

Supplementary Appendix

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This appendix has been provided by the authors to give readers additional information about the work.

Subcutaneous Administration of a Monoclonal Antibody to Prevent Malaria in Children

Supplementary Appendix

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Methods

INCLUSION CRITERIA

Individuals were required to meet all the following criteria to be eligible for study participation:

1. Is within the appropriate age range for the respective cohort:
 - a. Children: Aged ≥ 6 years and < 11 years.
 - b. Adults: Aged ≥ 18 years.
2. Able to provide proof of identity to the satisfaction of the study clinician completing the enrollment process.
3. In good general health and without clinically significant medical history.
4. Adult participants or parent and/or guardian of minor participants able to provide informed consent.
5. Willing to have blood samples and data stored for future research.
6. Resides in or near Kalifabougou or Torodo, Mali, and available for the duration of the study.
7. For the adult cohort, females of childbearing potential must be willing to use reliable contraception from 21 days prior to study day 0 through the final study visit as described below.
 - Reliable methods of birth control include 1 of the following: confirmed pharmacologic contraceptives via parenteral delivery or intrauterine or implantable device.
 - Nonchildbearing women will be required to report date of last menstrual period, history of surgical sterility (i.e., tubal ligation, hysterectomy) or premature ovarian insufficiency, and will have urine or serum pregnancy test performed per protocol.

EXCLUSION CRITERIA

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

1. Body weight < 15 kg or > 30 kg for children, or > 60 kg for adults.
2. Currently receiving or planning to receive SMC.
3. Any history of menses (for 6-10 year old cohort) or positive pregnancy test at screening (for adult cohort).
4. Behavioral, cognitive, or psychiatric disease that in the opinion of the investigator affects the ability of the subject to understand and comply with the study protocol.
5. Subject (for adult subjects) or parental (for minor subjects) study comprehension examination score of $< 80\%$ correct or per investigator discretion.
6. Hemoglobin, white blood cell, absolute neutrophil, or platelet count outside the local laboratory-defined limits of normal. (Subjects may be included at the investigator's discretion for "not clinically significant" values.)
7. Alanine transaminase (ALT) or creatinine (Cr) level above the local laboratory-defined upper limit of normal. (Subjects may be included at the investigator's discretion for "not clinically significant" values.)
8. Infected with HIV, hepatitis C virus (HCV), or hepatitis B virus (HBV).

9. Known or documented sickle cell disease by history. (Note: Known sickle cell trait is NOT exclusionary.)
10. Clinically significant abnormal electrocardiogram (ECG; QTc >460 or other significant abnormal findings, including unexplained tachycardia or bradycardia).
11. Evidence of clinically significant neurologic, cardiac, pulmonary, hepatic, endocrine, rheumatologic, autoimmune, hematological, oncologic, or renal disease by history, physical examination, and/or laboratory studies including urinalysis.
12. Receipt of any investigational product within the past 30 days.
13. Participation or planned participation in an interventional trial with an investigational product until the last required protocol visit. (Note: Past, current, or planned participation in observational studies is NOT exclusionary.)
14. Medical, occupational, or family problems as a result of alcohol or illicit drug use during the past 12 months.
15. History of a severe allergic reaction or anaphylaxis.
16. Severe asthma (defined as asthma that is unstable or required emergent care, urgent care, hospitalization, or intubation during the past 2 years, or that has required the use of oral or parenteral corticosteroids at any time during the past 2 years).
17. Salivary gland disorder diagnosed by a doctor (e.g., parotitis, sialadenitis, sialolithiasis, salivary gland tumors).
18. Pre-existing autoimmune or antibody-mediated diseases including but not limited to: systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, Sjogren's syndrome, or autoimmune thrombocytopenia.
19. Known immunodeficiency syndrome.
20. Known asplenia or functional asplenia.
21. Use of chronic (≥ 14 days) oral or IV corticosteroids (excluding topical or nasal) at immunosuppressive doses (i.e., prednisone >10 mg/day) or immunosuppressive drugs within 30 days of day 0.
22. Receipt of a live vaccine within the past 4 weeks or a killed vaccine or COVID-19 vaccine within the past 2 weeks prior to study agent administration.
23. Receipt of immunoglobulins and/or blood products within the past 6 months.
24. Previous receipt of an investigational malaria vaccine or monoclonal antibody in the last 5 years.
25. Known allergies or contraindication against artemether-lumefantrine.
26. Clinical signs of malnutrition.
27. Other condition(s) that, in the opinion of the investigator, would jeopardize the safety or rights of a subject participating in the trial, interfere with the evaluation of the study objectives, or render the subject unable to comply with the protocol.

SUBCUTANEOUS INJECTION PROCEDURE

In the age de-escalation and dose escalation study (Part A), adult participants were assigned in open-label fashion to receive L9LS at one of three doses: 300 mg administered SC, 600 mg administered SC, or 20 mg/kg of body weight administered IV (IV infusion procedure described below). Each participant in the 300 mg L9LS group received a single 2 ml SC injection of L9LS in the upper arm at the vial concentration of 150 mg/ml. Each participant in the 600 mg L9LS group received two, 2 ml SC injections of L9LS (one in each upper arm) at the vial concentration of 150 mg/ml.

In the randomized, placebo-controlled, pediatric phase of the age de-escalation and dose escalation study (Part A), each participant in the 150 mg L9LS/placebo arm received either a single 1 ml SC injection of L9LS (at the vial concentration of 150 mg/ml) or normal saline in the upper arm. Each participant in the 300 mg L9LS/placebo arm received either two, 1 ml SC injections of L9LS (at the vial concentration of 150 mg/ml) or normal saline, one in each upper arm. The faint yellow color of L9LS was obscured by covering all syringes with translucent yellow tape.

In the randomized, placebo-controlled trial for efficacy (Part B), each participant received two, 1 ml SC injections (one in each upper arm) to maintain blinding. Specifically, each participant in the placebo group received two, 1 ml injections of normal saline; each participant in the 300 mg L9LS group received two, 1 ml injections of L9LS at the vial concentration of 150 mg/ml; and each participant in the 150 mg L9LS group received two, 1 ml injections of L9LS diluted 1:1 with normal saline for a final concentration of 75 mg/ml. All subcutaneous injections were administered over a few seconds. The faint yellow color of L9LS was obscured by covering all syringes with translucent yellow tape.

All study products were prepared by unblinded pharmacists. The study participants, the clinical staff, and the study team were blinded to study treatment allocation for all pediatric subjects, except for designated individuals who administered the study agents and remained separate from the team of blinded investigators who conducted all subsequent follow-up study assessments.

INTRAVENOUS INFUSION PROCEDURE

The intravenous route of administration was only used in the high dose (20 mg/kg of L9LS) group of the open-label adult study for safety (Part A). The pharmacists prepared L9LS using infusion bags pre-filled with 100 mL of normal saline. The calculated L9LS volume was added to the infusion bag. Aseptic technique was used to place an intravenous catheter in an arm vein and the study product was infused over approximately 30 minutes. The average duration of infusion was 34 minutes. After infusion of the study agent, the intravenous administration set was flushed with approximately 30 mL of normal saline.

DETECTION OF *P. FALCIPARUM* BY MICROSCOPIC EXAMINATION OF THICK BLOOD SMEARS

Thick blood smears were analyzed by two independent readers who were unaware of the trial group assignments. A third reader examined blood smears when discrepancies occurred. A positive blood smear was defined as two independent readers both reporting the presence of any *P. falciparum* asexual parasites after counting 500 leukocytes or examining 40 high-power fields. A negative blood smear was defined as two independent readers both reporting the absence of *P. falciparum* asexual or sexual parasites after counting 2500 leukocytes or examining 200 high-power fields. The competency of blood smear readers is regularly assessed at the Mali Research and Training Center laboratory, which is certified by the College of American Pathologists.

PHARMACOKINETICS

L9LS anti-idiotypic (ID) antibody was spot coated at 125 µg/mL on 384-well standard bind plates and packaged by Meso Scale Discovery (MSD). Sample and reagent handling was performed on a Beckman Biomek i7 automated workstation. Plates were blocked for 1 hour with MSD Blocker A solution. Non-heat-inactivated test samples were serially diluted in assay diluent (MSD Diluent 100). Blocked plates were washed, and diluted test samples as well as MSD kitted standards and controls were added to the washed assay plates. Plates were incubated with shaking for 4 hours at room temperature. Plates were washed and Sulfo-Tag labeled mouse anti-human IgG detection antibody in assay diluent was applied to the plates and allowed to associate with complexed anti-ID/L9LS within the assay wells for 1 hour, shaking. The plates were washed to remove unbound detection antibody, and MSD Read Buffer containing Sulfo-Tag substrate was immediately added to the wells. Plates were read using the MSD Sector Imager S600. As current is applied to the plate, areas of well surface which form a full anti-ID/L9LS/Sulfo-Tag anti-human IgG complex emit light in an electrochemiluminescent (ECL) reaction. The amount of L9LS sandwiched by the anti-ID and anti-human IgG antibodies is directly proportional to the concentration of reactive L9LS. Serial dilutions of sample within the dynamic range of the standard curve were interpolated to assign a sample concentration. Analysis was performed using MSD Discovery Workbench Software and Microsoft Excel. The qualified detection range of the assay was 610 ng/mL to 10,000 µg/mL.

DRIED BLOOD SPOT RNA EXTRACTION AND *PLASMODIUM FALCIPARUM* 18S RIBOSOMAL RNA QRT-PCR

Samples for *Plasmodium* 18S rRNA qRT-PCR were collected onto Whatman® Protein Saver 903 cards and dried. The DBS cards were packed individually in sealed gas-impermeable plastic bags containing a humidity indicator card and desiccant and shipped to the University of Washington Malaria Molecular Diagnostic Laboratory (MMDL). Spots corresponding to ~50 µL of blood were excised by touchless laser cutting¹ and incubated in 2 mL of bioMérieux NucliSENS lysis buffer for 30 min at 55°C in a water bath. DBS specimens in lysis buffer were centrifuged 2000 x g for 10 min at 25°C and then lysis buffer was subjected to RNA extraction on the Abbott m2000sp (Abbott Molecular, Inc., Niles, IL) using a protocol specifying sampling of 1

mL of the lysis buffer/blood mix and a final elution volume of 88 μ L. The quantitative reverse transcription polymerase chain reaction (qRT-PCR) assay was performed using primer sets and probes targeting a *P. falciparum*-specific region of the asexual-stage 18S rRNAs, a pan-*Plasmodium* genus-specific region of the asexual 18S rRNAs, and the human endogenous TATA-Box binding protein (TBP) mRNA as a low copy number endogenous internal control. The Abbott m2000sp setup a 35 μ L qRT-PCR mastermix composed of the Bioline SensiFAST™ Probe Lo-ROX One-Step Kit (Thomas Scientific) and the following reagents: *P. falciparum*-specific probe 5'-[6-FAM]-ATTTATTCAGTAATCAAATTAGGAT-3'[Black Hole Quencher 1 PLUS] (BioSearch Technologies, Inc.), *P. falciparum*-specific primers (PfDDT1451F21: 5'-GCGAGTACACTATATTCTTAT-3' and PfDDT1562R21: 5'-ATTATTAGTAGAACAGGGAAA-3'; BioSearch Technologies, Inc.), pan-*Plasmodium* probe 5'-[CAL Fluor Orange 560]-ACCGTCGTAATCTTAACCATAAACTA[T(Black Hole Quencher-1)]GCCGACTAG-3'[Spacer C3] (BioSearch Technologies, Inc.), pan-*Plasmodium* primers (PanDDT1043F19: AAAGTTA[+A]GGGA[+G][+T]GAAGA and PanDDT1197R22: AA[+G]ACTTTGATTCTC[+A]TAAGG ([+X] denotes a locked nucleic acid; Qiagen), human TBP mRNA probe 5'-[Quasar 670]-CACAGGAGCCAAGAGTGAAGAACAGT-3'[Black Hole Quencher-2] (BioSearch Technologies, Inc.) and human TBP primers (forward: 5'-GATAAGAGAGCCACGAACCAC-3' and reverse: 5'-CAAGAACTTAGCTGGAAAACCC-3'; BioSearch Technologies, Inc.). The mastermix (35 μ L) was combined with extracted template (15 μ L), reverse transcription was performed for 10 minutes at 48°C, followed by denaturation for 2 minutes at 95°C and then 40 cycles of PCR (5 seconds at 95°C and 35 seconds at 54°C per cycle). The analytical sensitivity of the assay is 20 estimated parasites/mL. Absolute copy number was determined using a calibration curve of custom-made, quantified Armored RNA encoding full-length *P. falciparum* 18s rRNA (Asuragen) and converted to estimated parasites/mL as described {PMID 31017084}. Controls in every run were high, low, and negative DBS controls. The internal human TBP mRNA control was also evaluated for every DBS sample.

