.31	0.176	0.471
14	0.333	0.196	0.495

With 42 participants per arm, there is 90% power to detect the difference between two arms if the event rate is 0.01, 0.05, or 0.1 in one arm and no less than 0.24, 0.33, or 0.42 in the other arm, respectively, based on two-sided proportion test at significance level 0.05.

Table 7 shows the power for comparing two arms on the primary efficacy endpoint, namely, the time from receiving the second dose of L9LS (or placebo) to the first Pf blood-stage infection during extension study with each arm of 42 participants.

Table 7. Power for between-arm comparison on time to infection based on two-sided log rank test at significance level 0.05.

Infection rate in one arm	Infection rate in the other arm	Relative efficacy	Power (%)
0.5	0.2	0.6	79
0.5	0.15	0.7	90
0.6	0.24	0.6	89
0.6	0.18	0.7	96
0.7	0.28	0.6	96
0.7	0.21	0.7	99

9.4 Populations for Analyses

The following datasets will be considered in study analyses:

- Intention-to-treat (ITT) 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 (PP) 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 be additionally performed according to the actual product received.

9.4.1 Evaluable for toxicity

All participants will be evaluable for toxicity from the time of their first treatment with L9LS or placebo. The toxicity analysis will be ITT.

9.4.2 Evaluable for objective response

Not applicable.

9.4.3 Evaluable Non-Target Disease Response

Not applicable.

9.5 Statistical Analyses

9.5.1 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% CIs. Formal comparisons will use standard methods, contingency tables for categorical variables, t-tests for comparing means if data follow a normal distribution or geometric means if data after log transformation follow a normal distribution, or nonparametric analogs for comparing medians. 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 the amount of missing data is modest (e.g., <10%). We will examine the “missing completely at random” assumption if the amount of 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: Weight-stratified randomization will be implemented in the dose-escalation study and the efficacy study. In the efficacy study, 75 participants will be recruited into each weight group (15-19 kg, 20-25 kg, and 26-30 kg). Within each weight group, randomization will be 1:1:1 allocation to the 2 L9LS arms and the placebo arm. The total number of participants will be 75 in each arm (25 of weight 15-19 kg, 25 of weight 20-25 kg, and 25 of weight 26-30 kg). A similar stratified randomization will be applied to the dose-escalation study: six participants in each weight group, starting with 26-30 kg, followed by 20-25 kg, and then 15-19 kg, will be recruited and randomized 1:1 to receive L9LS at the dose of 150 mg or placebo; if no safety concerns arise, randomization into the L9LS at the dose of 300 mg or placebo ensues in the same manner. To limit the number of dropouts before L9LS administration, randomization and L9LS administration will occur as close in time as possible.

9.5.1.1 Year 2 Extension Study

The same analysis methods for safety, PK, and efficacy endpoints described for the original study (year 1) below will be applied to the year 2 extension study. There will be no multiplicity adjustment in extension study analyses. All comparisons will be conducted at two-sided significance level 0.05.

9.5.2 Analysis of the Primary Endpoints

Analysis for the primary endpoint for safety and tolerability is described in section 9.5.5.

Analysis for the primary efficacy endpoint, protective efficacy with Pf infection determined by blood smear, is described in section 9.5.4.

9.5.3 Analysis of the Secondary Endpoint(s)

Analysis of the secondary efficacy endpoints, incidence of Pf infection determined by RT-PCR, is described in section 9.5.4.

Analyses of the secondary endpoints for the PK of L9LS and the association of L9LS concentration with Pf infection risk are described in section 9.5.6.

9.5.4 Efficacy Analyses

The primary efficacy endpoint (efficacy study primary endpoint) is the incidence of malaria infection defined as blood smear–positive Pf infection between 1 week and 24 weeks after administration. The secondary efficacy endpoints are the incidence of malaria infection as determined by RT-PCR and the incidence of clinical malaria. The efficacy analyses will be MITT over participants in the efficacy study.

