Protocol Title: A Phase 1, Dose Escalation, Open-Label Clinical Trial with Experimental Controlled Human Malaria Infections (CHMI) to Evaluate Safety and Protective Efficacy of an Anti-Malaria Human Monoclonal Antibody, VRC-MALMAB0100-00-AB (CIS43LS), in Healthy, Malaria-Naive Adults (VRC 612)

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VACCINE RESEARCH CENTER

Protocol VRC 612
(NIH 20I0017)

Title: A Phase 1, Dose Escalation, Open-Label Clinical Trial with Experimental Controlled Human Malaria Infections (CHMI) to Evaluate Safety and Protective Efficacy of an Anti-Malaria Human Monoclonal Antibody, VRC-MALMAB0100-00-AB (CIS43LS), in Healthy, Malaria-Naive Adults

Abbreviated Title: A Phase 1 Trial to Evaluate CIS43LS in Healthy Adults

IND Sponsor
Vaccine Research Center (VRC)
National Institute of Allergy and Infectious Diseases (NIAID)
Bethesda, Maryland, US

IND 142,632

Investigational Product CIS43LS Manufacturer:
Vaccine Clinical Materials Program (VCMP) operated under contract
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ABBREVIATIONS

Abbreviation	Definition
ADA	Anti-Drug Antibody
AE	Adverse event
ALT	Alanine aminotransferase
ANA	Anti-nuclear antibody
AST	Aspartate aminotransferase
CBC	Complete blood count
cGLP	Current Good Laboratory Practices
cGMP	Current Good Manufacturing Practices
CHI	Controlled human infection
CHMI	Controlled human malaria infection
CMP	Comprehensive Metabolic Panel
CRF	Case Report Form
CRO	Contract Research Organization
CSP	Circumsporozoite protein
CTP	Clinical Trials Program
DoD	Department of Defense
DoDI	Department of Defense Instruction
DNA	Deoxyribonucleic Acid
DOT	Directly observed therapy
EKG	Electrocardiogram
ELISA	Enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIV	Human Immunodeficiency Virus
HRPP	Human Research Protections Program
HSBP	Human Subjects Protection Branch
ICH	International Conference on Harmonization
IND	Investigational New Drug Application
IV	Intravenous
LIMS	Laboratory Management Information System
mAb	Monoclonal Antibody
MCG	Micrograms
MedDRA	Medical dictionary for regulatory activities
MO	Medical Officer
NAT	Nucleic acid test
NHP	Non-Human Primate
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NIH CC	NIH Clinical Center

Abbreviation	Definition
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase Chain Reaction
Pf	*Plasmodium falciparum*
PfCSP	*Plasmodium falciparum* circumsporozoite protein
PfSPZ	*Plasmodium falciparum* whole-sporozoite
PI	Principal Investigator
PK	Pharmacokinetics
PSRT	Protocol Safety Review Team
QA	Quality Assurance
RBC	Red blood cells
rPfCSP	Recombinant *Plasmodium falciparum* circumsporozoite protein
SAE	Serious adverse event
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SC	Subcutaneous
SPZ	Sporozoite
SUSAR	Serious and unexpected suspected adverse reaction
ULN	Upper limit of normal
UMB	University of Maryland, Baltimore
U.S.	United States
UPnonAE	Unexpected Problem that is not an Adverse Event
VCMP	Vaccine Clinical Materials Program
VEC	Vaccine Evaluation Clinic
VIS	Volunteer Infection Studies
VIP	Vaccine Immunology Program
VRC	Vaccine Research Center
WBC	White blood cells
WHO	World Health Organization
WRAIR	Walter Reed Army Institute of Research

PRINCIPAL INVESTIGATOR PROTOCOL SIGNATURE PAGE

VRC 612: A Phase 1, Dose Escalation, Open-Label Clinical Trial with Experimental Controlled Human Malaria Infections (CHMI) to Evaluate Safety and Protective Efficacy of an Anti-Malaria Human Monoclonal Antibody, VRC-MALMAB0100-00-AB (CIS43LS), in Healthy, Malaria-Naive Adults

I, the Principal Investigator for the study site indicated below, agree to conduct the study in full accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct the study in compliance with United States (US) Health and Human Services (HHS) regulations (45CFR 46); applicable US Food and Drug Administration (FDA) regulations; standards of the International Conference on Harmonization Guidelines for Good Clinical Practice (E6); Institutional Review Board/Ethics Committee (IRB/EC) determinations; all applicable in-country, state, and local laws and regulations; and other applicable requirements (e.g., US National Institutes of Health) and institutional policies. I will comply with all requirements regarding the obligations of investigators as outlined in the Statement of Investigator (Form FDA 1572), which I have also signed. The protocol signature page will be signed for subsequent protocol approvals.

I agree to maintain all study documentation pertaining to the conduct of this study, including but not limited to, case report forms, source documents, laboratory test results, and medication inventory records, per FDA regulation (21 CFR 312.62) and all applicable requirements. No study records will be destroyed without prior authorization from VRC/NIAID.

Publication of the results of this study will be governed by the VRC/NIAID policies. Any presentation, abstract, or manuscript will be made available by the investigators to VRC Leadership for review prior to submission.

I have read and understand the information in this protocol and will ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed about the obligations incurred by their contribution to the study.

_____	_____
Name/Title of Principal Investigator	Study Site Name
_____	_____
Signature of Principal Investigator	Date

PRÈCIS

Title: **VRC 612**: A Phase 1, Dose Escalation, Open-Label Clinical Trial with Experimental Controlled Human Malaria Infections (CHMI) to Evaluate the Safety and Protective Efficacy of an Anti-Malaria Human Monoclonal Antibody, VRC-MALMAB0100-00-AB (CIS43LS), in Healthy, Malaria-Naïve Adults.

Design: This is the first study of the VRC-MALMAB0100-00-AB (CIS43LS) monoclonal antibody (mAb) targeting the *Plasmodium falciparum* (Pf) circumsporozoite protein (PfCSP) in healthy adults. This two-part, dose-escalation, adaptive design study will evaluate the safety, tolerability, pharmacokinetics (PK), and protective efficacy of CIS43LS. The primary hypothesis is that CIS43LS will be safe and tolerable when administered by either intravenous (IV) or subcutaneous (SC) routes. The secondary hypotheses are that CIS43LS will be detectable in human sera with a definable half-life and confer protection following a controlled human malaria infection (CHMI).

Study Product: VRC-MALMAB0100-00-AB was isolated and developed by the Vaccine Research Center (VRC), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH) and binds a unique and conserved epitope at the junction of the N- and repeat regions of PfCSP.

Subjects: Healthy subjects, 18-50 years of age, who are malaria-naive.

Study Plan: **Part A**: Part A evaluated the doses and routes as shown below in the Study Schema table in an open-label, dose escalation design.

Part B: Part B evaluated CIS43LS doses and routes prior to CHMI in Part A veteran subjects and new Part B enrollees.

Part C: Part C will evaluate CIS43LS doses and routes needed to reach a threshold of protection by assessing serum concentration prior to CHMI in a dose down design.

