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Low Dose Intravenous and Subcutaneous CIS43LS Monoclonal Antibody Protects Against Malaria: A Phase 1 Clinical Trial

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VRC 612 Part C Study Team

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SUMMARY

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Contributors

MRG and RLW were the principal investigator of VRC 612 and KEL was clinical site principal investigator for VRC 612 Part C. AHI, MO, NMB, MG, ZH, LAH, FM, IJG, ACC, JRM, JS, CC, SH, AM, MRG, EVC, EEC, RLW, JEL, LKD, and RAS contributed to the conception and design. KEL, AAB, KM, KSS, SJ, BS, and MA contributed to investigation and sample collection. AHI, MH, LS, LS, MC, LW, MN, KL, SHP, OT, AM, BL, SN, EVC, EEC, LKD, and RAS contributed to data analysis and interpretation. EEC and KEL have accessed and verified all the underlying data. All authors contributed to the writing and final approval of the manuscript.

Declaration of Interest

AHI and RAS are listed as inventors on pending patent application describing CIS43 and related antibodies. KEL, MRG, RLW and all other authors report no financial conflicts or conflicts of interest. Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the authors, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense. The investigators have adhered to the policies for protection of human subjects as prescribed in AR 70–25.

Background: Human monoclonal antibodies (mAb) may offer an important new tool to reduce malaria morbidity and mortality. In the first two parts of a three-part clinical trial, the antimalarial mAb CIS43LS conferred high level protection at doses of 20 or 40 mg/kg administered intravenously (IV) followed by controlled human malaria infection (CHMI). The ability of CIS43LS to mediate protection at lower doses or by subcutaneous (SC) route is unknown. Here, part three of the clinical trial is presented to provide data on optimizing the dose and administration route of CIS43LS.

Methods: Healthy, malaria-naïve participants aged 18–50 years received mAb at doses of 1 (n = 7), 5 (n = 4), and 10 (n = 3) mg/kg IV and 5 (n = 4) and 10 (n = 4) mg/kg SC. Safety analysis was performed as the study’s primary outcome on all participants who received product administration. Secondary outcomes were the pharmacokinetic properties and protective efficacy of CIS43LS after CHMI. Participants underwent CHMI by the bites of five mosquitoes infected with *Plasmodium falciparum* (Pf) 3D7 strain approximately 8 weeks following mAb inoculation. Six additional naïve control participants underwent CHMI simultaneously. Participants were followed daily from Days 7–18 and Day 21 with qualitative PCR used for Pf detection. Participants who tested positive were treated with atovaquone-proguanil and those who remained negative were treated at Day 21. Participants continued follow-up until 24 weeks.

Findings: CIS43LS administration was safe and well tolerated with no serious adverse events occurring. CIS43LS protected 18 of 22 participants. No participants developed parasitemia following dosing at 5 mg/kg IV (0/4), 5 mg/kg SC (0/4), 10 mg/kg IV (0/3), and 10 mg/kg SC (0/4). All 6 controls and 4 of 7 participants dosed at 1 mg/kg IV developed parasitemia following CHMI. PK analysis to determine antibody levels of CIS43LS at the time of CHMI revealed CIS43LS concentration ranges of 10·5 to 15·5, 41·7 to 64·1, and 113 to 120·5 ug/mL for 1, 5, and 10 mg/kg IV groups, and 33·2 to 44·5, 62·8 to 87·7 ug/mL for 5 and 10 mg/kg SC groups.

Interpretations: CIS43LS mediated protection at low doses and by the SC route providing evidence that this approach may be useful to prevent malaria across several clinical use cases.

INTRODUCTION

Despite decades of vaccine and therapeutics research, malaria remains a devastating illness which contributes to substantial disease with additional economic impact in the most vulnerable populations. Service disruptions related to the COVID-19 pandemic resulted in 241 million malaria infections and a 12% rise in annual deaths to 627,000 individuals in 2020.(1) The rise in partial artemisinin resistance in WHO African regions, difficulties in mass distribution of insecticide-treated bed nets, and the limited durable efficacy with the currently approved vaccine warrant urgent new interventions.

Malaria vaccine development has been complicated by the multi-staged life cycle of the *Plasmodium falciparum* (Pf) parasite and understanding the specific humoral and cell-mediated immune responses required for protection. Early in the life cycle, immediately following the bites of mosquitoes, a low number of Pf sporozoites are injected into the skin and migrate via the bloodstream to the liver. This is the stage at which antibody-mediated neutralization of the parasite may be possible prior to quiescent liver stage development.(2–4) Once liver stage infection has been initiated, radiation or genetically attenuated whole

organisms are another approach for T cell mediated protection in the liver (5–7) The most advanced subunit malaria vaccines, RTS,S adjuvanted with AS01,(8) and R21 adjuvanted with Matrix M[®],(9, 10) are comprised of the circumsporozoite protein (CSP). Recently, RTS,S was recommended by the WHO for widespread distribution in moderate to high transmission settings in individuals 5–17 months of age.(11) CSP is the most abundant protein enveloping the Pf sporozoite and is comprised of three regions: An N-terminal domain, a highly conserved central repeat region characterized by a large number of Asn-Ala-Asn-Pro (NANP) tetrapeptide repeats, and a C-terminal domain.(12) CSP is required for motility in the skin and infectivity in the liver, and thus, is an ideal target for vaccines and other immune based strategies such as monoclonal antibodies (mAbs) to neutralize sporozoites and limit malaria infection.(13)

