Supplementary Appendix

Supplement to: Wu RL, Idris AH, Berkowitz NM, et al. Low-dose subcutaneous or intravenous monoclonal antibody to prevent malaria. N Engl J Med 2022;387:397-407. DOI: 10.1056/NEJMoa2203067

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

LOW DOSE MONOCLONAL ANTIBODY BY SUBCUTANEOUS ROUTE FOR MALARIA PREVENTION

Supplementary Appendix

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VRC 614 Study Team

Joseph P. Casazza, Alicia T. Widge, Peter D. Crompton, Douglas Rosing, Christopher L. Case, Blake Warner, Olga Trofymenko, Abidemi Ola, Karen Parker, Priya Kamath, Laura Novik, Pamela Costner, Jamie Saunders, William Whalen, Xiaolin Wang, Jennifer Cunningham, Anita Arthur, Aba Eshun, Justine Jones, Jennifer Phipps, Preeti Apte, Renunda Hicks, LaShawn Requilman, Catina Evans, Pernell Williams, Jackie Stephens, Olga Vasilenko, Galina Yamshchikov, Stephanie Taylor, Charla Andrews, Mark O'Callahan, Thuy Nguyen, Hope Wilson, Kathy Zephir, Brenda Larkin; Department of Laboratory Medicine: Sanchita Das, Ijeoma Ikpeama, Lorenzo Walker; WRAIR Study Team: Tatyana Savransky, Matthew Burrows, Leah Perazzo, Ying Jin-Clark; Translational Research Program: Ruth Woodward, John-Paul Todd, and the Vaccine Production Program: Liz Scheideman, Brandi France, Rajoshi Chaudhuri, Shireen Khayat, Rachel Prucnal, Jin Huang, Jane Rouse, Thomas Smith III, Janel Holland-Linn, Nadia Amharref, Shamitha Shetty, Shing-Fen Kao, Elle Millender, Peifeng Chen, Robin Luedtke, Deonte Toliver, Flo Kaltovich, Manos Tzortzakakis, Daniel Gowetski, Yi-Hsin Fan, Prasanthi Bandi, Yoo-Jung Yang, Ivan Loukinov, Vibhuti Wadhwa, Kristin Leach, Krishna Gulla, Xin Wang, Farah Vejzagic, Vaidehi Bhagat, Erwin Rosales, Lana Hogan, Christine Anderson, Erin Reilly, Melissa Resto, Yile Li, Paula Lei, Migen Bimi, Lisa Kueltzo, Will Adams, and Benjamin Clarkson

Author Contributions

Designed the study:	**Gathered the data:**	**Analyzed the data:**
Ashly E. Lukoskie Andrew J. McDougal Azza H. Idris Christian Buettner Edmund V. Capparelli Emily E. Coates Floreliz Mendoza Jason G. Gall John R. Mascola Joseph R. Francica Judy A. Stein Julie E. Ledgerwood Kevin Carlton LaSonji A. Holman Lesia K. Dropulic Martin R. Gaudinski Neville K. Kisalu Nina M. Berkowitz Richard L. Wu Robert A. Seder Sandra Vazquez Somia Hickman Zonghui Hu	Allison Beck Andrezza Campos Chagas Christian Buettner Ellie Seo Floreliz Mendoza Ingelise J. Gordon Iris Pittman Jittawadee Murphy LaSonji A. Holman Lawrence T. Wang Leonid Serebryannyy Lesia K. Dropulic Marjaan Imam Martin R. Gaudinski Mike Castro Nina M. Berkowitz Patricia Morgan Renunda Hicks Richard L. Wu Rosa Silva Sandeep R. Narpala Sarah O'Connell Shinyi Telscher Wei Shi Wing-Pui Kong	Adrian McDermott Andrezza Campos Chagas Azza H. Idris Barbara J. Flynn Christian Buettner Edmund V. Capparelli Emily E. Coates Floreliz Mendoza Ingelise J. Gordon Jittawadee Murphy John R. Mascola Joseph R. Francica Julie E. Ledgerwood Lais Da Silva Pereira Larisa Strom LaSonji A. Holman Lawrence T. Wang Lesia K. Dropulic Martin R. Gaudinski Myra Happe Neville K. Kisalu Nina M. Berkowitz Richard L. Wu Robert A. Seder Sandeep R. Narpala Sarah O'Connell Sarah H. Plummer Zonghui Hu
Vouches for the data and analysis: **All authors**	**Who wrote the paper:** Azza H. Idris Christian Buettner Edmund V. Capparelli Emily E. Coates Larisa Strom Lesia K. Dropulic Martin R. Gaudinski Myra Happe Nina M. Berkowitz Richard L. Wu Robert A. Seder Seemal F. Awan Zonghui Hu	**Decided to Publish the paper:** Richard L. Wu Robert A. Seder