VRC 612 Study Schema

Site	Group	Subjects	CIS43LS Administration		CHMI
			Dose (mg/kg)	Route	
Part A					
VRC	1	6	5	IV	Cancelled
	2	6	5	SC	
	3	6	20	IV	
	4A	6	40	IV	
	4B	3	40	IV	N/A
	5	6	Control		Cancelled
Part B					
VRC	6	6	5	SC	X
	7	6[1]	20	IV	X
	8	6[1]	--	--	X
	9	6	40	IV	X
	10	6[1,2]	Control		X
Part C					
UMB	11	7	1	IV	X
	12	4	5	IV	X
	13	4	5	SC	X
	14	4	10	IV	X
	15	4	10	SC	X
	16	6[2]	Control		X
	Total	92[3]	[1] Group includes Part A subjects [2] Two (2) additional control subjects will be enrolled as CHMI back-ups [3] Up to 100 subjects may be enrolled if needed for additional safety or efficacy evaluations.		

Duration: Subjects who receive CIS43LS will be followed through 24 weeks after product administration. Control subjects will be followed through 8 weeks after CHMI.

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1. INTRODUCTION

Malaria is a mosquito-borne protozoan disease belonging to the genus Plasmodium that affects 250-500 million people, and kills approximately 500,000 individuals annually, with an enormous economic impact in the developing world, especially sub-Saharan Africa [1-3]. The five recognized species of Plasmodium that cause human malaria infection are *Plasmodium falciparum* (Pf), *P. vivax, P. ovale, P. malariae,* and *P. knowlesi*. Among these, Pf causes more deaths in children worldwide than any other single infectious agent. An estimated 30,000 travelers from North America, Europe, and Japan contract malaria per year. Although malaria is preventable with chemoprophylaxis and completely curable with early intervention, drug treatment is not readily accessible in many parts of the world. Additionally, the use of antimalarial drugs over time has been associated with the emergence of drug-resistant strains. Lack of compliance with preventive drug treatment by individuals travelling to endemic areas may also result in fatal malaria infection. The world's first malaria vaccine, RTS,S/AS01 (Mosquirix™), a recombinant protein-based vaccine targeting Pf, was approved for use by European regulatory authorities in 2015. It is currently being evaluated in immunization programs in sub-Saharan Africa despite having been found to provide only partial protection (of about 30-50%) against clinical malaria to children and infants [4, 5]. Therefore, the development of a safe and more effective malaria vaccine is an urgent priority and may take many additional years. Alternatively, the use of antibodies for passive prevention of malaria provides a new and more immediate approach for malaria prevention that, if successful, would have a major impact on improving public health worldwide. Recent development of highly potent human monoclonal antibodies that have changes made to improve their duration *in vivo* and protect in different pre-clinical models will provide the first assessment of this approach.

1.1. Study Rationale

1.1.1. CIS43LS Development

The Vaccine Research Center (VRC), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH) has been investigating broadly-neutralizing human monoclonal antibodies (bNAbs) targeted at a variety of pathogens that may be utilized in clinical applications including preventive and therapeutic measures. In the case of malaria, prevention by passive immunization has potential applications for use in travelers, military personnel, season control and elimination campaigns in endemic areas.

VRC investigators isolated the monoclonal antibody (mAb), termed CIS43, from a subject immunized with an attenuated *Plasmodium falciparum* (Pf) whole-sporozoite (SPZ) vaccine (PfSPZ Vaccine (Sanaria)). The mAb was then adapted using site-directed mutagenesis in the Fc region, resulting in an LS mutation (CIS43LS) that has been found to increase product half-life in plasma [6, 7], which will be critical for optimizing its use. This LS modification has been applied to multiple VRC clinical mAbs and has been shown to be safe and significantly increase the half-life in plasma [8, 9].

CIS43LS recognizes a unique and highly conserved epitope at the junction of the N- and central repeat regions of the *Plasmodium falciparum* (Pf) circumsporozoite protein (PfCSP). In a published study in two different mouse models of malaria infection, passive immunization with CIS43 conferred high-level protection (70-100%) following malaria challenge with

concentrations of antibody *in vivo* ranging between 10-200 mcg/ml respectively [10]. Similar protection data were observed with CIS43LS [unpublished data]. These data show that CIS43LS is highly protective *in vivo* and suggest further exploration is warranted for passive prevention of malaria in humans.

1.1.2. Study Design

This study is a proof-of-concept study to evaluate CIS43LS. The Part A doses (5, 20, 40 mg/kg intravenous (IV) and 5 mg/kg subcutaneous (SC)) were selected based on efficacy data from challenge studies performed in mice showing that the protective concentration of antibody *in vivo* is between 10-200 µg/ml in two different models of malaria infection. Pharmacokinetic (PK) data from non-human primate (NHP) studies with CIS43LS and our prior clinical experience in healthy adults with human mAbs targeting Human Immunodeficiency Virus (HIV, i.e., VRC01, VRC01LS, and VRC07-523LS) and Ebolavirus (mAb114) at the same doses and regimens [11-14] suggest that we would achieve a dynamic range of antibody concentrations *in vivo* in humans with this dose escalation regimen that should be ideal for determining the expected protective titer following controlled human malaria infection (CHMI). As this is the first study in humans to assess the protective efficacy of a malaria mAbs, it is important to assess protective efficacy with all doses given.

Treatments with VRC mAbs have been generally well tolerated at all dose levels evaluated (1 to 40 mg/kg). The design of the CHMI phase of the study was based on previous clinical trial experience in published Phase 1 malaria vaccine trials that involved human Pf controlled human infections (CHI) [15, 16]. To our knowledge, this is the first trial to perform CHMI following administration of a malaria mAb.

Beyond acquiring safety and tolerability data, the initial intended purpose of Part A was to determine whether a CIS43LS serum concentration level correlated with protection from malaria infection following CHMI. The dose escalation design was to evaluate safety and tolerability of the product while also ensuring that participants presented at the time of CHMI with a wide range of CIS43LS serum concentrations. As expected, based on review of available PK data, Group 1 participants who received 5 mg/kg IV of CIS43LS early in Part A had a lower serum concentration at the intended time of CHMI compared to subjects receiving 40 mg/kg IV nearer to this time.

Twenty-one subjects were enrolled into Part A beginning in January 2020. Safety data indicated that CIS43LS administration by the IV and SC routes was well tolerated; initial PK measurements showed dose linearity with a defined half-life within the expected range. Unfortunately, the Part A CHMI scheduled for March 17, 2020 was cancelled due to emerging cases of COVID-19 in Maryland and the Washington Metro area. The consequent travel restrictions, local and state stay-at-home orders, and interrupted NIH Clinical Center patient care services also altered follow-up visits. Thus, subjects who received study product in Part A were primarily followed via remote contact to continue safety oversight during the COVID-19 pandemic.

With protocol Version 4.0, the study design for Part B was finalized following review of preliminary safety and PK data from Part A and in accordance with the overall goal of attaining a wide range of CIS43LS serum concentrations in subjects to support evaluation of correlates of protection following the CHMI. Part B subjects provided additional safety and tolerability data

for CIS43LS while also contributing to the first efficacy data set. Part B groups were enrolled based on subject availability and scientific priority during the ongoing COVID-19 pandemic.

Newly enrolled participants in Part B received 40 mg/kg IV doses of CIS43LS prior to the CHMI while other participants who received CIS43LS in Part A were re-dosed with a single 20 mg/kg IV infusion to ensure subjects presented with a gradient of serum concentrations prior to the CHMI. A subset of Part A subjects who received 40 mg/kg IV also participated in the Part B CHMI without CIS43LS redosing to assess for durability after a distant infusion roughly 8 months prior to CHMI. Group 6 (5 mg/kg SC in Part B) was never enrolled in order to focus on the groups receiving CIS43LS by IV. Actual CIS43LS concentrations in blood were measured from samples collected 1 day prior to Part B CHMI for all subjects.