Recently, several human CSP-derived mAbs have been isolated from vaccinated individuals who were protected from malaria following controlled human malaria infection (CHMI) or natural malaria infection.(14, 15) These include mAb317 from protected RTS,S inoculated participants,(16) as well as CIS43(17) and L9 from participants vaccinated with an attenuated, whole-organism Pf sporozoite vaccine (Sanaria).(4, 13) CIS43 was notable as being the first antibody to show preferential binding to a region at the junction of the N-terminal and central repeat, and spanning an Asn-Pro-Asp-Pro (NPDP) tetrapeptide thereby defining a new site of neutralization on CSP. In addition to potency, an important feature of improving the clinical utility of mAbs to prevent malaria is to extend their durability *in vivo*. By substituting leucine and serine amino acid mutations (LS) within the CIS43 Fc region, the half-life of CIS43LS is extended to 56 days.(18) In the first in human clinical study testing a mAb against malaria, intravenous (IV) administration of CIS43LS was found to be safe, well-tolerated, and provided complete protection in all nine participants that underwent CHMI.(18) Of note, all participants, who advanced to CHMI, received doses of 20 and 40 mg/kg and were all administered by IV route. Importantly, two subjects that received a single administration of 40mg/kg IV were protected following CHMI 9 months later. The concentration of CIS43LS at the time of CHMI was approximately 50ug/ml. Thus, it is still not known whether protection can be achieved at lower concentrations of CIS43LS which will be important to define for clinical development. A critical aspect to the implementation of passive immunization with CIS43LS is to assess lower doses and perform pharmacokinetic analyses to establish the antibody concentration required for protection. Moreover, assessment of alternate routes of administration such as subcutaneous (SC) dosing will be important to enhance the feasibility of widespread clinical use. Here, to extend the previously reported findings, we report the results of the safety and optimization of dose and route analysis for the human anti-malaria mAb CIS43LS.

METHODS

Study Design:

VRC 612 Part C was the third part of a three-part first-in-human phase 1, adaptive, clinical trial evaluating the safety and protective efficacy of the antimalarial mAb CIS43LS. Parts A and B were initiated prior to the COVID-19 pandemic, and this influenced some changes to the original design of the study based on having to alter the timing of the CHMI. In Part C,

participants received a single open-label administration dose of either 1, 5, or 10 mg/kg IV, or 5 or 10 mg/kg SC. The study was sponsored by the Vaccine Research Center (VRC), of the National Institute of Allergy and Infectious Diseases (NIAID), at the National Institutes of Health (NIH) in Bethesda, MD, USA and was conducted at the University of Maryland, Baltimore (UMB) Center for Vaccine Development and Global Health in Baltimore, MD, USA. The clinical trial protocol was reviewed and approved by the National Institutes of Health Institutional Review Board (IRB) through a reliance agreement established with the UMB IRB. Controlled human malaria infection was conducted at the UMB Center for Vaccine Development and Global Health with entomologic support from the Walter Reed Army Institute of Research (WRAIR) in Silver Spring, Maryland.

Participants:

Eligible study participants were healthy adults, 18–50 years of age, with no previous malaria vaccinations or infections. Individuals were recruited with IRB-approved advertisements through print and electronic media. Participants were enrolled based on eligibility criteria. Participant eligibility was evaluated by clinical laboratory tests, self-reported medical history, and physical examination. Eligibility criteria are available in full on [ClinicalTrials.gov](https://clinicaltrials.gov) under identifier [NCT04206332](https://clinicaltrials.gov/ct2/show/study/NCT04206332). All participants provided written informed consent prior to enrollment. Participants were enrolled into open dose groups, in a sequential, dose-escalating manner.

Procedures:

Product Information—CIS43LS is a human IgG1 mAb that was developed and manufactured by the VRC. A recombinant Chinese hamster ovary DG44 clonal cell line¹⁴ developed by the Vaccine Production Program was transferred to the VRC pilot plant for clinical material manufacture. The study product was manufactured under current Good Manufacturing Practices at the VRC pilot plant operated by the Vaccine Clinical Material Program, Leidos Biomedical Research (Frederick, MD, USA). The study product was filled into single-dose vials at a concentration of 100 mg/ml.

Product Administration—Product administration was weight based (mg/kg). Participants assigned to an IV administration group received infusions, accompanied by an in-line filter, over 15–30 minutes. Participants assigned to a SC administration group had administration at sites deemed acceptable by the clinician and participant. Multiple SC administrations of pre-filtered study product were allowable with a maximum volume of 2.5 ml per injection site. All participants were observed in clinic for 4–6 hours following completion of product administration, to complete the PK sampling time points.

Safety Surveillance—Local and systemic reactogenicity parameters were recorded through 7 days after mAb infusion as solicited adverse events (AEs). Unsolicited AEs were recorded for 28 days following mAb infusion and for 28 days post-CHMI. AE grading utilized the Division of AIDS (DAIDS) Table for Grading.⁽¹⁹⁾ Serious AEs and new chronic medical conditions were recorded from product administration to the end of the trial.

Controlled Human Malaria Infection (CHMI)—All trial participants were exposed to *Anopheles stephensi* mosquitoes infected with Pf 3D7 strain, 48 to 56 days after product administration. Additionally, participants met standard infectivity criteria consisting of five qualifying bites as determined by mosquitoes taking a blood meal and having a salivary gland score of 2 or greater.⁽²⁰⁾ Evaluations for parasitemia by polymerase chain reaction (PCR) occurred on post-CHMI days 7 through 18 and concluded on day 21. All participants who were PCR positive were treated while those participants who remained negative for parasitemia underwent directly observed definitive antimalarial treatment on day 21.

Pharmacokinetics:

Serum concentrations of CIS43LS were quantified using CIS43LS anti-idiotypic capture Electrochemiluminescence Immunoassay (ECLIA) method on Meso Scale Discovery (MSD) platform up to 24 weeks post antibody administration as previously described.⁽¹⁸⁾ Pharmacokinetic properties including maximum serum concentration (C_{max}) and time to maximum serum concentration (T_{max}) were estimated by non-compartmental methods; linear trapezoid method was used to calculate area under the curve (AUC). Population PK analysis was performed to generate compartmental PK parameters including clearance (CL), half-life ($T_{1/2\beta}$), and volume of distribution (V_{ss}). Additional details on the quantification methods and PK assessment are described in the Supplementary Appendix (appendix p 2).

