Abbreviated Title: L9LS MAb in Malian Infants

Faculté de Médecine Pharmacie d'Odontostomatologie (FMOS) Protocol #: 2024/158/CE/USTTB

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Title: A Phase 1b, Randomized, Double-Blind, Placebo-Controlled Trial to Assess the Safety, Tolerability, and Pharmacokinetics of L9LS in Infants in Mali and to Evaluate the Impact of L9LS on Subsequent R21/Matrix-M^{TM} Vaccine Immunogenicity

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Investigational Agent:

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

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

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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*
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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Council on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (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 form(s), recruitment materials, and all participant materials will be submitted to the FMOS Ethics Committee (EC) for review and approval. Approval of both the protocol and the consent forms must be obtained before any participant 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; an EC 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: A Phase 1b, Randomized, Double-Blind, Placebo-Controlled Trial to Assess the Safety, Tolerability, and Pharmacokinetics of L9LS in Infants in Mali and to Evaluate the Impact of L9LS on Subsequent R21/Matrix-M™ Vaccine Immunogenicity

Study Description: This is an age-stratified, randomized, double-blind, placebo-controlled trial evaluating the safety, tolerability, and pharmacokinetics (PK) of 1-time intramuscular (IM) administration of the monoclonal antibody (MAb) L9LS to healthy Malian infants aged 1 to 12 months, followed by an assessment of the impact of L9LS on the immunogenicity of subsequent administration of the R21/Matrix-M™ vaccine. The study hypotheses are that L9LS will be safe and will not impact the immunogenicity of the R21/Matrix-M™ vaccine. During the beginning of the 6-month malaria season (approximately August and September at the study site), 180 participants will be enrolled and randomized 1:1 to 150 mg of L9LS (n=90) or normal saline placebo (n=90). Randomization of participants in each arm will be age-stratified (1 to 4 months, n=60; ≥5 to 8 months, n=60; ≥9 to 12 months, n=60). The safety of L9LS will be assessed within each of the 3 age strata. Participants will be followed at study visits 1, 3, 7, 14, 21, and 28 days later, and once every 4 weeks thereafter through study day 280 (40 weeks). Approximately 5 months after receiving L9LS or placebo, all participants will receive the R21/Matrix-M™ vaccine as 3 total doses given 4 weeks apart as per World Health Organization (WHO) recommendations and the anticipated Malian vaccination guidelines. Primary study assessments include medical history, physical examination, and blood collection to assess antibody responses to the R21/Matrix-M™ vaccine, L9LS PK, anti-drug antibody (ADA) assessments, identification of *Plasmodium falciparum* (Pf) infection by microscopic examination of thick blood smears and reverse transcription polymerase chain reaction (RT-PCR), and other research laboratory evaluations. Through their local provider, all participants 3 months and older will be offered 4 rounds of seasonal malaria chemoprevention (SMC) as a monthly 3-day treatment course of sulfadoxine-pyrimethamine plus amodiaquine (SPAQ), as it is the standard of care in Mali for malaria prevention in children 3 months to 5 years of age. Parents/guardians of participants may be re-contacted after their study participation has ended to answer questions regarding use of SMC.

Objectives:

Primary Objectives:

1. To evaluate the safety and tolerability of L9LS administered IM at 150 mg in healthy Malian infants.
2. To evaluate the PK of L9LS throughout the study at a dose of 150 mg administered IM to healthy Malian infants.

Secondary Objectives:

1. To evaluate the impact of L9LS or placebo on the immunogenicity of subsequent R21/Matrix-M™ vaccination in healthy Malian infants.
2. To evaluate the risk of hypersensitivity reactions [an adverse event of special interest (AESI)] following the R21/Matrix-M™ vaccine when administered 20, 24, and 28 weeks after L9LS/placebo administration.
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.

Exploratory Objectives:

1. To determine if IM administration of L9LS at 150 mg (compared to placebo) mediates protection against clinical malaria in healthy Malian infants 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.
2. To determine if IM administration of L9LS at 150 mg (compared to placebo) mediates protection against clinical malaria in healthy Malian infants 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.
3. To determine if IM administration of L9LS at 150 mg (compared to placebo) mediates protection against naturally occurring Pf infection in healthy Malian infants during a single malaria season as detected from microscopic examination of thick blood smears.
4. To determine if IM administration of L9LS at 150 mg (compared to placebo) mediates protection against naturally occurring Pf infection in healthy Malian infants during a single malaria season as detected by RT-PCR.
5. To correlate L9LS serum concentration with clinical malaria risk.
6. To correlate L9LS serum concentration with Pf infection risk.

7. To assess hemoglobin levels throughout the study in healthy Malian infants who receive IM administration of L9LS at 150 mg or placebo.

Endpoints:

Primary Endpoints:

1. Incidence and severity of local and systemic adverse events (AEs) occurring within 7 days after the administration of L9LS.
2. Measurement of L9LS in sera of recipients.

Secondary Endpoints:

1. Total IgG anti-NANP antibody titers measured by electrochemiluminescence immunoassay (ECLIA) 28 days and 84 days after the third R21/Matrix-M^{TM} vaccination.
2. Incidence and severity of hypersensitivity reactions occurring within 7 days after the administration of each dose of R21/Matrix-M^{TM} vaccine.
3. Measurement of ADA to L9LS in sera of recipients.

Exploratory Endpoints:

1. Incidence of clinical malaria (definition 1, see section 2.3.4) between 2 and 24 weeks after administration of L9LS or placebo.
2. Incidence of clinical malaria (definition 2, see section 2.3.4) between 2 and 24 weeks after administration of L9LS or placebo.
3. Pf blood-stage infection as detected by microscopic examination of thick blood smears obtained between 2 and 24 weeks after administration of L9LS or placebo.
4. Pf blood-stage infection as detected by RT-PCR between 2 and 24 weeks after administration of L9LS or placebo.
5. PK analysis of L9LS and the association of L9LS concentration with clinical malaria risk.
6. PK analysis of L9LS and the association of L9LS concentration with Pf infection risk.
7. Hemoglobin levels.

Study Population: 180 (accrual ceiling 360) Healthy Malian infants aged 1 to 12 months residing in Kalifabougou, Faladje, and Torodo

Phase: 1b

Description of Sites/Facilities Enrolling Participants:	The MRTC clinics in Kalifabougou, Faladje, and Torodo. All day 0 (study agent administration) activities and R21/ Matrix-M™ vaccination visits will be performed at Kalifabougou only. All 3 sites will conduct recruitment, screening, follow-up visits, and unscheduled study visits.
Description of Study Intervention:	The study agent will be administered as a 1-time IM injection of L9LS at a dose of 150 mg (1 mL) or matching normal saline placebo (1 mL).
Study Duration:	3 years
Participant Duration:	40 weeks

1.2 Schema

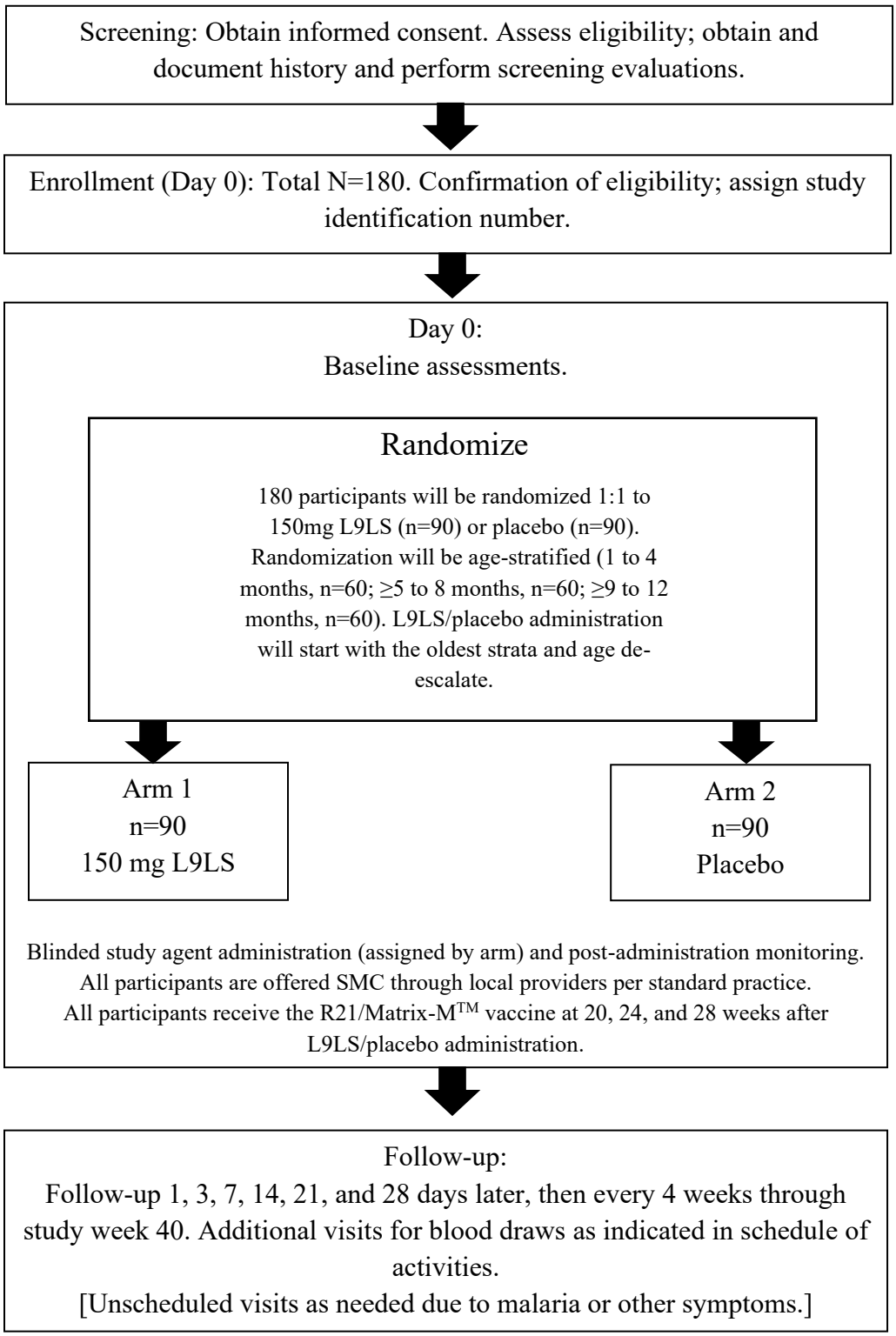


Figure 1. Study flow diagram.

1.3 Schedule of Activities (SOA).

Study Day		**Screening**	**Enrollment /Baseline** [1]	**1**	**3**	**7**	**14**	**21**	**28**	**56**	**84**	**112**	**140**	**147**	**168**	**175**	**196**	**203**	**224**	**252**	**280** [2]	**Illness Visit**	**ET**
Window (days)		-56 to 0	0	+1	±1	±2	±3	±3	±3	±7	±7	±7	±7	±1	±7	±1	±7	±1	±7	±7	±7		
Clinical Procedures/Evaluations																							
Study comprehension exam		X																					
Informed consent		X																					
Confirmation of identity, age, and residency		X	X																				
Physical exam [3]		X	X	X	X	X	X	X	X	X	X	X	X	X[13]	X	X[13]	X	X[13]	X	X	X	X	X
Length		X	X						X	X	X	X	X		X		X		X	X	X		
Weight		X	X						X	X	X	X	X		X		X		X	X	X		
Vital signs (temperature and pulse)		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Medical history [3]		X	X	X	X	X	X	X	X	X	X	X	X	X[13]	X	X[13]	X	X[13]	X	X	X	X	X
Concomitant medications [4]		X	X	X	X	X	X	X	X	X	X	X	X		X		X		X	X	X	X	X
Randomization			X																				
Study agent administration			X [5]																				
R21 administration [6]													X		X		X						
Adverse events assessment			X	X	X	X	X	X	X	X	X	X	X	X[13]	X	X[13]	X	X[13]	X	X	X	X	X	X
Laboratory Evaluations																							
Test	Tube	(Blood volume in mL)																					
HIV screen [7]	SST	(X)																					
CBC with differential	EDTA	1	1 [8]			1																1[12]	1[12]
Hemoglobin type [9]	EDTA	(X)																					

Study Day		**Screening**	**Enrollment /Baseline [1]**	**1**	**3**	**7**	**14**	**21**	**28**	**56**	**84**	**112**	**140**	**147**	**168**	**175**	**196**	**203**	**224**	**252**	**280 [2]**	**Illness Visit**	**ET**
Window (days)		-56 to 0	0	+1	±1	±2	±3	±3	±3	±7	±7	±7	±7	±1	±7	±1	±7	±1	±7	±7	±7		
ALT, Cr	SST	(X)	(X) [8]			(X)																(X)[12]	(X) [12]
Blood smear and dried blood spot for Pf RT-PCR [10]	-		(X)			(X)	0.2	0.2	(X)	0.2	(X)	0.2	(X)		0.2		(X)		(X)	0.2	(X)	0.2	0.2
Hemoglobin [11]									(X)		(X)		(X)				(X)				(X)		
PK studies	SST		2 [8]			2			2		2		2				2				2		
R21 serology	SST		(X)																2		(X)		
ADA	SST		(X)						(X)										(X)		(X)		
Daily volume (mL)		**1**	**3**	**-**	**-**	**3**	**0.2**	**0.2**	**2**	**0.2**	**2**	**0.2**	**2**	**-**	**0.2**	**-**	**2**	**-**	**2**	**0.2**	**2**		
Cumulative volume (mL)		**1**	**4**	**4**	**4**	**7**	**7.2**	**7.4**	**9.4**	**9.6**	**11.6**	**11.8**	**13.8**	**13.8**	**14**	**14**	**16**	**16**	**18**	**18.2**	**20.2**	**-**	**-**

Abbreviations: ADA, anti-drug antibodies; ALT, alanine transaminase; CBC, complete blood count; Cr, creatinine; EDTA, ethylenediaminetetraacetic acid; ET, early termination; HIV, human immunodeficiency virus; 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 (study agent administration) activities will be performed at Kalifabougou only. All 3 sites will conduct recruitment, screening, follow-up visits, and unscheduled study visits. All R21 vaccination visits will be performed at Kalifabougou only.
- At any time during the study, a participant may have an unscheduled illness visit if experiencing malaria symptoms or other symptoms. The participant may be referred for standard care according to local guidelines. Unscheduled illness visits may take place in Kalifabougou, Faladje, or Torodo, depending on the participant's residence.

Footnotes:

[1] If enrollment is within 2 days of screening, duplicate procedures will not be repeated.

[2] Participants may be re-contacted after completing study participation for a brief survey regarding use of SMC.

[3] Complete/comprehensive at screening; targeted/interim at other visits. Medical history will include past and current anti-malaria practices (eg, insecticide-treated bed nets and SPAQ chemoprophylaxis).

[4] Including all vaccines received.

[5] All other study procedures must be completed prior to study agent administration. Each participant 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, participants will be assessed for local reactogenicity (including pain/tenderness, swelling, redness, bruising, and pruritus at the site of injection) and systemic reactogenicity (including fever, irritability/fussiness, loss of appetite, diarrhea, vomiting, and rash). If a participant 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.

[6] The R21/Matrix-M vaccine will be given in accordance with national public health vaccination guidelines for dose and schedule.

[7] HIV screening of the infant will only be done if the mother does not have a documented HIV test result that was done during her pregnancy with the infant. If required, HIV screening of the infant will be performed according to international guidelines. HIV testing will be 2 RDTs. If the RDTs are both positive or discordant, the participant will be referred for confirmatory ELISA and further medical assessment with confirmatory testing. Pre- and post-test HIV counseling will be provided.

[8] Collected prior to study agent administration.

[9] Hemoglobin typing will be performed at screening using the EDTA tube and will be used in eligibility assessments.

[10] The blood smear and dried blood spots can be prepared from a heel stick, finger prick, or venipuncture. 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.

[11] Measured by Hemocue machine.

[12] Labs may be drawn at illness visits and early termination visits if deemed necessary after Investigator assessment.

[13] Focused medical history and physical exam to assess for serum sickness symptoms: fever, arthralgia, myalgia, skin eruptions, lymphadenopathy, marked discomfort, and/or dyspnea.