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 and compared by the logrank test across different arms. The protective efficacy of the study product will be estimated by the hazard ratio from the Cox proportional hazards model. These analyses will be carried out by R packages that account for interval censoring: package "interval" for deriving a nonparametric maximum likelihood estimation based on the Kaplan-Meier survival curve and logrank test, and package "icenReg" for Cox proportional hazards regression. To address the heterogeneity of the study population, 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 arms and the placebo arm in spite of randomization. An additional Cox regression will be performed with pre-existing parasitemia (Pf infection status determined by blood smear at baseline) as the stratification variable. The Holm method will be adopted to handle the issue of multiplicity in comparing the two dose arms against the placebo.

The secondary efficacy analysis will be based on the proportion of infection. The proportion of infection will be estimated for each arm and compared across arms based on Kaplan-Meier estimates assuming events occur at the time they are detected: the proportion of infection by one minus the Kaplan-Meier estimate in the arm, and the vaccine efficacy by one minus the relative risk of infection with melding method for constructing the melded 95% CI of the vaccine efficacy.[37]

Since this trial adopts weight-stratified randomization, participants cover the dose range from 5 mg/kg to 20 mg/kg. In addition to the above efficacy analyses that compare each dose arm with the placebo arm, an exploratory analysis will be additionally conducted to assess how dosage, in terms of mg of L9LS per kg of subject body weight, affects the protective efficacy.

The above analyses will apply to both the primary and the secondary efficacy endpoints.

9.5.5 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. In the rare case of subjects receiving a regimen different from assignment, an as-treated analysis will be additionally performed where subjects will be analyzed according to the product they actually receive.

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 AE(s) will be summarized by dose arm along with the exact 95% CIs of the AE rate. The number and percentage of subjects under each AE will be

additionally reported. 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.

9.5.6 Pharmacokinetics Analysis

PK analysis will be carried out for subjects in the dose-escalation study and in the efficacy study with blood samples collected at defined timepoints as listed in section 1.3. The PK analysis will be PP. The following PK analysis will be performed as needed.

Individual Subject Pharmacokinetic Analysis: A non-compartmental (NC) PK analysis will be performed on the L9LS 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 (Cmax) and time of maximal concentration (Tmax) 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 L9LS concentration falls below the quantitative limit (QL) of the assay, the sample with concentration below the QL will be assigned a L9LS 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 L9LS 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 Pharmacokinetic Analyses: Based on preclinical PK results for L9LS 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 (Vdss), 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.[38]

To assess the association of L9LS concentration with protection, we will perform a Cox proportional hazards regression for the time to the first infection with L9LS concentration as a time-varying covariate. A logistic regression analysis will be additionally performed to model the infection rate as a function of L9LS concentration via the method of generalized estimating equation (GEE) to account for repeated measures. The GEE estimation will only be performed over the scheduled visits with the binary outcome (infected or not) as the response and the L9LS concentration as the covariate. This analysis will be performed over all participants that receive L9LS including those aged 6-10 years in the dose-escalation study and those in the efficacy study.

9.5.7 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.

9.5.8 Planned Interim Analyses

Interim safety analysis will be performed when safety data are available from the age de-escalation and dose-escalation study subjects. The purpose of the interim analysis on safety data is to clear safety concerns for proceeding to the efficacy study. This interim analysis will not affect the power or type I error in the primary analysis on efficacy in the efficacy study.

9.5.9 Sub-Group Analyses

Not applicable.

9.5.10 Tabulation of individual Participant Data

Safety data will be presented by line listing and tables at the individual level, as described in section 9.5.5.

9.5.11 Exploratory Analyses

To explore the impact of L9LS 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 L9LS footprint.

For assessment of ADA in the year 2 extension study, the count and percentage (as well as the exact 95% CI) of participants with detectable ADA in each arm will be reported. The percentage of participants with detectable ADA will be compared between two arms via Fisher's exact test.

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 5.7. Following the process of Diallo and colleagues,[39] 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.