Anti-drug Antibody (ADA) analysis:

A three-tiered approach was used to detect anti-drug antibodies (ADAs) elicited post CIS43LS infusion. Detailed methods on the assay procedures could be found in the Supplementary Appendix (appendix p 3).

Outcomes:

The primary outcome was the safety and tolerability of CIS43LS administered at 1, 5, and 10 mg/kg by IV route and at 5 and 10 mg/kg by SC route. Secondary outcomes were the pharmacokinetic properties and protective efficacy of CIS43LS after CHMI approximately 8 weeks following product administration. The total duration of follow-up was 24 weeks.

Sample Size:

Sample size determination was based on feasibility and safety assessment. The sample size per group was designed to capture a range of doses to generate additional data to define a threshold concentration for protection. Additionally, sample size calculations for safety were determined by the ability to detect serious adverse events (SAEs). For a sample size of $n=4$, there was more than a 90% chance to observe at least one SAE if the true rate was no less than 0.438 and more than a 90% chance to observe no SAE if the true rate was no more than 0.025. For a sample size of $n=6$, there was more than a 90% chance to observe at least one SAE if the true rate was no less than 0.319 and more than a 90% chance to observe no SAE if the true rate was no more than 0.017.

Statistical Analysis:

In accordance with the trial protocol, the safety analysis included all participants who received product administration. Subjects with one or more AEs were analyzed by dose group. Subjects experiencing more than one AEs were counted once under the event of highest severity. The efficacy analysis included all enrolled participants who underwent CHMI. The primary efficacy analysis was based on a two-sided Barnard test on the proportion of infected participants among CIS43LS-recipients versus controls who underwent CHMI concurrently. The secondary efficacy analysis was based on time to parasitemia, where CIS43LS-recipients were compared with the controls via a log-rank test. Kaplan-Meier curves were provided for each group. Additionally, a Welch two Sample t-test was used to determine the association of CIS43LS serum concentration prior to CHMI and parasitemia.

Role of the Funding Source:

The VRC of the NIAID, NIH funded the study and its investigators, including study site investigators at UMB Center for Vaccine Development and Global Health in Baltimore, MD, USA, had complete control over study design, data collection, data analysis, data interpretation, and writing of the report.

RESULTS

Of the 47 individuals screened, 31 participants were enrolled from September 1, 2021, to October 29, 2021 (figure 1). Participants were enrolled in the open-label protocol to receive doses of 1, 5, or 10 mg/kg IV or 5 or 10 mg/kg SC. One participant assigned to the 10 mg/kg IV group withdrew, due to a new medical diagnosis, prior to product administration, and was replaced. The final study population comprised of 17 (57%) women and 13 (43%) men; median age was 32 years (range 20–49; appendix p 4). Eight participants were enrolled to be control participants and did not receive CIS43LS. Twenty-two participants, assigned to five different dose and route groups underwent study product administration. Fourteen participants received study product by IV infusion [1 mg/kg (n = 7), 5 mg/kg (n = 4), 10 mg/kg (n = 3)] and eight participants received study product by SC administration [5 mg/kg (n = 4) and 10 mg/kg (n = 4)] (Figure 1). Participants assigned to the SC administration groups received 2–4 injections based on weight, in the abdomen and separated by at least 2 inches. Participants were observed for 4–6 hours following product administration.

Twenty-eight participants (22 who had received CIS43LS and 6 control participants) underwent CHMI on November 2, 2021. CHMI occurred on days 54–56 following CIS43LS administration for all participants except for one participant in the 10 mg/kg IV dose group undergoing CHMI at 48 days following administration. The 21-day monitoring for parasitemia concluded on November 23, 2021. Twenty-seven participants completed study follow-up (One participant in the 10 mg/kg IV group withdrew for non-study related reasons, at post-CHMI Day 15, prior to follow-up completion, and received terminal anti-malarial therapy). Per the study protocol, this individual was included in all data analysis, including safety and efficacy, as well all available timepoints for pharmacokinetics and anti-drug antibody analysis. The final study follow-up visit occurred on February 28, 2022.

No safety concerns were identified following mAb infusion (figure 2, appendix p 5). Seventeen (17/22: 77.3%) mAb recipients had no solicited local AEs and five (5/22: 22.7%) had mild symptoms including three with pain, one with pain and redness at the infusion site, and one with pruritis at the injection site. Fifteen (15/22: 68.2%) had no solicited systemic AEs, five (5/22: 22.7%) had mild, and two (2/22: 9.1%) had moderate AEs. Malaise (n = 5) and headache (n = 5) were the most commonly reported symptoms, but no severe events were reported. There was no evidence of increased solicited symptoms by dose escalation or route of administration. There were three mild to moderate, related unsolicited AEs associated with IV infusion including hypertension, abdominal pain and dizziness. All were transient, lasting less than 24 hours in duration. No serious AEs attributed to infusion occurred. Following CHMI, there were three instances of mild, pruritic macular-papular lesions [Dose (protection status): 1 mg/kg IV (unprotected), 5 mg/kg SC (protected), and 10 mg/kg SC (protected)] at the site of mosquito bite challenge occurring after the mosquito bite swelling had resolved (Days 26, 10 and 19 post-CHMI respectively). The symptoms self-resolved after 3, 4 and 35 days, and occurred across dose groups and route of administration.