2 INTRODUCTION

2.1 Study Rationale

Plasmodium falciparum causes over 600,000 malaria deaths annually, mostly among young children in Africa. 1 Despite widespread use of mosquito-control measures, targeted chemoprevention, and case management with rapid diagnostic tests and anti-malarial drugs, little progress has been made in reducing malaria deaths in recent years, 1 a trend that could worsen with increasing resistance to anti-malarial drugs 2,3 and insecticides. 4 Thus, it is critical to develop new interventions to reduce malaria mortality.

In 2021, the WHO recommended the RTS,S/AS01 vaccine for children, 4 doses of which demonstrated 36% efficacy against malaria over 4 years in children aged 5 to 17 months. 5 In 2023, the WHO also recently endorsed the R21/Matrix-M™ vaccine for children. Where monthly SMC is standard of care during the 4 to 6-month malaria season, a 3-dose regimen of R21/Matrix-M showed 75% efficacy over 12 months in children aged 5 to 36 months, and a booster after 12 months maintained efficacy over the subsequent 6 months.6 This efficacy is similar to RTS,S/AS01when given seasonally. 7,8 The WHO also recommends malaria chemoprevention for high-risk populations, such as infants and young children who reside in areas of moderate to high malaria transmission. 1 Although malaria chemoprevention is safe and efficacious in children, 9 achieving high-level coverage of frequent dosing regimens is challenging. 10

Of particular concern are infants and children in the first year of life since malaria mortality peaks in children under 1 year of age in moderate to high transmission settings (Figure 2), 11 especially after 3 months of age when the protective effect of maternal antibodies and fetal hemoglobin begins to wane. 12 Since infants are not eligible for vaccination with RTS,S/AS01 or R21/Matrix-M™ until 5 months of age (3 doses given at months 5, 6, and 7), and vaccine-induced efficacy is not fully established until approximately 1 month after the third vaccine dose, children are particularly vulnerable to malaria from approximately 3 to 8 months of age. The standard of care for malaria prevention in this age group includes insecticide-treated nets (ITNs) and chemoprevention. For example, in areas of seasonal malaria, SMC is recommended for children aged 3 months to 5 years and consists of monthly treatment courses of SPAQ during the 4- to 6-month malaria season. As noted above, it is challenging to achieve high-level coverage of frequent chemoprevention dosing regimens in this population, 10 and the effectiveness of chemoprevention and ITNs is threatened by the emergence and spread of drug-resistant parasites 2,3 and insecticide-resistant mosquitos, 4 respectively. Therefore, new drugs, including monoclonal antibodies, that prevent malaria for up to 6 months after a single dose could improve prevention coverage in this vulnerable population.

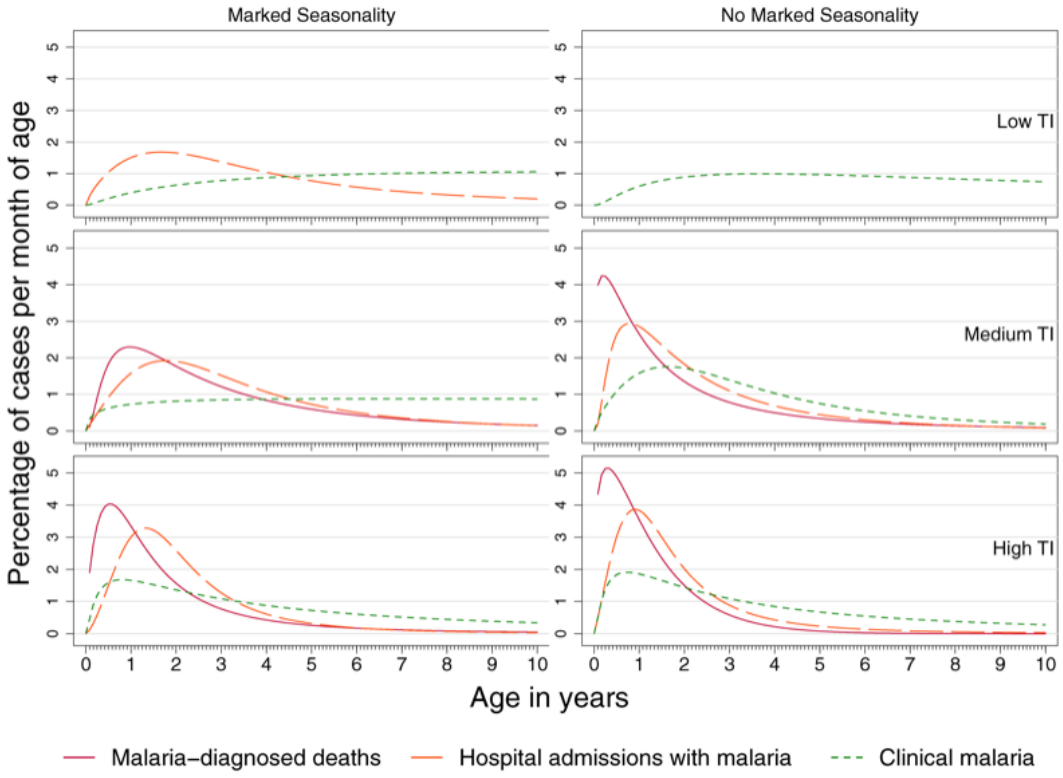


Figure 2. Age-patterns of P. falciparum malaria in Sub-Saharan Africa.

Percentage of uncomplicated clinical malaria, hospital admissions with malaria and malaria-diagnosed deaths per month of age in children under 10 years of age, by transmission intensity (TI) and seasonality of malaria transmission. 11

L9LS is a monoclonal antibody with an extended half-life that targets a highly conserved junctional Pf circumsporozoite protein (CSP) epitope. 13 In a phase 1 trial, adult participants received L9LS either intravenously or subcutaneously at a dose of 1 mg, 5 mg, or 20 mg/kg. Of the 17 participants who received a single dose of L9LS, 15 (88%) were protected after controlled human malaria infection. Parasitemia did not develop in any of the participants who received 5 or 20 mg/kg of intravenous L9LS. Parasitemia developed in 1 of 5 participants who received 1 mg/kg intravenously, 1 of 5 participants who received 5 mg/kg subcutaneously, and all 6 control participants through 21 days after the controlled human malaria infection. 14 A recently completed phase 2 trial assessed the safety and efficacy of subcutaneous administration of L9LS in children aged 6 to 10 years in Mali over a 6-month malaria season. L9LS was found to be safe, and compared to placebo, the efficacy of 300 mg of L9LS was 69.9% against Pf infection (adjusted 95% CI, 50.1-81.9; P<0.001) and 77.4% against clinical malaria (adjusted 95% CI, 55.0-88.7; P<0.001). 15 In addition, as described in section 2.4.1, there have been no evident safety concerns in an ongoing phase 2 trial of L9LS in Kenya involving children aged 5 months to 10 years.

These results provide a rationale for assessing the safety and efficacy of a single dose of L9LS in infants to provide protection against malaria before they become eligible for vaccination with RTS,S/AS01 or R21/Matrix-M ^{TM}. Since L9LS targets the PfCSP protein, the same protein included in the RTS,S/AS01 or R21/Matrix-M^{TM} vaccines, it is important to assess for potential interaction between L9LS and PfCSP-based vaccines. Since R21/Matrix-M^{TM} is more likely to be widely deployed in the coming years, this study will assess the safety, tolerability, and PK of a 1-time IM administration of L9LS to healthy Malian infants aged 1 to 12 months, followed by an assessment of the impact of L9LS on the immunogenicity of the R21/Matrix-M^{TM} vaccine in the same children. Of note, the anti–SARS-CoV-2 monoclonal antibody bamlanivimab minimally affected the endogenous immune response to COVID-19 vaccination. 16 Findings like these led the CDC to recommend against delaying COVID-19 vaccination for individuals who had received anti–SARS-CoV-2 monoclonal antibodies.

However, as enrollment in this protocol neared completion, a paper was published that described the first prospective randomized controlled trial in humans to specifically address the question of whether mAbs that target protein epitopes present in a vaccine would alter endogenous immune responses to that vaccine. 17 The trial assessed the potential interaction of various doses of a combination of two mAbs (casirivimab and imdevimab) that targets the SARS-CoV-2 spike protein with Moderna's mRNA-1273 SARS-CoV-2 vaccine, in healthy SARS-CoV-2 seronegative adults. All vaccinated participants developed neutralizing antibodies to SARS-CoV-2. However, neutralizing titers were decreased up to 4-fold when serum concentrations of casirivimab and imdevimab were as low as ~10 ug/mL at the time of vaccination. Minimal reduction in vaccine-induced neutralization titers was observed when mAb serum concentrations were <10 ug/mL at the time of vaccination. Across all mAb dose groups there was minimal effect on vaccine-induced total binding antibodies to the vaccine protein and T-cell responses to the spike protein. Co-administration of casirivimab and imdevimab plus mRNA-1273 was generally safe and well tolerated. A slight increase in treatment-emergent adverse events (TEAEs) was observed in the casirivimab and imdevimab plus vaccine groups versus the vaccine-only group. An increase in TEAEs related to mRNA-1273 vaccination was observed in participants treated with casirivimab and imdevimab, driven by increased reactions at the mRNA-1273 injection site. Treatment-emergent serious adverse events were rare and occurred at

similar rates between the casirivimab and imdevimab plus vaccine and the vaccine-only groups. [17]

In this protocol, the R21/Matrix-M™ vaccine will be administered at 20, 24, and 28 weeks after L9LS/placebo administration to ensure that study participants initiate the vaccine series during the recommended age range of 5 to 17 months. Preliminary population PK modeling of the phase 2 trial of L9LS in Malian children aged 6 to 10 years suggests that the serum concentration of L9LS 20 weeks after administering a 150 mg dose will be ≤10 µg/mL. As noted above, casirivimab/imdevimab serum concentrations of <10 ug/mL had minimal impact on vaccine immunogenicity; however it is not known whether this concentration of L9LS would impact immunogenicity following R21 vaccination.

Although L9LS binds to a specific epitope on PfCSP that is not contained in the RTS,S/AS01 or R21/Matrix-M™ vaccines (Figure 3) [13], in data on file, L9LS shows some binding to R21 in vitro. In rodents, the administration of L9LS prior to vaccination with R21 did not statistically significantly alter protective efficacy against malaria challenge; however, there was a clear trend toward lower R21 efficacy when high doses of L9LS were given prior to vaccination (corresponding to L9LS serum concentrations of >50 µg/mL at the time of vaccination) whereas R21 efficacy was unaffected at lower doses of L9LS when L9LS serum concentrations were <50 µg/mL at the time of R21 vaccination.

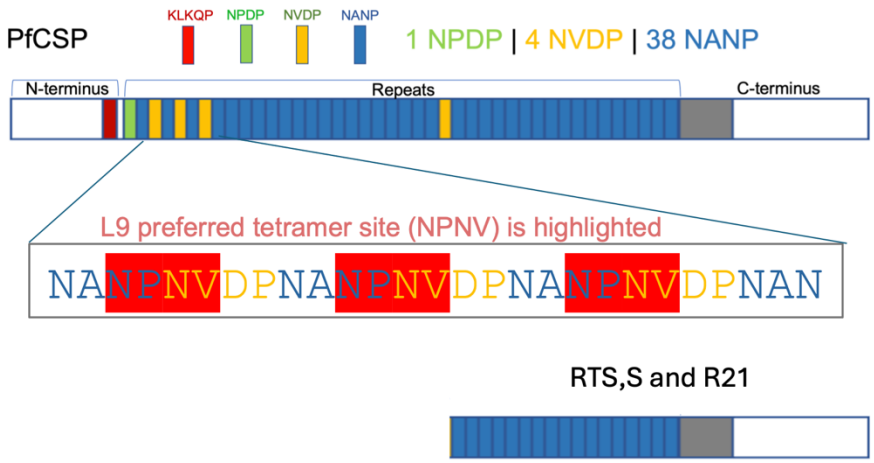


Figure 3. L9LS binds a PfCSP epitope that is not contained in the R21 and RTS,S vaccines.

L9LS binds with high affinity to a 27mer epitope of PfCSP that contains 3 NPNV motifs. This epitope is not contained in the R21 or RTS,S malaria vaccines, both of which only contain the C-terminus and 19 NANP repeats.

Pharmacodynamic modeling of the phase 2 trial of L9LS in Malian children aged 6 to 10 years is ongoing and therefore the relationship between L9LS serum concentration and protection from malaria in this population remains to be determined; however, in the phase 1 trial of L9LS in US adults, L9LS serum concentrations as low as 9.2 µg/mL were associated with protection from controlled Pf infection.[14]

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 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. 18 In a recent phase 2 trial involving healthy adults in Mali, the safety and efficacy of a single intravenous (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 Pf 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. 19 (NCT04329104; FMOS/FAPH Protocol Number: 2020/32/CE/FMOS/FAPH).

L9, the wild-type parent of L9LS, was isolated by sorting the PfCSP-reactive memory B cells obtained from a participant 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. Of note, this specific epitope is not contained in the R21/Matrix-M vaccine and thus there is little binding of L9LS to R21. 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 effective dose in 80% of the population and effective concentration in 80% of the population 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). 13 The unique preference of L9 for NPNV motifs was further underscored by the fact that all 4 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 1 or more NVDP motifs, these data suggest that L9 should bind all circulating strains of Pf malaria. 13

An important aspect of using MAbs to prevent malaria is to increase their durability in vivo. Thus, 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 2 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 identified 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 malaria 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 for Nonclinical Laboratory Studies.

2.2.1.2.1 TCR Study

The TCR assay screened a standard panel of normal human tissues (3 donors per tissue) and Sprague Dawley rat tissues (2 rats per tissue). Mammalian cells transfected to express CSP were used as a tissue-positive control. Two concentrations of L9LS were tested: 1.15 µg/mL (selected as the concentration which saturated the positive control tissue), and 11.5 µg/mL (10-fold excess). A negative control IgG1κ antibody (GR338422-1, no mammalian target antigen) was tested at the same 2 concentrations. L9LS exhibited scattered specific membrane binding in 3 of 3 human salivary gland tissue samples and localized to the epithelial cells lining the ducts and acini. Binding was rare (1 to 5% of these epithelial cells) at 1.15 µg/mL, and rare to occasional (5 to 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 subcutaneous (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 (eg, 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 (ie, 2-fold increase in both heart weight and heart-body weight compared to the control means), with normal heart histology. Spontaneous cardiomyopathies are occasionally observed for the Sprague Dawley strain of rat. Based on the singular incidence, this observation is not considered treatment related.

With reference to the US Food and Drug Administration (FDA) Center for Drug Evaluation and Research 2005 Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial

Clinical Trials for Therapeutics in Adult Healthy Volunteers, scaling based on body weight is appropriate for a MAb expected to distribute mainly in the vascular space. The rat IV NOAEL of 400 mg/kg x2 supports the IV clinical high dose with a 20-fold dose margin. The rat SC NOAEL of 100 mg/kg x2 supports the SC clinical dose with a 20-fold dose margin.

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

2.2.1.3 Clinical Experience

The first-generation anti-malaria MAb CIS43LS was shown to be safe and conferred complete protection against experimental controlled human malaria infections (CHMI) in a phase 1 study in healthy adult volunteers at the VRC. 18 In the phase 1 study, participants 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. 19 (ClinicalTrials.gov Identifier: NCT04329104; FMOS/FAPH Protocol Number: 2020/32/CE/FMOS/FAPH). Together, the phase 1 and 2 trials of CIS43LS provided proof of concept that a MAb with an extended half-life can safely confer protection against Pf infection.