10.1.1.2 Individual Informed Consent

Consent process: 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 and minor subjects' parents/guardians 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 adult subjects and parents/guardians of minor 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 adult subject or minor subject's parent/guardian 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. Adult subjects will provide consent by signing or fingerprinting (if illiterate) the consent form. Parents/guardians will provide permission for their child/ward to participate by signing or fingerprinting (if illiterate) the consent form.

Also, a study comprehension examination will be conducted to make sure that the study is understood by the potential adult subject or minor subject's parent/guardian 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 by the potential adult subject or

by the parent/guardian is mandatory to enroll the adult or their child/ward, respectively. 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 potential adult subject whose comprehension is questionable, or the child/ward of any individual whose comprehension is questionable, regardless of score, may be excluded from enrollment.

Minor participants will be included in all discussions about the study, and age-appropriate language will be used to describe the procedures and tests involved in this study, along with the risks, discomforts, and benefits of participation. However, children will not be required to provide assent as the age ranges included in this study typically do not have the ability to fully understand the nature of research.

The consent process will be documented in the adult or child subject's medical record.

10.1.2 Consent for Minors When They Reach the Age of Majority

When a pediatric subject reaches age 18, continued participation (including ongoing interactions with the subject or continued analysis of identifiable data) will require that consent be obtained from the now adult with the standard protocol consent document to ensure legally effective informed consent has been obtained.

If reconsent is not feasible, we request waiver of informed consent to continue to use data and/or specimens for those individuals who become lost to follow up or who have been taken off study prior to reaching the age of majority.

Requirements for Waiver of Consent consistent with 45 CFR 46.116(f)(3):

(1) The research involves no more than minimal risk to the subjects.
    a. Analysis of samples and data from this study involves no additional risks to subjects.
(2) The research could not practicably be carried out without the waiver or alteration.
    a. Considering the length of time between the minor's last contact with the research team and their age of majority, it will likely be very difficult to locate them again. A significant reduction in the number of samples analyzed is likely to impact the quality of the research.
(3) As the research involves using identifiable private information or identifiable biospecimens, the research could not practicably be carried out without using such information or biospecimens in an identifiable format.
    a. Though the purpose of future studies cannot yet be known, they often involve the correlation of clinical outcomes and clinical interventions with laboratory studies. Such information would be unavailable if access to medical record numbers was unavailable.

(4) The waiver or alteration will not adversely affect the rights and welfare of the subjects.
    a. Retention of these samples or data does not affect the welfare of subjects.
(5) Whenever appropriate, the subjects will be provided with additional pertinent information after participation.
    a. We only request a waiver of consent for those subjects who have been lost to follow-up or who have been taken off study prior to reaching the age of majority.

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 NIH researchers, EC review and approval will be obtained. This includes the NIH 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

During the study, the principal investigator and study team will be responsible for ensuring study activities are conducted in compliance with the protocol, ICH GCP, and applicable regulatory requirements. Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, and documentation according to study SOPs.

10.8 Data Handling and Record Keeping

10.8.1 Data Collection and Management Responsibilities

Study data will be maintained in 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 the EC as needed.

10.8.2 Study Records Retention

Study documents will be retained in accordance with regulatory and institutional requirements, ICH GCP guidelines, and the NIH Intramural Records Retention Schedule. 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 and Non-Compliance

It is the responsibility of the investigator to use continuous vigilance to identify and report deviations and non-compliance to the FMOS/FAPH EC and the NIH (as applicable) according to NIH HRPP Policy 801 (as described in section 8.6.1). All deviations must be addressed in study source documents and reported to the NIAID Program Official and sponsor. The investigator is responsible for knowing and adhering to the reviewing EC requirements.

10.9.1 NIH Definition of Protocol Deviation

The definition of a protocol deviation is provided in section 8.4.1.

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.

Human data generated in this study for future research will be shared as follows:

- De-identified or identified data with approved outside collaborators under appropriate agreements.
- De-identified results or data in publication and/or public presentations.