CIS43LS pharmacokinetic (PK) profile was analysed for all study participants, including 21 participants with 24 weeks of PK data and one participant with eight weeks of PK data. CIS43LS displayed dose-dependent linearity (figure 3, appendix p 7–8). The mean (\pm SD) C_{\max} serum concentrations after a single intravenous CIS43LS infusion were 383.7 \pm 30.8 ug/ml, 223.5 \pm 53.3 ug/ml, 42.2 \pm 10.4 for 10 mg/kg IV, 5 mg/kg IV and 1 mg/kg IV dose groups, respectively. The mean (\pm SD) C_{\max} serum concentrations after a single subcutaneous administration were 104 \pm 19.2 ug/ml for 10 mg/kg SC group and 53.6 \pm 8.5 ug/ml for 5 mg/kg SC group. T_{\max} occurred during the immediate post infusion period following intravenous administration and between 14 to 16 days after subcutaneous administration. Based on the population PK analysis, the overall CIS43LS clearance (CL) was 33.6 mL/day (Bootstrap (BS) 95% confidence interval (CI), 30.7 to 36.5 mL/day), half-life ($T_{1/2\beta}$) was 80 days (BS 95% CI, 76.5–83.2 days) and the volume of distribution (V_{ss}) was 3.8 liters (BS 95% CI, 3.5 to 4.1 liters) (table 1). The population PK estimate for subcutaneous bioavailability (F) was 63% (BS 95% CI, 57 to 70 %). CIS43LS concentrations week 24 after a single dose ranged from 16.7–21.6 and 31.5–45.7 ug/ml for 5 and 10 mg/kg IV groups and 14.5–21.7 and 23.3–29.4 ug/ml for 5 and 10 mg/kg SC groups. We also performed simulation PK modelling to predict serum concentration profile of various dosages IV and SC. At 48 weeks the median (and 90% prediction intervals (PI)) of CIS43LS for intravenous groups were 0.96 ug/ml (0.54–1.51) for 1 mg/kg IV group, 4.78 ug/ml (2.71–7.55) for 5 mg/kg IV and 9.56 ug/ml (5.42–15.10) for 10 mg/kg IV group (appendix p 8). Following SC administration, the median (and 90% PI) predicted concentrations 48 weeks post dose were 3.12 ug/ml (1.54–6.29) for 5 mg/kg SC dose, and 6.24 ug/ml (3.07–12.58) for 10 mg/kg SC group (appendix p 8).

We assessed anti-drug antibodies using a three-tiered approach in all study participants enrolled in the trial including parts A and B of the study that assessed higher doses of CIS43LS administered by IV route.(18) We did not detect ADAs in any of the samples following CIS43LS administration (appendix p 9–10).

All CHMI participants obtained five qualifying bites determined by mosquitoes taking a blood meal with a salivary gland rating of 2 or greater. The median salivary score was 2.9 (IQR: 2.60–3.25) for mosquitoes applied to control participants as compared to 3.0 (IQR: 2.67–3.25) for mosquitoes applied to CIS43LS recipients (appendix p 11–13).

Following CHMI, four of the 22 CIS43LS recipients and all 6 control participants developed parasitemia as determined by PCR through day 21 following CHMI ($P=0.0005$ by two-sided Barnard test). No participants developed parasitemia following dosing at 5 mg/kg SC (0/4), 5 mg/kg IV (0/4), 10 mg/kg SC (0/4), and 10 mg/kg IV (0/3). 4 of 7 participants of the lowest dose group, 1 mg/kg IV, developed parasitemia ($P=0.10$ by two-sided Barnard test). The median prepatent period for participants infused 1 mg/kg mAb was 12.0 (range 11–12) days vs. 7.5 (range 7–9) days in controls ($P=0.012$) (figure 4). At the time of CHMI, serum concentrations of CIS43LS ranged from 10.5–15.5 ug/ml for the 1 mg/kg IV, 33.2–44.5 ug/ml for the 5 mg/kg SC, 41.7–64.1 ug/ml for the 5 mg/kg IV, 62.8–87.7 ug/ml for the 10 mg/kg SC, and 113–120.5 ug/ml for the 10 mg/kg IV dose groups (table 2). Based on this study's sample size ($n=22$), to attain a 90% or greater probability of protection from parasitemia the concentration of CIS43LS would need to be greater than or equal to 22.5 ug/mL (95% CI, 12.1 to 41.7 ug/ml).

DISCUSSION

In a previous study, the long-acting monoclonal antibody CIS43LS prevented malaria in a small number of participants when administered by IV infusion at doses of 20 and 40 mg/kg.(18) Herein, we advance our knowledge of the pharmacokinetics and malaria prevention efficacy by establishing that a single dose of CIS43LS at 5–10 mg/kg, administered by SC or IV route, provides high-level protection against CHMI approximately eight weeks (48–56 days) after antibody administration. The success of a single, low dose mAb administered by a SC route offers a new tool in the armamentarium against this parasite.

The mAb administration was well tolerated and safe with no significant dose-dependent symptoms or associated reactions with the route of delivery. While the numbers are low, taken together with the first two parts of this trial, CIS43LS is promising in terms of potential clinical use. The ease of SC administration, recently also demonstrated for L9LS,(21) allows for widespread application as well as age de-escalation into pediatric populations, which may not be amenable to IV dosing.