ANTIGEN GENOTYPING VIA 4CAST AMPLICON SEQUENCING

We used Illumina-based amplicon sequencing to assess allelic diversity in PfcSP, PfAMA1, PfSERA2, and PfTRAP (4CAST)² gene fragments in dried bloodspot samples of study participants who showed qRT-PCR-positivity for *P. falciparum* at enrollment. We processed samples from enrollment (prior to artemether-lumefantrine administration), day 0, day 3, day 7, day 14, and day 28 timepoints for these participants. We extracted total bloodspot DNA via KingFisher Flex instrument using KingFisher Ready DNA Ultra 2.0 Prefilled Plates (ThermoFisher Scientific) and subsequently applied selective whole-genome amplification (sWGA) to DNA extracts using primers Pf1-10 from Oyola et al. 2016.³ We used AMPure XP magnetic beads (Beckman Coulter) to exchange post-reaction sample buffer to 10 mM Tris-HCl + 0.1 mM EDTA (likewise via Kingfisher Flex) and performed PCR amplification steps as detailed in La Verriere et al. 2022² but with the following adjustments: PCR1 used an initial incubation step at 95°C (3 min); 29 amplification cycles at 98°C (20 s), 57°C (15 s), and 62°C (30 s); and a final extension step at 72°C (1 min). PCR1 products underwent Exonuclease I digestion (ThermoFisher Scientific) and subsequent 1/3 dilution in nuclease-free water. PCR2 used an initial incubation step at 95°C (3 min); 10 amplification cycles at 98°C (20 s), 65°C (30s), and 72°C (30 s); and a final extension

step at 72°C (1 min). We applied double-sided size selection (AMPure XP) prior to sequencing pooled libraries via 500-cycle (2 x 251 bp) Illumina MiSeq Reagent Kit v2 and resolved microhaplotypes from sequence output using the malaria amplicon processing pipeline available at <https://github.com/broadinstitute/malaria-amplicon-pipeline.git>. This pipeline wraps around DADA2, an amplicon denoising algorithm that tests whether nucleotide variation around abundant sequence types is statistically consistent with expected error distribution or a consequence of true biological variation in the sample set.⁴ Pipeline settings were run in default as described in LaVerriere et al.² except for applying 30 bp 3'-trimming to all input reads (raising average base quality prior to error inference). Finally, we discarded microhaplotypes supported by <200 read-pairs or by <10% total read-pairs within a locus. Samples were classified as polyclonal if any 4CAST locus exhibited two or more distinct microhaplotypes following filtering. Successful 4CAST assays most often yielded data from all four loci, but low parasitemia samples sometimes yielded data from a subset of loci. We analyzed all samples that yielded at least one microhaplotype for at least one locus to evaluate the possibility that monoclonal post-enrollment samples represent recrudescence as opposed to new infection. Monoclonal post-enrollment samples which exclusively contain alleles also observed at enrollment are likely to represent recrudescence if the matching allele set (i.e., the strain) occurs infrequently in the background population. We applied a simple estimate of background frequency for strains of interest by counting the number of times the strain occurs among enrollment samples divided by the total number of strains that occur among enrollment samples (Strain_Tot_Enroll). We multiplied Max_UA and Haps_per_UA to estimate Strain_Tot_Enroll because polyclonality among enrollment inhibits direct counting of all strains. Max_UA represents the maximum number of unique alleles (UA) observed among enrollment samples for any of the 4CAST loci. Haps_per_UA represents the average number of distinct multi-locus haplotypes associated with each UA among monoclonal enrollment samples (considering only UAs present among monoclonal enrollment samples). We did not assess the likelihood that polyclonal post-enrollment samples represent recrudescence (from polyclonal enrollment samples) because no complete allele matching to enrollment occurred for polyclonal post-enrollment samples. In such cases it may be useful to permute background frequencies of individual alleles as opposed to estimating the background frequency of linked allele sets as described above.

STATISTICAL ANALYSIS

For Part A, the prespecified primary endpoint was the incidence and severity of local and systemic adverse events occurring within 7 days after the administration of L9LS. For Part B, the prespecified primary efficacy endpoint was *P. falciparum* blood-stage infection as detected by microscopic examination of thick blood smears between 1 and 24 weeks after the administration of L9LS or placebo. Based on historical data we assumed that the *P. falciparum* infection rate under placebo would be no less than 40% during the 24-week observation period.⁵ Assuming a drop-out rate of 10% and using a total two-sided significance level of 0.05 with 0.025 allocated to each of the two comparisons, we determined that 75 participants enrolled in each arm would give the efficacy trial 90% power in each comparison (placebo versus 150 mg of L9LS; and placebo versus 300 mg of L9LS) to detect protective efficacy if the underlying efficacy were greater than or equal to 70% if at least 40% of participants in the placebo group became infected.

As prespecified in the protocol, the primary efficacy analysis was based on time to first *P. falciparum* infection and used the modified intention-to-treat dataset that included all randomized participants who received the study agent, including those who withdrew or were lost to follow-up. The P values reported for the primary efficacy endpoint were based on the log-rank test comparing each L9LS arm with the placebo arm. Protective efficacy was estimated by the hazard ratio from the Cox proportional hazards model that accounted for interval censoring. Time-to-event efficacy was calculated as $\text{Efficacy (\%)} = (1 - \text{HR}) \times 100$, in which HR is the hazard ratio of infection between study arms. A prespecified secondary efficacy analysis was based on the proportion of participants infected with *P. falciparum* over the 6-month study period. The proportions of infection were estimated for each arm and compared across arms based on Kaplan-Meier estimation. Proportional efficacy was calculated as $\text{Efficacy (\%)} = (1 - \text{RR}) \times 100$, in which RR is the relative risk of infection between study arms.

The same efficacy analyses (time-to-event and proportional) were applied to the pre-specified secondary endpoints of clinical malaria.

In the primary efficacy analyses, the Holm method was applied separately to the primary efficacy endpoint (time to first *P. falciparum* infection) and secondary efficacy endpoint (time to first clinical malaria episode) to control for multiplicity in comparing each L9LS dose group with the placebo group. The adjusted 95% confidence intervals were reported accordingly for both efficacy endpoints.

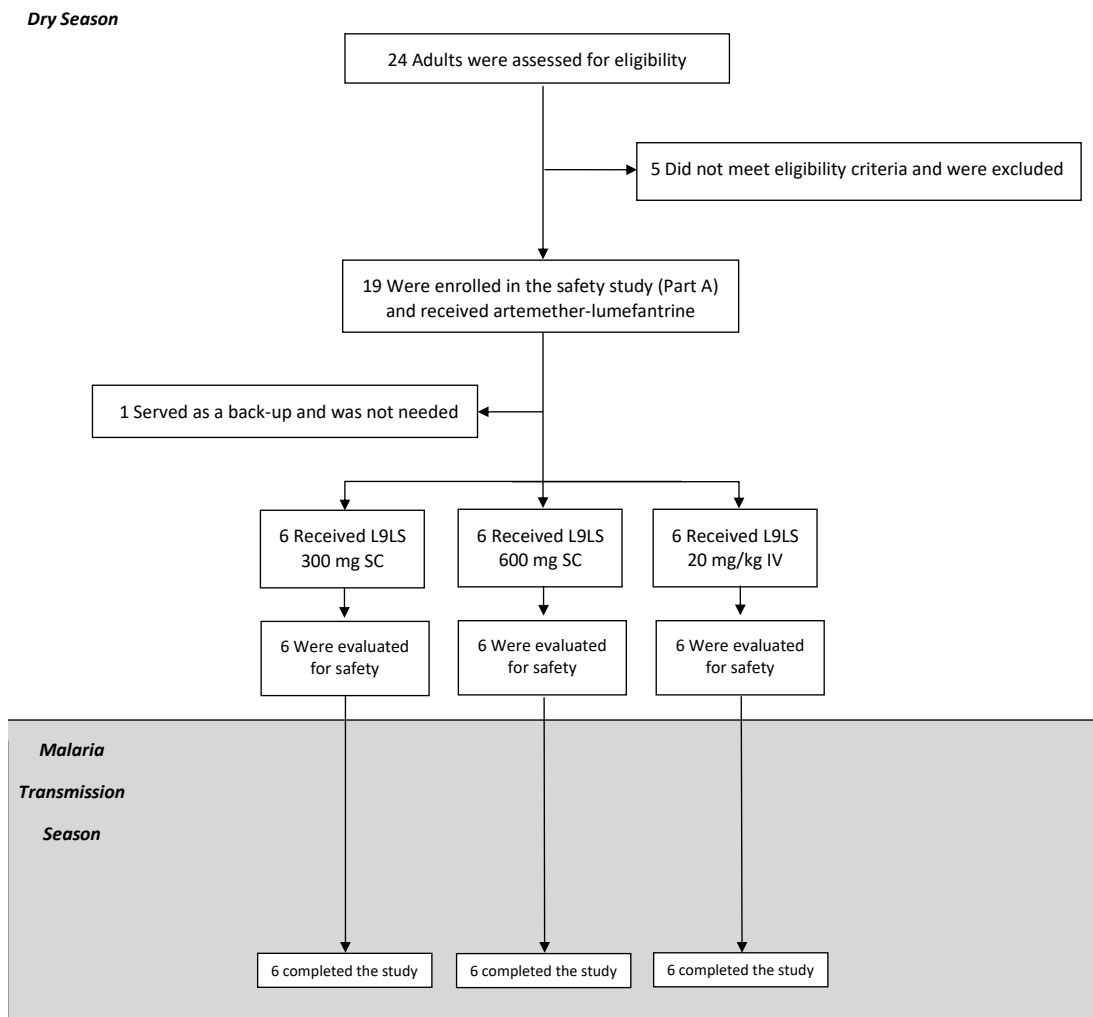
In exploratory analyses, the association between the weight-based dose of L9LS and protective efficacy against *P. falciparum* infection and clinical malaria was evaluated with the Cox proportional hazards model with the weight-based dose of L9LS as a covariate. The efficacy of L9LS against clinical malaria was also assessed via Andersen-Gill's recurrent event analysis to account for the repeated incidence of clinical malaria within participants. In further exploratory analysis we investigated the protective efficacy of L9LS over time by a piece-wise constant hazard model that expanded the Cox proportional hazards model to allow the hazard to vary over time but to be constant within each specified time interval. We partitioned the follow-up period into three time intervals: 0-50 days, 51-100 days and 101-168 days post product administration. Like the original efficacy analysis, only infections occurring 7 to 168 days post product administration were counted. One participant who had no additional visits after product administration was included in this analysis and was censored on day zero. Secondary and exploratory analyses were limited to point estimates with 95% confidence intervals, the widths of which were not adjusted for multiplicity and may not be used in place of hypothesis testing.

Missing values were rare, therefore complete case analysis was used. Analyses were performed with JMP version 16.2.0 and RStudio 1.4.1717 or 1.3.1056 with R 4.1.1.

Supplemental Results and Figures

Screening, Enrollment and Follow-up of Adult Participants (Figure S1)

The adult component of Part A was an open-label, dose-escalation trial to evaluate the safety and side-effect profile of L9LS. Before the malaria season, 24 adults 18 to 55 years of age were assessed for eligibility. Five participants were excluded because they did not meet eligibility criteria, and the remaining 19 adults were enrolled and received artemether–lumefantrine 7 to 21 days before administration of L9LS or placebo, to clear any possible *P. falciparum* blood-stage infection. One of these participants served as a back-up and was not needed because the sample size had been met. Between March 30 and April 23, 2022, a total of 18 adult participants received a single administration of L9LS in three escalating dose groups: 300 mg SC (6 participants), 600 mg SC (6 participants), and 20 mg per kilogram IV (6 participants). All 18 participants completed study visits through day 196.