Data will be shared at the time of publication or shortly thereafter.

10.10.2 Genomic Data Sharing Compliance

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 (GWAS), single nucleotide polymorphisms (SNP) arrays, and genome sequence, transcriptomic, epigenomic, and gene expression data.

10.11 Collaborative Agreements

Not applicable.

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 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 Concentrations vs. Time Curve
Cave	Average Concentrations
CBC	Complete Blood Count
CFA	Communauté Financière Africaine
CFR	Code of Federal Regulations
cGMP	Current Good Manufacturing Practices
CHMI	Controlled Human Malaria Infection
CI	Confidence Interval
CL	Clearance
Cmax	Maximum Concentration

CONSORT	Consolidated Standards of Reporting Trials
Cr	Creatinine
CRF	Case Report Form
CRS	Cytokine Release Syndrome
CSO	Clinical Safety Office
CSP	Circumsporozoite Protein
CTM	Clinical Trials Management
DCR	Division of Clinical Research
DS	Drug Substance
DSMB	Data and Safety Monitoring Board
EC	Ethics Committee
EC80	Effective Concentration in 80% of the population
ED80	Effective Dose in 80% of the population
ELISA	Enzyme-Linked Immunosorbent Assay
FDA	Food and Drug Administration
FMOS/FAPH	Faculté de Médecine Pharmacie d'Odontostomatologie
GCP	Good Clinical Practice
GEE	Generalized Estimating Equation
GLP	Good Laboratory Practices
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HRPP	Human Research Protection Program
ICH	International Council on Harmonisation
IND	Investigational New Drug Application
ISM	Independent Safety Monitor
ITT	Intention-To-Treat
IV	Intravenous(ly)
LIG	Laboratory of Immunogenetics
MAb	Monoclonal Antibody
MITT	Modified Intention-To-Treat
MRTC	Malaria Research and Training Center
NC	Non-Compartmental
NCT	National Clinical Trial
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

PCR	Polymerase Chain Reaction
PE	Protective Efficacy
Pf	*Plasmodium falciparum*
PK	Pharmacokinetic(s)
PP	Per-Protocol
Q	Intercompartmental Clearance
QL	Quantitative Limit
QTc	Corrected QT Interval
RDT	Rapid Diagnostic Test
REDCap	Research Electronic Data Capture
REF	Reportable Event Form
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SAE	Serious Adverse Event
SAR	Suspected Adverse Reaction
SC	Subcutaneous
SERF	Safety Expedited Report Form
SMC	Seasonal Malaria Chemoprevention
SMM	Sponsor Medical Monitor
SOA	Schedule of Activities
SOP	Standard Operating Procedure
SUSAR	Serious and Unexpected Suspected Adverse Reaction
SRCP	Safety Review and Communication Plan
TCR	Tissue Cross-Reactivity
Tmax	Maximal Concentration
UP	Unanticipated Problem
UPnonAE	Unanticipated Problem that is not an Adverse Event
US	United States
USD	United States Dollar
USTTB	University of Sciences, Techniques, & Technologies of Bamako
VCMP	Vaccine Clinical Materials Program
Vd	Volume of Distribution
Vdss	Volume of Distribution at Steady-State
VRC	Vaccine Research Center
WHO	World Health Organization

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APPENDIX A: MRTC URINE LABORATORY NORMAL VALUES

Urine Dip/Urinalysis - Adults

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).