Supplemental Methods:

Generation of L9LS anti-idiotype (ID) antibody

Mouse mAbs specific for the L9 idiotype were generated as previously described.[1] Briefly, L9 Fab was generated by LysC digestion and female Balb/c mice (Jackson Laboratory) were intramuscularly immunized twice with 20 μg of L9 Fab in 100 μL PBS and Ribi adjuvant (Millipore Sigma) at 0 and 4 weeks. Ten days post-boost, ELISA was used to test serum reactivity against L9 Fab and mice with high L9 reactivity were selected for cell fusion. Three days before cell fusion, L9-reactive mice were boosted IV with 20 μg of L9 Fab in 100 μL PBS prior to spleen excision. Splenocytes were purified and fused with myeloma cells Sp2/0 (ATCC) in a 2:1 ratio according to established fusion protocols.[2] Ten days post-fusion, triplicate hybridoma supernatants were screened for L9 Fab reactivity. PCR was performed to amplify the heavy and light chain antibody genes of positive hybridomas, which were cloned into mouse IgG1 expression vectors (Gene Synthesis), expressed in Expi293 cells (Thermo Fisher Scientific), and purified over protein A (GE Healthcare). ELISA with several top candidates showed dose-dependent binding to L9 but not to CIS43 IgG (Figure S1). Further comparison of anti-ID candidates resulted in the selection of clone 3-1 which had a specific signal to L9LS with low background noise signal. The 3-1 anti-idiotype clone was subcloned into a mouse IgG2a backbone and is referred to as L9LS anti-idiotype (ID) antibody in all subsequent studies.

Quantification of L9LS Serum Concentrations

L9LS anti-idiotype (ID) antibody diluted to 3 ug/mL was applied to the uncoated 96-well plate surface followed by overnight incubation, a wash, and blocking with 5% MSD Blocker A solution. 8-point serially diluted reference standard and test samples were then assayed in duplicates. SULFO-TAG labelled anti-human IgG detection antibody at concentration of 2 ug/mL was added and

subsequently incubated for 1 hour. Plates were washed and MSD GOLD READ buffer B solution containing electrochemiluminescence (ECL) was applied. The plates were read using MSD SECTOR S 600 instrument. Emitted light, proportional to the amount of anti-ID:L9LS:SULFO-TAG labelled anti-human IgG complex in the presence of the ECL substrate, was quantified by MSD Sector instrument. All calculations were performed from 5-parameter logistical standard curves generated in Excel and GraphPad Prism Software (GraphPad Software, La Jolla, CA, USA) with an assay quantitation range between 1 and 2000 µg/mL.

Population Pharmacokinetics (PK) Analysis

A population PK analyses was performed to determine compartmental PK parameters including clearance (CL), volume of distribution ($V_{dss)}$ and half-life ($T_{1/2\beta}$) with the PK program NONMEM 7.3 (ICON^{R}). A two-compartment model was used to fit the data using the First Order Conditional Estimation Method with Interaction (FOCEI). The number of participants was too small for a robust broad population PK covariate analysis. Therefore, the impact of subjects' size was accounted for using allometric scaling normalized to 70 kg with dose level being the only covariate explored. The final pharmacokinetic model selection was based on changes in the objective function, a goodness-of-fit statistic generated by NONMEM, and graphically by goodness of fit plots. Bootstrapping (1000 replicates) using Wings for NONMEM (ver 7.4) was performed to calculate 95% confidence intervals of the PK parameter estimates for the final population PK model. Monte Carlo simulations, with 2500 replicates, were performed using the final population PK model to generate predicted L9LS profiles.