L9LS was 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 2-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 measured at the time of the CHMI. 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. 14

Unlike CIS43LS, which was administered IV in the Mali phase 2 trial, L9LS was administered SC in children based on its potential higher potency and higher concentration. As a follow-up to the VRC phase 1 trial of L9LS, a phase 2 trial was conducted to evaluate the safety and efficacy of L9LS administered SC in healthy children aged 6 to 10 years in Mali, where Pf malaria infection is endemic. Unlike children aged 1 to 5 years in Mali, who receive SMC as standard of care, SMC is not currently recommended in children aged 6 to 10 years in Mali, although it is recommended for this age group in neighboring Senegal and is being considered by other

countries where malaria is seasonal. In the first year of the study, Part A assessed safety and tolerability at 3 dose levels in adults, followed by 2 dose levels in children. In Part B, children were randomly assigned to receive 150 or 300 mg of L9LS (weight-based dose range of 5 to 20 mg/kg), or placebo. The primary efficacy endpoint was time to first infection detected on blood smear performed every 2 weeks for 24 weeks. A secondary efficacy endpoint was time to first clinical malaria episode. In Part B, 225 children were randomized and 75 were assigned to each group. L9LS was well-tolerated, and no serious AEs occurred. Infections occurred in 30 (40.0%), 36 (48.0%), and 61 (81.3%) children who received 300 or 150 mg of L9LS, or placebo, respectively. Compared to placebo, L9LS efficacy against infection (Figure 4) was 69.9% for 300 mg (adjusted 95% CI, 50.1-81.9; P<0.001) and 66.0% for 150 mg (adjusted 95% CI, 45.0-79.0; P<0.001); and efficacy against clinical malaria (Figure 5) was 77.4% for 300 mg (adjusted 95% CI, 55.0-88.7; P<0.001) and 67.0% for 150 mg (adjusted 95% CI, 39.0-82.0; P<0.001). Thus, SC administration of L9LS to children was safe and protective against Pf infection and clinical malaria over 6 months (ClinicalTrials.gov number, NCT05304611). 15 A second-year extension of this trial is ongoing to evaluate the safety and tolerability of a second SC dose of L9LS administered at 150 mg and 300 mg (compared to placebo), and to evaluate the PK and ADA risk of a second dose of L9LS.

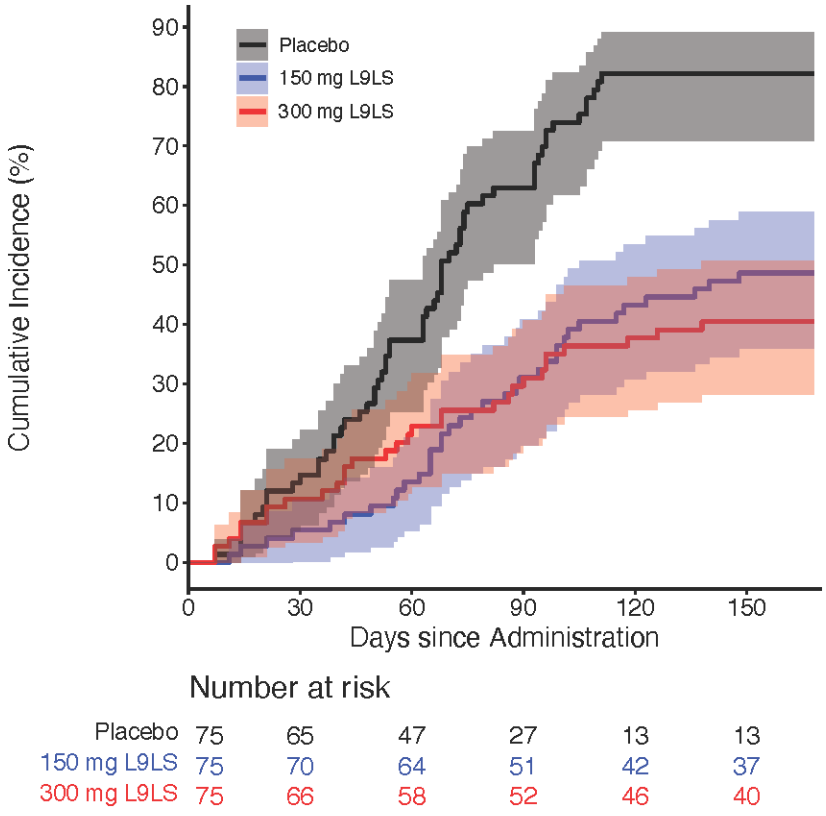


Figure 4. Efficacy of L9LS against P. falciparum infection among Malian children aged 6 to 10 years.

Shown is the cumulative incidence of the first Pf blood-stage infection during a 6-month malaria season (irrespective of symptoms being present) after a single subcutaneous injection of 150 mg of L9LS, 300

mg of L9LS, or placebo. Pf infections were detected by microscopic examination of thick blood smears collected during scheduled trial visits and unscheduled illness visits. Blood smears were collected before the administration of L9LS or placebo on day 0 and then on days 3, 7, 14, 21, and 28 and every 2 weeks thereafter for a total of 24 weeks. Only blood smears collected between weeks 1 and 24 were included in the efficacy analysis. Shaded areas indicate the 95% confidence intervals.

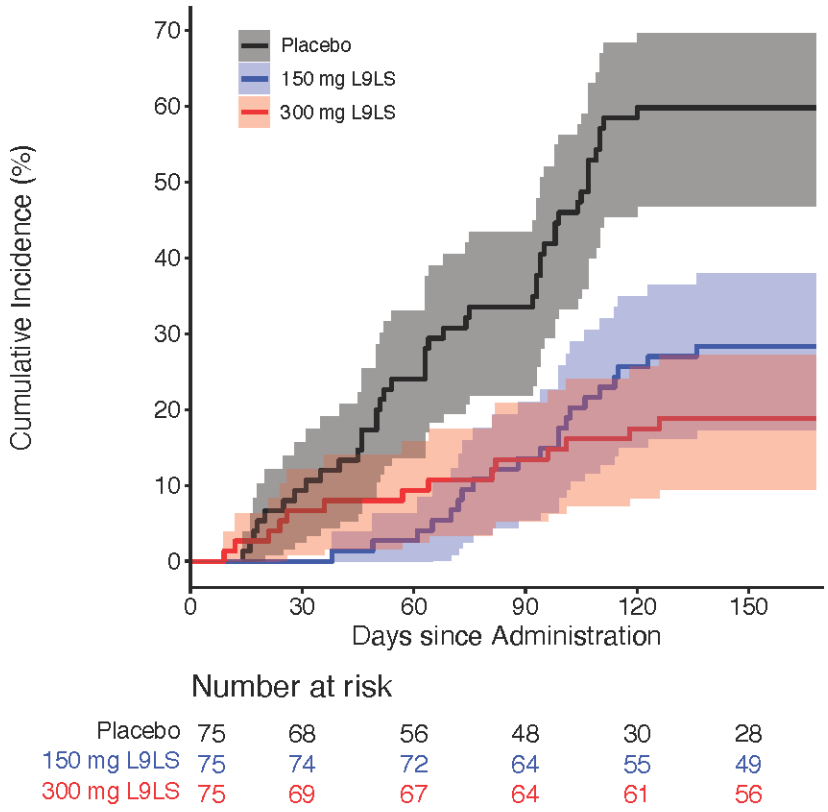


Figure 5. Efficacy of L9LS against clinical malaria among Malian children aged 6 to 10 years.

Shown is the cumulative incidence of the first clinical malaria episode due to Pf infection during a 6-month malaria season after a single subcutaneous injection of 150 mg of L9LS, 300 mg of L9LS, or placebo. The pre-specified definition of clinical malaria was an illness accompanied by measured axillary fever ≥37.5°C, or history of fever (subjective or objective) in the previous 24 hours, and *P. falciparum* asexual parasitemia >5,000 parasites/μL as detected from microscopic examination of thick blood smear. Clinical malaria episodes were detected during scheduled trial visits and unscheduled illness visits. Only clinical malaria episodes occurring between weeks 1 and 24 were included in the efficacy analysis. Shaded areas indicate the 95% confidence intervals.

In addition, an ongoing phase 2 trial in Kenya, initiated in September 2022, is evaluating the safety and tolerability of 1-time SC administration of L9LS in healthy children aged 5 months to 10 years, as well as the safety and protective efficacy of up to 2 doses of L9LS against Pf infection among children aged 5 to 59 months, in a setting of perennial high transmission, at 6-month intervals over 48 months of follow-up. Before study agent administration, all subjects are given dihydro-artemisinin-piperaquine to clear any preexisting Pf blood-stage infection. Part 1 of

this trial is an age de-escalation and dose-escalation study to assess the safety and tolerability of L9LS in children aged 5 months to 10 years at a dose range of 5-20 mg/kg. Part 2 of the trial is an efficacy study in which a total of 324 children 5 to 59 months are randomized to receive a 10 to 20 mg/kg dose of L9LS or placebo by SC administration. Participants are followed over 12 months with monthly blood smear microscopy and RT-PCR and twice-monthly symptomatology and care-seeking behavior questionnaires. Dosing is based on 3 weight bands; all doses are administered SC with fixed doses of 75 mg L9LS, 150 mg L9LS, or 225 mg L9LS, resulting in a range of 10 to 20 mg/kg. Participants in the L9LS arm are randomized 1:1 at baseline to receive either a second L9LS injection or a placebo injection to evaluate the additional efficacy of a second dose administered 6 months after the first dose. Those in the placebo arm receive a second injection of placebo (ClinicalTrials.gov number, NCT05400655). A summary of currently available safety data from this ongoing trial is provided in section 2.4.1. The complete unblinded safety and efficacy results from this trial are expected to be available in Q4 2024.

2.3 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.

2.3.1 Measurement of anti-NANP IgG antibody titers

A secondary objective of this study is to evaluate the immunogenicity of the R21/Matrix-M™ vaccine in healthy Malian infants after receiving 150 mg of L9LS or placebo. The endpoint for this objective is total IgG anti-NANP antibody titers measured by ECLIA, using the Meso Scale Discovery LLC–based automation platform, 28 days and 84 days after the third R21/Matrix-M™ vaccination. The measurement of total IgG anti-NANP antibody titers aligns with the primary immunogenicity endpoint for the R21/Matrix-M™ vaccine trials in Africa, and importantly, total IgG anti-NANP antibody titers are the only known correlate of R21/Matrix-M™ vaccine efficacy. 20 An L9LS anti-idiotype antibody that prevents L9LS from binding to PfCSP will be used to distinguish and quantify R21 vaccine-induced IgG responses. Anti-NANP R21 serology detecting only the IgG3 subtype present in the sample will also be performed as an exploratory endpoint.

2.3.2 PK Analysis

Serum concentrations of L9LS will be measured with an L9LS anti-idiotype antibody and a Meso Scale Discovery LLC–based automation platform using similar methodology as previously described for other VRC MAb products. 21 In brief, L9LS PK is measured in serum using an

MSD ECLIA 384-well plate assay with an optimized concentration of the L9LS anti-idiotype antibody, followed by emitter-coupled logic signal measurement with the MSD Sector Imager 600. The assay is qualified to meet assay precision and linearity criteria and was found to have a lower limit of detection (LLOD) of 610 ng/mL at a minimum required dilution of 1:100.

2.3.3 Detection of Anti-Drug Antibodies

Assays for detection of ADAs 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. 21 In brief, L9LS ADA is detected in serum using a 3-tier MSD assay. Tier 1 is a screening assay that involves bridging incubation with test serum, labeled L9LS, and biotinylated L9LS, followed by binding to a streptavidin-coated plate. The tier 1 assay is qualified to meet assay precision criteria and demonstrated a LLOD of 0.00019 µg/mL using L9LS anti-idiotype antibody as surrogate ADA that is identified as tier 1 positive. Tier 2 is a competition assay that involves pre-incubation of test serum with unlabeled L9LS, followed by the bridging assay used in Tier 1. The tier 2 assay is qualified to meet assay precision criteria and demonstrated a LLOD of 0.00038 µg/mL using L9LS anti-idiotype antibody as surrogate ADA that is identified as tier 2 positive. Tier 3 is a surrogate functional assay that involves incubating test serum on L9LS-coated plates followed by incubation with labeled PfCSP. The tier 3 assay is qualified to meet assay precision criteria and demonstrated a LLOD of 0.3906 µg/mL using L9LS anti-idiotype antibody as surrogate ADA that is identified as tier 3 positive.

2.3.4 Measures of MAb-Mediated Protection Against Pf infection and Clinical Malaria

In exploratory analysis, L9LS-mediated protection against naturally occurring Pf infection will be assessed and compared to control participants. The exploratory endpoint defining L9LS-mediated protection from Pf infection is the absence of Pf parasites in blood samples obtained from L9LS-recipients collected between 2 and 24 weeks after administration of L9LS or placebo. The criteria for a case of Pf infection are 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. Giemsa-stained thin blood films will also be prepared and examined by trained personnel to determine the *Plasmodium* species present. Thick and thin 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 an additional exploratory endpoint, L9LS-mediated protection against clinical malaria will be assessed and compared to control participants between 2 and 24 weeks after administration of L9LS or placebo. The 2 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. 7,22

Clinical malaria will be assessed by both passive surveillance (ie, unscheduled sick visits) and active surveillance (eg, medical history and physical exam at all scheduled visits, and blood smear at most 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, as part of another exploratory endpoint, *Plasmodium* 18S ribosomal ribonucleic acid (rRNA) RT-PCR, will be performed by Dr. Sean Murphy, University of Washington. [23] 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. Dried blood spots are collected on filter paper, sealed in plastic envelopes with desiccant, and stored at -80°C in Mali and then at room temperature in the US.

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

2.4 Risk/Benefit Assessment

2.4.1 Known Potential Risks

Risks of L9LS: As summarized below, L9LS safety data is derived from 4 clinical studies, 1 completed and 3 ongoing. Although all 4 studies contribute to the safety database with 753 cumulative individuals having been exposed to L9LS globally, children aged 5 months to 11 years who reside in Mali or Kenya comprise the majority of L9LS exposures.

VRC 614 (NCT05019729) was a phase 1, dose escalation, open-label trial with experimental CHMI conducted in 2021 that evaluated the safety and efficacy of L9LS in healthy malaria-naive adults in the US. Twenty-three subjects received 1 dose of L9LS administered at 1, 5, or 20 mg/kg IV (n=13 subjects), 5 mg/kg SC (n=5 subjects), or 5 mg/kg IM (n=5 subjects). L9LS was safe and well-tolerated upon IV, SC, and IM administration, and there were no serious adverse events (SAEs). [14] Safety, tolerability, and PK data for the 5 mg/kg IM group (n=5 subjects), is detailed in section 4.3.

Protocol 2022/34/CE/USTTB (NCT05304611) is an ongoing 2-part, phase 2, dose-escalation, and age de-escalation trial initiated in 2022 that is evaluating the safety and tolerability of a 1-time SC (300 or 600 mg) or IV (20 mg/kg) administration of L9LS in healthy Malian adults (n=18) that is open-label, and SC (150 mg or 300 mg) administration of L9LS or placebo in healthy Malian children (n=36) is double-blind. The second part of the trial is evaluating the protective efficacy of L9LS SC (150 or 300 mg) versus placebo against Pf infection and clinical malaria over a 6-month malaria season in healthy children 6 to 10 years of age (n=225). The year 1 data is unblinded and overall, L9LS administrations were well-tolerated with all reported reactogenicity being mild to moderate in severity. There were no SAEs experienced during year 1. An ongoing year 2 extension of this study initiated in 2023 is evaluating the safety, PK, and ADA risk of a second dose of L9LS in the same children at the same dose range. For year 2, which remains blinded, 235 participants were randomized and dosed with study product (150 mg L9LS or 300 mg L9LS) or placebo. All product administrations are complete and follow up visits are ongoing. Overall, product administrations during year 2 were well-tolerated with all reported reactogenicity being mild to moderate in severity. There have been no SAEs reported during year 2.