The Part B CHMI was conducted on October 20, 2020, with 9 recipients of CIS43LS plus 6 control subjects undergoing challenge. None of the 9 CIS43LS recipients developed parasitemia during the 21-day post-CHMI monitoring period, whereas 5 of 6 control subjects became parasitemic on day 8 or 9. This indicated that CIS43LS could protect human subjects in a CHMI model. The range of CIS43LS serum concentrations at the time of CHMI was approximately 50 to 500 mcg/mL. The lack of infections in the CIS43LS groups over a range of serum concentrations was unexpected because the preclinical mouse data would not have predicted a serum concentration of 50 mcg/mL to be protective. The lack of infections also prevented completion of a regression analysis to determine the protective serum concentration threshold. This threshold was still felt to be scientifically important, therefore a third part to the trial is needed in order to assess for this critical endpoint.

With protocol Version 6.0, Part C is being added to the trial with the objective of identifying the lowest dose of CIS43LS that could confer protection following CHMI. Part C will be conducted at the University of Maryland, Baltimore (UMB) Center for Vaccine Development and Global Health. UMB will perform subject screening, enrollment, product administration, and the CHMI. The sample size for Part C groups is informed by Part B data that suggested more data from specific lower dose groups is needed to define the lower than initially predicted serum concentration threshold. Therefore, Part C will allocate greater numbers of subjects to the 1 mg/kg group in order to accumulate sufficient data.

1.2. Background

1.2.1. Previous Human Experience

There was no human experience with CIS43LS prior to this trial. Preliminary VRC 612 Part A and Part B data are provided in Section 2.3. Prior clinical experience in completed and ongoing Phase 1 and 2 trials of healthy adults with human mAbs manufactured and formulated by the VRC that recognize pathogen-specific epitopes (i.e., VRC01, VRC01LS, VRC07-523LS, and mAb114) [11-14] are used to summarize the general safety risk associated with mAbs.

Treatments with these mAbs have been generally well tolerated, with no reported deaths or serious adverse events (SAEs) assessed as related to the study products. Typical for mAbs, the predominant local reactogenicity complaint has been mild pain/tenderness, although reports of mild injection site pruritus, redness and swelling have occurred at modestly higher frequencies with SC administration. Malaise, muscle pain, and headache have been the most frequently reported solicited complaints noted in the 3 days post product administration and these have also

been mostly transient and mild in severity. Urticaria and infusion reactions comprised of chills, rigors, myalgia, headache, and/or fever have been reported after IV infusions at product doses of 10 to 40 mg/kg; these reactions have been transient, resolved without sequelae within 24 hours of onset, and treated with over-the-counter analgesics and antipyretics.

1.2.2. Controlled Human Infections (CHI)

Controlled human infection (CHI) trials (also referred to as Volunteer Infection Studies (VIS)) result in the experimental infection of healthy subjects with the infectious agent of choice and are an unparalleled tool in infectious disease research. CHI trials allow for the accelerated evaluation of novel drugs and vaccines for potential efficacy, while also providing the opportunity to prospectively study clinical disease progression. The first controlled human malaria infection (CHMI) was performed in the mid-1980s [17]. This process involves the deliberate infection with malaria parasites either by mosquito bite or direct injection. VRC has successfully conducted three Phase 1 CHMI studies: VRC 312 [15], VRC 314 [18] and VRC 612 Part B [9].

According to a comprehensive review of CHI trials using a variety of pathogens, an estimated 6000 subjects have received CHI [19]. Only four possibly-related SAEs have been published, of which two were observed following malaria vaccination and CHMI [19]. The first cardiac event of possible myocarditis, reported in 2008 [20], prompted recommendation by a panel of experts that consideration of cardiac risk be required for clinical challenge trials and that individuals identified as at increased cardiovascular risk be excluded from malaria clinical challenge trials [21]. A second cardiac event was observed in 2013 following malaria vaccination and CHMI [22]. VRC was also informed of a possible 3rd, unpublished, myocarditis event in 2014. As a result of these events and the recommendations, evidence of increased cardiovascular disease risk or an electrocardiogram (ECG) with clinically significant abnormalities will preclude trial enrollment as described in Section 4.1.2.

All challenge phases in this study will be performed with Pf strain 3D7, sensitive to Chloroquine and Malarone (atovaquone and proguanil), via mosquito bite, which is the natural route of exposure and transmission in the field. While subjects will be monitored closely throughout study duration, monitoring intensity will increase following exposure to Pf-infected mosquitoes to ensure rapid diagnosis and treatment. Appropriate drug therapy will be used to treat and cure any subject with a confirmed Pf infection after diagnosis by PCR, the most sensitive assessment for detecting early malaria infection, in order to minimize the risk of developing a serious complication. The Pf strain to be used for CHMI in this study can be effectively treated with and cured by the antimalarial medications to be used in this trial [23, 24].

There is negligible risk of transmitting malaria to a person in the community since subjects are exposed to malaria-infected mosquitos only during the CHMI at a contained facility.

1.3. Laboratory Assessments of CIS43LS

Some laboratory assessments in this study are also designed to characterize the investigational product. This includes PK analysis and evaluation for anti-drug antibody (ADA) development after product exposure. These are further described in the subsequent sections. Other assays may also be completed from stored samples at a later date if additional assessments are needed.

The VRC's Vaccine Immunology Program (VIP), formerly NVITAL, Gaithersburg, MD, will process blood and store coded samples, and will either perform sample testing or ship coded samples to designated research laboratories at the VRC or other approved collaborators. See APPENDIX I for schedules, volumes and tube types to be used for research sample collection. Research assays will be performed on samples from both study product recipients and infectivity controls at baseline and throughout the study.

Tube types for clinical labs are according to institutional requirements and are shown in the Schedule of Evaluations to estimate blood volumes. Different tubes for clinical evaluations may be used to meet site requirements. Research sample tube types and blood volumes must be used as shown or as otherwise instructed by the IND Sponsor. In some instances, coded samples may be transported directly by study staff to the laboratory of an approved collaborator.

1.3.1. Pharmacokinetic (PK) Analysis

Concentrations of CIS43LS will be measured by Meso Scale Discovery (MSD) based automation platform and similar methodology as previously described for other VRC mAb products [12].

1.3.2. Detection of Anti-Drug Antibody

Assays for detection of ADA will be performed at specified timepoints following product administration and CHMI compared to baseline status using a similar methodology as previously described for other VRC mAb products [12]. A three-level tiered approach will be used to screen, confirm, and functionally characterize for ADA in the clinical serum samples according to the Food and Drug Administration (FDA) guidance. Screening and confirmation will involve a Meso Scale Discovery (MSD) electrochemiluminescence (ECL) bridging assay. In the absence of an adequate in vivo sporozoite entry inhibition assay, a competitive target binding assay of CIS43LS to its cognate epitopes will be used as a proxy for sporozoite recognition and neutralization.