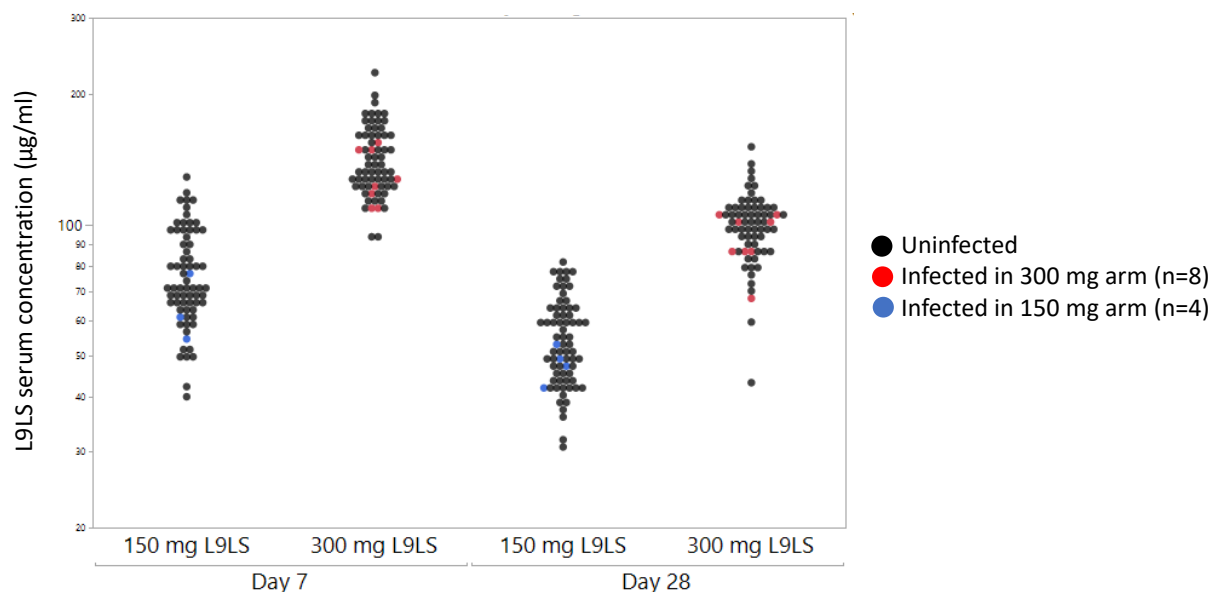


Post-hoc analysis of L9LS pharmacokinetics, *P. falciparum* infection detection by qRT-PCR, and *P. falciparum* genotyping through study day 28 (Figures S2 and S3).

In the efficacy trial, it was notable that the risk of infection (Fig. 2 in main text) and clinical malaria (Fig. 3 in main text) was similar in the placebo and L9LS 300 mg arms during the first 28 days of the trial. Moreover, during the first 28 days of the trial, 8 participants who received 300 mg of L9LS were infected compared to only 4 infected participants who received 150 mg of L9LS. Thus we conducted a post-hoc analysis to investigate the potential mechanisms underlying the lack of dose effect on protection during this period.

First, to determine if reduced L9LS bioavailability was associated with higher rates of early infections in the 300 mg L9LS arm we measured serum concentrations of L9LS at pre-specified time points 7 and 28 days after L9LS administration (Fig. S2). Among participants who received 300 mg of L9LS and were not infected between days 7 and 28, the mean (\pm SD) concentration of L9LS was 140.3 ± 1.2 $\mu\text{g/ml}$ and 97.5 ± 1.2 $\mu\text{g/ml}$ on days 7 and 28, respectively. Among the 8 participants who received 300 mg of L9LS and were infected between days 7 and 28, the mean (\pm SD) concentration of L9LS was 127.4 ± 1.2 $\mu\text{g/ml}$ and 91.0 ± 1.2 $\mu\text{g/ml}$ on days 7 and 28, respectively. As expected, L9LS concentrations were generally lower in the 150 mg L9LS arm compared to the 300 mg L9LS arm at both time points. These data suggest that the higher rate of early infections in the 300 mg L9LS arm was not due to reduced L9LS bioavailability.

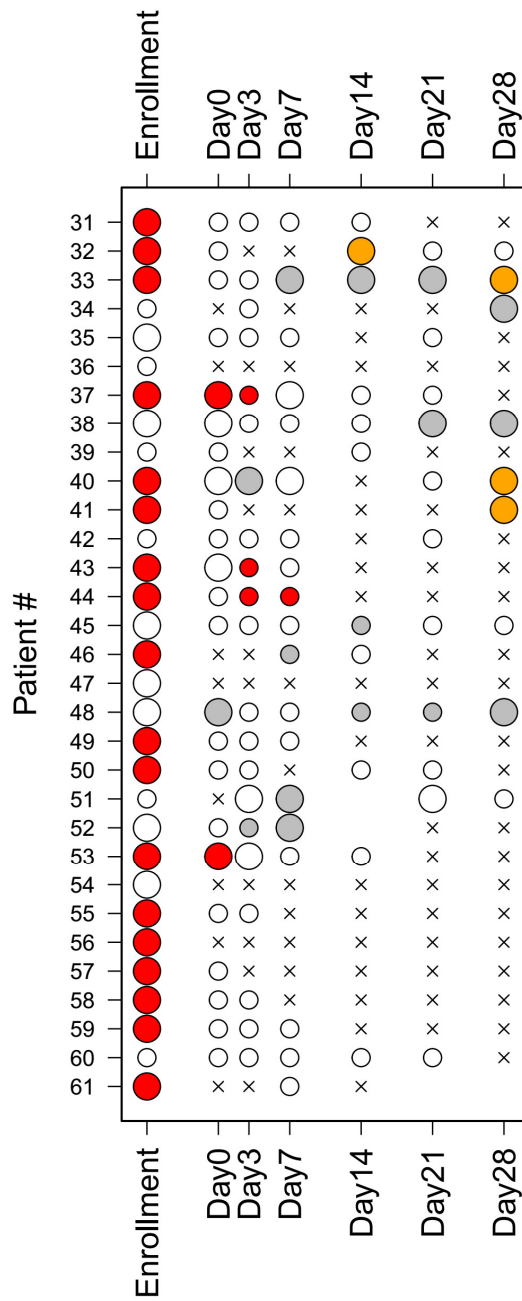
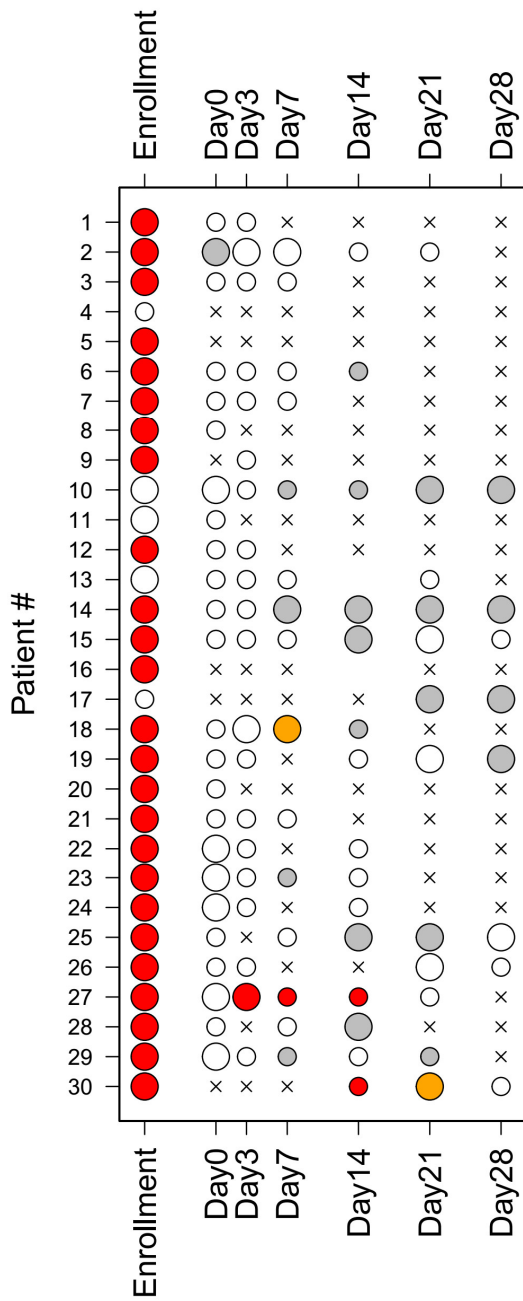
Figure S2. L9LS serum concentrations by study day and study arm. Shown are L9LS serum concentrations on study days 7 and 28 by dose group. Black circles represent participants who received 150 mg or 300 mg of L9LS and were not infected between study days 7 and 28. Red circles represent participants who received 300 mg of L9LS and were infected between study days 7 and 28 (n=8). Blue circles represent participants who received 150 mg of L9LS and were infected between study days 7 and 28 (n=4). The day 7 sample was not available for 1 infected participant in the 150 mg arm; and among the 67 uninfected participants in the 300 mg arm, samples were not available for 7 participants at day 7, and 5 participants at day 28.



Next, we investigated whether there were differences in the baseline prevalence of submicroscopic *P. falciparum* infection in the placebo, 150 mg L9LS, and 300 mg L9LS arms. Despite balanced randomization of baseline characteristics, including *P. falciparum* detected on blood smear at enrollment prior to artemether-lumefantrine administration (Table 1 in main text), the prevalence of submicroscopic infection at enrollment detected with the more sensitive qRT-PCR assay differed across study arms. Infections were detected at enrollment by qRT-PCR in 27 of 75 participants (36.0%) who received placebo, 29 of 75 (38.7%) who received 300 mg of L9LS, and 18 of 75 (24.0%) who received 150 mg of L9LS. Among the 22 participants who had infections detected by blood smear between days 7 and 28, infections were also detected by qRT-PCR at enrollment in 7 of 10 (70%) in the placebo arm, 5 of 8 (62.5%) in the 300 mg L9LS arm, and 4 of 4 (100%) in the 150 mg L9LS arm. The odds of detecting infection by blood smear between days 7 and 28 were higher among participants who were also infected by qRT-PCR at baseline compared to those who were uninfected at baseline (OR = 6.6; 95% CI 2.31-21.7, by Fisher's Exact Test). These data suggest that submicroscopic infection at baseline may be a marker of higher re-infection risk. Indeed, an observational study conducted among children at the same site indicated that baseline infection is a marker of earlier re-infection risk following artemether-lumefantrine administration (Figs. S4 and S5).

Finally, we genotyped infections that had been detected at enrollment by qRT-PCR and by blood smear between days 7 and 28 (Fig. S3) to determine whether early infections were recrudescence following the administration of artemether-lumefantrine, which has been reported to occur in a small proportion of individuals in clinical trials following directly observed administration of artemether-lumefantrine.⁶ Genotyping suggested that 6 of 22 (27.3%) infections that occurred across the 3 study arms between days 7 and 28 were recrudescence infections; this included 3 of 10 (30.0%) in the placebo arm, 2 of 8 (25.0%) in the 300 mg L9LS arm, and 1 of 4 (25.0%) in the 150 mg L9LS arm. The probability (p) that such cases represent new infections instead of recrudescence can be estimated based on strain frequencies in the background population. We estimated these probabilities for participants #27 (p = 0.025), #30 (p = 0.147), #37 (p = 0.025), #43 (p = 0.147), #44 (p = 0.025), and #53 (p = 0.025) using enrollment samples to represent the background population.

Figure S3. Antigen genotyping via 4CAST amplicon sequencing in study participants who had *P. falciparum* infection detected by qRT-PCR at enrollment. Each row represents longitudinal sampling of one participant, with timepoints ascending from left to right. Filled circles indicate samples for which 4CAST sequencing was successful (4CAST+). Circles represent qRT-PCR-positive samples. Small versus large circle size indicates inferred level of parasitemia (≤ 2000 parasites/ml or > 2000 parasites/ml). Fill color indicates the fraction of alleles that matches the enrollment allele set (red: 100%; orange: $< 100\%$ but $> 0\%$; grey: 0%). Timepoints with red fill color that are not preceded (left) by grey or orange fill color therefore may represent cases of recrudescence without additional sources of infection during the elapsed time interval.



qPCR- 4CAST- × qPCR+ 4CAST- ○ qPCR+ 4CAST+ ●
 ● All alleles at timepoint match enrollment
 ○ Subset of alleles at timepoint match enrollment
 × No alleles at timepoint match enrollment

Taken together, we hypothesize that new infections were possible before L9LS reached maximum serum concentrations ~7 days after subcutaneous administration, resulting in patent blood-stage infections 7-21 days later when the prophylactic tail of artemether-lumefantrine administered at enrollment had waned. The higher incidence of early infections in the 300 mg L9LS arm compared to the 150 mg L9LS arm may be related to the higher prevalence of baseline submicroscopic infection in the former, a marker of higher re-infection risk, and a cause of recrudescence infections in some individuals.

Historical observational data demonstrating an association between baseline asymptomatic *P. falciparum* infection before the malaria season and risk of re-infection during the ensuing malaria season (Figures S4 and S5)

In 2011 we initiated an ongoing observational cohort study of malaria in Kalifabougou, Mali,^{5,7} the same site of the present phase 2 trial of L9LS. Before the malaria season in May 2012, 593 asymptomatic children aged 2-13 years were screened for *P. falciparum* infection using a rapid diagnostic test (RDT) that has a sensitivity comparable to that of blood smear⁸. All individuals found to be infected with *P. falciparum* by RDT (n=104) were treated with a standard 3-day course of artemether-lumefantrine, the first daily dose of which was directly observed by study staff. Dried blood spots collected from RDT-negative participants at the same timepoint (n=489) were later analyzed by PCR to retrospectively identify PCR-negative participants (n=434). Subsequently, dried blood spots were collected every two weeks from July through December 2012 during the 6-month malaria season. All dried blood spots were tested by PCR for *P. falciparum*.⁵ We found that the risk of *P. falciparum* infection was higher among children who were asymptotically infected before the malaria season (Fig. S4), independent of age, sex, and hemoglobin type (Fig. S5).