Protocol KEMRI 4413 (NCT05400655) is an ongoing 2-part, double-blinded, placebo-controlled, phase 2, dose-escalation, and age de-escalation trial that started in 2022 that is evaluating the safety and tolerability of a 1-time SC administration of L9LS (5, 10, or 20 mg/kg) or normal saline placebo in healthy Kenyan children aged 5 to 59 months and 5 to 10 years (Part 1), as well as the protective efficacy of 1 or 2 SC administrations of L9LS (at the 10-20 mg/kg dose level range) or normal saline placebo against Pf infection among children aged 5 to 17 months and 18 to 59 months at enrollment, in a setting of perennial high transmission (Part 2). Part 1 is unblinded and Part 2 remains blinded. In Part 1 participants were randomized in a 3:1 ratio to receive L9LS or placebo. A total of 27 participants aged 5 to 10 years received L9LS (n=9 in each dose group of 5, 10, and 20 mg/kg) and 27 participants aged 5 to 59 months received L9LS (n=9 in each dose group of 5, 10, and 20 mg/kg). Product administrations were well tolerated. Overall, there were no clear trends or groupings of AEs by dose group and no SAEs were reported in Part 1. In the Part 2 efficacy study, all 324 enrolled participants have received the first and 302 the second (administered 6 months after dose 1) product administrations. Twenty-nine SAEs have been reported during this period, including 1 death that was considered unrelated to the study (thermal burn); all other SAEs were grade 3 and all were deemed unlikely related to the investigational product. Of note, based on data from the Mali trial suggesting that L9LS doses higher than 20 mg/kg may be needed to protect children from malaria for 12 months, the Kenya trial was extended for a second year to assess the safety and efficacy of L9LS at a dose range of 30 to 40 mg/kg. An initial safety study included 24 children aged 5 to 71 months, with 12 participants in 2 dose groups (30 mg/kg and 40 mg/kg) in a 3:1 ratio of L9LS to placebo. All participants are being followed for a total of 3 months. The safety study only includes children aged 5 to 71 months because this is the age range of children who will be invited to continue in the year 2 extension to assess L9LS efficacy at the higher dose range. Twelve participants received a SC dose of 30 mg/kg, and 12 received a SC dose of 40 mg/kg. Day 7 safety laboratory tests are available for 11 of 12 participants in the 30-mg/kg dose group (1 participant withdrew consent prior to the day 7 blood draw) and 12 of 12 participants in the 40-mg/kg group.

Of these 23 participants, 1 participant in the 30-mg/kg group had a grade 1 leukopenia that was not considered clinically significant and was associated with an upper respiratory tract infection (URTI), and 1 participant in the 40-mg/kg group had a mild grade 1 neutropenia, which was also not considered clinically significant. No solicited local or systemic reactogenicity was reported in the 30-mg/kg group. In the 40-mg/kg group, 3 of 12 participants had transient grade 1 swelling at the injection site, and 1 participant had a grade 3 fever (onset on day 1 and maximum temperature of 38.6 ℃ measured at home on day 3, when the fever also resolved). During the febrile illness, this participant had 2 negative blood smears for malaria, a negative blood culture, and 2 full hemograms (taken 2 days apart) which were essentially normal apart from mild anemia with a hemoglobin of 8.6 g/dL, which was treated with hematinics. No focus of infection was found, but the child had a papular rash 2 days after the fever had resolved. One participant in the 40-mg/kg group also had grade 2 nausea 2 days after dosing (considered possibly related). The 3 injection site swellings were all no longer present on day 1 and were considered probably related, but also possibly related to study procedures, namely the SC injection technique.

Regarding unsolicited AEs in the 30-mg/kg group, 3 participants had grade 1 URTIs, 1 participant had a grade 1 gastroenteritis, 1 participant had a grade 1 papular rash with no other associated symptoms, and 1 participant had generalized pruritus 1 day after dosing. A URTI that began on the day of dosing and the generalized pruritus were considered possibly related and the

other unsolicited AEs unlikely related. In the 40-mg/kg group, 3 participants had a grade 1 papular rash without other associated symptoms, 1 participant had a grade 1 URTI, 1 had mild anemia, and 1 participant had conjunctivitis. All the unsolicited AEs in the 40-mg/kg dose group were considered unlikely related. Table 1 summarizes AEs of these 23 participants through the first 7 days after dosing.

Table 1. Participant AEs through the first 7 days following dosing of L9LS from protocol KEMRI 4413 (NCT05400655).

	30 mg/kg	**40 mg/kg**
Enrolled	12	12
Completed Day 7 safety labs	11*	12
Day 7 lab abnormalities	1 grade 1 leukopenia	1 grade 1 neutropenia
Solicited local events (w/in 7 days)	0	3 (transient swelling)
Solicited systemic events (w/in 7 days)	0	-1 grade 3 pyrexia** -1 grade 2 nausea
Unsolicited adverse events	-3 grade 1 URTIs -1 grade 1 gastroenteritis 1 grade 1 papular rash	-1 grade 1 URTI -1 grade 1 conjunctivitis 3 grade 1 papular rashes -1 grade 1 anemia
* 1 participant withdrew consent on day 5 ** Fever onset day 1, end day 3; 2 negative blood smears, negative blood culture, 2 normal hemograms, mild anemia (8.6 g/dL); papular rash appeared 2 days after fever resolution		

Protocol 2023/109/CE/USTTB (NCT05816330) is a phase 2 trial that started in 2023 and is evaluating the safety and tolerability of a 1-time SC administration of L9LS, as well its protective efficacy against Pf infection over a 6-month malaria season. The study is randomized, double-blind, placebo-controlled, sex-stratified (2:1 female to male ratio), and weight-stratified (n=288 total) with 2 treatment arms, L9LS 900 mg SC (n=216) and placebo (n=72), to assess safety and protective efficacy of L9LS and placebo. Product administration is complete for all study participants and the study remains blinded. Overall, product administrations were well-tolerated with all reported reactogenicity being mild to moderate in severity. One SAE was reported, which was considered unrelated in blinded investigation (lumbar disc herniation that led to hospitalization).

Transient decreases in white blood cell (WBC) and neutrophil counts up to grade 2 severity have been observed in phase 2 trials of L9LS, both among L9LS and placebo recipients. One participant who received L9LS in the Mali phase 2 pediatric trial had a transient, asymptomatic WBC increase up to grade 4 severity, with a normal physical exam, and without associated clinical sequelae. To date, the transient laboratory abnormalities do not appear to represent a safety concern given the absence of clinical findings and because occurrences of these types of transient laboratory abnormalities have been observed across L9LS and placebo arms and without dose-related trends among L9LS recipients.

As noted above, in a preclinical TCR study, L9LS produced membrane and cytoplasmic binding in a portion of ductal and acinar epithelial cells in the human salivary gland which is of unclear clinical significance. Binding occurred in only 1 to 5% of cells and was scattered without evidence of clustering. Histopathology findings suggest 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 L9LS trials have excluded any population at an increased risk for salivary gland dysfunction (eg, parotitis, sialadenitis, sialolithiasis, salivary gland tumors, Sjogren's syndrome), although exclusions on this basis have been unusual. In addition, L9LS trials have prospectively monitored 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 trials. 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. 14 In addition, the ongoing phase 2 trials of L9LS in Malian adults and children, and Kenyan children, have included prospective monitoring for signs and symptoms that may be indicative of salivary gland hypofunction, and none have been noted to date.

Overall, 368 adults and 651 children aged 5 months to 10 years of age have been enrolled into the L9LS clinical program, of which approximately 753 participants have received L9LS. In total, 27 participants have been enrolled into phase 1 trials and 992 into phase 2 trials. Adult participants have received L9LS as a single IV, SC, or IM dose covering a 1- to 20-mg/kg weight-adjusted dose range. An estimated 496 pediatric participants have received at least 1 SC dose of L9LS covering a 5- to 40-mg/kg weight-adjusted dose range. L9LS has been well tolerated without evident safety concerns.

Risks of MAb Administration: Administration of MAbs may cause immune reactions such as acute anaphylaxis, serum sickness, and the generation of ADAs. 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. 24 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, pruritus) 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; 24 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. 24 Most administration-related events occur within the

first 24 hours after initiation of MAb administration. These reactions have not been observed with L9LS.

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. 24 In general, and with due consideration of the needs dictated by individual participant 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. 25 These reactions have not been observed with L9LS.

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

Risks of administering the R21/Matrix-M™ vaccine after L9LS administration: There is a theoretical risk that circulating L9LS could bind the R21/Matrix-M™ vaccine and cause a type III hypersensitivity reaction mediated by antigen-antibody complexes that cause fever, rash, arthralgias, and lymphadenopathy. In general, type III hypersensitivity reactions are self-limiting and are not associated with long-term complications. The risk of a type III hypersensitivity reaction involving L9LS and R21/Matrix-M™ is expected to be low based on studies that have assessed the safety of co-administering MAbs with vaccines that target the same pathogen. For example, the anti-rabies MAb SII RMAb (Rabishield), which is licensed in India for rabies post-exposure prophylaxis, was shown to be safe when co-administered with a rabies vaccine. 26,27 Similarly, a recent trial demonstrated the safety of co-administering a MAb combination that targets the SARS-CoV-2 spike protein (casirivimab and imdevimab) with Moderna's mRNA-1273 SARS-CoV-2 vaccine, in healthy SARS-CoV-2 seronegative adults. 17 In that trial, up to 1200 mg of casirivimab and imdevimab was assessed, resulting in a serum concentration of ~120 µg/mL at the time of vaccination with mRNA-1273.

In this protocol, the R21/Matrix-M™ vaccine series will start 20 weeks after L9LS/placebo administration when the serum concentration of L9LS is expected to be ≤10 µg/mL; therefore, type III hypersensitivity reactions are not expected in L9LS recipients following R21/Matrix-M™ vaccination. However, because L9LS may still be circulating at the time of vaccination, if a type III hypersensitivity reaction were to occur, it would likely manifest within 7 days of R21/Matrix-M™ vaccination.

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: 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.

Risks of Loss of Confidentiality: Because this study involves collecting personally identifiable information about participants, there is a risk of breach of confidentiality. To minimize this risk, samples will be labeled with a study number without identifiers. Data will be stored in a secure manner, such as in locked cabinets or password protected computer files. More information on protection of confidentiality is described in section 10.3.

2.4.2 Known Potential Benefits

Participants may not receive direct health benefit from study participation. Depending on whether L9LS confers protective efficacy, participants 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 addition, all participants in this study will receive the R21/Matrix-M™ vaccine, which is a standard vaccine being given in Mali. It is not currently available in Kalifabougou, Faladje, and Torodo outside of this study, but will become available in the future. This vaccine should help protect participants against malaria.

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

2.4.3 Assessment of Potential Risks and Benefits

The study population lives in an area where malaria is endemic, and they are among those at highest risk of severe malaria and death. As described above, it is possible that some participants may benefit from study participation by receiving some protection during the malaria season. In general, MAbs for other clinical indications have an excellent safety profile, and for L9LS specifically there have been no safety concerns to date. 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 participant eligibility criteria and monitoring after study product administration.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
1. To evaluate the safety and tolerability of L9LS administered IM at 150 mg in healthy Malian infants. 2. To evaluate the PK of L9LS throughout the study at a dose of 150 mg administered IM to healthy Malian infants.	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.	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.

Secondary		
1. To evaluate the impact of L9LS or placebo on the immunogenicity of subsequent R21/Matrix-M™ vaccination in healthy Malian infants. 2. To evaluate the risk of hypersensitivity reactions (an AESI) following the R21/Matrix-M™ vaccine when administered 20, 24, and 28 weeks after L9LS/placebo administration. 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. Total IgG anti-NANP antibody titers measured by ECLIA 28 days and 84 days after the third R21/Matrix-M™ vaccination. 2. Incidence and severity of hypersensitivity reactions occurring within 7 days after the administration of each dose of R21/Matrix-M™ vaccine. 3. Measurement of ADA to L9LS in sera of recipients.	1. Total IgG anti-NANP antibody titers associated strongly with R21/Matrix-M™ vaccine efficacy. 2. There is a theoretical risk that L9LS could bind the R21/Matrix-M™ vaccine and cause a hypersensitivity reaction. 3. ADA to L9LS may impact the PK and activity of L9LS.
Tertiary/Exploratory		
1. To determine if IM administration of L9LS at 150 mg (compared to placebo) mediates protection against clinical malaria in healthy Malian infants 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. 2. To determine if IM administration of L9LS at 150 mg (compared to placebo) mediates protection against clinical malaria in healthy Malian infants during a single	1. Incidence of clinical malaria (definition 1, see section 2.3.4) between 2 and 24 weeks after administration of L9LS or placebo. 2. Incidence of clinical malaria (definition 2, see section 2.3.4) between 2 and 24 weeks after administration of L9LS or placebo. 3. Pf blood-stage infection as detected by microscopic examination of thick blood smears obtained between 2 and 24 weeks after administration of L9LS or placebo. 4. Pf blood-stage infection as detected by RT-PCR between 2 and 24 weeks	1. Infants in Mali are at high risk for clinical malaria. 2. Infants in Mali are at high risk for clinical malaria. 3. Microscopic examination of thick blood smears is the gold standard for malaria diagnosis. 4. RT-PCR is more sensitive than blood smear for detecting Pf blood-stage infection. 5. Concentrations of L9LS in blood will help assess durability of L9LS and will allow for correlation

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. 3. To determine if IM administration of L9LS at 150 mg (compared to placebo) mediates protection against naturally occurring Pf infection in healthy Malian infants during a single malaria season as detected from microscopic examination of thick blood smears. 4. To determine if IM administration of L9LS at 150 mg (compared to placebo) mediates protection against naturally occurring Pf infection in healthy Malian infants during a single malaria season as detected by RT-PCR. 5. To correlate L9LS serum concentration with clinical malaria risk. 6. To correlate L9LS serum concentration with Pf infection risk. 7. To assess hemoglobin levels throughout the study in healthy Malian infants who receive IM administration of L9LS at 150 mg or placebo.	after administration of L9LS or placebo. 5. PK analysis of L9LS and the association of L9LS concentration with clinical malaria risk. 6. PK analysis of L9LS and the association of L9LS concentration with Pf infection risk. 7. Hemoglobin levels.	with clinical malaria risk. 6. Concentrations of L9LS in blood will help assess durability of L9LS and will allow for correlation with Pf infection risk. 7. Infants in Mali are at high risk for anemia.

4 STUDY DESIGN

4.1 Overall Design

This is an age-stratified, randomized, double-blind, placebo-controlled phase 1b trial evaluating the safety, tolerability, and PK of 1-time IM administration of L9LS to healthy Malian infants aged 1 to 12 months, followed by an assessment of the impact of L9LS on the immunogenicity of the R21/Matrix-M^{TM} vaccine. The study hypotheses are that L9LS will be safe and will not

impact the immunogenicity of the R21/Matrix-M^{TM} vaccine. At the beginning of the 6-month malaria season (approximately August and September), 180 participants will be enrolled and randomized 1:1 to 150 mg of L9LS (n=90) or placebo (n=90). Randomization of participants aged 1 to 12 months in each L9LS dose arm will be age-stratified (1 to 4 months, n=60; ≥5 to 8 months, n=60; ≥9 to 12 months, n=60) and enrollment will be age de-escalated starting with participants aged ≥9 to 12 months. The study is powered to assess safety of L9LS within each of the 3 age strata, since a fixed dose of 150 mg of L9LS will provide a different mg/kg dose range across the age range of participants (Table 2). Participants will be followed for safety to assess AEs at study visits 1, 3, 7, 14, 21, and 28 days later, and once every 4 weeks thereafter through study day 280 (40 weeks). Twenty weeks after L9LS/placebo administration, all participants will receive the R21/Matrix-M^{TM} vaccine according to WHO recommendations and the anticipated local standard of care and Malian vaccination guidelines (ie, 3 doses, 4 weeks apart). The R21/Matrix-M^{TM} vaccine will be provided by the study team in accordance with the recommended schedule in Mali. Within 7 days after each dose of R21/Matrix-M^{TM}, participants will have a scheduled visit to be assessed for hypersensitivity reactions, an AESI. Participant's parent/guardian will be instructed to bring the participant in for an unscheduled visit if he or she develops fever or rash. The participant will be evaluated by the study team to assess for signs and symptoms of hypersensitivity reactions through a focused medical history and physical exam. Primary study assessments include medical history, physical examination, and blood collection to assess antibody responses to the R21/Matrix-M^{TM} vaccine, L9LS PK, ADA assessments, identification of Pf infection by microscopic examination of thick blood smears and RT-PCR, and other research laboratory evaluations. During the malaria season, the study team will work with local providers to ensure that all subjects 3 months and older are offered SMC as a monthly 3-day treatment course of SPAQ, the standard of care in Mali for malaria prevention in children 3 months to 5 years of age.

Parents/guardians of participants may be re-contacted after the conclusion of their study participation to answer questions regarding use of SMC. The survey will include questions regarding whether or not a course of SMC was administered, if the course was completed, and the reason(s) for completion, partial-completion, or non-completion.