1.4. Measures of mAb-Mediated Protection and Parasitemia

CIS43LS-mediated protection will first be assessed after CHMI and compared to infection in control subjects. The endpoint defining mAb-mediated protection for the CHMI is the absence of Pf parasites in blood samples obtained from CIS43LS-recipients collected from Day 7 through Day 21 post-CHMI. The criteria for a case of malaria is confirmation of parasitemia either by the Malaria real-time PCR assay or blood smear. The Malaria real-time PCR assay targets the 18S rRNA (ribosomal RNA) gene of Pf parasites, which is a DNA target. It is the default means of monitoring for parasitemia in this protocol, and a single positive PCR result will confirm infection [15, 25, 26]. The malaria real-time PCR assay will be performed by the NIH Clinical Center Department of Laboratory Medicine for Part B or the UMB Division of Malaria Laboratories Research for Part C.

A blood smear will be performed by the NIH Clinical Center Microbiology Service or UMB Malaria Group personnel per their departmental SOPs for any subject who develops symptoms likely due to malaria despite not having a positive PCR result. With traditional microscopy, positivity is defined as the presence of two or more parasites in 0.5 mcL of blood. Laboratory personnel performing PCR and blood smear testing will be blinded to subject group and

treatment received or lack thereof, in the case of controls. Research blood samples may also be used for parasite genome analysis.

2. STUDY PRODUCT AND CHMI

2.1. Study Product: CIS43LS

MAb VRC-MALMAB0100-00-AB (CIS43LS) was discovered and developed by the VRC, NIAID, NIH. CIS43LS binds a unique and conserved epitope at the junction of the N- and central repeat regions of the PfCSP. The study product was manufactured under current Good Manufacturing Practice (cGMP) by the Vaccine Clinical Materials Program (VCMP) operated under contract by Leidos Biomedical Research, Inc., Frederick, MD.

2.2. Preclinical Experience

To assess CIS43LS as a candidate for clinical trials, recombinant, research grade mAb was evaluated on binding properties, auto-reactivity, pharmacokinetics, two mouse models of *in vivo* protection following challenge, and PK studies in NHPs were performed. In mice, CIS43LS conferred 100% protection following mosquito bite challenge. In NHP PK studies, CIS43LS exhibited substantially longer half-life, both in blood and in skin biopsy samples as compared to CIS43 antibody without the LS mutation.

Preclinical toxicology studies were conducted to assess safety in compliance with current Good Laboratory Practices (cGLP) in Sprague Dawley rats using CIS43LS. The toxicology pre-clinical material was tested in the final formulation buffer and manufactured using the same VCMP manufacturing process as used to produce clinical trial material. There were no findings of toxicologic significance attributed to CIS43LS from the results.

2.2.1. *In vitro* Studies

Differences in the binding properties of CIS43LS and CIS43 were assessed. Binding of CIS43LS and CIS43 to a specific target antigen expressed on malaria parasites was determined by ELISA. CIS43LS and CIS43 exhibited similar binding to recombinant *Plasmodium falciparum* circumsporozoite protein (rPfCSP). Avidity and stoichiometry of CIS43LS and CIS43 binding to rPfCSP were also comparable as assessed by isothermal titration calorimetry (ITC).

CIS43LS autoreactivity was assessed using the anti-nuclear antibody (ANA) HEp2 cell staining system from Zeus Scientific (Branchburg, NJ). Antibodies were tested at 25 and 50 mcg/mL. At 25 mcg/mL, test antibodies were scored in comparison to negative and positive control samples. Neither CIS43 nor CIS43LS shows an autoreactivity signal by ANA staining. Autoreactivity was also assessed by an anti-cardiolipin QUANTA Lite ACA IgG III ELISA (Inova Diagnostics, San Diego, CA). Using a similar control schema to that described for ANA HEp2 Cell staining, anti-cardiolipin reactivity was negative by ELISA.

2.2.2. *In vivo* Studies

a. Protection studies in C57BL/6 mice and Rhesus macaques

To assess protection following passive transfer of mAb, naïve C57BL/6 mice received 300 mcg by IV inoculation of CIS43, CIS43LS, or negative control antibody. Chimeric parasites comprising *Plasmodium berghei* (Pb) sporozoites (SPZ) which naturally infect mice that expressed *Plasmodium falciparum* CSP (Pb-PfCSP SPZ) were administered by one of two routes

(injected by tail vein or by exposure to bites by infected mosquitos). In the two mouse challenge models, passive transfer of CIS43 and CIS43LS yielded nearly indistinguishable results. Administration of either CIS43 or CIS43LS reduced liver parasite burden by about two logs when challenged by IV route, which delivers the parasites directly into the liver and bypasses the normal route of infection through the skin. Moreover, passive transfer of CIS43 or CIS43LS conferred 100% sterile protection in mice as assessed by parasitemia in blood following a natural challenge by five mosquito bites in the skin. This type of challenge is similar to what is used in the human CHMI.

To confirm the improved half-life of CIS43LS and inform clinical protocol development, particularly concerning the timing of CHMI, a 20-week PK study was conducted in NHPs (Rhesus macaques). Two animals per group received CIS43 or CIS43LS, administered by IV route at a dose of 10 mg/kg. CIS43LS exhibited substantially longer half-life, both in blood and skin biopsy samples than CIS43. In blood, CIS43LS maintained a titer of more than 35 mcg/mL on the last sample day (day 140). In contrast, CIS43 dropped to approximately 30 ug/mL by Day 49. Skin biopsy data were concordant.

b. Toxicology Studies in Sprague Dawley Rats

A study of repeat dosing at 10-day intervals by IV and SC administration of CIS43LS in male and female Sprague Dawley rats (SRI Study No. M416-19) was conducted in compliance with cGLP to evaluate toxicity and toxicokinetics. The test article and control (formulation buffer) were administered on Days 1 and 11 by IV administration at doses of 0, 40 or 400 mg/kg/day (Groups 1−3, respectively) or by SC administration at doses of 5 or 50 mg/kg/day (Groups 4−5, respectively). Necropsies were performed on Days 12 and 46. Endpoints included clinical observations, body weight, food consumption, body temperature, dose site irritation, clinical pathology, and organ weights.

All animals survived to their scheduled sacrifice timepoint. No test article-related changes were observed in clinical observations, body weights, food consumption, dose site irritation, clinical chemistry, coagulation, and organ weights. Body temperature was normal (≤ 101.0°F) for all males at 24 hours post-dose. Elevated body temperatures ranging from 101.1 to 102.1°F were seen from one or more females in high-dose IV or both SC treated groups for at least 24 hours after dosing on Days 1 and/or 11. Body temperature returned to normal levels within 48 hours post-dose. These findings are considered test article-related although reversible and at levels considered to be non-adverse.

Statistically significant and dose-dependent changes in some hematology parameters were observed on Days 12 and 46. The changes included increased platelet count (1.4 fold) and absolute reticulocyte count (1.2 fold) in Group 5 females; and decreased percent monocyte (by 30.6 to 32.2%) in Group 3 and 4 females on Day 12. On Day 46, increased absolute neutrophils (2.0 to 2.3 fold) in Group 3 and 4 males and decreased hemoglobin (by 6.1 to 7.6%) and/or hematocrit (by 5.6 to 7.6%) in all treated-group females were observed. The changes in neutrophils and monocytes seen after SC administration are consistent with an immune response and are considered test article-related although reversible and at levels considered to be non-adverse. Without changes in red blood cell (RBC) counts, the findings in platelet count, reticulocytes, hemoglobin or hematocrit are not considered to be biologically meaningful or toxicologically adverse. In summary, no findings of toxicologic significance were attributed to CIS43LS from the results available as of this report.