An important objective of this trial was to identify the lowest serum concentration of CIS43LS that could confer protection following CHMI. Previously, protection was established in all nine participants who received at least one dose of 20mg/kg or 40 mg/kg CIS43LS given by the IV route, limiting the ability to discern the serum concentration required for protection using regression analysis.(18) The lowest concentration of CIS43LS in two of the participants that were protected 9 months following 40 mg/kg IV was 50 ug/ml. In this study, by including a dosing arm of 1 mg/kg IV, a range of protective and break-through infection concentrations were observed. This dosing arm established that 3 of 7 participants (42.9%) remained aparasitemic following CHMI in this low dose arm. The 1 mg/kg IV group participants had a mean serum level of 12 ug/ml (range 10.5–15.5 ug/ml)

at the time of challenge. Serum levels for doses of 5–10 mg/kg ranged from 33.2–120.5 ug/ml, with dose-dependent increases in concentration as well as increased levels in the IV vs. SQ route of administration (table 2). Based on the small sample size in this controlled study, the minimum concentration of CIS43LS required for a 90% or greater probability of protection from parasitemia was 22.5 ug/ml (95%CI, 12.1 to 41.7 ug/ml). Of note, CIS43LS concentrations in the lowest dosing group (1 mg/ml) were similar in both parasitemic and aparasitemic participants; 11.88 vs. 12.17 ug/ml respectively. While the exact mAb threshold for protection is unclear, the CHMI model itself is imprecise in terms of the amount of sporozoites delivered via 5 mosquito bites and requires neutralization of 100% of sporozoites to prevent infection. Thus, the 1 mg/mL dose range may be variably sufficient to neutralize all sporozoites and may depend upon the burden administered. The functional durability of CIS43LS was not assessed beyond 8-week CHMI time point, in this trial. However, PK levels at week 24 remain above 14.5 ug/ml for the 5 and 10 mg/ml IV and SC groups, which suggests a level of protection that may extend to 6 months post-inoculation.

A critical factor in achieving the required therapeutic levels is antibody half-life. Previously, in Part A and B of the study, the half-life was calculated as 56 days with a clearance of 44 days. As this study was done in 2020, unanticipated interruptions in sampling occurred as a result of the SARS-CoV-2 pandemic. Here with additional sampling, the half-life estimate of CIS43LS is approximately 80 days with clearance of 35 days. This long-half life will be important for clinical translation as the increased durability of CIS43LS makes SC dosing more feasible and reduces cost of goods over a treatment period.

The implications for the protection of a mAb administered SC and the potential for long-lasting durability are far-reaching. Both CIS43LS, and a second promising mAb, L9LS, which targets the minor repeats, primarily located within the junctional region of PfCSP,(13) are being tested in ongoing field studies in Mali ([clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT05304611) registration [NCT05304611](https://clinicaltrials.gov/ct2/show/study/NCT05304611); [NCT04329104](https://clinicaltrials.gov/ct2/show/study/NCT04329104)) and Kenya ([NCT05400655](https://clinicaltrials.gov/ct2/show/study/NCT05400655)). CIS43LS, administered by IV, was recently found to confer high level, dose dependent protection in Malian adults against intense, seasonal Pf malaria. The 40 mg/kg IV-dosed CIS43LS efficacy after six months was 88.2% while the 10 mg/kg dose efficacy was 75%.(22) If long acting mAbs prevent infection over 6–12 months after a single administration by SC route, they could offer a powerful new tool in preventing malaria morbidity and mortality against seasonal and perennial infection in infants and children as well as pregnant women. Moreover, passively administered antibodies following R21 immunization resulted in enhanced protective efficacy, compared to either intervention alone, in a murine model.(23) This approach may offer a template towards an effective sequential strategy in our quest to provide protection against malaria through infancy and childhood.

The limitations of this trial include a small sample size and a single CHMI time point at 8 weeks. Additional trials are needed to explore the durability of CIS43LS in mediating long-term protection and the correlative pharmacokinetic threshold. The conserved nature of the junctional epitope, as well as low mutational rate of eukaryotes compared to viruses, suggests that breakthrough infection may be less likely. However, careful analysis of breakthrough falciparum strains in field trials will be critical. The results presented demonstrate protection at low dose administration and validate the successful route of

SC administration. Furthermore, our results support the bioengineering of next-generation vaccines, inclusive of junctional epitopes.(24, 25) Future research may assess protection against malaria in pregnant women and children. These advances, coupled with next-generation vaccines, strengthen the armamentarium against malaria and allow for targeted malaria elimination efforts.

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Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Sharing

Data reporting the study's primary and secondary protocol-specified objectives will be available as de-identified data on ClinicalTrials.gov approximately one year following the study completion date (23 February, 202022) under the study registration number [NCT04206332](https://ClinicalTrials.gov). The study protocol and informed consent form are also available on ClinicalTrials.gov. Additional de-identified data may be made available upon request to

the corresponding author for investigators whose proposed use of the data has been approved by the NIH IRB.

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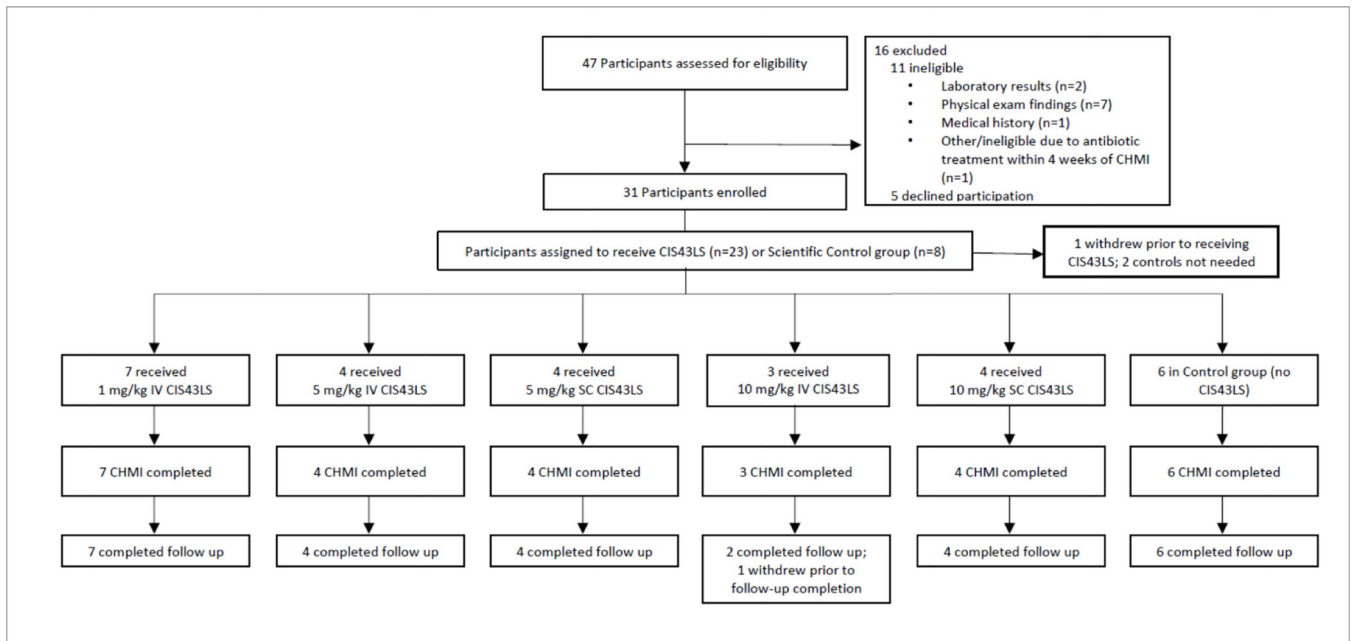