Interim safety data from the ongoing phase 2 trials of L9LS in Mali and Kenya 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 3 MRTC clinics, 1 in Torodo, 1 in Faladje, and 1 in Kalifabougou. The day 0 visit (L9LS/placebo administration) and all R21/ Matrix-M^{TM} vaccination visits will take place at Kalifabougou for all participants, 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 13 km north of Kalifabougou, and Faladje is located 28 km north of Kalifabougou. The economy is based on subsistence farming. Kalifabougou, Faladje, and Torodo are similar in terms of geographic, demographic, and epidemiological characteristics, and both typically experience intense seasonal Pf transmission from July through December each year. 28

4.2 Scientific Rationale for Study Design

This study was designed to test L9LS in the setting of naturally occurring Pf infection and in a population that could potentially benefit from a novel therapeutic for malaria prevention.

The study will use randomization and a double-blind design to minimize bias in participant 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 and Route of Administration

The dose of L9LS used in this study was selected based on preclinical data, data generated in the phase 1 VRC study, data generated in the ongoing phase 2 trial of 6 to 10-year-old children in Mali, and data generated from an interim efficacy analysis in infants and children in Kenya. This study will evaluate a fixed dose of 150 mg of L9LS in infants in a range of participant weights (3.5 to approximately 11 kg) that will provide a weight-based dosing range of approximately 13.6 to 42.9 mg/kg, mirroring the weight-based dosing range in previous and ongoing trials.

Compared to weight-based dosing, fixed dosing will provide a greater range of L9LS concentrations in vivo for determining a protective titer (exploratory endpoint). Fixed dosing is also simpler to administer, reduces the risk of dosing errors, and is generally more cost effective—factors that are relevant to the potential use of L9LS in malaria-endemic regions.

In this trial, infants aged 1 to 12 months of age weighing at least 3.5 kg (infants weighing less than 3.5 kg will be excluded) will receive a 150-mg (1-mL) dose of L9LS IM. Based on WHO weight-for-age tables (Figure 6), the estimated weights and weight-based L9LS dose ranges for female and male study participants are shown in Table 2.

Weight-for-age GIRLS
Birth to 5 years
(percentiles)

World Health Organization

Year: Month	Months	3rd	15th	Median	85th	97th
0: 0	0	2.4	2.8	3.2	3.7	4.2
0: 1	1	3.2	3.6	4.2	4.8	5.4
0: 2	2	4.0	4.5	5.1	5.9	6.5
0: 3	3	4.6	5.1	5.8	6.7	7.4
0: 4	4	5.1	5.6	6.4	7.3	8.1
0: 5	5	5.5	6.1	6.9	7.8	8.7
0: 6	6	5.8	6.4	7.3	8.3	9.2
0: 7	7	6.1	6.7	7.6	8.7	9.6
0: 8	8	6.3	7.0	7.9	9.0	10.0
0: 9	9	6.6	7.3	8.2	9.3	10.4
0:10	10	6.8	7.5	8.5	9.6	10.7
0:11	11	7.0	7.7	8.7	9.9	11.0
1: 0	12	7.1	7.9	8.9	10.2	11.3

Weight-for-age BOYS
Birth to 5 years
(percentiles)

World Health Organization

Year: Month	Months	3rd	15th	Median	85th	97th
0: 0	0	2.5	2.9	3.3	3.9	4.3
0: 1	1	3.4	3.9	4.5	5.1	5.7
0: 2	2	4.4	4.9	5.6	6.3	7.0
0: 3	3	5.1	5.6	6.4	7.2	7.9
0: 4	4	5.6	6.2	7.0	7.9	8.6
0: 5	5	6.1	6.7	7.5	8.4	9.2
0: 6	6	6.4	7.1	7.9	8.9	9.7
0: 7	7	6.7	7.4	8.3	9.3	10.2
0: 8	8	7.0	7.7	8.6	9.6	10.5
0: 9	9	7.2	7.9	8.9	10.0	10.9
0:10	10	7.5	8.2	9.2	10.3	11.2
0:11	11	7.7	8.4	9.4	10.5	11.5
1: 0	12	7.8	8.6	9.6	10.8	11.8

Figure 6. WHO weight-for-age tables (percentiles) for girls and boys from birth to 12 months.

Note, weight-for-age tables from birth to 5 years were truncated at 12 months.
(https://www.who.int/tools/child-growth-standards/standards/weight-for-age)

Table 2. Estimated weight ranges and L9LS weight-based dosing ranges for female and male infants by age at enrollment.

	Females		**Males**	
Age (months)	**Estimated median weight (range)—kg[1]**	**Estimated median L9LS dose (range)—mg/kg**	**Estimated median weight (range)—kg[1]**	**Estimated median L9LS dose (range)—mg/kg**
1	4.2 (3.5 – 5.4)	35.7 (27.8 – 42.9)	4.5 (3.5 – 5.7)	33.3 (26.3 – 42.9)
2	5.1 (4.0 – 6.5)	29.4 (23.1 – 37.5)	5.6 (4.4 – 7.0)	26.8 (21.4 – 34.1)
3	5.8 (4.6 – 7.4)	25.9 (20.3 – 32.6)	6.4 (5.1 – 7.9)	23.4 (19.0 – 29.4)
4	6.4 (5.1 – 8.1)	23.4 (18.5 – 29.4)	7.0 (5.6 – 8.6)	21.4 (17.4 – 26.8)
5	6.9 (5.5 – 8.7)	21.7 (17.2 – 27.3)	7.5 (6.1 – 9.2)	20.0 (16.3 – 24.6)
6	7.3 (5.8 – 9.2)	20.5 (16.3 – 25.9)	7.9 (6.4 – 9.7)	19.0 (15.5 – 23.4)
7	7.6 (6.1 – 9.6)	19.7 (15.6 – 24.6)	8.3 (6.7 – 10.2)	18.1 (14.7 – 22.4)
8	7.9 (6.3 – 10.0)	19.0 (15.0 – 23.8)	8.6 (7.0 – 10.5)	17.4 (14.3 – 21.4)
9	8.2 (6.6 – 10.4)	18.3 (14.4 – 22.7)	8.9 (7.2 – 10.9)	16.9 (13.8 – 20.8)
10	8.5 (6.8 – 10.7)	17.6 (14.0 – 22.1)	9.2 (7.5 – 11.2)	16.3 (13.4 – 20.0)
11	8.7 (7.0 – 11.0)	17.2 (13.6 – 21.4)	9.4 (7.7 – 11.5)	16.0 (13.0 – 19.5)
12	8.9 (7.1 – 11.3)	16.9 (13.3 – 21.1)	9.6 (7.8 – 11.8)	15.6 (12.7 – 19.2)

[1]Infants weighing less than 3.5 kg will be excluded, otherwise weight range estimates encompass 3rd to 97th percentiles based on WHO weight-for-age tables (Figure 6).

In the phase 2 Mali trial involving children aged 6 to 10 years who weighed between 15 and 30 kg, a fixed dose of 300 mg of L9LS provided efficacy against clinical malaria of 77.4% (adjusted 95% CI, 55.0-88.7; P<0.001), representing a weight-base dose range of 10 to 20 mg/kg. An interim analysis of the phase 2 Kenya L9LS trial involving infants and children aged 5 months to 5 years suggests that infants may require a weight-based dose that is higher than 20 mg/kg to achieve ≥80% protective efficacy against clinical malaria over 6 months. Specifically, in the Kenya trial, 324 children 5 to 59 months of age were randomized to receive L9LS or placebo by SC administration. L9LS dosing was based on 3 weight bands with fixed doses of 75 mg, 150 mg, or 225 mg, resulting in a weight-based dose range of 10 to 20 mg/kg. A pre-specified interim

analysis showed that 6-month efficacy against clinical malaria among children aged 5 to 17 months was 60% (adjusted 95% CI, 27-79; P=0.003). Based on these data, and data from the Mali trial suggesting that L9LS doses higher than 20 mg/kg may be needed to protect children from malaria for 12 months, it was proposed that the Kenya trial be extended for a second year to assess the safety and efficacy of L9LS at a dose range of 30- to 40-mg/kg. An initial safety study included 24 children aged 5 to 71 months, with 12 participants in each dose group in a 3:1 ratio of L9LS to placebo. To date, 12 subjects have received a SC dose of 30 mg/kg, and 12 have received a SC dose of 40 mg/kg. As described in detail in section 2.4.1, there have been no safety concerns to date at this dose range.

The 150-mg dose of L9LS (1 mL) will be administered IM rather than SC because IM dosing is the standard route of administration of most Expanded Program for Immunization vaccines to infants and is therefore a more familiar route of administration for healthcare workers in malaria-endemic regions.

Of note, in the phase 1 VRC trial of L9LS 14, 5 healthy US volunteers received a single IM dose of L9LS at a dose of 5 mg/kg (the safety, tolerability, and PK data for IM injections was not published). The volume of L9LS injected IM at a single site for these 5 individuals ranged from 2.51 mL to 3.12 mL. IM administration of L9LS in these 5 individuals was safe and well tolerated. Specifically, in terms of local reactogenicity, 1 of 5 individuals reported mild pain/tenderness at the injection site and none experienced pruritus, redness, swelling, or bruising. In terms of systemic reactogenicity, 1 of 5 reported mild headache, and none reported malaise, muscle aches, chills, nausea, joint pain, or fever. One of 5 individuals had a grade 1 alanine transaminase (ALT) elevation 8 days post-administration that was deemed not related and resolved within 7 days.

The PK of L9LS at a dose of 5 mg/kg was similar in those who received it SC (n=5), IM (n=5), or IV (n=4), as shown in Figure 7 below.

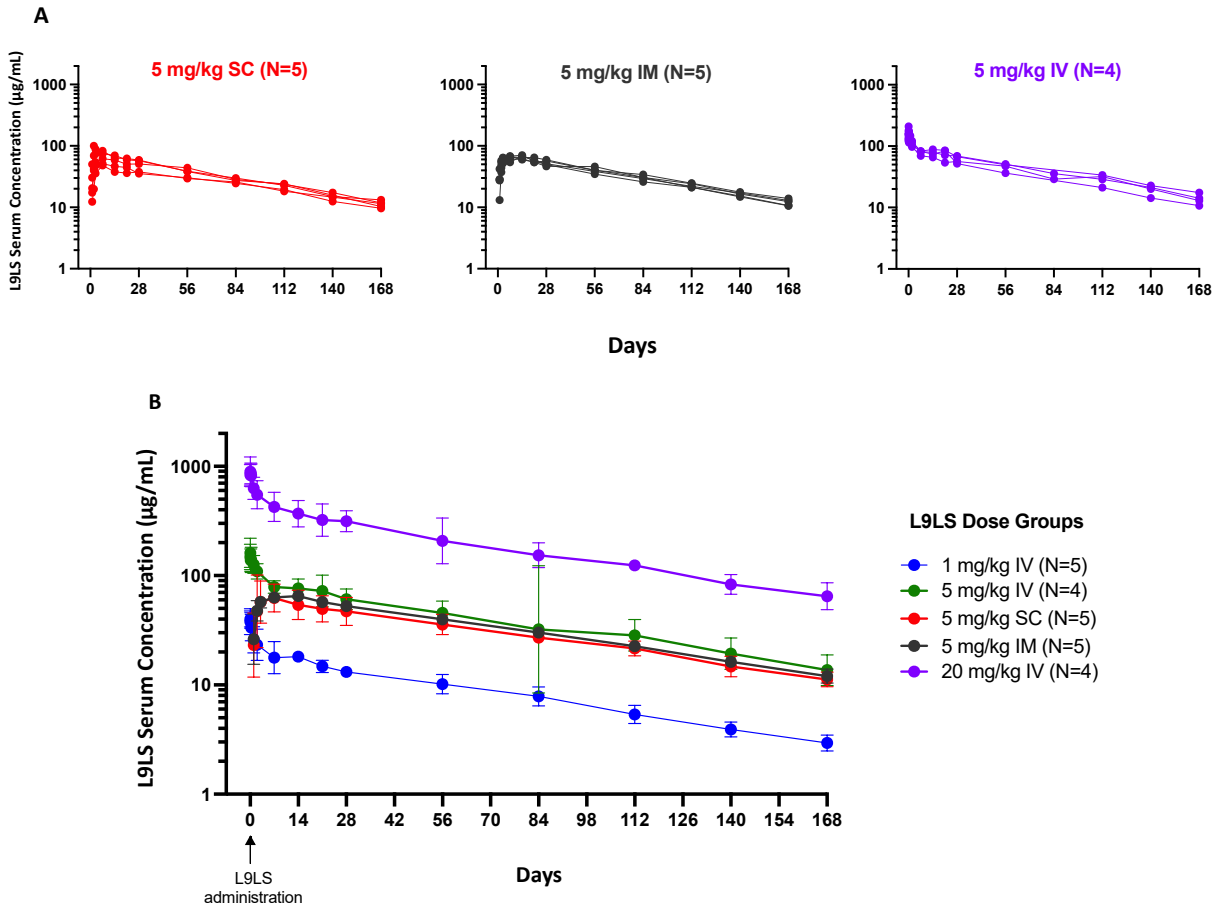


Figure 7. Serum concentrations of L9LS in the phase 1 trial involving healthy US adults.

Panel A shows the serum concentrations of L9LS over time in individual trial participants who received a single 5 mg/kg dose of L9LS SC, IM, or IV. Panel B shows the geometric mean serum concentrations of L9LS with 95% confidence intervals (indicated by I bars) for each dose group after administration of a single dose of L9LS.

Also of note, the MAb Beyfortus™ (nirsevimab-alip) is approved for the prevention of RSV in neonates, infants, and children up to 24 months of age. Beyfortus™ is administered as an IM injection at a dose of 50 mg (0.5 mL) if less than 5 kg in body weight, and 100 mg (1 mL) if greater than or equal to 5 kg in body weight. In a phase 3 trial, 1 mL IM injections of Beyfortus™ were safe and well tolerated. 29

5 Study Population

5.1 Inclusion Criteria

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

1. Age ≥1 to 12 months at enrollment.
2. Born at ≥37 weeks gestation (at term).
3. Parent and/or guardian able to provide proof of identity to the satisfaction of the study clinician completing the enrollment process.

4. In good general health and without clinically significant medical history.
5. Parent and/or guardian able to provide informed consent.
6. Willing to have blood samples and data stored for future research.
7. Resides in or near Kalifabougou, Faladje, or Torodo, Mali, and available for the duration of the study.

5.2 Exclusion Criteria

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

1. Body weight <3.5 kg.
2. Behavioral, cognitive, or psychiatric disease in the parent and/or guardian that in the opinion of the investigator affects the ability of the parent and/or guardian to understand and comply with the study protocol.
3. Any fever (≥ 37.5°C, regardless of route) or acute illness within 7 days prior to randomization.
4. Clinically significant congenital anomaly or documented or suspected serious medical illness (eg, history of epilepsy), serious congenital anomaly, or immediate life-threatening condition in the infant that may interfere with the ability to complete study requirements, as judged by the examining clinician.
5. Prior history of a suspected or actual acute medically life-threatening event.
6. Receipt of any blood products, monoclonal or polyclonal antibody/immunoglobulin (for example, hepatitis B immune globulin, intravenous immunoglobulin), or anticipated use during the study.
7. Any suspected or actual life-threatening acute or chronic illnesses known in the mother during her pregnancy.
8. Parental study comprehension examination score of <80% correct or per investigator discretion.
9. Hemoglobin, WBC, absolute neutrophil, or platelet count outside the local laboratory-defined limits of normal. (Participants may be included at the investigator's discretion for "not clinically significant" values.)
10. ALT or creatinine (Cr) level above the local laboratory-defined upper limit of normal. (Participants may be included at the investigator's discretion for "not clinically significant" values.)
11. Mother and/or infant infected with HIV.
12. Sickle cell disease by testing. (Note: Known sickle cell trait is NOT exclusionary.)
13. Evidence of clinically significant neurologic, cardiac, pulmonary, hepatic, endocrine, rheumatologic, autoimmune, hematological, oncologic, or renal disease by history, physical examination, and/or laboratory studies.
14. Receipt of any investigational product within the past 30 days.
15. 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.)
16. History of a severe allergic reaction or anaphylaxis.