Taken together, these studies support the continued development of CIS43LS for clinical evaluation. The data confirm the predicted longer half-life effect of the LS mutation and show that there is no functional difference in terms of *in vitro* binding or *in vivo* protection compared to CIS43. No safety, efficacy, or manufacturability concerns were noted that precluded proceeding to further toxicology studies, GMP manufacturing, IND filing, and clinical investigations.

2.3. Clinical Experience

Part A and B data from this trial is the full extent of current human experience with CIS43LS. See Section 1.2.1 for more information about similar VRC mAb products.

VRC 612 was initiated in January 2020 with accrual into Part A. Part B of the trial began in September 2020. As of February 15, 2021, 25 subjects received 29 administrations of CIS43LS. While most subjects (n=21) received only a single dose, 4 subjects originally dosed in Part A received a second dose of 20 mg/kg IV in Part B. CIS43LS administrations were given by IV (n=25) and SC (n=4) routes. At the time of this summary, subjects are in follow up; data quality assurance and monitoring are ongoing.

Human experience data collected thus far, including frequency and severity of solicited and unsolicited adverse events, are summarized below. Overall, VRC 612 data demonstrate that CIS43LS is safe and well tolerated upon IV and SC administration. There have been no reported SAEs related to study product and no study pause criteria met.

Local Reactogenicity: Mild pain/tenderness at the injection site was the most frequently reported solicited local symptom, reported by 4/25 (16%) IV recipients and 3/4 (75%) SC recipients. All pain events resolved within the 7-day solicited period. One event of moderate bruising began on Day 4 in an IV recipient and continued through 4 days after the solicited period to Day 11. No other local symptoms were reported.

Systemic Reactogenicity: Mild headache was the most frequently reported solicited systemic symptom, reported by 5/25 (20%) IV recipients and 1/4 (25%) SC recipients. Mild myalgia was reported by 3/25 (12%) IV recipients and 2/4 (50%) SC recipients. Other events reported by IV recipients include mild (2/25, 8%) and moderate (1/25, 4%) malaise, moderate nausea (1/25, 4%), mild joint pain (1/25, 4%), and mild fever (1/25, 4%). All events resolved within the 7-day solicited period with the exception of one report each of moderate malaise and mild headache by an IV recipient that began on Day 4 and lasted until Day 10, and one report of mild myalgia by an IV recipient that began on the day of product administration (Day 0) and lasted until Day 10.

Unsolicited AEs: Of the 25 subjects who received CIS43LS, 9 subjects (36%) had at least one AE with the highest severity of mild for 5 (20%) subjects, moderate for 3 (12%) subjects and severe for 1 (4%) subject. The most frequently reported unsolicited AE by 3 of 21 (14%) subjects was upper respiratory tract infection (URTI), which was assessed as not related to the study product for all subjects. Three mild AEs were assessed as related to study product including one event each of blood creatinine increase lasting 7 days, transient asymptomatic neutropenia lasting 6 days and dizziness lasting 1 day. All related events resolved with no residual effects.

2.4. Controlled Human Malaria Infection (CHMI)

Preparation of infected mosquitoes for CHMI will be performed according to a Type II Master File 033797, Malaria Challenge Model, Standard Operating Procedures for the Pf model used. To prepare infected mosquitoes, Pf asexual and sexual erythrocytic stage parasites will be grown in normal human erythrocytes using standard culture medium containing 10% normal human serum. The blood and serum for culture are purchased from a Food and Drug Administration (FDA)-accredited blood bank. Each shipment carries a certificate of analysis certifying that the blood products are negative or non-reactive for immunologic evaluation and infectious disease testing.

3. STUDY OBJECTIVES

3.1. Primary Objectives

- To evaluate the safety and tolerability of CIS43LS administered IV at 1, 5, 10, 20, and 40 mg/kg in healthy, malaria-naive adults
- To evaluate the safety and tolerability of CIS43LS administered SC at 5 and 10 mg/kg in healthy, malaria-naive adults

3.2. Secondary Objectives

- To evaluate the pharmacokinetics of CIS43LS at each dose level throughout the study
- To determine if IV or SC administration of CIS43LS mediates protection against infectious *P.* falciparum following CHMI
- To estimate the lowest dose of CIS43LS administered IV and SC that confers protection against infectious *P. falciparum* following CHMI

3.3. Exploratory Objectives

- To determine whether anti-drug antibody (ADA) to CIS43LS can be detected in sera of recipients at specific time points throughout the study
- To assess for IgG1 allotypes and allotype-specific effects on CIS43LS pharmacokinetics
- To explore and characterize the humoral and cellular response to CIS43LS
- To assess durability of mAb-mediated protection of CIS43LS

4. STUDY DESIGN AND CLINICAL PROCEDURES

Parts A and B of this open-label, dose escalation study were conducted at the VRC Vaccine Evaluation Clinic (VEC) in the NIH Clinical Center (NIH CC) and the CHMI was conducted at the Walter Reed Army Institute of Research (WRAIR) insectary with the oversight of NIH CC staff. All of Part C (including the CHMI) will be conducted at the UMB Center for Vaccine Development and Global Health. The primary hypothesis is that CIS43LS will be safe and tolerable when administered by either IV or SC routes. The secondary hypotheses are that CIS43LS will be detectable in human sera with a definable half-life and will confer protection following a CHMI.

Part A: The study began with subject enrollment into Group 1. The dose escalation safety reviews proceeded as described in Section 4.5. Part A safety data show that CIS43LS is safe and tolerable at all dose groups. Preliminary PK data suggest that a gradient of serum concentrations will be present across groups at the time of CHMI.

Part B further evaluated the CIS43LS doses and routes needed to reach a threshold of protection by assessing serum concentrations prior to CHMI in Part A veteran subjects as well as in new enrollees.

Part C will evaluate CIS43LS doses and routes needed to reach a threshold of protection by assessing serum concentration prior to CHMI in a dose down design.

The overall study schema is as follows:

Table 1: Study Schema

VRC 612 Study Schema					
Site	**Group**	**Subjects**	**CIS43LS Administration**		**CHMI**
			Dose (mg/kg)	**Route**	
Part A					
VRC	1	6	5	IV	Cancelled
	2	6	5	SC	
	3	6	20	IV	
	4A	6	40	IV	
	4B	3	40	IV	N/A
	5	6	Control		Cancelled
Part B					
VRC	6	6	5	SC	X
	7	6[1]	20	IV	X
	8	6[1]	--	--	X
	9	6	40	IV	X
	10	6[1,2]	Control		X
Part C					
UMB	11	7	1	IV	X
	12	4	5	IV	X
	13	4	5	SC	X
	14	4	10	IV	X
	15	4	10	SC	X
	16	6[2]	Control		X
	Total	92[3]	[1] Group includes Part A subjects [2] Two (2) additional control subjects will be enrolled as CHMI back-ups [3] Up to 100 subjects may be enrolled if needed for additional safety or efficacy evaluations.		

4.1. Study Population

Subjects will be screened to confirm eligibility requirements for participation using the VRC 500 screening protocol. The screening and education process required prior to enrollment is designed to ensure that subjects comprehend the purpose, details and risks/benefits of the study. Eligibility will be re-assessed prior to Part B enrollment for any Part A subjects who are invited to participate in Part B.