Figure 1: VRC 612 Part C Trial CONSORT Diagram.

Participants were enrolled according to dose-escalation protocol starting with the lowest dose group of 1 mg/kg IV. Of the 31 participants enrolled in Part C, 22 received one dose of CIS43LS and 8 were assigned to the control group and did not receive CIS43LS. One participant in the 10 mg/kg IV CIS43LS group withdrew consent prior to receiving CIS43LS due to a new medical diagnosis. 28 participants underwent controlled human infection in November 2021: 22 of these participants had received CIS43LS and 6 were control participants (the other 2 control participants were enrolled as back-ups and were not needed). One participant in the 10 mg/kg IV CIS43LS group withdrew consent after Controlled Human Malaria Infection (CHMI) but prior to follow-up completion due to personal reasons. All other participants completed follow-up visits. IV denotes intravenous, and SC subcutaneous route of CIS43LS administration.

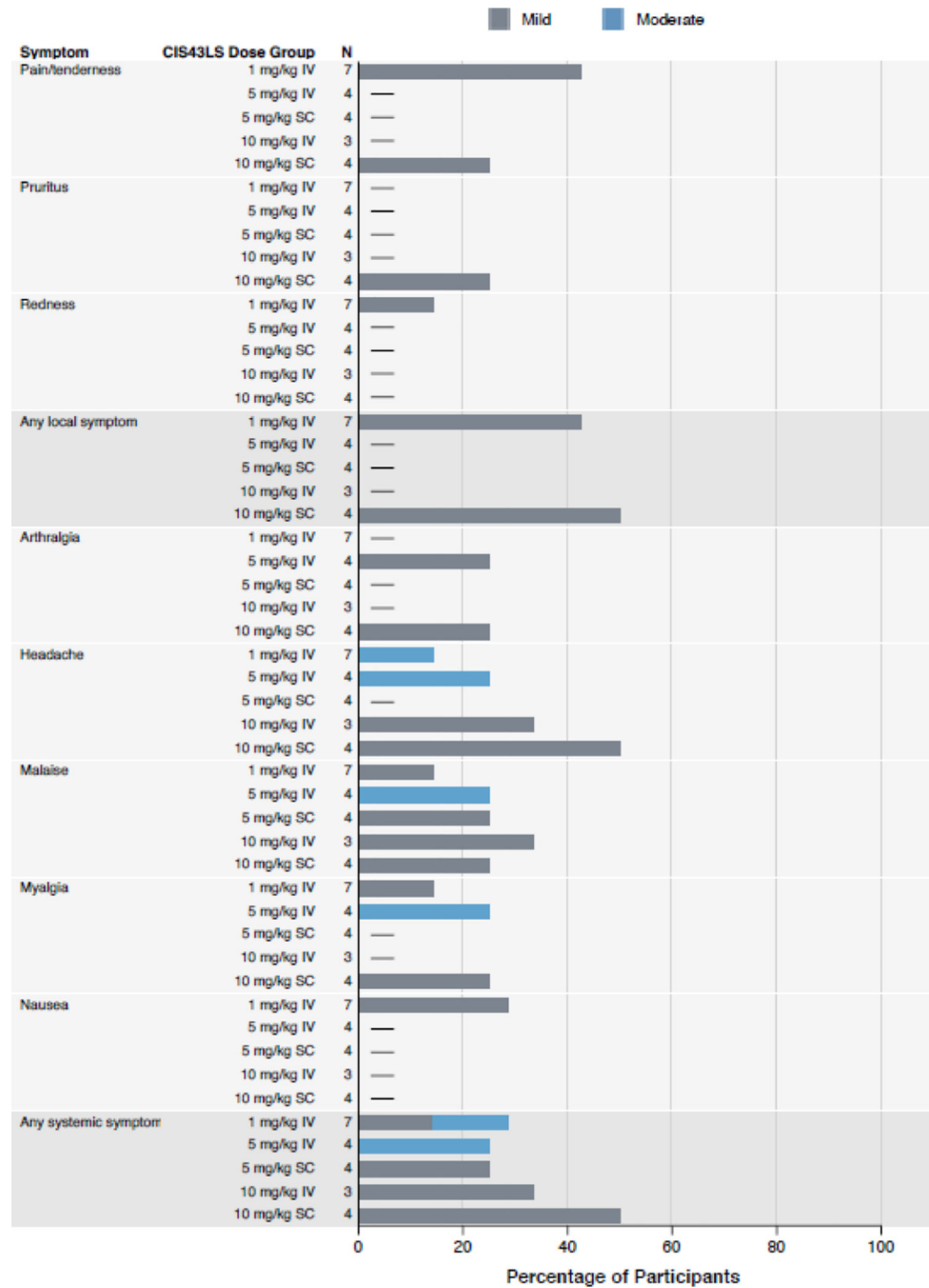


Figure 2: Maximum local and systemic solicited reactogenicity.