17. Salivary gland disorder diagnosed by a doctor (eg, parotitis, sialadenitis, sialolithiasis, salivary gland tumors).
18. Pre-existing autoimmune or antibody-mediated diseases including but not limited to systemic lupus erythematosus 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 (ie, prednisone >10 mg/day) or immunosuppressive drugs within 30 days of day 0.
22. Previous receipt of the R21/Matrix-M™ vaccine.
23. Previous receipt of an investigational malaria vaccine or monoclonal antibody
24. Previous receipt of L9LS monoclonal antibody by the mother.
25. Clinical signs of malnutrition.
26. Other condition(s) that, in the opinion of the investigator, might jeopardize the safety or rights of an individual participating in the trial, interfere with the evaluation of the study objectives, or render the participant unable to comply with the protocol.

5.3 Inclusion of Vulnerable Participants

- **Children:** L9LS is currently being evaluated in ongoing phase 2 clinical trials in Mali (6- to 10-year-old children) and Kenya (children aged 5 months to 5 years) to evaluate safety and protective efficacy of L9LS in healthy children exposed to seasonal and perennial malaria transmission, respectively. Unblinded safety and efficacy results from year 1 of the Mali trial indicate that SC administration of L9LS is safe, well tolerated, and efficacious against clinical malaria. Unblinded and blinded data from the Kenya trial indicate that L9LS is safe and well tolerated. Therefore, this protocol will proceed with administration of L9LS in healthy infants aged 1 to 12 months, an age group that is particularly vulnerable to severe malaria (Figure 2). In Mali, SMC is recommended for children aged 3 months to 5 years; therefore, all participants 3 months and older in this trial will be offered SMC as standard of care through their health care provider.

  Infants who weigh less than 3.5 kg are excluded because they are near the 3rd percentile in weight-for-age for 1 month old infants according to WHO growth standards (Figure 6), and a dose of 150 mg in infants weighing less than 3.5 kg would exceed 42 mg/kg. The maximum volume of study agent that will be administered to children in this study is 1 mL (150 mg of L9LS).
- **Illiterate Individuals:** We anticipate that many parents/guardians of individuals eligible for this study will be illiterate in French, so the study team will translate the consent orally into local languages when appropriate, as described in section 10.1.

5.4 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 will not impact the lifestyle of the participants.

5.6 Screen Failures

Screen failures are defined as participants 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 participants, to meet the Consolidated Standards of Reporting Trials 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 (eg, abnormal 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 participant number.

5.7 Strategies for Recruitment and Retention

Study participants will be selected based on the eligibility criteria described in sections 5.1 and 5.2. The total target sample size across all study sites is 180 participants (accrual ceiling 360). We expect to enroll all participants within the first 2 months of the study from August through September. Participant selection will not be limited based on sex, race, or ethnicity.

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

5.7.1 Costs

There are no costs associated with participation in this trial.

5.7.2 Compensation

Parents or guardians of minor participants 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: the screening visit, enrollment visit (injection) and R21 vaccination visits.
- 3,000 CFA Franc for all other visits, including completion of SMC survey.

The total compensation amount for participants will be 73,000 CFA Franc (valued at approximately USD $130). Payment will be provided in cash after the completion of each visit.

Participants along with their parents/guardians 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

For the majority of infants, the anterolateral aspect of the thigh (ie, vastus lateralis muscle) is the recommended site for IM injection because it provides comparatively larger muscle mass than

the deltoid. 30 For all intramuscular injections, the needle should be long enough to reach the muscle mass and prevent L9LS from seeping into subcutaneous tissue, but not so long as to involve underlying nerves, blood vessels, or bone. For the majority of infants, a 1-inch needle is sufficient to penetrate the thigh muscle. 30 Intramuscular injections are administered at a 90-degree angle to the skin into the anterolateral aspect of the thigh. The needle gauge for intramuscular injection is 22-25 gauge. 30

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

6.1.2 Dosing and Administration

Study agent will be dependent on study arm assignment (Table 3). Procedures for administration are described in section 6.1.2.3.

Table 3. Study agent assignment and dosing by arm.

Arm	Total Subjects	Study Agent and Dose
1	90	150 mg L9LS IM
2	90	Placebo (normal saline) IM

6.1.2.1 Dose Limiting Toxicity

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

6.1.2.2 Dose Modifications

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

6.1.2.3 Drug Administration

Prior to study agent administration on day 0, participants will undergo vital signs measurement and a targeted physical examination (as needed based on signs, reported symptoms, or interim medical history). For all participants, the total volume of assigned study agent will be 1 ml that will be administered by a single syringe.

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 participants by designated individuals who remain separate from the team of blinded investigators who conduct all subsequent follow-up study assessments.

Participants will be observed for at least 1 hour following completion of study product administration and vital signs will be recorded for 1 hour (+/-15 minutes) after study agent administration. Prior to discharge from the clinic, vital signs will be recorded, and participants will be assessed for local reactogenicity (including pain/tenderness, swelling, redness, bruising, and pruritus at the site of study product injection) and systemic reactogenicity (including fever, irritability/fussiness, loss of appetite, vomiting, diarrhea, and rash). Any participant 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 participant will be referred to the district hospital to evaluate for safety and possible treatment. Clinicians will follow any solicited symptoms that are ongoing until they have resolved.

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Acquisition and Accountability

Acquisition: L9LS 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 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 participants or used for *in vitro* experimental studies. These vials will be disposed of in accordance with institutional or pharmacy policy.

6.2.2 Formulation, Appearance, Packaging, and Labeling

L9LS was manufactured under current Good Manufacturing Practice by the Vaccine Clinical Materials Program operated under contract by Leidos Biomedical Research, Inc., Frederick, MD. 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 IM 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 participants IM 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 ("Caution: New Drug - 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 (eg, 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 IM administration, the vials will be allowed to 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. 31

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. 31

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

6.2.4 Preparation

Study product will be prepared by an unblinded pharmacist. One syringe will be prepared for each subject for a total of one 1-mL IM injection. 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 IM injection order, the dose and study arm code will be included in the pharmacy order. To prepare an IM injection, the pharmacist will do the following:

- For participants assigned to L9LS:
    1) Retrieve a single vial to prepare the full dose. After the vial is thawed, the vial 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 using a 5-micron filter needle. The filter needle must be discarded prior to dispensing and replaced with a needle suitable for IM injection at the time of administration.
- For participants assigned to placebo:
    1) Withdraw 1 mL of normal saline placebo into the syringe using a 5-micron filter needle. A new filter needle must be used to prepare each syringe. The filter needle must be discarded prior to dispensing and replaced with a needle suitable for IM injection at the time of administration.

6.3 Measures to Minimize Bias: Randomization and Blinding

6.3.1 Randomization

Participants will be randomized into 1 of 2 arms. Randomization lists for 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 9.4.1.

6.3.2 Blinding

This trial will be conducted with a double blind for all participants. 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 participants, the clinical staff, and the study team will be blinded to study treatment allocation for all participants, with the exception of designated individuals who administer the study agents and remain separate from the team of blinded investigators who conduct all subsequent follow-up study assessments.

The pharmacy team at the study site where administration is taking place will be unblinded, and they are responsible for maintaining security of study treatment assignments.

Data will remain blinded for the original protocol until the last participant completes the final study visit of the original protocol. Blinded participants will then be informed about their study treatment assignment.

Intentional unscheduled (clinically driven) unblinding: Emergency unblinding (eg, needed for participant welfare/treatment) should be limited to those who need the information to accomplish the participant management. There will be no delay in intentional unblinding performed in the interest of participant safety.

In no case is unblinding to be significantly delayed for approval, and the default will be to unblind any reasonable request made in the interest of participant safety.

Unintentional unblinding: If unintentional unblinding of study agent assignment occurs, the principal investigator will create a plan for ongoing management of the participant(s) involved and for preventing the recurrence of a similar incident, as appropriate. If the study team determines that the unintentional unblinding may have a significant impact on the study plan (eg, if the treatment codes for multiple participants were accidentally broken), the need for a protocol amendment will be addressed as soon as possible.

Management of unscheduled unblinding: All cases of unscheduled unblinding (ie, intentional and unintentional) will be handled according to SOPs and 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 participant. 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 SMM and EC.

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

6.4 Study Intervention Compliance

Study intervention administration will be documented by study staff.

6.5 Concomitant Therapy

All concomitant prescription and nonprescription (including over-the-counter, herbal, or traditional) medications taken during study participation will be recorded. For this protocol, a

prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician.

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

- Live or killed vaccines 1 week before or after study agent administration.
- 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 (ie, 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 individual participant or cohort (ie, pausing), or it may be discontinued for all participants and enrollment suspended (ie, halting). Pausing and halting are described in sections 8.4.5 and 8.4.6. Participants who have already received the study agent at the time of a pause or halt will continue planned follow-up under the protocol.

7.2 Participant Discontinuation/Withdrawal from the Study

Participants are free to withdraw from participation in the study at any time upon request.

Criteria and procedures for withdrawal and replacement of a participant by the investigator are provided in section 8.4.3.

Plans for managing the involuntary withdrawal of a participant are provided in section 8.4.3. The reason for participant discontinuation or withdrawal from the study will be recorded on the case report form (CRF).

Participants 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

A participant will be considered lost to follow-up if they fail to return for 3 consecutive scheduled visits and are unable to be contacted by the study site staff.

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

- The site will attempt to contact the parent/guardian of the participant 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 participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the parent/guardian (where possible, 3 telephone calls).

These contact attempts should be documented in the participant's medical record or study file.

- Should the parent/guardian continue to be unreachable, the participant will be considered to have withdrawn from the study with a primary reason of 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.

8 STUDY ASSESSMENTS AND PROCEDURES

The following study assessments and procedures will be performed according to the schedule provided in section 1.3.

8.1 Screening Procedures

Screening will be performed at the Kalifabougou, Faladje, and Torodo study sites. The study staff will explain the study to the prospective participant's parent/guardian, complete the study comprehension examination (completed by parent/guardian), obtain consent (section 10.1), and assess eligibility. Consent will be obtained before any study-related procedures are performed. At this time, the participant will be assigned a unique study identification number in the clinical database. The identification number will link participant samples and data collected throughout the study.

The following screening procedures and evaluations must be performed within -56 to 0 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 review of maternal pregnancy history and delivery of infant participant.
- Complete review of medication use including complete immunization history.
- Complete physical examination, including length, weight, head circumference, and middle upper arm circumference (MUAC).
- Vital signs (temperature and pulse).
- Blood collection via venipuncture for screening evaluations:
    - HIV screening of the infant will only be done if the mother does not have a documented HIV test result that was done during her pregnancy with the infant. If required, HIV screening of the infant will be performed according to international guidelines. HIV testing will be 2 RDTs. If the RDTs are both positive or discordant, the participant will be referred for confirmatory ELISA and further medical assessment with confirmatory testing. Pre- and post-test HIV counseling will be provided.
    - Hemoglobin typing.
    - Complete blood count (CBC) with differential.
    - ALT.
    - Cr.

A prospective participant 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 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.

Enrollment: If the individual is eligible and parents/guardians agree 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 L9LS/placebo 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 participant 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 including the use of insecticide-treated bed nets and SPAQ 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 length, weight, head circumference, and MUAC) will be done at screening. A targeted physical examination based on signs, reported symptoms, and medical history will be conducted at subsequent study visits. Length and weight will also be recorded on day 0 prior to study agent administration.

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 for L9LS or placebo, as described in section 6.1.

R21/Matrix-M™: The vaccine will be administered according to WHO recommendations and the local standard of care following the anticipated Malian guidelines.

Illness Visit: A participant's parent/guardian will be instructed to bring the participant in for an unscheduled visit if he or she has symptoms of malaria or other symptoms. The participant will be evaluated by the study team and referred for standard care according to local guidelines. At an illness visit, the participant 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 participant has a malarial infection, we will share these results with the participant's parent/guardian 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 or finger/heel prick will be performed with single-use needles or lancets. 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 participants by the NIH Clinical Center:

- Pediatric participants: 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 2.3.4) to identify Pf infection and clinical malaria as exploratory endpoints. Thin blood films will also be prepared and examined by trained personnel to determine the *Plasmodium* species present. This evaluation will be performed centrally in the laboratory in Bamako, unless the participant 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 an exploratory 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 participants 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. This evaluation will be performed by Dr. Daniel Neafsey at the Harvard School of Public Health.

R21/Matrix-M^{TM} vaccine serology: ECLIA to detect total IgG against PfCSP NANP will be performed on serum samples collected 28 and 84 days after the third dose of R21/Matrix-M^{TM} vaccine.

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 hemoglobin genotyping, Fc receptor genotyping, 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

Any clinically relevant test results will be shared with the participant's parent/guardian throughout the study. Results of research procedures or evaluations (including incidental findings) will be shared with the participant's parent/guardian and with their primary provider if they are medically actionable. Such results will be discussed with the participant along with guidance for appropriate clinical follow-up.

Hemoglobin genotyping results will be returned to the parents/guardians of participants and the participant's clinical provider for further management and follow-up if clinically indicated.

8.2.4.4 Return of Secondary Genomic Research Results

The data being generated in this trial are insufficient for conducting secondary analyses. Therefore, no secondary genomic research results will be returned to participants.

8.2.4.5 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 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.

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 participant, including any abnormal sign (eg, abnormal physical exam or laboratory finding),

symptom, or disease, temporally associated with the participant's participation in the research, whether or not considered related to the research.

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

Per US FDA guidance:

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

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

The sponsor is responsible for making the causality judgment.

Serious Adverse Event: An SAE:

- is an AE that results in death.
- is an AE that is a life-threatening event (places the participant 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 (eg, elective correction of an unchanged baseline skin lesion), or due to social circumstance (eg, 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.

- is a medically important event.

  NOTE: Medical and scientific judgment should be exercised. Events that significantly jeopardize the participant 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 (UP): A UP is any incident, experience, or outcome that meets all the following criteria:

1. Unexpected in terms of nature, severity, or frequency in relation to:
   a. the research (including but not limited to risks) as described in the EC-approved research protocol and informed consent document, investigator's brochure, or other study documents; **and**
   b. the characteristics of the participant population being studied; **and is**
2. Possibly, probably, or definitely related (see "Causality" below) to participation in the research **and**
3. Suggests the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized, per the documents currently approved by the EC.

   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 (eg, 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
  - participant 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 (eg, particulate, cloudiness, temperature excursion), even if there is no known or proven issue (ie, 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 participant, 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 participants 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 participants 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 participant. 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 participants.
2. **Continuing noncompliance:** A pattern of recurring noncompliance that either has resulted, or, if continued, may result in harm to participants or otherwise materially compromise the rights, welfare and/or safety of participants, 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 (eg, due to lack of understanding, knowledge, or commitment), or

intentional (eg, due to deliberate choice to ignore or compromise the requirements of any applicable regulation, organizational policy, or determination of the EC).

Adverse Event of Special Interest (AESIs): AESIs are study-specific events identified and specified in detail within the protocol that are of particular concern due to population, study intervention, class effect, etc. Reporting is handled in a protocol/study-specific manner and is generally enhanced or upgraded beyond the general standard.

A Type III hypersensitivity reaction associated with the administration of the R21/Matrix-M™ vaccine, characterized by symptoms such as fever, arthralgia, myalgia, skin eruptions, lymphadenopathy, marked discomfort, and/or dyspnea, is classified as an AESI in this protocol.

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 (eg, on the clinical chart/progress notes/clinical laboratory record), recorded (eg, in the study-specified CRF/research database), and reported (eg, 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 participant, 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 4.

AESIs will be reported by the expedited SAE mechanism as detailed in Table 5 and section 8.4.2.3.2 below, but this does not make the event an SAE if it does not otherwise qualify.

Table 4. Standard event recording, assessment, and reporting timeframes.

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

AESIs	End of participant participation in study, or if study personnel become aware thereafter

Table 5. AESI list and reporting.

Event description	How event will be reported
Type III hypersensitivity reaction occurring within 7 days following administration of each R21/Matrix-MTM vaccine, characterized by symptoms such as fever, arthralgia, myalgia, skin eruptions, lymphadenopathy, marked discomfort, and/or dyspnea.	ESIs will be reported to the sponsor in the same manner as SAEs (section 8.4.2.3.2).