Figure S4. Shown is the cumulative incidence of the first *P. falciparum* blood-stage infection during the 6-month malaria season (irrespective of symptoms being present) for children who were infected with *P. falciparum* by RDT at baseline and treated with artemether-lumefantrine (n=104), and children who were PCR negative for *P. falciparum* at baseline and not treated (n=434). The p value was based on the log-rank test. Shaded areas indicate the 95% confidence intervals.

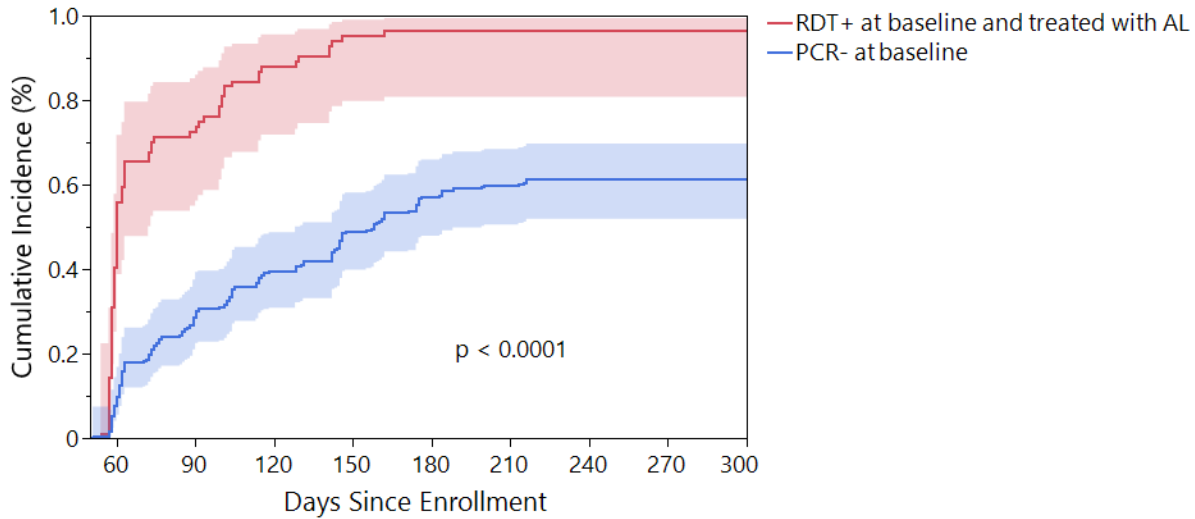
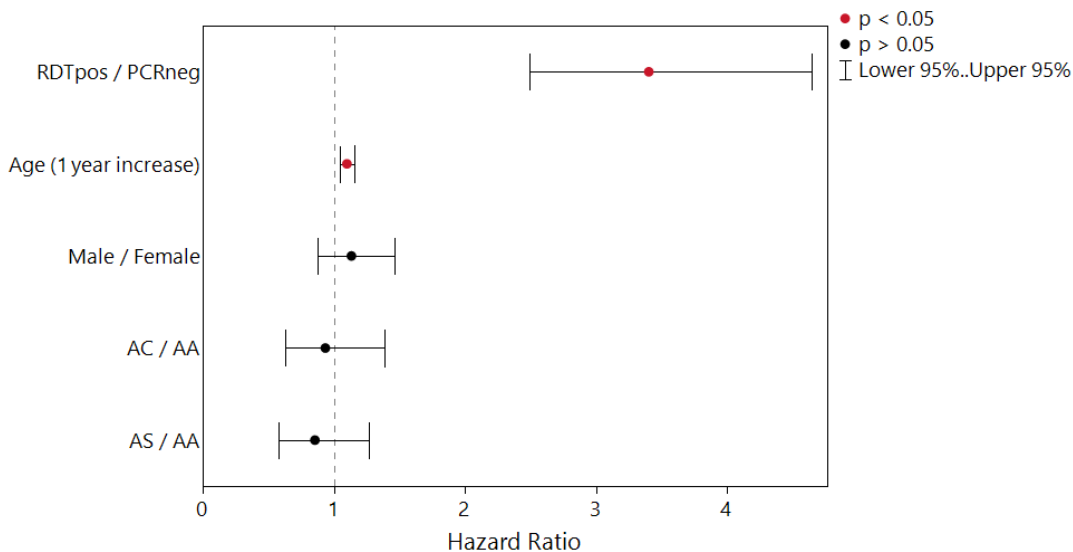
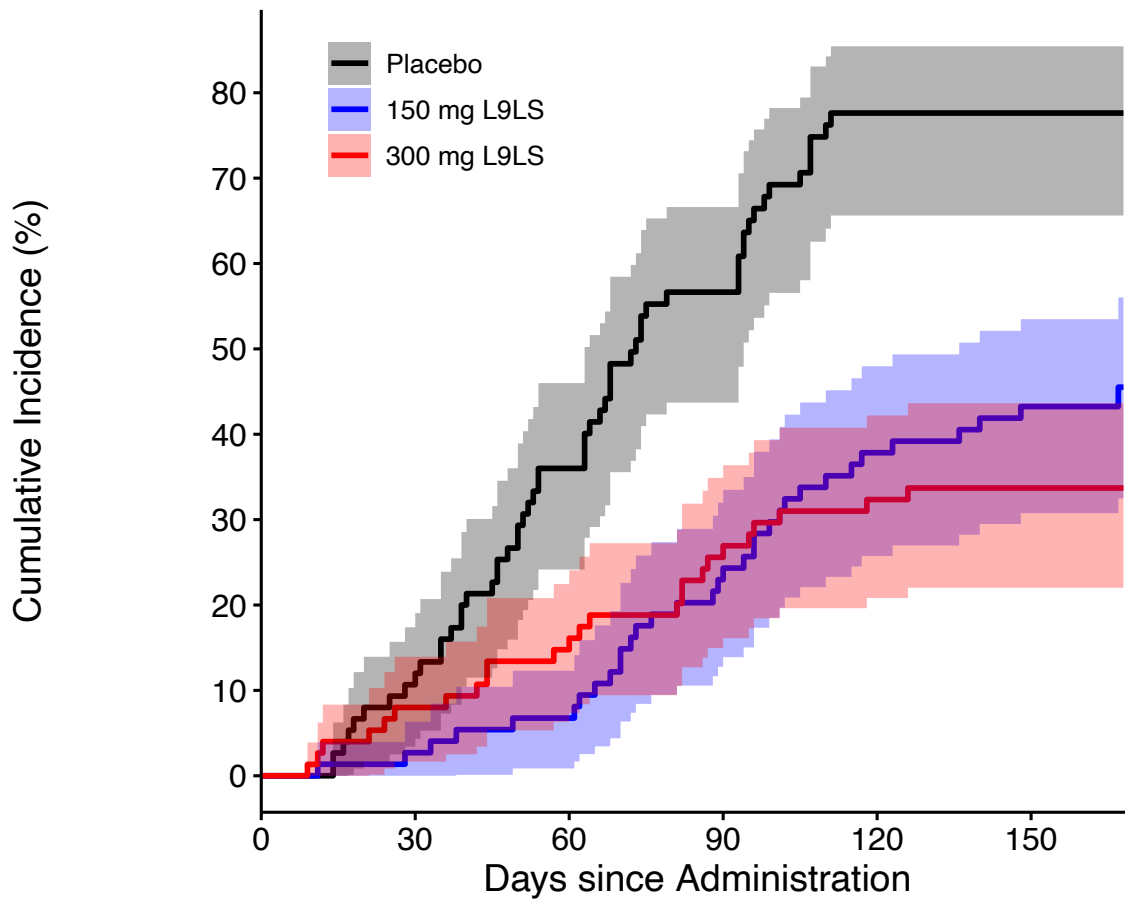


Figure S5. Shown are the results of a Cox proportional-hazards model of the effect of *P. falciparum* infection status in May 2012 on the risk of *P. falciparum* infection (irrespective of symptoms) during the ensuing 2012 malaria season, adjusted for the covariates age, sex, and hemoglobin type. Hazard ratios and 95% confidence intervals are represented by solid circles and horizontal bars, respectively. Red circles are statistically significant ($p < 0.05$), black circles are nonsignificant. The dashed vertical line represents a hazard ratio of 1, meaning no effect. Abbreviations: AA, hemoglobin type AA; AC, hemoglobin type AC; AS, hemoglobin type AS; PCR, polymerase chain reaction; RDT, rapid diagnostic test.



Time to Event Efficacy against Clinical Malaria by the Second Definition (Figure S6).

Shown is the cumulative incidence of the first clinical malaria episode due to *P. falciparum* 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 (second definition) was an illness accompanied by any level of *P. falciparum* asexual parasitemia as detected from microscopic examination of thick blood smear that resulted in the administration of anti-malarial treatment. 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.



Number at risk

Placebo	75	67	47	31	16	16
150 mg L9LS	75	72	69	57	46	39
300 mg L9LS	75	68	63	55	50	45

Proportional Efficacy against *P. falciparum* infection, and Clinical Malaria by the First and Second Definitions

In the secondary efficacy analysis that was based on the Kaplan–Meier estimate of the proportion of participants infected with *P. falciparum* over the 24-week trial period, the efficacy [(1–relative risk)×100] of 300 mg of L9LS as compared with placebo was 50.8% (unadjusted 95% CI, 28.7 to 65.7), and the efficacy of 150 mg of L9LS as compared with placebo was 40.8% (unadjusted 95% CI, 18.4 to 56.7). In the secondary efficacy analysis that was based on the Kaplan–Meier estimate of the proportion of participants experiencing at least one episode of clinical malaria by definition 1 (axillary fever $\geq 37.5^{\circ}\text{C}$, or history of fever in the previous 24 hours, and *P. falciparum* asexual parasitemia $>5,000$ parasites/ μL) over the 24-week trial period, the efficacy [(1–relative risk)×100] of 300 mg of L9LS as compared with placebo was 68.5% (unadjusted 95% CI, 38.8 to 83.3), and the efficacy of 150 mg of L9LS as compared with placebo was 52.6% (unadjusted 95% CI, 19.6 to 71.4). In the secondary efficacy analysis that was based on the Kaplan–Meier estimate of the proportion of participants experiencing at least one episode of clinical malaria by definition 2 (an illness accompanied by any level of *P. falciparum* asexual parasitemia that resulted in the administration of anti-malarial treatment) over the 24-week trial period, the efficacy [(1–relative risk)×100] of 300 mg of L9LS as compared with placebo was 56.6% (unadjusted 95% CI, 32.7 to 71.4), and the efficacy of 150 mg of L9LS as compared with placebo was 41.4% (unadjusted 95% CI, 16.2 to 58.6).

Distribution of Body Weight and Weight-based Dosing of L9LS for Participants in the Efficacy Study (Part B) (Figures S7 and S8)

In Part B, 225 children were randomly assigned (in a 1:1:1 ratio) by block randomization to receive 150 mg of L9LS, 300 mg of L9LS, or placebo (75 participants in each group) by subcutaneous injection. Randomization of participants in each arm was weight-stratified (26-30 kg, n=75; 20-25 kg, n=75; 15-19 kg, n=75). The body weight distribution for each arm in Part B is shown in Figure S7. The distribution of weight-based dosing of L9LS (mg of L9LS administered per kilogram of body weight) for all study participants who received L9LS in Part B is shown in Figure S8.

Figure S7. The body weight distribution for each study arm in the Efficacy study (Part B).