References:

1. Kisalu NK, Pereira LD, Ernste K, et al. Enhancing durability of CIS43 monoclonal antibody by Fc mutation or AAV delivery for malaria prevention. JCI Insight 2021;6.
2. Greenfield EA. Single-Cell Cloning of Hybridoma Cells by Limiting Dilution. Cold Spring Harb Protoc 2019;2019.

Supplemental Figures:

Figure S1. Specificity of mouse IgG_1 for the idiotype of L9.

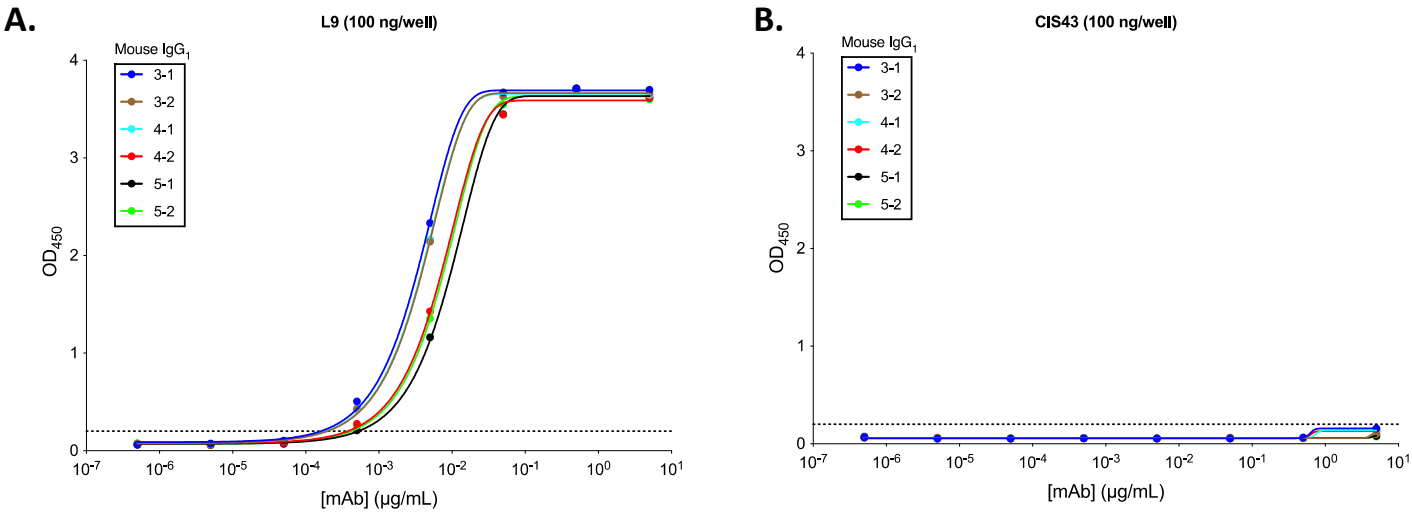

Figure S1. Specificity of Mouse IgG_1 for the Idiotype of L9. Binding of varying concentrations of L9 anti-idiotypic mAbs (id3-1, id3-2, id4-1, id4-2, id5-1, id5-2) to **A)** L9 – and **B)** CIS43 – coated plates.

Figure S2. L9LS Serum Concentrations by Dose Groups Following a Single Administration.