4.1.1. Inclusion Criteria

A subject must meet all of the following criteria to be included:

1. Able and willing to complete the informed consent process
2. Able to provide proof of identity to the satisfaction of the study clinician completing the enrollment process
3. Available for clinical follow-up through the last study visit
4. 18 to 50 years of age
5. In good general health without clinically significant medical history
6. Physical examination without clinically significant findings within the 56 days prior to enrollment
7. Weight ≤ 115 kg (for all groups except Groups 5, 10, and 16) and < 100 kg for Group 15
8. Adequate venous access if assigned to an IV group or adequate subcutaneous tissue if assigned to a SC group
9. Willing to have blood samples collected, stored indefinitely, and used for research purposes
10. Agrees to participate in a controlled human malaria infection (CHMI) and to comply with post-CHMI follow-up requirements (except Group 4B)
11. Agrees to refrain from blood donation to blood banks for 3 years following participation in CHMI (except Group 4B)
12. Agrees not to travel to a malaria endemic region during the entire course of study participation

Laboratory Criteria within 56 days prior to enrollment:

13. WBC 2,500-12,000/mm3
14. WBC differential either within institutional normal range or accompanied by the Principal Investigator (PI) or designee approval
15. Platelets = 125,000 – 500,000/mm3
16. Hemoglobin within institutional normal range or accompanied by the PI or designee approval
17. Creatinine ≤ 1.1 x upper limit of normal (ULN)
18. Alanine aminotransferase (ALT) ≤1.25 x ULN

19. Negative for HIV infection by an FDA approved method of detection

Laboratory Criteria documented any time prior to enrollment:

20. Negative sickle cell screening test
21. Negative troponin test (except Group 4B)
22. Electrocardiogram (ECG) without clinically significant abnormalities (examples may include: pathologic Q waves, significant ST-T wave changes, left ventricular hypertrophy, any non-sinus rhythm excluding isolated premature atrial contractions, right or left bundle branch block, advanced A-V heart block). ECG abnormalities determined by a cardiologist to be clinically insignificant as related to study participation do not preclude study enrollment (except Group 4B)
23. No evidence of increased cardiovascular disease risk; defined as >10% five-year risk by the non-laboratory method [27] (except Group 4B)

Criteria Specific to Women:

24. Postmenopausal for at least 1 year, post-hysterectomy or bilateral oophorectomy, or if of childbearing potential:
    a. Negative beta-human chorionic gonadotropin (β-HCG) pregnancy test (urine or serum) on day of enrollment, and prior to product administration and CHMI, and
    b. Agrees to use an effective means of birth control through the duration of study participation

4.1.2. Exclusion Criteria

A subject will be excluded if one or more of the following conditions apply:

1. Woman who is breast-feeding or planning to become pregnant during study participation
2. Previous receipt of a malaria vaccine
3. History of malaria infection
4. History of severe infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) defined per FDA guidance [28]
5. Active SARS-CoV-2 infection
6. Any history of a severe allergic reaction with generalized urticaria, angioedema or anaphylaxis prior to enrollment that has a reasonable risk of recurrence during the study
7. Hypertension that is not well controlled
8. Receipt of any investigational study product within 28 days prior to enrollment (note: Emergency Use Authorization COVID-19 vaccine is not exclusionary)
9. Receipt of any live attenuated vaccines within 28 days prior to enrollment
10. Bleeding disorder diagnosed by a doctor (e.g. factor deficiency, coagulopathy, or platelet disorder requiring special precautions) or significant bruising or bleeding difficulties with intramuscular injections or blood draws

11. History of a splenectomy, sickle cell disease or sickle cell trait
12. History of skeeter syndrome or anaphylactic response to mosquito-bites (except Group 4B)
13. Known intolerance to chloroquine phosphate, atovaquone or proguanil (except Group 4B)
14. Use or planned use of any drug, including antibiotics, with antimalarial activity within 4 weeks prior to CHMI
15. History of psoriasis or porphyria, which may be exacerbated after treatment with chloroquine (except Group 4B)
16. Anticipated use of medications known to cause drug reactions with chloroquine or atovaquone-proguanil (Malarone) such as cimetidine, metoclopramide, antacids, and kaolin (except Group 4B)
17. Any other chronic or clinically significant medical condition that in the opinion of the investigator would jeopardize the safety or rights of the volunteer, including but not limited to: diabetes mellitus type I, chronic hepatitis; OR clinically significant forms of: drug or alcohol abuse, asthma, autoimmune disease, psychiatric disorders, heart disease, or cancer

4.2. Inclusion of Vulnerable Subjects

4.2.1. Children

Children are not eligible to participate in this clinical trial because the study product has not been previously evaluated in adults. If the product is assessed as safe for further study, other protocols specifically designed for children may be conducted.

4.2.2. Participation of Site Employees

4.2.2.1. VRC Site

NIH employees and members of their immediate families may participate in this protocol. We will follow the Guidelines for the Inclusion of Employees in NIH Research Studies and will give each employee a copy of the "NIH Information Sheet on Employee Research Participation" and a copy of the "Leave Policy for NIH Employees Participating in NIH Medical Research Studies."

Neither participation nor refusal to participate will have an effect, either beneficial or adverse, on the participant's employment or work situation. For the VRC site, the NIH information sheet regarding NIH employee research participation will be distributed to all potential subjects who are NIH employees. The employee subject's privacy and confidentiality will be preserved in accordance with applicable policies at the study site. For any employee subjects, consent will be obtained by an individual who is independent of the employee's team. At NIH, if the individual obtaining consent is a co-worker to the subject, independent monitoring of the consent process will be included through the Bioethics Consultation Service. Protocol study staff will be trained on obtaining potentially sensitive and private information from co-workers or subordinates.

4.2.2.2. UMB Site

The UMB site will follow institutional policies related to study participation of students or University employees. Neither participation nor refusal to participate will have an effect, either beneficial or adverse, on the subject's educational, employment or work situation.

4.3. Clinical Procedures and Evaluations

Evaluation of study product safety will include laboratory studies, medical history, physical assessment by clinicians, and subject self-assessment. The study schedule is presented in the Schedule of Evaluations, APPENDIX I. Total blood volume drawn from each subject will not exceed the NIH Clinical Center Guidelines (Part A and Part B) or UMB Center for Vaccine Development and Global Health limits (Part C).

4.3.1. Recruitment and Retention

Study enrollments will be conducted at the NIH Clinical Center (Part A and Part B) and at the UMB Center for Vaccine Development and Global Health (Part C). Study subjects will be recruited through on-site and off-site IRB-approved advertising done through the VRC's screening protocol, VRC 500 (NCT 01375530). Effort will be made to include women and minorities in proportions similar to that of the community from which they are recruited.

4.3.1.1. Costs

There are no costs to subjects for their participation in this trial.

4.3.1.2. Compensation

4.3.1.2.1. NIH Compensation

Subjects will be compensated for time and inconvenience in accordance with the standards for compensation of the Clinical Research Volunteer Program. Compensation for study product administration in the IV groups accompanied by the same day PK blood draws will be $430; product administration visits in the SC group without PK blood draws will be $375. Compensation will be $200 for scheduled visits that include blood draws, $85 for clinic visits that do not include a blood draw or procedure, $55 for SARS-CoV-2 testing in Parts A and B of the study by nasopharyngeal swab, and $25 for timely completion of the electronic diary card. Compensation for a CHMI visit including the pre-CHMI visit the prior day is $400.

The total amount of compensation varies depending upon group and the visits completed. Compensation may also vary depending upon the number of days required for the daily evaluations in the period after a CHMI as those who become parasitemic early will require fewer days of in-clinic evaluation visits than those who have delayed or no parasitemia. CHMI control subjects will be compensated similarly for comparable visit types.