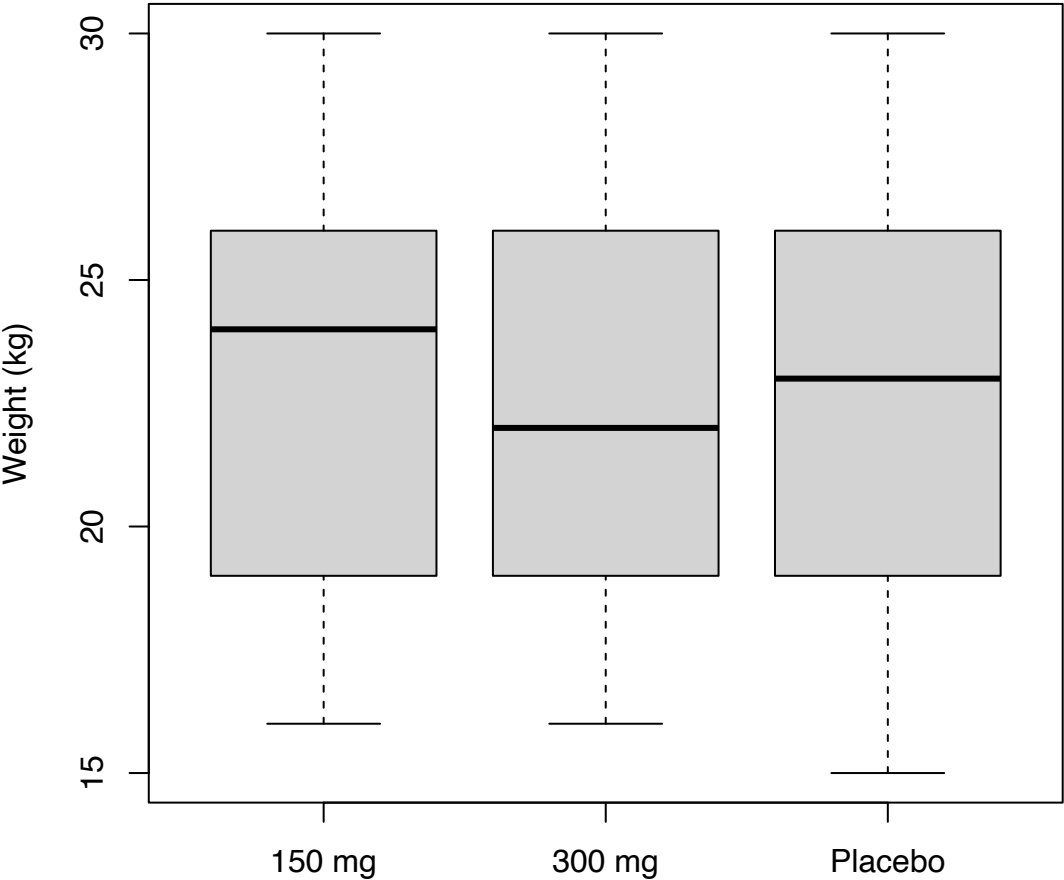
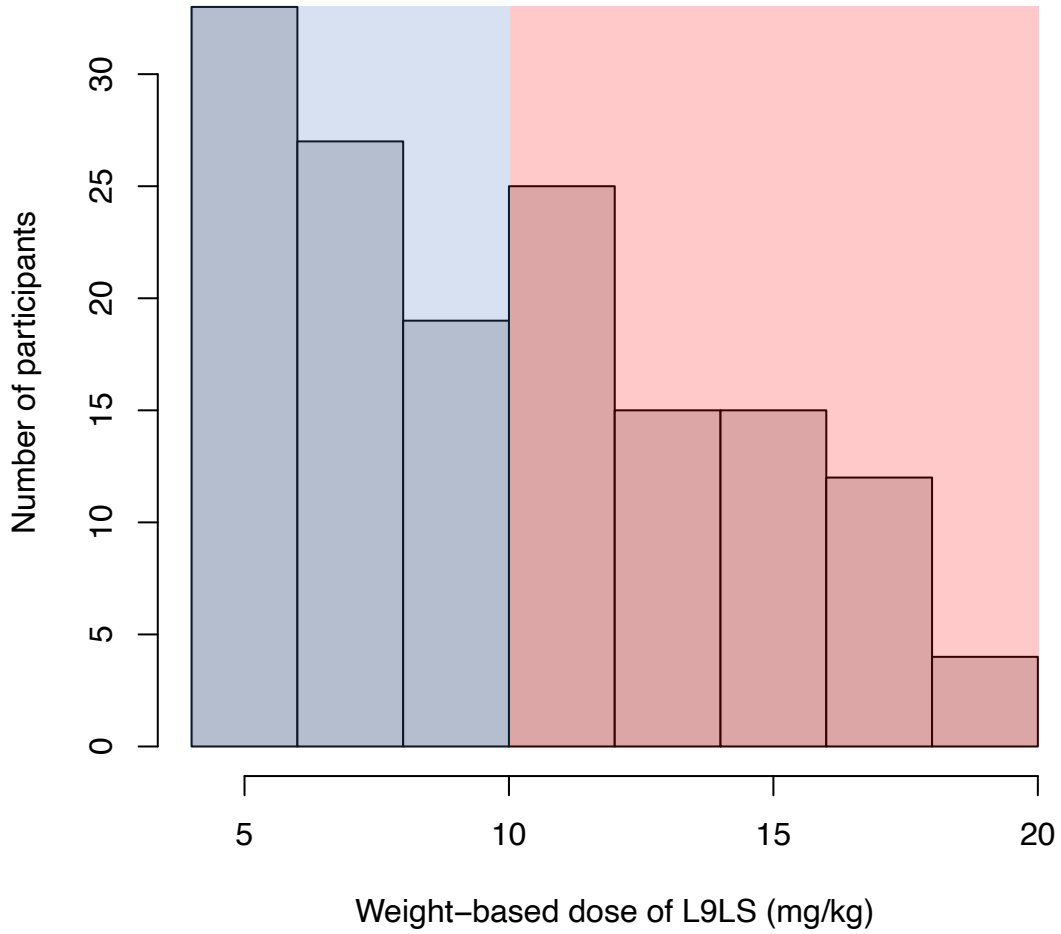


Figure S8. The distribution of weight-based dosing of L9LS for participants who received 150 mg of L9LS (blue) or 300 mg of L9LS (pink) in the Efficacy Study (Part B).



Relationship between Weight-based Dosing of L9LS and Efficacy against *P. falciparum* infection and Clinical Malaria (Figures S9 – S11)

A pre-specified exploratory analysis examined the relationship between the weight-based dose of L9LS (mg of L9LS administered per kilogram of body weight) and the protective efficacy against *P. falciparum* infection (Fig. S9), clinical malaria by definition 1 (Fig. S10), and clinical malaria by definition 2 (Fig. S11).

Figure S9.

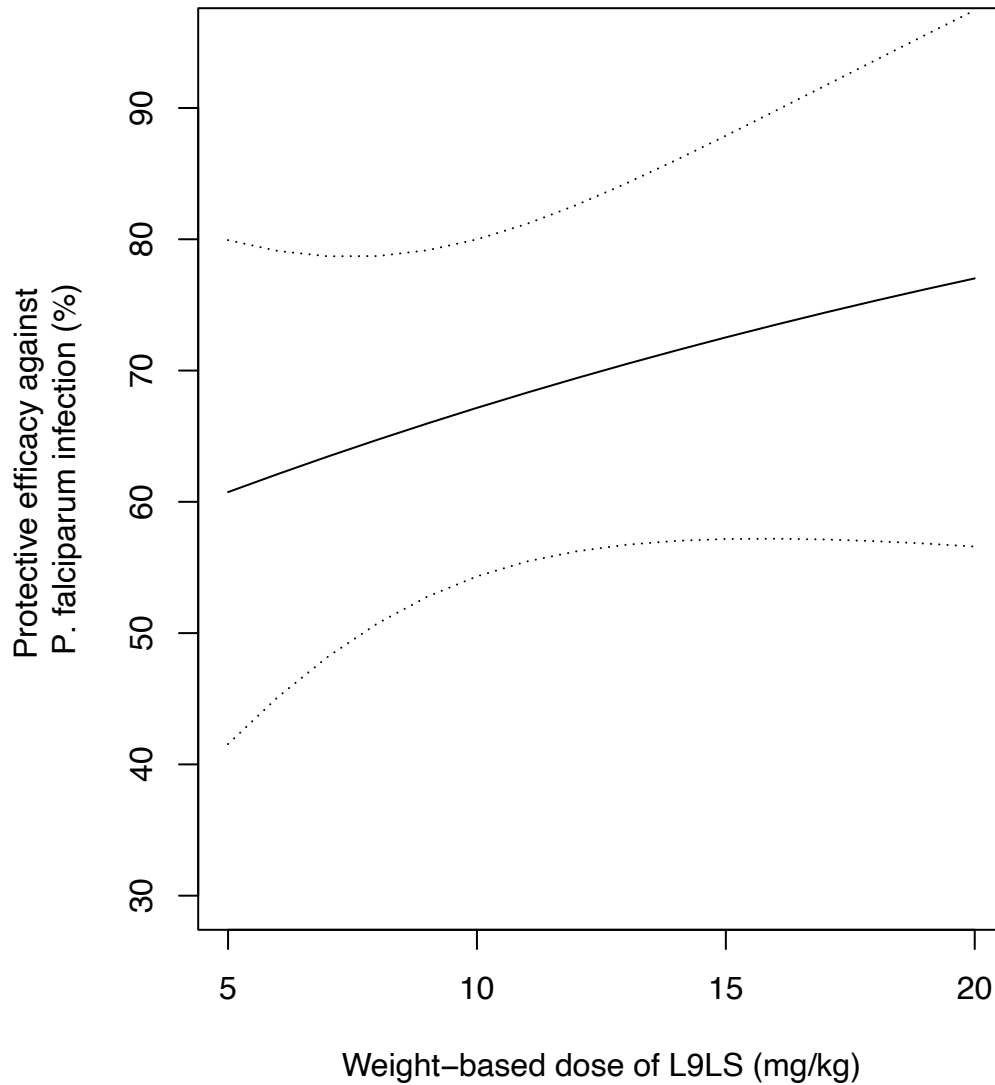


Figure S10.

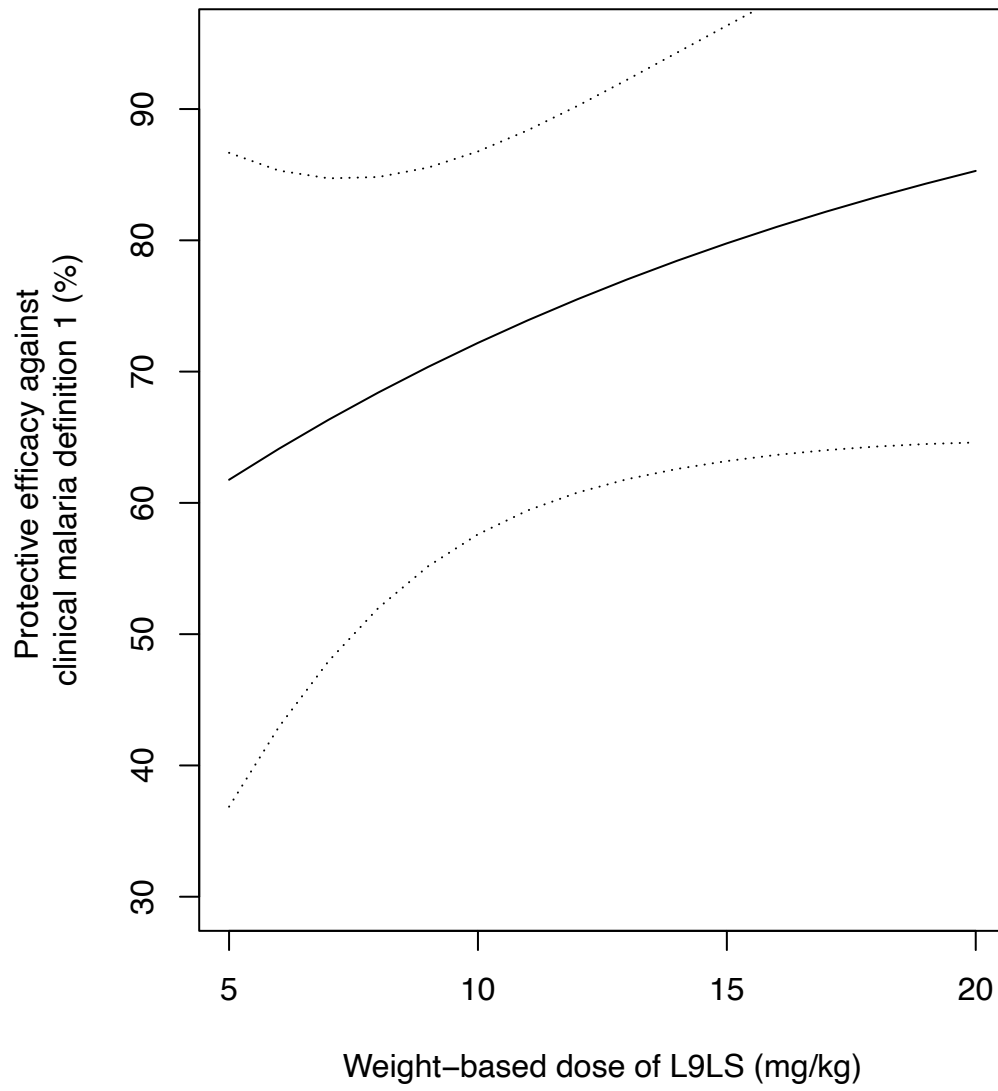
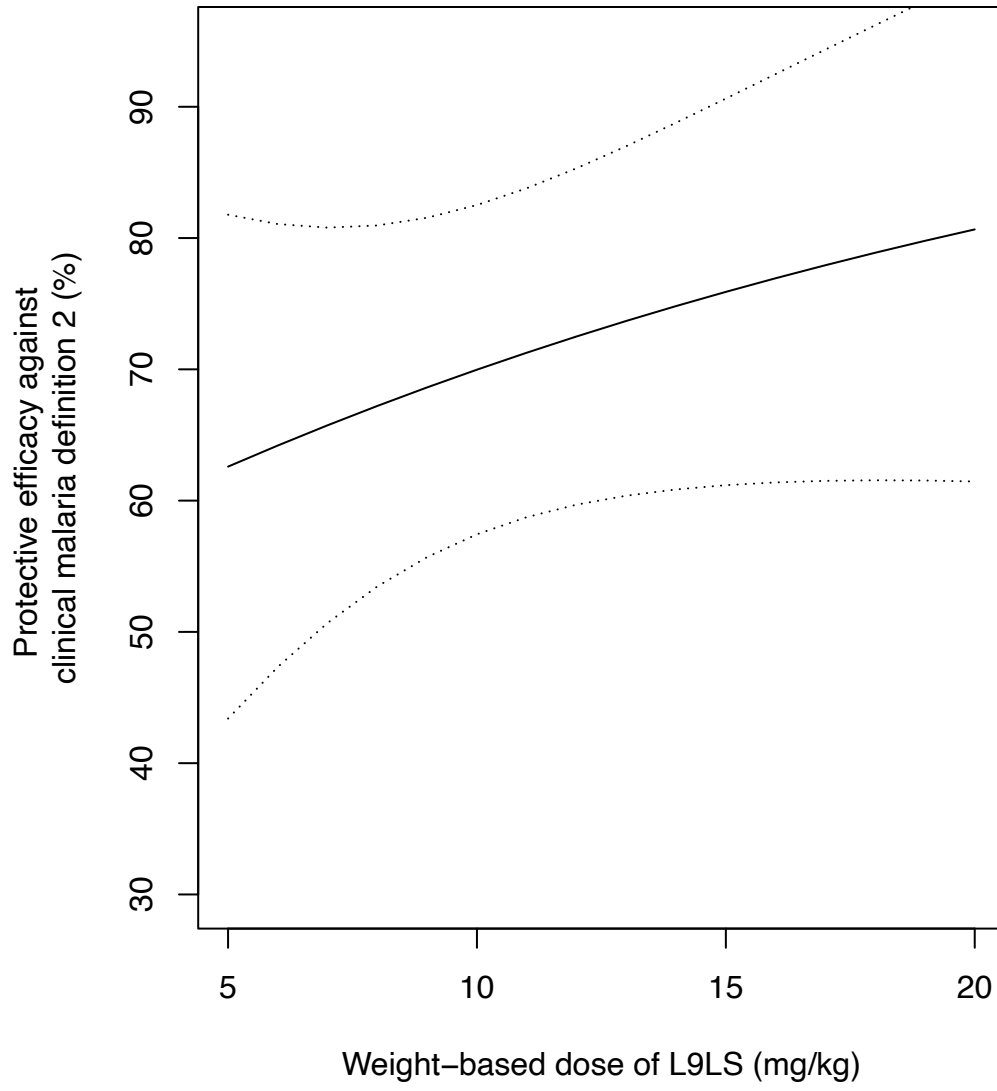