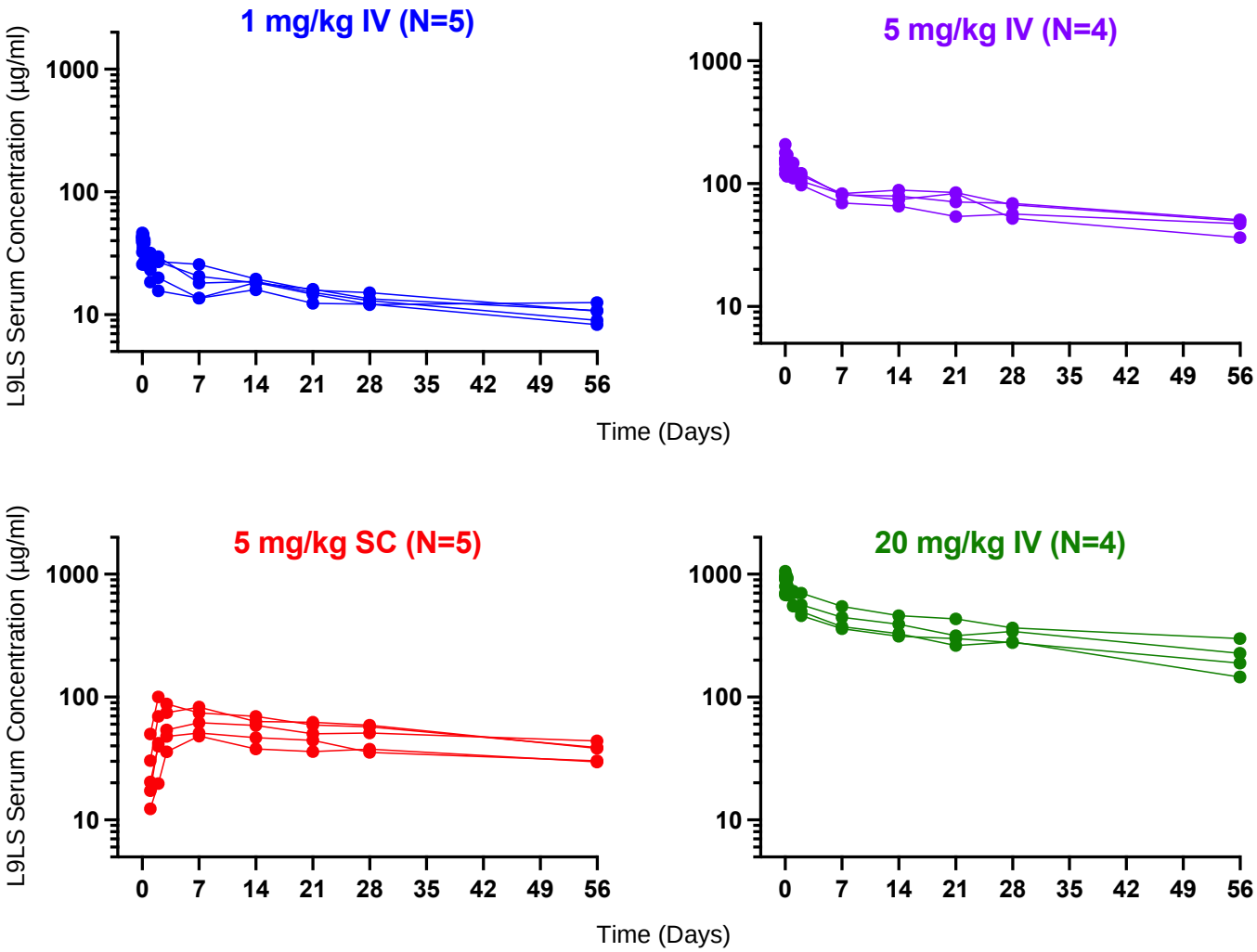

Figure S2. L9LS Serum Concentrations by Dose Group Following a Single Administration. Serum concentrations of L9LS for individual study participants in each dose group. Arrows indicate L9LS administration.

Figure S3. Average Salivary Gland Ratings (SGR) Comparison.