8.4.2.1 Investigator Assessment of Adverse Events

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

8.4.2.1.1 Severity Grading

The investigator will grade the severity of fever (by non-oral temperature reading) and each blood laboratory testing AE according to the "Mali Adverse Event Grading Scale" provided in APPENDIX B. Other events may be graded using the tables provided in APPENDIX A and APPENDIX C. Events that are not gradable using these tables will be graded according to the "Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events" which can be found at: https://rsc.niaid.nih.gov/sites/default/files/corrected-grading-table-v-2-1-with-all-changes-highlighted.pdf.

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 (eg, clinical jaundice)
- indicate a possible organ toxicity (eg, elevated serum Cr)
- result in additional/repeat testing or medical intervention (procedures/treatments) (eg, 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 (eg, 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 (eg, 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 (eg, 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
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 participant 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 (eg, start and stop dates, event type, relationship, and grade), and **must be updated if necessary** (eg, 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 participant 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 (eg, the participant 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 participant safety concern, the SMM should be consulted by email or phone no later than when reports are made to the CTM.

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 Participant

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

- An individual participant'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 participant or to the integrity of the study data.
- A change in the participant's condition as follows:
    - Loss of the ability of the parent/guardian to provide informed consent.

- Withdraws permission to have blood samples or data stored for future research.

- The investigator determines that continued participation in the study would not be in the best interest of the participant.

8.4.3.1 Re-enrollment and Unplanned Procedure Repetition

Unless otherwise specified within this protocol (eg, rescreening as described in section 5.6), each person who is a participant in this study may be enrolled and may pass through each step and process outlined in the protocol, only **ONCE** (ie, participants 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 Clinical Team Advisor.

8.4.3.2 Replacement of Withdrawn Participants or Participants Who Discontinue Study Agent

Participants withdrawn prior to study agent administration will be replaced.

All participants 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 Clinical Team Advisor in Mali

The Clinical Team Advisor 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. A Clinical Team Advisor 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 participants. The Clinical Team Advisor will conduct independent safety monitoring and recommend appropriate action regarding AEs and other safety issues. The Clinical Team Advisor 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 Clinical Team Advisor prior to or at the same time they are submitted to the EC or CSO unless otherwise specified herein. The Clinical Team Advisor will be notified immediately if any pausing rule is met. The principal investigators will also notify the Clinical Team Advisor if intentional or unintentional unblinding occurs. If the Clinical Team Advisor is unblinded to the study agent given to an individual subject during medical

management, the Clinical Team Advisor will report that unblinding to the DSMB Executive Secretary. The Clinical Team Advisor will not have access to unblinded data.

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.

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. The principal investigator will submit the written DSMB summary report with recommendations to the EC.

8.4.5 Pausing Rules

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

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

- A participant 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 participant 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 participants for any safety issue. The study safety oversight bodies (eg, DSMB, Clinical Team Advisor) 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 Clinical Team Advisor. 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 Clinical Team Advisor, 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 participant(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

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

8.4.6 Halting Rules for the Protocol

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

The halting rules are:

- Two or more participants 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 (eg, DSMB, Clinical Team Advisor) may recommend a halt to the CSO.

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

Participants who do not resume study agent/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 EC according to NIH Human Research Protection Program (HRPP) Policy 801, as described in section 8.6.1.

8.6 ADDITIONAL REPORTING REQUIREMENTS

8.6.1 Reporting to the FMOS EC

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

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

- Actual or suspected noncompliance. Determinations by the FMOS 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 participant 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 participant 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

UPs, major protocol deviations, and deaths will be reported to the NIAID clinical director according to institutional timelines.

9 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypothesis

The study hypotheses are that L9LS will be safe and will not impact the immunogenicity of the R21/Matrix-M^{TM} vaccine. The primary objectives are to evaluate the safety, tolerability, and PK of L9LS administered IM at 150 mg in healthy Malian aged 1 to 12 months, and a secondary objective is to evaluate the immunogenicity of the R21/Matrix-M^{TM} vaccine in healthy Malian infants who received 150 mg of L9LS or placebo.

Primary Endpoints:

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

Secondary Endpoints:

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

- Total IgG anti-NANP antibody titers measured by ECLIA 28 days and 84 days after the third R21/Matrix-M^{TM} vaccination.
- Measurement of ADA to L9LS in sera of recipients.

Exploratory Efficacy Endpoints:

- Incidence of clinical malaria (definition 1, see section 2.3.4) between 2 and 24 weeks after administration of L9LS or placebo.
- Incidence of clinical malaria (definition 2, see section 2.3.4) between 2 and 24 weeks after administration of L9LS or placebo.
- Pf blood-stage infection as detected by microscopic examination of thick blood smears obtained between 2 and 24 weeks after administration of L9LS or placebo.
- Pf blood-stage infection as detected by RT-PCR between 2 and 24 weeks after administration of L9LS or placebo.
- Hemoglobin levels throughout the study after administration of L9LS or placebo.

9.2 Sample Size Determination

9.2.1 Sample Size Considerations for Safety Events

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 (eg, AEs) within each arm under each age group. With sample size n=30 in each arm of an age group, there is over 90% chance to observe at least 1 AE if the true rate is at least 0.074 and over a 90% chance to observe no AE if the true rate is no more than 0.003. Probabilities of observing 0 or more than 1 AE within an arm are presented in Table 6 for a range of possible true event rates. The precision of AE rate estimation is outlined in Table 7.

Table 6. Probability of events for different safety scenarios within an arm (n=30).

True event rate	Pr(0)	Pr(>=1)	Pr(>1)

0.005	0.86	0.14	0.01
0.01	0.74	0.26	0.036
0.02	0.545	0.455	0.121
0.035	0.343	0.657	0.283
0.05	0.215	0.785	0.446
0.1	0.042	0.958	0.816

Abbreviations: Pr, probability.

Table 7. 95% exact confidence interval for the event rate based on the potential number of observed events within an arm (n=30).

Number of observed events	95% Confidence interval	
	Lower bound	Upper bound
0	0	0.116
1	0.001	0.172
2	0.008	0.221
3	0.021	0.265
4	0.038	0.307
5	0.056	0.347
6	0.077	0.386

9.2.2 Sample Size Considerations for R21/Matrix-M™ Immunogenicity

The trial will consist of 2 arms. Participants in the L9LS arm will receive 150 mg of L9LS and those in the placebo arm will receive normal saline. Five months after L9LS/placebo administration, all participants will begin the R21/Matrix-M™ vaccine series administered as 3 doses 4 weeks apart. The study endpoint for immunogenicity is the antibody response (anti-NANP IgG) to the R21/Matrix-M™ vaccine 28 and 84 days after the third dose of R21/Matrix-M™. A study hypothesis is that L9LS will not impact the immunogenicity of the R21/Matrix-M™ vaccine. This will be evaluated via a non-inferiority test. The null hypothesis is H0: The geometric mean of the Ab response in the L9LS arm < half the geometric mean of the Ab response in the placebo arm; the alternative hypothesis is H1: The geometric mean of the Ab response in the L9LS arm >= half the geometric mean of the Ab response in the placebo arm. According to a phase 2 trial that compared the immunogenicity and efficacy of 5 µg R21/25 µg Matrix-M (low-dose arm) to 5 µg R21/50 µg Matrix-M (high-dose arm), at 28 days after the third R21/Matrix-M™ vaccination antibody titers were 6133 EU (95% CI 5161–7289) in the low-dose group compared to 11,438 EU (9985–13 102; p<0.0001) in the high-dose arm (Figure 8). Despite the reduction in antibody titre of approximately 50% in the low-dose arm, 12-month efficacy of the low- and high-dose arms compared to the rabies vaccine control arm was 71% (95% CI 59–79; p<0.0001) and 77% (67–84; p<0.0001), respectively, and there was no significant difference in clinical malaria incidence between the low- and high-dose arms at either 6 or 12 months of follow-up (Figure 9). 22

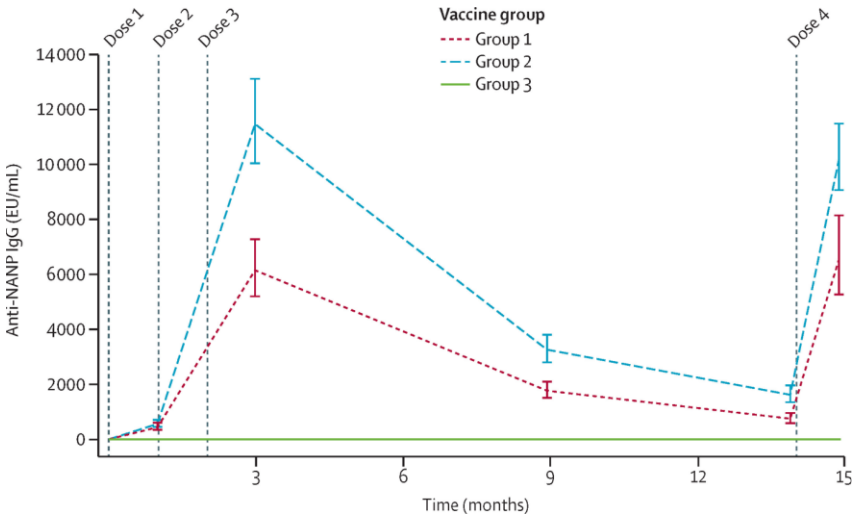


Figure 8. Antibody responses to R21/MM.

Geometric mean antibody titres (95% CI). Anti-NANP antibodies were measured by ELISA at baseline; 28 days after first vaccination; 28 days, 6 months, and 1 year after the third vaccination; and 28 days after the booster (fourth) dose administered 1 year after the third dose. Group 1 received 5 µg R21/25 µg MM, group 2 received 5 µg R21/50 µg MM, and group 3, the control group, received Rabivax-S. MM=Matrix-M. NANP=Asn-Ala-Asn-Pro. 22

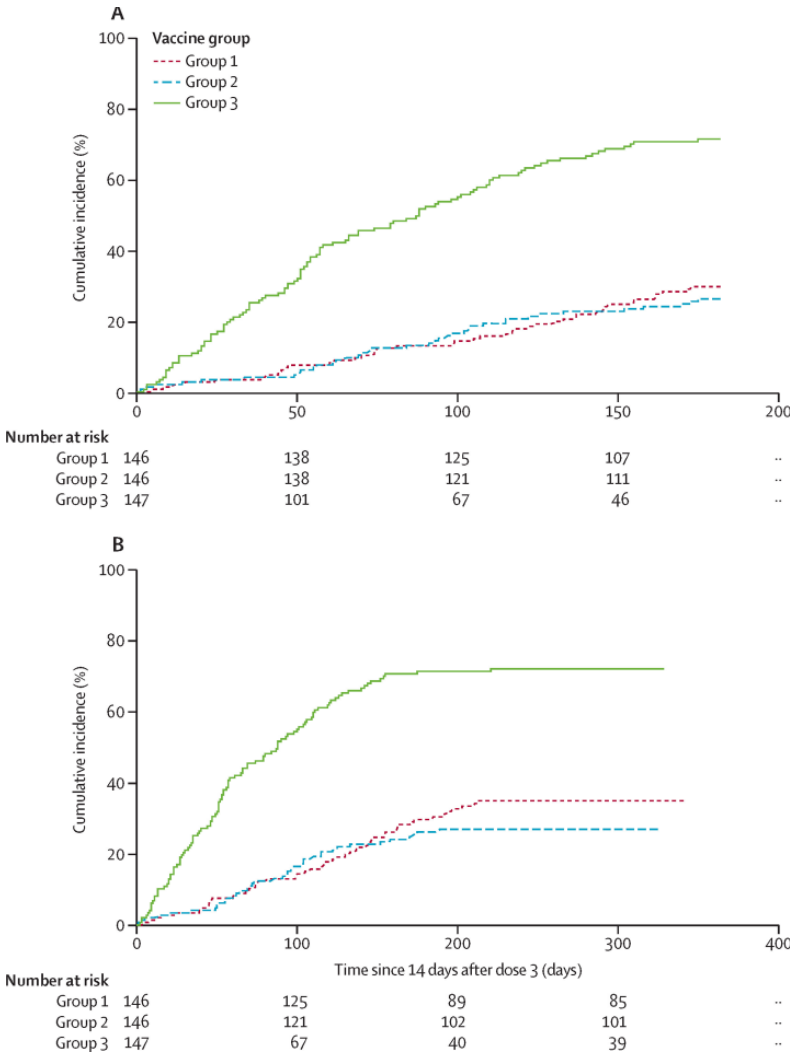


Figure 9. Kaplan-Meier estimates of the time to first episode of clinical malaria.

The primary analysis was based on a modified intention-to-treat population. Group 1 received 5 µg R21/25 µg MM, group 2 received 5 µg R21/50 µg MM, and group 3, the control group, received rabies vaccinations (Rabivax-S). (A) Data beginning from 14 days to 6 months after third vaccination. (B) Data beginning from 14 days to 12 months after third vaccination. MM=Matrix-M. 22

Based on these observations, the noninferiority margin of 0.5 is considered. Table 8 shows the required total sample size for the non-inferiority test to attain 90% power at one-sided alpha level of 0.025, assuming a margin of 0.5 and a coefficient of variation (CV) of 0.3. (Datoo et al. indicates the standard deviation of the log-scaled antibody endpoint around 0.3) 20 at varying values of the projected reduction in the geometric mean of the antibody response in the L9LS arm compared to the placebo arm. It is expected that L9LS will not interfere with R21/Matrix-M^{TM}, resulting in a reduction of the immunogenicity endpoint by no more than 42%. A total sample size of 168 is deemed sufficient. The sample size calculation assumes a lognormal distribution of the antibody response and is achieved via R package "PowerTOST". It is effectively based on the comparison of the log-scaled antibody response via a two-sample t-test.

Accounting for a 7% loss to follow-up rate, a total of 180 participants will be randomized: 90 to the placebo arm and 90 to the L9LS arm.

Table 8. The total sample size required for noninferiority test with 90% power at one-sided alpha level of 0.025 and noninferiority margin 0.5.

Margin of noninferiority in reduction	Projected reduction	Total sample size
0.5	0.42	168
0.5	0.40	112
0.5	0.35	56
0.5	0.30	36

To accommodate for a possibly higher variability in antibody response in this study, Table 9 shows the maximum reduction in the geometric mean of antibody response (the L9LS arm relative to the placebo arm) at which noninferiority can be declared with 90% power, at different levels of variability expressed as the CV of the antibody response. For example, if the CV is 1, this study will have 90% power to declare noninferiority if the reduction in the geometric mean of antibody response is no more than 23%.

Table 9. The maximum reduction in antibody response at which noninferiority can be declared with 90% power at one-sided alpha level of 0.025 and noninferiority margin of 0.5.

CV	Maximum reduction
0.5	0.36
1	0.23
1.5	0.13
2	0.05

Abbreviations: CV, coefficient of variation of antibody response

Of note, the lower bound of 0.5 agrees with WHO guidance (WHO Expert Committee on Biological Standardization, 67th report. Geneva: World Health Organization; 2017) (WHO technical report series; no. 1004) which states, “When it is proposed to demonstrate non-inferiority between vaccine groups based on geometric mean titer (GMT) or geometric mean concentration ratios for antibody titres or concentrations it is suggested that the lower bound of the 95% confidence interval around the ratio (test versus reference vaccine) should not fall below 0.67. Under certain circumstances NRAs [national regulatory authorities] may consider allowing a lower bound (for example, 0.5) or alternative criteria.” 32

9.3 Populations for Analyses

The following datasets will be considered in study analyses:

- Intention-to-treat (ITT) analysis dataset will include all participants who receive assignment and will be analyzed according to the initial randomization assignment.
- Modified intention-to-treat (MITT) analysis dataset will include all randomized participants that receive L9LS or placebo and later receive R21 and will be analyzed according to the initial randomization assignment.
- Per-protocol (PP) analysis dataset will include all randomized participants who receive the assigned product, L9LS or placebo, followed by 3 doses of R21 at the specified time points, and have anti-NANP IgG antibody titers measured 28 days and 84 days after the third dose of R21, and will be analyzed according to the initial randomization assignment.

In cases where participants receive an intervention other than the randomly assigned, an as-treated analysis will be additionally performed according to the actual product received.

9.3.1 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.

9.3.2 Evaluable for Objective Response

Not applicable.

9.3.3 Evaluable Non-Target Disease Response

Not applicable.