Figure S11.



Recurrent Event Analysis of Clinical Malaria (Figures S12 and S13)

According to the first definition of clinical malaria, the mean number of clinical malaria cases per person-year was 2.014, 0.794, and 0.544 in the placebo arm, the 150 mg L9LS arm, and the 300 mg L9LS arm, respectively. The protective efficacy was 62% (unadjusted 95% confidence interval: 39-76%) for 150 mg of L9LS and 73% (unadjusted 95% confidence interval: 53-85%) for 300 mg of L9LS based on Andersen-Gill's recurrent event analysis. According to the second definition of clinical malaria, the mean number of clinical malaria cases per person-year was 3.391, 1.333, and 0.897 in the placebo arm, the 150 mg L9LS arm, and the 300 mg L9LS arm, respectively. The protective efficacy was 62% (unadjusted 95% confidence interval: 46-73%) for 150 mg of L9LS and 74% (unadjusted 95% confidence interval: 61-82%) for 300 mg of L9LS based on Andersen-Gill's recurrent event analysis. Shown below is the cumulative number of clinical malaria cases by definition 1 (Fig. S12) and definition 2 (Fig. S13).

Figure S12.

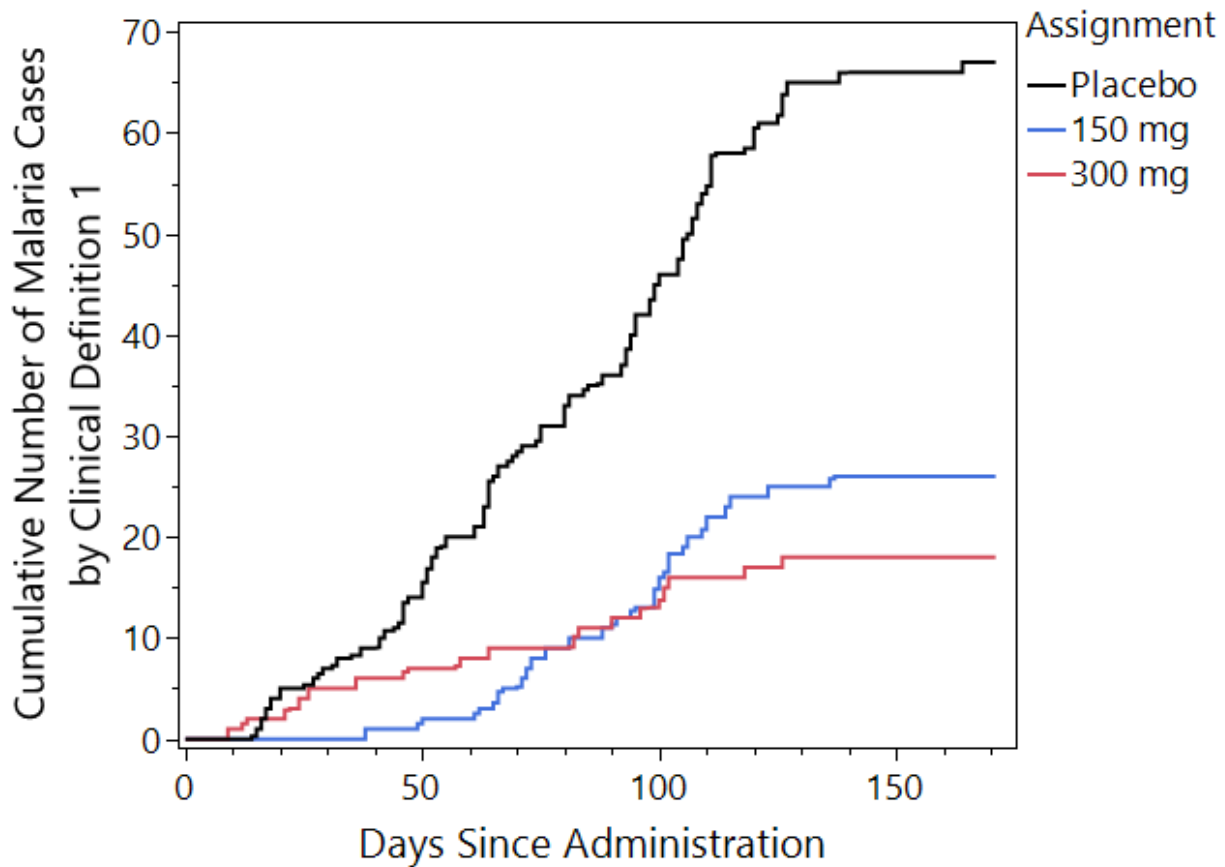
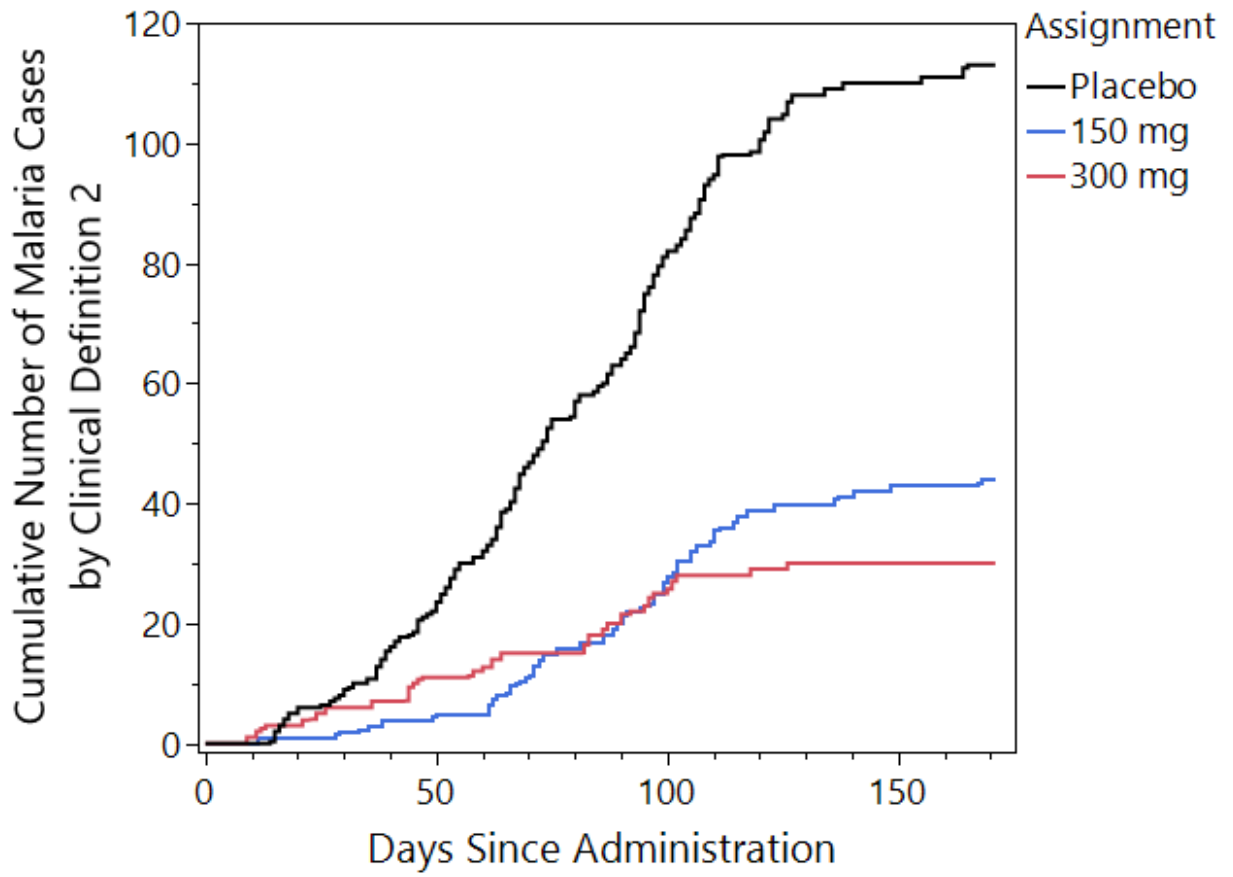


Figure S13.



Supplemental Tables

Table S1. Characteristics of the adult participants in the dose escalation study (Part A) at baseline, according to dose group.

Characteristic	L9LS 300 mg SC (N=6)	L9LS 600 mg SC (N=6)	L9LS 20 mg/kg IV (N=6)
Age, median (range)—yr	30 (18-49)	29 (18-44)	31 (19-36)
Sex—no. (%)			
Female	3 (50.0)	4 (66.7)	4 (66.7)
Male	3 (50.0)	2 (33.3)	2 (33.3)
Weight, median (range)—kg	55 (40-60)	57 (51-60)	55 (51-60)
Site—no. (%)			
Kalifabougou	6 (100)	6 (100)	6 (100)
Torodo	0	0	0
Any <i>Plasmodium</i> species detected on blood smear at enrollment— no. (%)	1 (16.7)	2 (33.3)	0

<i>Plasmodium falciparum</i>	1 (16.7)	2 (33.3)	0
<i>Plasmodium malariae</i>	0	0	0
<i>Plasmodium ovale</i>	0	0	0
Hemoglobin genotype—no. (%)			
HbAA	5 (83.3)	6 (100)	3 (50)
HbAS	1 (16.7)	0	2 (33.3)
HbAC	0	0	1 (16.7)
HbCC	0	0	0
HbSC	0	0	0

Table S2. Characteristics of the pediatric participants in the dose escalation study (Part A) at baseline, according to dose group.