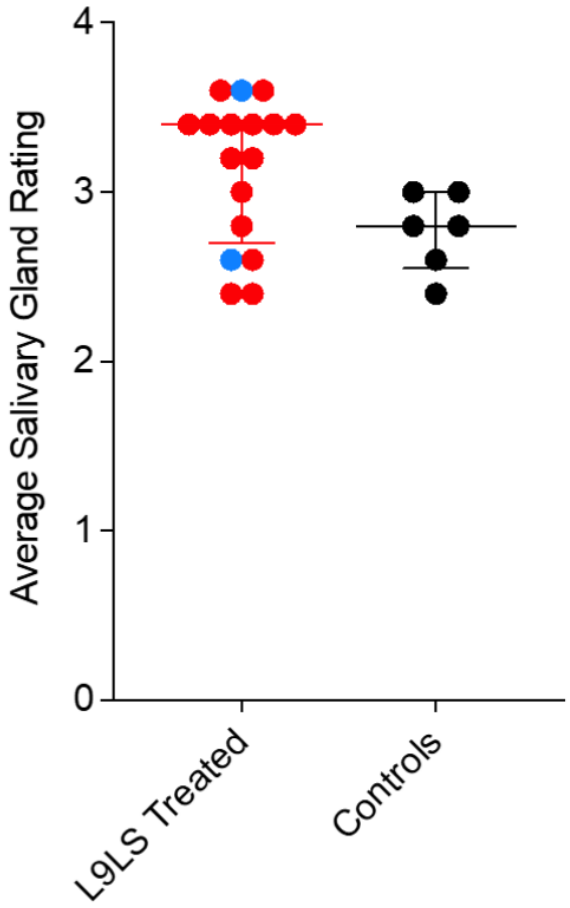

Figure S3. Average Salivary Gland Ratings (SGR) Comparison. Average salivary gland ratings (SGR) for both L9LS treated and control subjects are shown. Note the 2 subjects among the treated cohort that developed parasitemia are highlighted in blue. Horizontal lines depict the median salivary score with 25 – 75% interquartile ranges for L9LS treated 3.4 (2.8 – 3.4) vs. controls 2.8 (2.65 – 2.95), respectively.

Supplemental Tables:

Table S1. Baseline Demographic Characteristics of VRC 614 Study Participants.

TABLE S1. BASELINE DEMOGRAPHIC CHARACTERISTICS OF VRC 614 STUDY PARTICIPANTS.

CATEGORY	SUBCATEGORY	1 mg/kg IV (N=5)	5 mg/kg SC (N=5)	5 mg/kg IV (N=4)	20 mg/kg IV (N=4)	CONTROLS (N=9)
GENDER no.(%)	**MALE**	1 (20.0%)	1 (20.0%)	3 (75.0%)	4 (100.0%)	3 (33.3%)
	FEMALE	4 (80.0%)	4 (80.0%)	1 (25.0%)	0 (0.0%)	6 (66.7%)
AGE† no.(%)	**18-20**	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	21-30	5 (100.0%)	4 (80.0%)	4 (100.0%)	4 (100.0%)	9 (100.0%)
	31-40	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	41-50	0 (0.0%)	1 (20.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	MEAN (SD)	23.4 (2.7)	26.4 (9.3)	25.3 (3.3)	24.3 (3.2)	22.9 (0.9)
	RANGE	[21.0, 28.0]	[21.0, 43.0]	[22.0, 29.0]	[22.0, 29.0]	[22.0, 24.0]
RACE no.(%)	**ASIAN**	0 (0.0%)	1 (20.0%)	0 (0.0%)	1 (25.0%)	0 (0.0%)
	BLACK OR AFRICAN AMERICAN	0 (0.0%)	1 (20.0%)	0 (0.0%)	0 (0.0%)	2 (22.2%)
	WHITE	3 (60.0%)	3 (60.0%)	4 (100.0%)	3 (75.0%)	5 (55.6%)
	MULTIRACIAL	1 (20.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (11.1%)
	NOT REPORTED	1 (20.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (11.1%)
ETHNICITY no.(%)	**NON-HISPANIC/LATINO**	4 (80.0%)	3 (60.0%)	4 (100.0%)	4 (100.0%)	6 (66.7%)
	HISPANIC/LATINO	1 (20.0%)	2 (20.0%)	0 (0.0%)	0 (0.0%)	3 (33.3%)
WEIGHT (kg)	**MEAN (SD)**	62.6 (7.6)	59.2 (10.4)	72.2 (16.4)	76.7 (2.9)	75.6 (22.0)
	RANGE	[54.4, 75.2]	[52.0, 77.3]	[56.7, 94.3]	[74.8, 80.9]	[54.3, 120.5]
EDUCATION no.(%)	**COLLEGE/UNIVERSITY**	4 (80.0%)	5 (100.0%)	3 (75.0%)	4 (100.0%)	9 (100.0%)
	ADVANCED DEGREE	1 (20.0%)	0 (0.0%)	1 (25.0%)	0 (0.0%)	0 (0.0%)

† Age represents age at initial enrollment day.

Table S2. Maximum Local Reactogenicity for L9LS Recipients by Dose Group.