4.3.1.2.2. UMB Compensation

Subjects will be compensated for time and inconvenience in accordance with the standards for compensation of the UMB Center for Vaccine Development and Global Health. Compensation for study product administration in the IV groups accompanied by the same day PK blood draws will be $400; product administration visits in the SC group without PK blood draws will be

$175. Compensation will be $75 for scheduled in-person visits, with or without blood draws; and $10/day for timely completion of the 7-day diary card, to a total of up to $70. Compensation for a CHMI visit including the pre-CHMI visit the prior day is $325.

The total amount of compensation varies depending upon group and the visits completed. Compensation may also vary depending upon the number of days required for the daily evaluations in the period after a CHMI as those who become parasitemic early will require fewer days of in-clinic evaluation visits than those who have delayed or no parasitemia. CHMI control subjects will be compensated similarly for comparable visit types.

4.3.2. Screening

Screening for this study will be completed through the VRC's screening protocol, VRC 500 (NIH 11-I-0164). Subjects will be recruited through Institutional Review Board (IRB)-approved advertising. Screening evaluations are performed to confirm eligibility and will include medical history review, physical exam, and the clinical laboratory tests detailed in APPENDIX I. Women presumed to be of reproductive potential will be given a pregnancy test. A baseline electrocardiogram (EKG) will be performed. Additional assessments of health may be conducted at screening based on clinical judgment. Pre-exposure research blood samples may be collected anytime during screening through enrollment and will not be subject to the "56-day prior to enrollment" restriction.

Informed consent documents will be reviewed during screening. Counseling related to potential risks of the study product, pregnancy prevention, and avoiding exposure to malaria will be performed. An Assessment of Understanding (AoU) will be completed in association with enrollment into VRC 612. Records will be kept documenting the reason that screened subjects do not enroll.

4.3.3. Enrollment and Study Day 0

In this study, enrollment is defined as the assignment of a study identification number and a study group in the clinical database. A clinician will discuss the target dates and timing of the study product administration, CHMI, and sample collections before completing an enrollment to help ensure that the subject can comply with the projected schedule. Informed consent must be obtained prior to enrollment. For CIS43LS recipients, enrollment will occur at Visit A1R or B1R (Day -28 to Day 0) and may occur on the same day as product administration at Visit A2 or B2 (Day 0) in advance of product administration. For CHMI control subjects, Visit A1R or B1R (Day -56 to Day -1) is the enrollment day, which will be within 56 days prior to the CHMI.

For CIS43LS recipients, Day 0 is defined as the day of product administration. If Day 0 does not coincide with enrollment, then the enrollment day may be referred to by a negative number of days (i.e., Day -1). For calculating elapsed days following Day 0, each subsequent calendar date is labeled by the next sequential "Study Day" as shown in APPENDIX I. Since there may be more than one research sampling timepoint of interest per study day, each sample collection timepoint has its own "Visit Number." For this reason, there may be more than one visit number recorded on the same calendar date.

The study started in Part A with subject enrollment directly into Group 1. Additional dose groups (Groups 2, 3, 4A, and 4B) opened per the dose escalation rules as outlined in Section 4.5, and subjects were directly enrolled into the subsequent open groups. Subjects in Group 5 were

able to choose to participate in the control group (and will be offered to enroll into Group 10 as Part B CHMI controls) and undergo CHMI without receiving product administration. Part B subjects will also be directly enrolled. Because this is an open-label design, subjects will know what group they are in on the day of enrollment.

Medical history and Day 0 evaluations (prior to product administration, as applicable per study group) are the baseline for subsequent safety assessments except that the screening evaluations will be the baseline for those only done at screening.

In Part C, the groups will be enrolled directly starting with the lowest dose group (Group 11) and then enrollments will continue into the additional dose groups. Group 16 may be filled at any time in order to ensure an adequate number of control participants for CHMI.

4.3.4. Product Administration

All product administrations will be completed according to the assigned group.

On the day of, and prior to product administration, vital signs (temperature, blood pressure, heart rate and respiratory rate) will be recorded, a targeted physical examination (based on signs, reported symptoms or interim medical history) may be conducted as needed, and women of childbearing potential must have a negative pregnancy test.

If a subject is assigned to an IV administration group, the IV access will be placed in an arm vein in an aseptic manner. A different site may be used for collection of PK blood samples; however, the same site may be used after flushing the line if another site is not available. CIS43LS will be administered with approximately 100 mL of normal saline IV over about 15-30 minutes, with a target of 30 minutes for the initial infusion for each subject. Infusions lasting longer than 30 minutes are allowed. If the subject experiences side effects during the infusion, the rate of infusion may be slowed or stopped to alleviate the symptoms.

If a subject is assigned to a SC administration group, the SC administration site(s) to be used must be assessed as acceptable by the clinician and the subject. The preferred SC administration site is the abdomen, but the upper arm or thigh may be used. Given the weight criterion in this study group, the maximum volume needed to administer a 10 mg/kg SC dose is not expected to exceed 10 mL. The SC dose will be administered by standard needle in a maximum volume of about 2.5 mL per injection site. Up to 6 SC injection sites may be used if deemed necessary by the clinician. SC administration sites should be at least 2 inches apart.

Procedures for CIS43LS preparation and administration are described in Section 7.

4.3.5. Post-Product Administration Follow-up

In all study groups in Part A, the first subject in each dose group to receive a unique dose level (5, 20, or 40 mg/kg) or route will be observed for at least 4 hours following completion of initial product administration. All other subjects (including those in Parts B and C) will be observed for at least 2 hours following completion of product administration. Collection of PK samples will be conducted according to the Schedule of Evaluations for the subject's study group.

Prior to discharge from the clinic, subjects will be assessed for local and systemic reactogenicity and vital signs will be recorded. Any subject who is assessed as being unwell or has ongoing

reactogenicity symptoms will be asked to remain in the clinic until evaluation and discharge by a study clinician. This includes the possibility of an overnight inpatient stay to evaluate for safety.

4.3.6. Solicited Adverse Events (Reactogenicity)

Each subject will be given a 7-day diary (paper and electronic-based available), a thermometer, and a measuring device. Subjects will use the diary to record their highest temperature, local and systemic symptoms, and concomitant medications daily for 7 days after each product administration. Subjects will be provided training on diary completion and proper usage of the thermometer to measure temperature and the measuring device to measure injection site symptoms. Completion of diary training will be noted in the source documents. While the electronic diary is preferred, subjects will have the option to use a paper diary. The paper diary, if used, will be transcribed into the study database and stored in the subject file for monitoring purposes. When neither paper nor electronic diary is available from the subject, the study clinician will document the source of reactogenicity information recorded in the study database.

The signs and symptoms solicited by the diary will include systemic events of temperature, feeling unusually tired or unwell, muscles aches, headache, chills, nausea, and joint pain, and local events at the product administration site of pain/tenderness, swelling, redness, bruising, and pruritus. Subject diaries will be reviewed by a clinician for accuracy and completeness at follow-up visits. Clinicians will follow any solicited symptoms that are ongoing after 7 days until they have resolved.