Characteristic	L9LS 150 mg SC (N=9)	L9LS 300 mg SC (N=9)	Placebo (N=18)
Age, median (range)—yr	8 (7-10)	8 (6-10)	8 (6-10)
Sex—no. (%)			
Female	7 (77.8)	3 (33.3)	8 (44.4)
Male	2 (22.2)	6 (66.7)	10 (55.6)
Weight, median (range)—kg	23 (16-30)	22 (17-29)	23 (15-29)
Site—no. (%)			
Kalifabougou	9 (100)	9 (100)	18 (100)
Torodo	0	0	0
Any <i>Plasmodium</i> species detected on blood smear at enrollment— no. (%)	0	5 (55.6)	0
<i>Plasmodium</i> <i>falciparum</i>	0	4 (44.4)	0

<i>Plasmodium malariae</i>	0	0	0
<i>Plasmodium ovale</i>	0	1 (11.1)	0
Hemoglobin genotype—no. (%)			
HbAA	9 (100)	8 (88.9)	16 (88.9)
HbAS	0	0	2 (11.1)
HbAC	0	1 (11.1)	0
HbCC	0	0	0
HbSC	0	0	0

Table S3. Solicited maximum local and systemic reactogenicity within 7 days after administration of L9LS among adult participants in the dose escalation study (Part A), according to dose group.

Symptom and Severity¹	L9LS 300 mg SC (N=6)	L9LS 600 mg SC (N=6)	L9LS 20 mg/kg IV (N=6)
	<i>number of participants (percent)</i>		
Local reactogenicity			
Pain			
None	6 (100)	6 (100)	6 (100)
Tenderness			
None	6 (100)	6 (100)	6 (100)
Pruritis			
None	6 (100)	6 (100)	6 (100)
Swelling			
None	3 (50.0)	5 (83.3)	6 (100)
Mild	3 (50.0)	1 (16.7)	0
Redness			
None	6 (100)	6 (100)	6 (100)
Bruising			
None	6 (100)	6 (100)	6 (100)

Any local symptom			
None	3 (50.0)	5 (83.3)	6 (100)
Mild	3 (50.0)	1 (16.7)	0
Systemic reactogenicity			
Fever			
None	6 (100)	6 (100)	6 (100)
Malaise			
None	6 (100)	6 (100)	6 (100)
Muscle aches			
None	6 (100)	6 (100)	6 (100)
Headache			
None	6 (100)	6 (100)	6 (100)
Chills			
None	6 (100)	6 (100)	6 (100)
Nausea			
None	6 (100)	6 (100)	6 (100)
Joint Pain			
None	6 (100)	6 (100)	6 (100)
Any systemic symptom			
None	6 (100)	6 (100)	6 (100)

¹For participants reporting multiple episodes of a given adverse event, the event type is counted once per participant at the maximum severity. There was no moderate (Grade 2), severe (Grade 3) or life-threatening (Grade 4) solicited local or systemic reactogenicity reported within 7 days after administration of L9LS among adult participants in the dose escalation study (Part A).

Table S4. Solicited maximum local and systemic reactogenicity within 7 days after administration of L9LS among pediatric participants in the dose escalation study (Part A), according to dose group.

Symptom and Severity¹	L9LS 150 mg SC (N=9)	L9LS 300 mg SC (N=9)	Placebo (N=18)
	<i>number of participants (percent)</i>		
Local reactogenicity			
Pain			
None	9 (100)	9 (100)	18 (100)
Tenderness			
None	9 (100)	9 (100)	18 (100)
Pruritis			
None	9 (100)	9 (100)	18 (100)
Swelling			
None	9 (100)	9 (100)	18 (100)
Redness			
None	9 (100)	9 (100)	18 (100)
Bruising			
None	9 (100)	9 (100)	18 (100)
Any local symptom			

None	9 (100)	9 (100)	18 (100)
Systemic reactogenicity			
Fever			
None	9 (100)	9 (100)	18 (100)
Malaise			
None	9 (100)	9 (100)	18 (100)
Muscle aches			
None	9 (100)	9 (100)	18 (100)
Headache			
None	9 (100)	9 (100)	17 (94.4)
Moderate	0	0	1 (5.6)
Chills			
None	9 (100)	9 (100)	18 (100)
Nausea			
None	9 (100)	9 (100)	18 (100)
Joint Pain			
None	9 (100)	9 (100)	18 (100)
Any systemic symptom			
None	9 (100)	9 (100)	17 (94.4)
Moderate	0	0	1 (5.6)

¹For participants reporting multiple episodes of a given adverse event, the event type is counted once per participant at the maximum severity. There was no mild (Grade 1), severe (Grade 3) or life-threatening (Grade 4) solicited local or systemic reactogenicity reported within 7 days after administration of L9LS or placebo among pediatric participants in the dose escalation study (Part A)

Table S5. Adverse events among adult participants in the dose escalation study (Part A) from the date of L9LS administration through the 28-week study period, according to study arm.¹

Adverse Event System Organ Class ² Adverse Event Preferred Term	All Study Arms	L9LS Arm 1: 300 mg SC		L9LS Arm 2: 600 mg SC		L9LS Arm 3: 20 mg/kg IV	
		G1 Mild	G2 Moderate	G1 Mild	G2 Moderate	G1 Mild	G2 Moderate
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	15						
RESPIRATORY DISORDER ³	12	1 (0) 0%	5 (4) 66.7%	0 (0) 0%	1 (1) 16.7%	0 (0) 0%	5 (4) 66.7%
COUGH	3	0 (0) 0%	0 (0) 0%	0 (0) 0%	2 (1) 16.7%	0 (0) 0%	1 (1) 16.7%
INFECTIONS AND INFESTATIONS	9						
MALARIA ⁴	4	0 (0) 0%	1 (1) 16.7%	0 (0) 0%	2 (2) 33.3%	0 (0) 0%	1 (1) 16.7%
RHINITIS	4	0 (0) 0%	1 (1) 16.7%	0 (0) 0%	2 (2) 33.3%	0 (0) 0%	1 (1) 16.7%
PARONYCHIA	1	0 (0) 0%	0 (0) 0%	0 (0) 0%	1 (1) 16.7%	0 (0) 0%	0 (0) 0%
NERVOUS SYSTEM DISORDERS	8						
HEADACHE	8	0 (0) 0%	4 (3) 50%	0 (0) 0%	3 (2) 33.3%	0 (0) 0%	1 (1) 16.7%
GASTROINTESTINAL DISORDERS	7						
GASTRITIS ⁵	4	1 (1) 16.7%	0 (0) 0%	0 (0) 0%	1 (1) 16.7%	0 (0) 0%	2 (2)
ABDOMINAL PAIN	2	0 (0) 0%	0 (0) 0%	1 (1) 16.7%	0 (0) 0%	0 (0) 0%	1 (1) 16.7%
DYSPEPSIA	1	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	1 (1) 16.7%
BLOOD AND LYMPHATIC SYSTEM DISORDERS	5						
NEUTROPENIA	4	1 (1) 16.7%	0 (0) 0%	1 (1) 16.7%	0 (0) 0%	2 (1) 16.7%	0 (0) 0%
LEUKOPENIA	1	0 (0) 0%	0 (0) 0%	1 (1) 16.7%	0 (0) 0%	0 (0) 0%	0 (0) 0%
CARDIAC DISORDERS	5						
TACHYCARDIA	5	2 (1) 16.7%	0 (0) 0%	1 (1) 16.7%	1 (1) 16.7%	1 (1) 16.7%	0 (0) 0%
GENERAL DISORDERS AND ADMN SITE CONDITIONS	1						
PYREXIA	1	0 (0) 0%	0 (0) 0%	1 (1) 16.7%	0 (0) 0%	0 (0) 0%	0 (0) 0%
INVESTIGATIONS	1						
HAEMOGLOBIN DECREASED	1	0 (0) 0%	0 (0) 0%	0 (0) 0%	1 (1) 16.7%	0 (0) 0%	0 (0) 0%

¹This table presents all unsolicited adverse events among adult participants in the dose escalation study (Part A) from the date of L9LS administration through the end of the 28-week study period. It also includes all adverse events related to laboratory assessments collected for 14 days after L9LS administration. All solicited adverse events are excluded from this table and can be found in Table S3. As pre-specified in the protocol, all 18 adult participants who received L9LS in the dose escalation study (Part A) were included in the safety analysis. Each field has the format # (X) %, where # is the number of adverse events, X is the number of participants with one or more episodes of the given event, and % is the number of participants with one or more episodes of the given event divided by the total number of participants who received L9LS multiplied by 100. For participants reporting multiple episodes of a given event, the event type is counted once per participant at the maximum severity.

²Adverse events were coded using the Medical Dictionary for Regulatory Activities (MedDRA), version 24.1. Events were grouped by System Organ Classes (SOCs) and line listed by Preferred Term (PT).

³All cases of the Preferred Term “Respiratory disorder” were documented by study investigators as “upper respiratory illness” which was coded as the MedDRA Lowest Level Term (LLT) “Upper respiratory disorder”.

⁴Participants with any signs or symptoms of malaria (e.g. fever, headache, myalgias, nausea, vomiting) and a positive blood smear (any level of parasitemia) and/or a positive rapid diagnostic test (RDT) were diagnosed with malaria and provided with standard treatment following the recommendations of the Mali National Malaria Control Program. All malaria cases among adults in the dose escalation study (Part A) were *P. falciparum*.

⁵The Preferred Terms “Gastritis” and “Ulcer” were combined into the Preferred Term “Gastritis” since study investigators described gastritis-like syndromes as “Gastritis” and “Ulcer” interchangeably.

Table S6. Adverse events among pediatric participants in the dose escalation study (Part A) from the date of L9LS or placebo administration through the 28-week study period, according to study arm.¹

Adverse Event System Organ Class ² Adverse Event Preferred Term	All Study Arms	L9LS Arm 1: 150 mg SC			L9LS Arm 2: 300 mg SC		L9LS Arm 3: Placebo	
		G1 Mild	G2 Moderate	G4 Pot Life Threatening	G1 Mild	G2 Moderate	G1 Mild	G2 Moderate
INFECTIONS AND INFESTATIONS	70							
MALARIA ³	51	0 (0) 0%	10 (7) 77.8%	0 (0) 0%	0 (0) 0%	11 (7) 77.8%	0 (0) 0%	30 (13) 72.2%
RHINITIS	12	0 (0) 0%	5 (4) 44.4%	0 (0) 0%	0 (0) 0%	4 (3) 33.3%	0 (0) 0%	3 (3) 16.7%
PHARYNGITIS	3	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	3 (3) 16.7%
BACTERIAL INFECTION	1	0 (0) 0%	1 (1) 11.1%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
CONJUNCTIVITIS	1	0 (0) 0%	1 (1) 11.1%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
EAR INFECTION	1	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	1 (1) 5.6%
TONSILLITIS	1	0 (0) 0%	1 (1) 11.1%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	28							
RESPIRATORY DISORDER ⁴	19	0 (0) 0%	6 (4) 44.4%	0 (0) 0%	0 (0) 0%	6 (4) 44.4%	0 (0) 0%	7 (6) 33.3%
COUGH	9	0 (0) 0%	2 (2) 22.2%	0 (0) 0%	0 (0) 0%	3 (2) 22.2%	0 (0) 0%	4 (3) 16.7%
NERVOUS SYSTEM DISORDERS	23							
HEADACHE	21	1 (1) 11.1%	5 (5) 55.6%	0 (0) 0%	0 (0) 0%	4 (3) 33.3%	0 (0) 0%	11 (7) 38.9%
DIZZINESS	2	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	2 (1) 5.6%	0 (0) 0%
GASTROINTESTINAL DISORDERS	17							
ABDOMINAL PAIN	11	1 (1) 11.1%	0 (0) 0%	0 (0) 0%	0 (0) 0%	1 (1) 11.1%	5 (4) 22.2%	4 (3) 16.7%
DIARRHOEA	2	0 (0) 0%	1 (1) 11.1%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	1 (1) 5.6%
VOMITING	2	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	1 (1) 5.6%	1 (1) 5.6%
FOOD POISONING	1	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	1 (1) 5.6%
NAUSEA	1	1 (1) 11.1%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
BLOOD AND LYMPHATIC SYSTEM DISORDERS	13							
LEUKOPENIA	6	2 (1) 11.1%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	4 (3) 16.7%	0 (0) 0%
NEUTROPENIA	6	2 (2) 22.2%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	3 (3) 16.7%	1 (1) 5.6%
LEUKOCYTOSIS	1	0 (0) 0%	0 (0) 0%	1 (1) 11.1%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
GENERAL DISORDERS AND ADMN SITE CONDITIONS	4							
PYREXIA	4	2 (2) 22.2%	0 (0) 0%	0 (0) 0%	1 (1) 11.1%	0 (0) 0%	1 (1) 5.6%	0 (0) 0%
INVESTIGATIONS	4							
BLOOD PRESSURE SYSTOLIC DECREASED	3	0 (0) 0%	1 (1) 11.1%	0 (0) 0%	0 (0) 0%	0 (0) 0%	1 (1) 5.6%	1 (1) 5.6%
HAEMOGLOBIN DECREASED	1	1 (1) 11.1%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
CARDIAC DISORDERS	3							
TACHYCARDIA	2	1 (1) 11.1%	1 (1) 11.1%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
BRADYCARDIA	1	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	1 (1) 5.6%	0 (0) 0%
METABOLISM AND NUTRITION DISORDERS	1							
DECREASED APPETITE	1	0 (0) 0%	1 (1) 11.1%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%

¹This table presents all unsolicited adverse events among pediatric participants in the dose escalation study (Part A) from the date of L9LS or placebo administration through the end of the 28-week study period. It also includes all adverse events related to laboratory assessments collected for 14 days after L9LS or placebo administration. All solicited adverse events are excluded from this table and can be found in Table S4. As pre-specified in the protocol, all 36 pediatric participants who received study agent in the dose escalation study (Part A) were included in the safety analysis. Each field has the format # (X) %, where # is the number of adverse events, X is the number of participants with one or more episodes of the given event, and % is the number of participants with one or more episodes of the given event divided by the total number of participants who received the study agent multiplied by 100. For participants reporting multiple episodes of a given event, the event type is counted once per participant at the maximum severity.