TABLE S2. MAXIMUM LOCAL REACTOGENICITY FOR L9LS RECIPIENTS BY DOSE GROUP					
SYMPTOM INTENSITY	1 mg/kg IV (N=5)	5 mg/kg SC (N=5)	5 mg/kg IV (N=4)	20 mg/kg IV (N=4)	ALL GROUPS (N=18)
PAIN/TENDERNESS					
None	2(40%)	3(60%)	3(75%)	4(100%)	12(66.7%)
Mild	3(60%)	2(40%)	1(25%)	0(0%)	6(33.3%)
Moderate	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Severe	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
PRURITUS					
None	5(100%)	4(80%)	4(100%)	4(100%)	17(94.4%)
Mild	0(0%)	1(20%)	0(0%)	0(0%)	1(5.6%)
Moderate	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Severe	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
SWELLING					
None	5(100%)	5(100%)	4(100%)	4(100%)	18(100%)
Mild	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Moderate	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Severe	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
REDNESS					
None	5(100%)	4(80%)	4(100%)	4(100%)	17(94.4%)
Mild	0(0%)	1(20%)	0(0%)	0(0%)	1(5.6%)
Moderate	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Severe	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
BRUISING					
None	4(80%)	5(100%)	4(100%)	4(100%)	17(94.4%)
Mild	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Moderate	1(20%)	0(0%)	0(0%)	0(0%)	1(5.6%)
Severe	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
ANY LOCAL SYMPTOM					
None	2(40%)	3(60%)	3(75%)	4(100%)	12(66.7%)
Mild	2(40%)	2(40%)	1(25%)	0(0%)	5(27.8%)
Moderate	1(20%)	0(0%)	0(0%)	0(0%)	1(5.6%)
Severe	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)

Table S3. Maximum Systemic Reactogenicity for L9LS Recipients by Dose Group.

TABLE S3. MAXIMUM SYSTEMIC REACTOGENICITY FOR L9LS RECIPIENTS BY DOSE GROUP					
SYMPTOM INTENSITY	1 mg/kg IV (N=5)	5 mg/kg SC (N=5)	5 mg/kg IV (N=4)	20 mg/kg IV (N=4)	ALL GROUPS (N=18)
MALAISE					
None	4(80%)	2(40%)	3(75%)	4(100%)	13(72.2%)
Mild	1(20%)	3(60%)	1(25%)	0(0%)	5(27.8%)
Moderate	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Severe	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
MUSCLE ACHES					
None	5(100%)	5(100%)	4(100%)	4(100%)	18(100%)
Mild	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Moderate	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Severe	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
HEADACHE					
None	4(80%)	3(60%)	3(75%)	4(100%)	14(77.8%)
Mild	1(20%)	1(20%)	1(25%)	0(0%)	3(16.7%)
Moderate	0(0%)	1(20%)	0(0%)	0(0%)	1(5.6%)
Severe	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
CHILLS					
None	5(100%)	4(80%)	3(75%)	4(100%)	16(88.9%)
Mild	0(0%)	1(20%)	1(25%)	0(0%)	2(11.1%)
Moderate	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Severe	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
NAUSEA					
None	5(100%)	3(60%)	4(100%)	4(100%)	16(88.9%)
Mild	0(0%)	1(20%)	0(0%)	0(0%)	1(5.6%)
Moderate	0(0%)	1(20%)	0(0%)	0(0%)	1(5.6%)
Severe	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
JOINT PAIN					
None	5(100%)	5(100%)	3(75%)	4(100%)	17(94.4%)
Mild	0(0%)	0(0%)	1(25%)	0(0%)	1(5.6%)
Moderate	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Severe	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
TEMPERATURE					
None	5(100%)	5(100%)	4(100%)	4(100%)	18(100%)
Mild	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Moderate	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Severe	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
ANY SYSTEMIC SYMPTOM					
None	4(80%)	2(40%)	3(75%)	4(100%)	13(72.2%)
Mild	1(20%)	2(40%)	1(25%)	0(0%)	4(22.2%)
Moderate	0(0%)	1(20%)	0(0%)	0(0%)	1(5.6%)
Severe	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)

Table S4. Pharmacokinetic Parameters for L9LS.

TABLE S4. PHARMACOKINETIC (PK) PARAMETERS FOR L9LS.