Urine Dip/Urinalysis - Pediatrics

Urine	Reference Ranges
Protein	< 10 mg/L
Blood (Microscopic) – RBC/HPF	< 25 WBC/µg None

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

APPENDIX B: MALI LABORATORY ADVERSE EVENT GRADING SCALE, ADULTS

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 – 216.00	> 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.[40,41]

[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: MALI LABORATORY ADVERSE EVENT GRADING SCALE, CHILDREN

Hematology and Biochemistry Values[1, 2]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-threatening (Grade 4)
Hemoglobin (Male/Female) - gm/dL	7.50 – 8.40	6.10 – 7.49	5.00 – 6.09	< 5.00 g/dL
WBC Increase - 10^3/µL	14.50 – 16.09	16.10 – 20.09	20.10 – 30.00	>30.00
WBC Decrease - 10^3/µL	2.50 – 3.30	1.50 – 2.49	1.00 – 1.49	< 1.00 with or without fever
Neutrophil/Granulocyte Decrease[3] - 10^3/µL	0.75 – 0.99	0.50 – 0.74	< 0.50	< 0.50 with fever
Platelets Decreased - 10^3/µL	100.0 – 120.9	70.0 – 99.9	25.0 – 69.9	< 25.0
Creatinine (Male/Female) - µmol/L	95.00 – 119.99	120.00 – 149.99	150.00 – 200.00	> 200.00 and requires dialysis
Liver Function Tests/ALT - U/L	75.0 – 150.9	151.0 – 300.9	301.0 – 600.0	> 600.0

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. [40,41]

APPENDIX D: MALI VITAL SIGNS ADVERSE EVENT GRADING SCALE, CHILDREN

Vital Signs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Temperature	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	39.0 – 40 102.1 – 104	> 40 > 104
Tachycardia - beats per minute; at rest + calm	121 – 135	136 -- 150	>150	ER visit or hospitalization for arrhythmia
Bradycardia - beats per minute[4]; at rest + calm	55 -- 59	50 – 54	< 50	ER visit or hospitalization for arrhythmia
Hypertension (systolic) -mm Hg; at rest + calm	131 – 140	141 – 150	> 150	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) -mm Hg; at rest + calm	81 – 90	91 – 95	> 95	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) -mm Hg; at rest + calm	75 – 79	70 – 74	< 70	ER visit or hospitalization for hypotensive shock

APPENDIX E: DOSE ESCALATION PEDIATRIC STUDY EXTENSION

Study Extension Rationale

Pediatric participants who are enrolled in the Dose Escalation study may be followed with additional study visits through the end of the malaria season to 1) determine whether they become infected with *P. falciparum* (as detected by RT-PCR from dried blood spots collected every 4 weeks), and 2) extend the PK analysis of L9LS to approximately 9 months (as determined by measuring the concentration of L9LS in blood samples collected every 4 weeks). The Pediatric Dose Escalation component of the study had 2 dose groups (150 mg of L9LS or placebo and 300 mg of L9LS or placebo). Following all subjects in the Pediatric Dose Escalation group will allow for exploratory analyses that assess the correlation between L9LS serum concentrations and *P. falciparum* infection risk over a 9-month period. Since these participants received study agent earlier than participants in the efficacy study, they are expected to have lower serum L9LS concentrations at the end of the malaria season compared to participants in the efficacy study, which will allow for correlations of malaria risk over a broader range of L9LS concentrations.

Study Extension Procedures

Pediatric participants who are enrolled in the Dose Escalation study will be offered participation in the extension study visits and undergo an informed consent process (see section 10.1.1.2) with the ICF addendum. Those that agree to participate will be enrolled and begin monthly Extension study visits at the MRTC clinic in Kalifabougou or Torodo. At each study visit, up to 1.0 mL of blood will be collected by venipuncture for blood smear, Pf RT-PCR, possible parasite genotyping in the case of a positive malaria parasite infection (see section 8.2.3), and measurement of L9LS concentrations in serum. If a participant develops symptoms of malaria or other symptoms, they will be asked to return for an illness visit (see section 8.2.1).
AEs related to the blood collection procedures and all SAEs will be collected and followed through resolution.

Participants will attend monthly study visits through Day 252, for a total of 2 monthly extension visits.

Compensation

Subjects will be compensated 3,000 Communauté Financière Africaine (CFA) Franc for each study visit for the time and inconvenience of participation. Payment will be provided in cash after the completion of each visit.

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 sections 8-10 for additional information related to safety definitions and reporting, data evaluation, and human subject protection procedures.