²Adverse events were coded using the Medical Dictionary for Regulatory Activities (MedDRA), version 24.1. Events were grouped by System Organ Classes (SOCs) and line listed by Preferred Term (PT).

³Participants with any signs or symptoms of malaria (e.g. fever, headache, myalgias, nausea, vomiting) and a positive blood smear (any level of parasitemia) and/or a positive rapid diagnostic test (RDT) were diagnosed with malaria and provided with standard treatment following the recommendations of the Mali National Malaria Control Program. Among children in the dose escalation study (Part A), two malaria cases were *Plasmodium ovale*, and all others were *P. falciparum*.

⁴All cases of the Preferred Term “Respiratory disorder” were documented by study investigators as “upper respiratory illness” which was coded as the MedRA Lowest Level Term (LLT) “Upper respiratory disorder”.

Table S7. Adverse events in the efficacy study (Part B) from the date of L9LS or placebo administration through the 24-week study period, according to study arm.¹

Adverse Event System Organ Class ² Adverse Event Preferred Term	All Study Arms	L9LS Arm 1: 150 mg SC		L9LS Arm 2: 300 mg SC		Placebo	
		G1 Mild	G2 Moderate	G1 Mild	G2 Moderate	G1 Mild	G2 Moderate
INFECTIONS AND INFESTATIONS	335						
MALARIA ³	220	0 (0) 0%	50 (36) 48.0%	0 (0) 0%	42 (33) 44.0%	0 (0) 0%	128 (59) 78.7%
RHINITIS	107	0 (0) 0%	29 (19) 25.3%	1 (1) 1.3%	37 (25) 33.3%	2 (1) 1.3%	38 (26) 34.7%
TONSILLITIS	3	0 (0) 0%	3 (3) 4.0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
PHARYNGITIS	2	0 (0) 0%	1 (1) 1.3%	0 (0) 0%	0 (0) 0%	0 (0) 0%	1 (1) 1.3%
URINARY TRACT INFECTION	2	0 (0) 0%	1 (1) 1.3%	0 (0) 0%	1 (1) 1.3%	0 (0) 0%	0 (0) 0%
TINEA CAPITIS	1	0 (0) 0%	1 (1) 1.3%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	211						
RESPIRATORY DISORDER ⁴	142	0 (0) 0%	46 (35) 46.7%	0 (0) 0%	49 (36) 48.0%	0 (0) 0%	47 (33) 44.0%
COUGH	69	1 (0) 0%	18 (13) 17.3%	1 (1) 1.3%	21 (17) 22.7%	2 (1) 1.3%	26 (21) 28.0%
NERVOUS SYSTEM DISORDERS	96						
HEADACHE	96	2 (2) 2.7%	32 (24) 32.0%	1 (1) 1.3%	33 (25) 33.3%	2 (1) 1.3%	26 (20) 26.7%
GASTROINTESTINAL DISORDERS	80						
ABDOMINAL PAIN	65	9 (8) 10.7%	12 (11) 14.7%	6 (1) 1.3%	16 (11) 14.7%	6 (4) 5.3%	16 (12) 16.0%
VOMITING	7	1 (1) 1.3%	0 (0) 0%	2 (2) 2.7%	0 (0) 0%	2 (2) 2.7%	2 (2) 2.7%
FOOD POISONING	5	0 (0) 0%	1 (1) 1.3%	0 (0) 0%	3 (3) 4.0%	0 (0) 0%	1 (1) 1.3%
TOOTHACHE	2	0 (0) 0%	1 (1) 1.3%	0 (0) 0%	0 (0) 0%	0 (0) 0%	1 (1) 1.3%
DIARRHOEA	1	0 (0) 0%	0 (0) 0%	0 (0) 0%	1 (1) 1.3%	0 (0) 0%	0 (0) 0%
GENERAL DISORDERS AND ADMN SITE CONDITIONS	33						
PYREXIA	29	7 (7) 9.3%	2 (2) 2.7%	10 (10) 13.3%	3 (3) 4.0%	5 (5) 6.7%	2 (1) 1.3%
CHILLS	4	0 (0) 0%	0 (0) 0%	3 (3) 4.0%	0 (0) 0%	1 (1) 1.3%	0 (0) 0%
INVESTIGATIONS	23						
BLOOD PRESSURE SYSTOLIC DECREASED	16	5 (4) 5.3%	1 (1) 1.3%	4 (4) 5.3%	0 (0) 0%	4 (3) 4.0%	2 (2) 2.7%
PLATELET COUNT DECREASED	3	0 (0) 0%	0 (0) 0%	1 (1) 1.3%	1 (1) 1.3%	1 (1) 1.3%	0 (0) 0%
ALT INCREASED	2	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	2 (2) 2.7%	0 (0) 0%
BLOOD PRESSURE DIASTOLIC INCREASED	2	1 (1) 1.3%	0 (0) 0%	1 (1) 1.3%	0 (0) 0%	0 (0) 0%	0 (0) 0%
CARDIAC DISORDERS	17						
TACHYCARDIA	14	1 (1) 1.3%	0 (0) 0%	5 (3) 4.0%	1 (1) 1.3%	7 (6) 8%	0 (0) 0%
BRADYCARDIA	3	0 (0) 0%	0 (0) 0%	1 (1) 1.3%	1 (1) 1.3%	1 (1) 1.3%	0 (0) 0%
BLOOD AND LYMPHATIC SYSTEM DISORDERS	11						
NEUTROPENIA	7	1 (1) 1.3%	0 (0) 0%	3 (3) 4.0%	0 (0) 0%	3 (3) 4.0%	0 (0) 0%
LEUKOCYTOSIS	2	0 (0) 0%	0 (0) 0%	0 (0) 0%	1 (1) 1.3%	1 (1) 1.3%	0 (0) 0%
LEUKOPENIA	2	0 (0) 0%	0 (0) 0%	2 (2) 2.7%	0 (0) 0%	0 (0) 0%	0 (0) 0%
EAR AND LABYRINTH DISORDERS	1						
EAR PAIN	1	0 (0) 0%	0 (0) 0%	0 (0) 0%	1 (1) 16.7%	0 (0) 0%	0 (0) 0%
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	1						
WOUND	1	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	1 (1) 1.3%
METABOLISM AND NUTRITION DISORDERS	1						
DECREASED APPETITE	1	0 (0) 0%	1 (1) 1.3%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%

¹This table presents all unsolicited adverse events in the efficacy study (Part B) from the date of L9LS or placebo administration through the end of the 24-week study period. It also includes all adverse events related to laboratory assessments collected for 14 days after study agent administration. All solicited adverse

events are excluded from this table and can be found in Table 2 in the main text. As pre-specified in the protocol, all 225 participants who received study agent in the efficacy study (Part B) were included in the safety analysis. Each field has the format # (X) %, where # is the number of adverse events, X is the number of subjects with one or more episodes of the given event, and % is the number of participants with one or more episodes of the given event divided by the total number of participants who received the study agent multiplied by 100. For participants reporting multiple episodes of a given event, the event type is counted once per participant at the maximum severity.

²Adverse events were coded using the Medical Dictionary for Regulatory Activities (MedDRA), version 24.1. Events were grouped by System Organ Classes (SOCs) and line listed by Preferred Term (PT).

³Participants with any signs or symptoms of malaria (e.g. fever, headache, myalgias, nausea, vomiting) and a positive blood smear (any level of parasitemia) and/or a positive rapid diagnostic test (RDT) were diagnosed with malaria and provided with standard treatment following the recommendations of the Mali National Malaria Control Program. Included in malaria adverse events are 3 cases of *P. ovale* in the placebo arm, 3 cases of *P. ovale* in the L9LS 300 mg arm, and 1 case of *P. ovale* in the L9LS 150 mg arm; 1 case of *P. malariae* in the placebo arm, 3 cases of *P. malariae* in the L9LS 300 mg arm, and 2 cases of *P. malariae* in the L9LS 150 mg arm; 2 cases of mixed *P. falciparum* and *P. ovale* in the L9LS 150 mg arm; 1 case of mixed *P. falciparum* and *P. malariae* in the L9LS 150 mg arm, and 1 case of mixed *P. falciparum* and *P. malariae* in the placebo arm. All remaining malaria cases were *P. falciparum* alone.

⁴All cases of the Preferred Term “Respiratory disorder” were documented by study investigators as “upper respiratory illness” which was coded as the MedRA Lowest Level Term (LLT) “Upper respiratory disorder”.

Table S8. Representativeness of study participants.

Category	Details
Disease under investigation	Malaria, a mosquito-borne disease caused by <i>Plasmodium</i> parasites.
Special considerations related to:	
Sex and gender	Although malaria affects both males and females, gender roles and gender dynamics give rise to different vulnerabilities, such as exposure patterns. Though males may be more vulnerable than females to exposure, females may be more vulnerable than males to the consequences of malaria, particularly during pregnancy.
Age	Infants and children under 5 years of age account for approximately 80% of all malaria deaths in Sub-Saharan Africa, as young children have not had time to develop partial clinical immunity that can develop over years of repeated infections.
Race or ethnic group	Anyone can get malaria; however, Africans carry a disproportionately high share of the global malaria burden given the high transmission rates of <i>Plasmodium falciparum</i> in this region.
Geography	Malaria occurs mostly in low and lower middle income tropical and subtropical areas of the world. Africa is the most affected due to a combination of factors: an efficient mosquito vector, parasite species (<i>Plasmodium falciparum</i>), weather conditions, limited resources, and socio-economic instability. Outside of Africa, malaria transmission can occur in South Asia, parts of Central and South America, the Caribbean, Southeast Asia, the Middle East, and Oceania.
Other considerations	An estimated 30,000 travelers from North America, Europe, and Japan contract malaria every year.
Overall representativeness of this trial	The participants in the trial had a near equal ratio of males to females. Gender, race and ethnicity characteristics were self-reported by the participants during the screening process. On the intake survey, they were asked their gender with options being male or female. By design, all participants in the efficacy trial (Part B) were healthy children 6-10 years of age living in the rural communities of Kalifabougou or Torodo, Mali and exposed to seasonal malaria. All participants were African which is representative of the study site population, and representative of the race/geography that bears the greatest burden of malaria. Although older male and female African children do not represent the populations most susceptible to severe malaria and death in Africa because they have generally acquired some degree of clinical immunity, their safety and efficacy data enable further trials in infants and younger children residing in malaria endemic areas.

Tables S9 and S10. Post-hoc analysis of L9LS efficacy against *P. falciparum* infection and clinical malaria over time.

We conducted a post-hoc efficacy analysis that allows for variable efficacy over time via the piece-wise constant hazard model, which expands the Cox proportional hazard model to allow the hazard to change over time but to be constant within each specified time interval. The tables below show the efficacy of each L9LS dose group compared to placebo for *P. falciparum* infection (Table S9) and clinical malaria definition 1 (Table S10) over three time periods: 0-50 days, 51-100 days, and 101-168 days following study product administration. These data suggest that efficacy against infection and clinical malaria decreases over time in the 150 mg L9LS arm, while efficacy is maintained in the 300 mg L9LS arm over the 6-month study period. The trend toward lower efficacy for 300 mg of L9LS compared to placebo against infection and clinical malaria during the first time window is consistent with the observation that the risk of infection and clinical malaria was similar in the placebo and 300 mg L9LS arms during the first 28 days of the trial (as shown in Figures 2 and 3 in main text).

Table S9. Protective efficacy against *P. falciparum* infection

L9LS Dose	Time Window (days)	Protective Efficacy (%)	Lower Bound of 95% CI	Upper Bound of 95% CI
150mg	0-50	71	32	88
150mg	51-100	64	37	79
150mg	101-168	47	-50	81
300mg	0-50	43	-13	71
300mg	51-100	76	54	87
300mg	101-168	78	22	94

Table S10. Protective efficacy against clinical malaria (definition 1)

L9LS Dose	Time Window (days)	Protective Efficacy (%)	Lower Bound of 95% CI	Upper Bound of 95% CI
150mg	0-50	88	47	97
150mg	51-100	58	12	80
150mg	101-168	53	-19	82
300mg	0-50	61	0	85
300mg	51-100	80	47	93
300mg	101-168	84	40	95

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