PK PARAMETERS BY DOSE GROUP

DOSE GROUP	C_{max} (mcg/mL)	T_{max} (d)	C_{28D} (mcg/mL)	C_{56D} (mcg/mL)	$AUC_{0\text{-}56D}$
	Mean (Std Dev)				
1 mg/kg IV (N=5)	41.5 (4.7)	0.1 (0.1)	13.2 (1.2)	10.3 (1.7)	699 (110)
5 mg/kg SC (N=5)	68.9 (22.3)	5.9 (2.2)	48.1 (11.0)	36.2 (6.1)	2152 (423)
5 mg/kg IV (N=4)	164.8 (31.1)	0.1 (0.1)	61.2 (8.2)	46.0 (6.7)	2884 (459)
20 mg/kg IV (N=4)	914.2 (146.5)	0.1 (0.05)	316.8 (43.8)	215.2 (64.8)	16632 (3435)

PK PARAMETERS FOR ALL GROUPS (N=18)

PK PARAMETER	VALUE	BOOTSTRAP (BS) MEDIAN	BS 95% CIS
$T_{1/2\beta}$= (days)	56	55	47 to 66
CL (mL/day)	46.1	47	43.1 to 53.0
Vdss (L)	3.67	3.66	3.31 to 4.09

Cmax= maximum serum concentration; Tmax= time to maximum serum concentration; C_{28D}, C_{56D} = concentration on day 28 and 56, respectively; $AUC_{0\text{-}56D}$= area under the curve from day 0 to day 56.
$T_{1/2\beta}$= beta half-life; CL=clearance, Vdss=volume of distribution
Bootstrap 95% confidence intervals (CIs) for population PK parameters

Table S5. Serum L9LS at Time of CHMI.

TABLE S5. SERUM L9LS CONCENTRATION AT TIME OF CHMI.

SUBJECT	L9LS DOSE/ROUTE	L9LS SERUM CONCENTRATION @ CHMI (µg/mL)	TIME FROM LAST ADMINISTRATION† (DAYS)	INFECTION STATUS **
1	1 mg/kg IV	9.2	43	Negative
2	1 mg/kg IV	10.3	42	Negative
3	1 mg/kg IV	11.1	41	Negative
4	1 mg/kg IV	11.5	28	Negative
5	1 mg/kg IV	10.95	22	**Positive**
6	5 mg/kg SC	28.6	36	Negative
7	5 mg/kg SC	40.8	36	Negative
8	5 mg/kg SC	29.6	35	Negative
9	5 mg/kg SC	41.1	35	**Positive**
10	5 mg/kg SC	56.35	21	Negative
11	5 mg/kg IV	45.2	29	Negative
12	5 mg/kg IV	60.3	28	Negative
13	5 mg/kg IV	55.05	21	Negative
14	20 mg/kg IV	387.9	20	Negative
15	20 mg/kg IV	299	20	Negative
16	20 mg/kg IV	256	20	Negative
17	20 mg/kg IV	333.7	14	Negative

†Time from last administration is an exact number of days between administration of L9LS and the date of CHMI for each study participant. **Infection status indicates study participants final PCR-confirmed outcome post CHMI.

Table S6. Controlled Human Malaria Infection Mosquito Score / Salivary Gland Rating and Outcome.