Percent of participants (x-axis) who reported a local or systemic symptom (y-axis) in the seven days following product administration. There were no reported local symptoms of bruising or swelling, or systemic symptoms of chills or elevated temperature for any participant following administration.

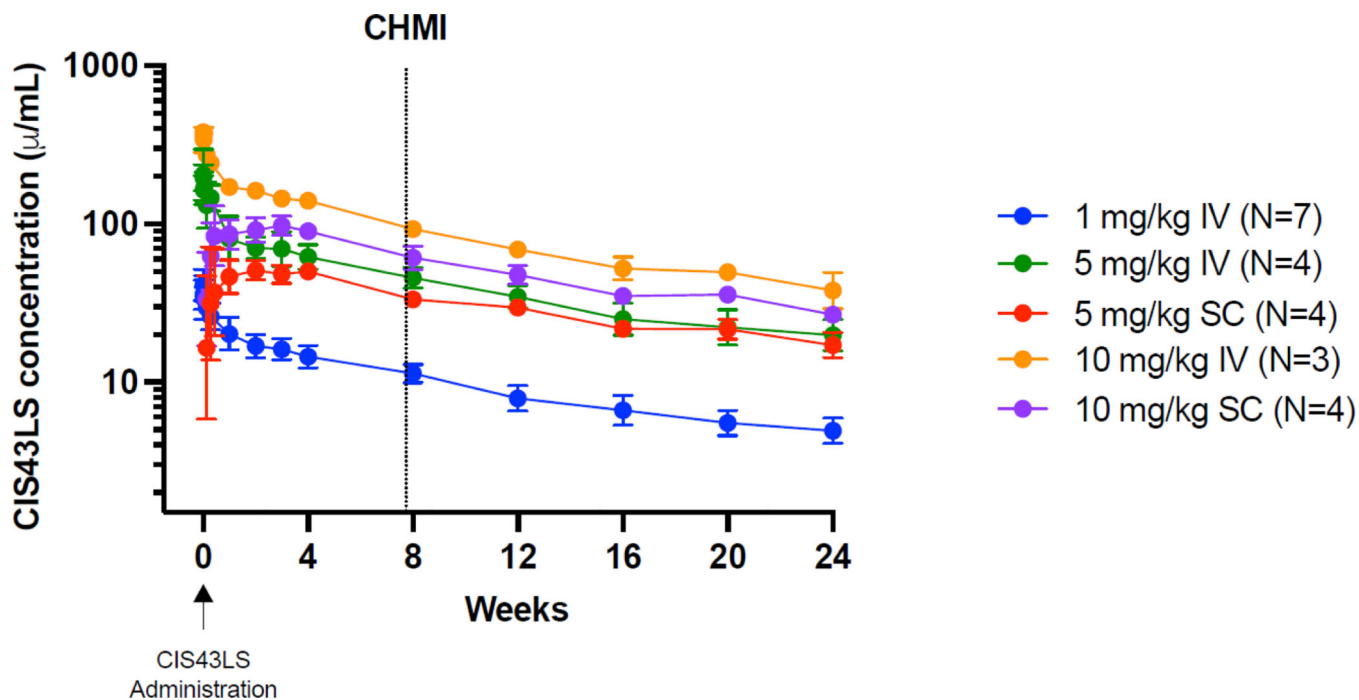


Figure 3: Serum concentrations of CIS43LS.

Geometric mean serum concentrations with standard deviations (indicated by bars) are displayed for each study group after a single administration of CIS43LS at Day 0. The dose and route of administration for each group is specified in the key. IV denotes intravenous, and SC subcutaneous route of CIS43LS administration. Vertical dotted line indicates CHMI that occurred on November 21, 2022. Time from CIS43LS administration to the date of CHMI for all study participants was between 54 to 56 days, with the exception of one study participant in 10 mg/kg IV group which was 48 days.