9.4 Statistical Analyses

9.4.1 General Approach

In general, descriptive statistics will be tabulated by treatment arm for endpoints of interest. This will include point estimates (mean, geometric mean, median, or proportions) and their respective 95% 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 (eg, <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 (ie, 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: Age-stratified randomization will be implemented. Sixty participants will be recruited into each age group starting with the oldest age group (≥9 to 12 months, followed by ≥5 to 8 months, followed by 1 to 4 months). Within each age group, randomization will be conducted at 1:1 allocation to either the L9LS arm or the placebo arm using block

randomization. The total number of participants will be 90 in each arm (30 of age 1 to 4 months, 30 of age ≥5 to 8 months, and 30 of age ≥9 to 12 months). To limit the number of dropouts before L9LS administration, randomization and L9LS administration will occur as close in time as possible.

9.4.2 Analysis of the Primary Endpoints

9.4.2.1 Safety Analysis

Safety analysis will be conducted on the ITT as well as MITT cohort where participants who are randomized but do not receive study products are excluded. Because of blinding and the brief length of time between assignment and administration, such cases are expected to be few in number. In the rare case of participants receiving a regimen different from assignment, an as-treated analysis will be additionally performed where participants 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 participants 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 participants under each AE will be additionally reported. For participants experiencing more than 1 AE, the subjects will be counted once under the event of highest severity.

Comparisons between the L9LS arm and placebo arm will be additionally performed in terms of the proportions of solicited AEs, related AEs, and SAEs.

9.4.2.2 Pharmacokinetics Analysis

PK analysis will be carried out with blood samples collected at defined timepoints as listed in section 1.3. The PK analysis will be MITT. The following PK analysis will be performed as needed.

Individual Participant Pharmacokinetic Analysis: A non-compartmental (NC) PK analysis will be performed on the L9LS concentration data generated from each participant. Individual participant and dosing arm concentration-versus-time profiles will be constructed in linear and semi-log scales. In the NC analysis, the maximum concentration and time of maximal concentration (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 participant's L9LS concentration falls below the quantitative limit (QL) of the assay, the sample with concentration below the QL will be assigned an 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 (eg, $Cave_{0\text{-}16WK}$ = ($AUC_{0\text{-}16WK}$) / 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. 33

9.4.3 Analysis of the Secondary Endpoint(s)

9.4.3.1 Immunogenicity Analysis

The analysis of immunogenicity will primarily be performed on the PP cohort. Total IgG anti-NANP antibody titers, measured by ECLIA 28 and 84 days after the third R21/Matrix-M™ vaccination, will be reported by the GMT along with 95% confidence intervals in L9LS and placebo arm, separately. The total IgG anti-NANP antibody titer will be compared between the 2 arms via a noninferiority test,

H0: The geometric mean of the Ab response in the L9LS arm < half the geometric mean of the Ab response in the placebo arm, versus

H1: The geometric mean of the Ab response in the L9LS arm >= half the geometric mean of the Ab response in the placebo arm.

With one-sided alpha level of 0.025 and noninferiority margin of 0.5; that is, noninferiority will be declared if the two-sided 95% confidence interval of the ratio of IgG anti-NANP antibody titers in the L9LS arm to that in the placebo arm is above 0.5. This noninferiority test will be conducted at both 28 days and 84 days after the third R21/Matrix-M™ vaccination.

In addition, we will assess the association of the total IgG anti-NANP antibody titers after R21/Matrix-M™ vaccination with the weight-based L9LS dose, defined as the milligram L9LS dose per kg of weight. This assessment will employ a linear regression model, where the response variable is the total IgG titer (with log transformation), and the regressors include the arm and the weight-based L9LS dose.

9.4.3.2 ADA Analysis

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 2 arms via Fisher's exact test.

9.4.4 Exploratory Analyses

9.4.4.1 Efficacy Analysis

Efficacy will be primarily evaluated over the PP cohort. The exploratory efficacy analysis will be based on time to the first infection, as detected by microscopic examination of thick blood smears or by RT-PCR, or clinical malaria episode between 2 and 24 weeks after administration of L9LS or placebo. The survival patterns will be described by Kaplan-Meier curves for each arm. The protective efficacy will be estimated 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 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. These regressors may include time of enrollment, pre-existing parasitemia, and possibly those

covariates that are significantly different between the study product arms and the placebo arm despite randomization.

Additional exploratory 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 1 minus the Kaplan-Meier estimate in the arm, and L9LS efficacy by 1 minus the relative risk of infection with melding method for constructing the melded 95% CI of L9LS efficacy. 34 In addition, exploratory efficacy analysis will compare the cumulative incidence of clinical malaria between study arms.

In addition to the above efficacy analyses, association analyses will be conducted to assess how the weight-based dose, as well as L9LS concentration (as a time-varying covariate), affects the protective efficacy on clinical malaria and Pf infection.

9.4.4.2 Other Exploratory Analyses

Hemoglobin levels will be documented in each arm, presenting the mean, standard deviation, and the 95% CI at various timepoints throughout the study. Additionally, a two-sample t-test will be conducted to compare hemoglobin levels between the L9LS and placebo arms throughout the study.

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 participants. 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.

9.4.5 Baseline Descriptive Statistics

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

9.4.6 Planned Interim Analyses

Not applicable.

9.4.7 Sub-Group Analyses

The aforementioned statistical analyses will also be conducted within each of the 3 age groups: 1 to 4 months, ≥5 to 8 months, and ≥9 to 12 months.

9.4.8 Tabulation of individual Participant Data

Safety data will be presented by line listing and tables at the individual level.

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, 35 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

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 participants' 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 parents/guardians of potential participants 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 participant'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. 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 participant'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 parent/guardian is mandatory to enroll their child/ward. 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, the child/ward of any individual whose comprehension is questionable, regardless of score, may be excluded from enrollment.

The consent process will be documented in the participant's medical record.

10.2 Study Discontinuation and Closure

The study may be temporarily suspended or permanently terminated if there is sufficient reasonable cause. In addition to the reporting described in that section, the principal investigator(s) will promptly contact the study participants, 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 participant, except as necessary for monitoring by the FMOS EC, FDA, NIAID, Office for Human Research Protections (OHRP), the VRC, or the sponsor's designee.

To further protect the privacy of study participants, 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 participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.4 Future use of Stored Specimens and Data

Participants 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: 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 EC.

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.

Additionally, participants 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 participant and to the EC. This decision will not affect the individual's participation in this protocol or any other protocols at NIH.

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 participant; 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 participants' records and source documents (participants' 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 (eg, consent forms, DFdiscover abstracts) and pertinent hospital or clinical records readily available for inspection by the local EC, FDA, the site monitors, and the NIAID staff for confirmation of the study data.

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

10.7 Quality Assurance and Quality Control

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

10.8 Data Handling and Record Keeping

10.8.1 Data Collection and Management Responsibilities

Study data will be maintained in CRFs and collected directly from participants during study visits and telephone calls or will be abstracted from participants' 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 participant 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. Relocation of research records will not proceed without written permission from OCRPRO/NIAID.

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 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 as specified in the protocol quality management plan and/or monitoring plan. The investigator is responsible for knowing and adhering to the reviewing EC requirements.

10.9.1 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 NIH Data Management and Sharing Policy and NIH Genomic Data Sharing Policy Compliance

This study will comply with the NIH Data Management and Sharing Policy, which applies to all new and ongoing NIH-funded research in the Intramural Research Program, as of January 25, 2023, that is associated with a ZIA, with a clinical protocol that undergoes scientific review and/or will involve genomic data sharing.

10.10.2 NIH Public Access Policy Compliance

This study will comply with the 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.

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
AESI	Adverse Events of Special Interest
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
CHMI	Controlled Human Malaria Infection
CI	Confidence Interval
CL	Clearance
COVID-19	Coronavirus Disease 2019
Cr	Creatinine
CRF	Case Report Form
CSO	Clinical Safety Office
CSP	Circumsporozoite Protein
CTM	Clinical Trials Management
CV	Coefficient of Variation
DSMB	Data and Safety Monitoring Board
EC	Ethics Committee
ECLIA	Electrochemiluminescence immunoassay
ELISA	Enzyme-Linked Immunosorbent Assay
EPI	Expanded Program for Immunization
FDA	Food and Drug Administration
FMOS	Faculté de Médecine Pharmacie d'Odontostomatologie
GCP	Good Clinical Practice
GEE	Generalized Estimating Equation
GMT	Geometric Mean Titer
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HRPP	Human Research Protection Program
ICH	International Council on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IM	Intramuscular(ly)
IND	Investigational New Drug Application
ITN	Insecticide-treated Nets
ITT	Intention-To-Treat
IV	Intravenous(ly)

LIG	Laboratory of Immunogenetics
LLOD	Lower Limit of Detection
MAb	Monoclonal Antibody
MITT	Modified Intention-To-Treat
MRTC	Malaria Research and Training Center
MUAC	Middle Upper Arm Circumference
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
Pf	*Plasmodium falciparum*
PK	Pharmacokinetic(s)
PP	Per-Protocol
Q	Intercompartmental Clearance
QL	Quantitative Limit
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
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SC	Subcutaneous
SERF	Safety Expedited Report Form
SMC	Seasonal Malaria Chemoprevention
SMM	Sponsor Medical Monitor
SOA	Schedule of Activities
SOP	Standard Operating Procedure
SPAQ	Sulfadoxine-pyrimethamine plus Amodiaquine
SUSAR	Serious and Unexpected Suspected Adverse Reaction
SRCP	Safety Review and Communication Plan
TCR	Tissue Cross-Reactivity
TI	Transmission Intensity
Tmax	Maximal Concentration
UP	Unanticipated Problem
UPnonAE	Unanticipated Problem that is not an Adverse Event
URTI	Upper Respiratory Tract Infection
US	United States

USD	United States Dollar
USTTB	University of Sciences, Techniques, & Technologies of Bamako
Vd	Volume of Distribution
Vdss	Volume of Distribution at Steady-State
VRC	Vaccine Research Center
WBC	White Blood Cell
WHO	World Health Organization

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APPENDIX A: MALI LABORATORY ADVERSE EVENT GRADING SCALE, CHILDREN

Table based on Local Laboratory Normal Range Reference Sheet. 36

Lab parameter	Unit	Grade 1	Grade 2	Grade 3	Grade 4
Hemoglobin	g/dL	6.0-7.0	5.0-5.9	<5.0	<5.0 with life-threatening signs
White blood cells Increase	$X10^3/\mu L$	17.0-20.0	20.1-30.0	>30.0	>30.0 with clinical signs
White blood cells decrease		2.5-4.0	1.5-2.4	1.0-1.4	<1.0
Neutrophils	$X10^3/\mu L$	1.0-1.3	0.7-0.9	<0.7	<0.7 with clinical signs
Platelets	$X10^3/\mu L$	80.0-130.0	50.0-79.9	<50.0	<50.0 with clinical signs
Lymphocytes	$X10^3/\mu L$	10.9-15.00	15.01-25.00	25.01-30.00	>30
Creatinine*	µmol/L	40.7-48.1	48.2-66.6 OR Increase to 1.3 to <1.5 x participant's baseline (Day 0)	66.7-125.8 OR Increase to 1.5 to <2.0 x participant's baseline (Day 0)	>125.8 OR Increase to ≥2.0 x participant's baseline (Day 0)
ALT	IU/L	51-60	61-80	81-100	>100

Abbreviations: ALT, alanine transaminase.

*Creatinine grading that is based on a fold-change increase from the participant's baseline (day 0) only applies if the creatinine level is grade 1 or higher in severity (ie, >40.7 μmol/L).

APPENDIX B: MALI VITAL SIGNS ADVERSE EVENT GRADING SCALE, INFANTS

Vital Signs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fever (°C axillary)	37.5 – 38.5 °C	38.6 – 39.5 °C	39.6 – 40 °C	40.1 °C and above
Hypothermia (°C axillary)	35.0 - 35.4 Resolving with routine measures to maintain normal temperature	34.5 – 34.9 Requiring and responding to intervention needed in addition to routine measures to maintain normal temperatures	34.0 – 34.4 Persistent despite intervention	<34.0
Tachycardia - beats per minute; at rest + calm	150 – 175	>175 – 200	>200	ER visit or hospitalization for arrhythmia
Bradycardia - beats per minute[4]; at rest + calm	75 – 79	70 – 74	60 – 69	<60 ER visit or hospitalization or need for CPR

APPENDIX C: SOLICTED AE GRADING

Local reaction	Mild Grade 1	Moderate Grade 2	Severe Grade 3	Life Threatening Grade 4
Pain at injection site	Minor disturbance Child moves arm normally	Pain when moving the affected limb, young child avoids moving the affected arm or leg	Cries/ protests when the affected limb is moved, pain disturbs sleep	Older child complains of severe pain, refuses to move the affected limb; young child cries inconsolably when limb is moved
Tenderness at injection site	Minor reaction to touch	Cries/ protests on touch	Cries/ protests when the affected limb is moved	Older child complains of severe pain, young child cries inconsolably when injection site is touched
Swelling at injection site	< 10 mm	10-25 mm	26-50 mm or	>50 mm
Redness at injection site	< 10 mm	10-25 mm	26-50 mm or	>50 mm
Bruising at injection site	< 10 mm	10-25 mm	ulceration	Necrosis
Pruritus at injection site*	Localized itching, no effect on activity	Frequent scratching, child cries more than usual	Constant scratching, disturbs sleep	Cries inconsolably because of pruritus

* For Infants under 6 months, Pruritus at injection site should be NA because it is too subjective. Infants cannot express Pruritus.

Relatedness: Most local reactions will be 'definitely related' unless there is another explanation like an insect bite or a burn at the injection site.

Systemic Reaction	Mild Grade 1	Moderate Grade 2	Severe Grade 3	Life threatening Grade 4
Fever (axillary temperature) In Degree Celsius	37.5 –38.5 °C	38.6–39.5 °C	39.6–40 °C	40.1 °C and above
Irritability/Fussiness	Crying more than usual, no effect on normal activity	Crying more than usual, interferes with normal activity	Crying a lot, prevents normal activity	Inconsolable, crying that cannot be comforted
Loss of Appetite*	Loss of appetite without decreased oral intake	Loss of appetite associated with decreased oral intake without significant weight loss	Loss of appetite associated with significant weight loss	Life-threatening consequences OR Aggressive intervention indicated (eg, tube feeding, total parenteral nutrition)
Diarrhea* *≥ 1 year of age*	Transient of intermittent episodes of unformed stools OR Increase ≤ 3 stools over baseline per 24-hour period	Persistent episodes of unformed to watery stools OR Increase of 4 to 6 stools over baseline per 24-hour period	Increase of ≥ 7 stools per 24-hour period OR IV fluid replacement indicated	Life-threatening consequences (eg, hypotensive shock)
< 1 year of age	Liquid stools (more unformed than usual) but usual number of stools	Liquid stools with increased number of stools OR Mild dehydration	Liquid stools with moderate dehydration	Life-threatening consequences (eg, liquid stools resulting in severe dehydration, hypotensive shock)
Vomiting	Transient or intermittent AND No or minimal interference with oral intake	Frequent episodes with no or mild dehydration	Persistent vomiting requiring rehydration	Life-threatening consequences (eg, hypotensive shock)

Systemic Reaction	Mild Grade 1	Moderate Grade 2	Severe Grade 3	Life threatening Grade 4
Rash* *Specify type, if applicable*	Localized rash (not at injection site)	Diffuse rash OR Target lesions	Diffuse rash AND Vesicles or limited number of bullae or superficial ulcerations of mucous membrane limited to one site	Extensive or generalized bullous lesions OR Ulceration of mucous membrane involving two or more distinct mucosal sites OR Stevens-Johnson syndrome OR Toxic epidermal necrolysis
Type III hypersensitivity reaction (ie, serum sickness reaction)*†	Mild signs and symptoms	Moderate signs and symptoms AND Intervention indicated (eg, antihistamines)	Severe signs and symptoms AND Higher level intervention indicated (eg, steroids or IV fluids)	Life-threatening consequences (eg, requiring pressor or ventilator support)

* Grading for these reactions were taken directly from "Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events" which can be found at: https://rsc.niaid.nih.gov/sites/default/files/corrected-grading-table-v-2-1-with-all-changes-highlighted.pdf.

† Definition: A disorder characterized by fever, arthralgia, myalgia, skin eruptions, lymphadenopathy, marked discomfort, and/or dyspnea.