TABLE S6. CONTROLLED HUMAN MALARIA INFECTION MOSQUITO SCORE / SALIVARY GLAND RATING AND OUTCOME							
SUBJECT	**L9LS DOSE/ROUTE**	**MOSQUITO SCORING**			**SALIVARY GLAND RATING***		**DAY OF POSITIVE PCR**
		Total No. Used	No. Fed	Qualifying Bites†	Average	Raw Ratings	**
1	1 mg/kg IV	5	5	5	2.4	2,3,2,3,2	Negative
2	1 mg/kg IV	10	6	5	2.8	NF,NF,2,3,4,NF,0,NF,3,2	Negative
3	1 mg/kg IV	5	5	5	3.4	4,3,4,4,2	Negative
4	1 mg/kg IV	8	5	5	3.4	NF,NF,4,3,2,NF,4,4	Negative
5	1 mg/kg IV	8	5	5	2.6	NF,NF,2,3,4,NF,2,2	8
6	5 mg/kg SC	6	6	5	3	2,4,3,2,0,4	Negative
7	5 mg/kg SC	8	7	5	3.6	NF,0,3,4,4,0,4,3	Negative
8	5 mg/kg SC	9	7	5	3.2	NF,NF,0,2,3,0,3,4,4	Negative
9	5 mg/kg SC	7	7	5	3.6	0,0,3,3,4,4,4	9
10	5 mg/kg SC	6	5	5	3.6	NF,3,4,3,4,4	Negative
11	5 mg/kg IV	7	5	5	3.4	NF,NF,3,4,4,4,2	Negative
12	5 mg/kg IV	11	7	5	3.4	NF,NF,NF,3,0,NF,4,3,0,3,4	Negative
13	5 mg/kg IV	6	6	5	3.4	0,3,3,4,4,3	Negative
14	20 mg/kg IV	8	7	5	2.4	NF,0,0,2,2,3,2,3	Negative
15	20 mg/kg IV	8	6	5	2.6	NF,NF,3,2,3,3,0,2	Negative
16	20 mg/kg IV	6	5	5	3.2	0,2,3,4,4,3	Negative
17	20 mg/kg IV	7	7	5	3.4	4,0,4,3,0,3,3	Negative
18	Control	7	6	5	2.6	NF,2,3,4,0,2,2	9
19	Control	6	6	5	2.8	3,3,2,2,0,4	12
20	Control	7	5	5	2.4	NF,2,2,2,3,NF,3	21
21	Control	6	6	5	2.8	3,2,4,2,0,3	9
22	Control	5	5	5	3	2,3,3,3,4	7
23	Control	6	5	5	3	NF,2,4,3,3,3	14

CHMI was carried out using *Anopholes Stephensi* mosquitoes infected with *Plasmodium falciparum* strain 3D7. *Salivary gland rating is based on number of sporozoites (spzs) observed after dissection. NF = mosquito did not feed, 0 = no spzs observed, 1 = 1-10 spzs, 2 = 11-100 spz, 3 = 101 – 1000 spzs, 4 = >= 1000 spzs.

†A qualifying bite is defined as a bite with a mosquito bearing a salivary gland rating of 2 or greater. All challenged subjects were required to have 5 qualifying bites.

**Volunteers were treated with Malarone, on day of positive PCR, or at day 21 if remained negative.

Table S7. Representativeness of Study Participants.

TABLE S7. REPRESENTATIVENESS OF STUDY PARTICIPANTS	
CATEGORY	
DISEASE, PROBLEM, OR CONDITION UNDER INVESTIGATION	Malaria, a mosquito-borne disease caused by Plasmodium parasites.
SPECIAL CONSIDERATIONS RELATED TO:	
SEX AND GENDER	Although malaria affects both men and women, gender roles and gender dynamics give rise to different vulnerabilities, such as exposure patterns. Though men may be more vulnerable than women to exposure, women may be more vulnerable than men to the consequences of malaria, particularly during pregnancy.
AGE	Infants and children under 5 accounted for 80% of all malaria deaths in Sub-Saharan Africa- as young children have not had time to develop partial immunity that can develop over years of exposure.
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 *Plasmodium falciparum* 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 (*Plasmodium falciparum*), weather conditions, scarce 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 per year.
OVERALL REPRESENTATIVENESS OF THIS TRIAL	The participants in the trial had a near equal ratio of men to women. Gender, race and ethnicity characteristics were self-reported by the participants during the screening process. On the intake survey, they were asked “Designated Gender at Birth” with options being male or female. Additionally, they were asked “Gender Identity” with options being male, female, transgender or other (specify). For additional information regarding the NIH mission on inclusion of women and minorities in clinical research, please visit: https://grants.nih.gov/policy/inclusion/women-and-minorities.htm. By design, all trial participants were young, healthy and malaria naïve adults living in the United States. The proportion of African Americans enrolled in the trial (11%) was slightly smaller than the total population distribution of African Americans in the United States. In contrast, the proportion of Asians enrolled in the trial (7.4%) was slightly larger than the total population distribution of Asians in the United States. Although the trial participants collectively are not representative of the populations burdened the most by malaria, their safety and efficacy data enable field studies to be conducted in regions in which malaria is endemic.