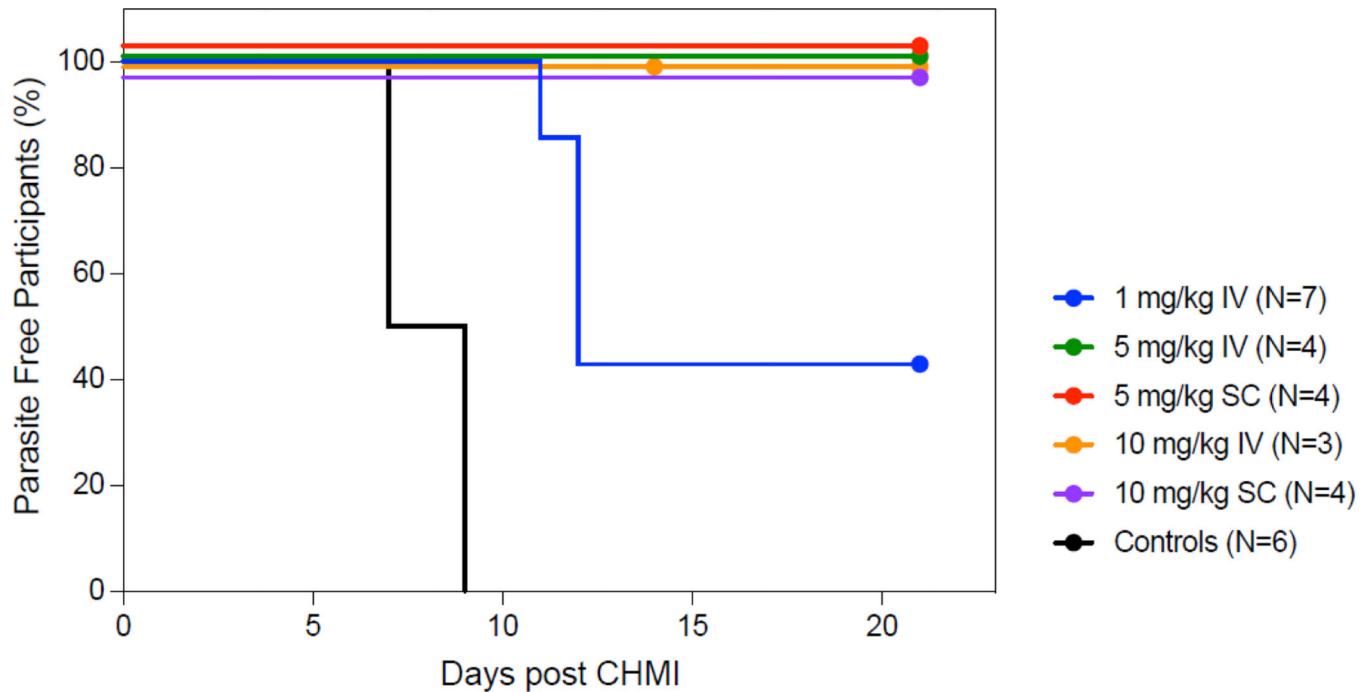


Figure 4: Parasitemia after Controlled Human Malaria Infection.

Kaplan-Meier analysis showing the time to parasitemia as measured by PCR. KP curves are provided for each arm. Note that one participant in the 10 mg/kg IV group (orange) withdrew at day 14 prior to completion of follow up. The proportion of infected subjects of all mAb treated recipients versus controls has a p value of 0.0005 by the Barnard test. Comparison of the 1 mg/kg IV recipients with controls gives p value of 0.10 with Barnard test.

Table 1.

Pharmacokinetic (PK) Parameters for CIS43LS

PK Parameters by Dose Group					
Dose Group	C _{max} (ug/ml)	T _{max} (d)	C _{28D} (ug/ml)	C _{84D} (ug/ml)	AUC _{0-168D}
Mean (Std Dev)					
1 mg/kg IV (N=7)	42.2 (10.4)	0.02 (0)	14.67 (2.57)	8.01 (1.59)	1731.5 (270.8)
5 mg/kg IV (N=4)	223.5 (53.3)	0.03 (0.02)	62.68 (11.45)	34.95 (5.63)	7346.6 (1375.3)
5 mg/kg SC (N=4)	53.6 (8.5)	14.32 (10.58)	50.23 (5.05)	29.8 (2.4)	5441.6 (499)
10 mg/kg IV (N=3)*	383.7 (30.8)	0.08 (0.09)	140.73 (8.44)	68.90 (0.71)	14866.8 (1255.78)
10 mg/kg SC (N=4)	104 (19.2)	16.6 (9.2)	90.2 (8.2)	48.1 (6.4)	9665 (1106.3)

PK Parameters for All Dose Groups (n = 22)			
PK Parameter	Value	Bootstrap (BS) Median	BS 95% CIs
T _{1/2β} (Days)	80	79.8	76.5 to 83.2
CL (ml/day)	33.8	33.8	30.7 to 36.5
V _{ss} (L)	3.79	3.79	3.50 to 4.07
F (%)	63	63	57 to 70

C_{max}= maximum serum concentration; T_{max}= time to maximum serum concentration; C_{28D}, C_{84D} = concentration on day 28 and 84, respectively.

AUC_{0-168D} = area under the curve from day 0 to day 168

T_{1/2β}= beta half-life; CL=clearance; V_{ss}=volume of distribution; F = subcutaneous bioavailability

Bootstrap 95% confidence intervals (CIs) for population PK parameters

* Analysis includes participant assigned to 10 mg/kg IV dose group who withdrew from the study at Day 15 post-CHMI

Table 2.

Serum CIS43LS At Time of CHMI

Subject	CIS43LS Dose/Route	CIS43LS Serum Concentration at CHMI ($\mu\text{g/mL}$)	Time From Last Administration (Days)	Infection Status
1	1 mg/kg IV	10.6	54	Yes
2	1 mg/kg IV	13.1	56	Yes
3	1 mg/kg IV	10.5	55	No
4	1 mg/kg IV	11	54	Yes
5	1 mg/kg IV	10.5	55	No
6	1 mg/kg IV	12.8	54	Yes
7	1 mg/kg IV	15.5	54	No
8	5 mg/kg IV	64.1	54	No
9	5 mg/kg IV	41.7	56	No
10	5 mg/kg IV	51.4	56	No
11	5 mg/kg IV	50.3	55	No
12	5 mg/kg SC	39.5	56	No
13	5 mg/kg SC	33.2	55	No
14	5 mg/kg SC	41.5	56	No
15	5 mg/kg SC	44.5	55	No
16	10 mg/kg IV	120.5	48	No
17	10 mg/kg IV	118.7	55	No
18*	10 mg/kg IV	113.0	55	No
19	10 mg/kg SC	71.9	56	No
20	10 mg/kg SC	82.2	55	No
21	10 mg/kg SC	87.7	56	No
22	10 mg/kg SC	62.8	56	No

Serum concentration after a single dose of CIS43LS at the time of CHMI. Time from last administration is an exact number of days between administration of CIS43LS and the date of CHMI for each study participant. Infection status indicates study participants who received CIS43LS and developed PCR-confirmed parasitemia post-CHMI.

* Indicates volunteer who withdrew at Day 15 following CHMI.