Diary data will be available in real-time to clinicians for subjects who use the electronic diary. Subjects using a paper diary will be encouraged to contact the clinic as soon as possible for any moderate or severe side effects that they experience in the 7 days post product administration. A study clinician may contact the subject by phone if any moderate or severe side effect is reported. Events that may require a clinic visit include rash, urticaria, fever of 38.6˚C (Grade 2) or higher lasting greater than 24 hours or significant impairment in the activities of daily living (such as those consistent with Grade 2 or higher impairment). Additionally, other clinical concerns may prompt a study visit based on the judgment of a study clinician.

4.3.7. CHMI Procedures

The interval between administration of study product and CHMI will vary depending on when a subject is enrolled. The CHMI will only occur following an evaluation of Day 7 safety data post product administration and may be administered at any time ≥ 8 days through 12 weeks post product administration. Some flexibility around the interval between the time of CIS43LS administration and CHMI is permitted given the need to work with each subject's personal schedule, availability of the CHMI facility and post-CHMI follow-up.

Prior to scheduling the CHMI, at least two emergency contact numbers will be confirmed and verified as authentic for each subject who will participate in the challenge. The clinical study team will review each subject's adherence to the schedule and safety follow-up to date. This review will attempt to identify any likelihood of unreliability on the part of the subject during the CHMI phase of the study. Any subject expressing inability to comply with study requirements may be deemed unsuitable for CHMI and will be excluded from the CHMI phase of the study. Any subject who receives study product, however, will be encouraged to remain in the study for safety follow-up visits.

Women of childbearing potential must have a negative pregnancy test within 2 days prior to CHMI. Use of antibiotics with antimalarial activity by CHMI participants should be avoided at least 4 weeks prior to and during the CHMI unless medically warranted.

CHMI will be conducted by experienced insectary and clinical staff at WRAIR and UMB. Mosquitoes infected via membrane feeds in preparation for the CHMI contain sporozoites in their salivary glands and up to 5 at a time will be allowed to feed on each subject under controlled conditions. Following exposure, the mosquitoes will be immediately dissected to confirm the presence of a blood meal and to determine the infectivity rate and the salivary gland score. Additional mosquitoes will be allowed to feed until 5 bites showing presence of a blood meal and a minimum 2+ salivary gland score has been achieved for each subject.

On the day of CHMI, subjects will be provided with counseling and written recommendations to reduce risk of natural mosquito exposure beginning 5 days after CHMI and continuing until completion of treatment.

4.3.8. Post-CHMI Follow-Up

The study team will be in contact with subjects at least 2 times in the first 7 days after the CHMI to provide close monitoring of clinical status. Blood samples to evaluate for malaria parasitemia will be collected daily and run on PCR from days 7 through 18 post-CHMI. Regular parasitemia evaluations will stop after treatment is initiated for positive cases of parasitemia. In the absence of malaria infection, all subjects who have not already started treatment will be provided with directly observed definitive antimalarial treatment at Day 21.

The COVID-19 pandemic demonstrated that large scale disruption of clinical trial activities is possible. While such large-scale disruptions are not expected at the time of Part B or Part C CHMI, the protocol is revising the post-CHMI follow-up duration from 28 days to 21 days as a risk mitigation procedure. A shorter follow-up time limits travel and public exposure to both subjects and trial staff in the event social distancing is required, while concomitantly ensuring the scientific integrity of efficacy data from both control subjects and CIS43LS recipients.

During the post-CHMI follow-up period, subjects may participate in their normal daily activities. A study physician will be available 24 hours a day by telephone or in person to the site staff in case needed for consultation about subject signs and symptoms. Any subject who is assessed as being unwell during the post-CHMI follow-up will be asked to remain in the clinic until evaluation and discharge by a study clinician. This includes the possibility of an overnight inpatient stay to evaluate for safety.

A troponin level will be obtained at baseline during screening for all CHMI participants. If a subject in post-CHMI follow-up complains of chest pain or has other signs and/or symptoms consistent with a cardiovascular event, another troponin level will be obtained and other cardiovascular evaluations will be performed, as medically indicated. Consultation with the NIH CC Cardiology Service (for Parts A and B) and UMB Cardiology Service (for Part C) will be obtained when indicated by the signs and symptoms.

Regardless of CHMI outcome and following parasitemia evaluations, subjects who received CIS43LS will continue to follow the Schedule of Evaluations, as outlined in APPENDIX I.

4.3.9. Parasitemia Management

Following CHMI, a case of malaria parasitemia will be defined as either a single positive PCR result or a thick blood smear that meets the criteria for positivity. This allows for cases of malaria to be identified before gametocytes can develop and thus before detection of gametocytes by blood smear are detectable. Malaria infection will be treated when the criterion for a case is met.

Regardless of PCR or blood smear results, all CHMI participants will receive directly observed antimalarial treatment at Day 21 post CHMI, if they have not already been treated for parasitemia by this timepoint. Subjects may also be provided with treatments like antiemetics and ibuprofen as needed for management of symptoms.

Refer to the Schedule of Evaluations, APPENDIX I**,** for the safety laboratory evaluations that are required to be performed at the onset of treatment and 2 days later.

Malaria infections in all subjects will be treated promptly and administered as directly observed therapy (DOT) by a clinician until the specified course of treatment. Treatment regimens subsequently described are known to be effective against the Pf strain being used in the CHMI.

- First line of treatment: a standard atovaquone/proguanil (Malarone®) regimen:

  Four Malarone Tablets (adult strength tablet = 250 mg atovaquone/100 mg proguanil; total daily dose 1 g atovaquone/400 mg proguanil hydrochloride) taken as a single dose daily for 3 consecutive days with food or a milky drink.

- Alternative treatment: a standard chloroquine regimen:

  Total of 1500 mg chloroquine base (2500 mg salt) given orally in divided doses: 600 mg base (1000 mg salt) initially, followed by 300 mg base (500 mg salt) given 6, 24, and 48 hours later]

Alternative medications and regimens known to be effective in curing the Pf strain administered for the CHMI may be used in the event of allergies, intolerances or lack of availability of treatments listed above.

Following treatment for a positive case of malaria, cure will be documented by a PCR negative result at 26 days (± 5 days) after the completion of treatment.

4.3.10. Follow-Up through End of Study

Study follow-up will continue via clinical visits through 24 weeks after the product administration or 8 weeks post final CHMI, whichever is most stringent. The visit schedule is based on intervals of time after product administration or CHMI. The schedule of visits, allowable windows for completing the visits, and evaluations performed at each visit are shown in the Schedule of Evaluations, APPENDIX I. Out of window study visits will be discouraged and recorded as protocol deviations but may be permitted at the discretion of the PI in the interest of obtaining safety and PK evaluations following exposure to the investigational study product or conduct of a CHMI.

Any subject who receives investigational product will be required to follow the product administration schedule for a complete safety and research evaluation through study duration. Subjects who undergo CHMI will be required to follow the CHMI Schedule of Evaluations to

completion. When visits from both schedules occur on the same day, all required components of the required visits for each schedule must be completed.

Subjects who receive study product but do not receive CHMI as scheduled are expected to continue follow-up according to the schedule for IV or SC group through 24 weeks, except that research sample collections may be discontinued for pregnant women or others in which it is contraindicated.

Refer to Section 4.6 for criteria for discontinuing product administration and/or study participation.

4.4. Concomitant Medications

Only routine prescription medications will be entered in the database at the time of enrollment. Subsequently, concomitant medications are only updated or recorded in the study database if there is an occurrence of an adverse event (AE) that requires expedited reporting or if the subject develops a new chronic condition that requires ongoing medical management. Treatment with antimalarial drugs will be recorded on a Malaria Endpoint Case Report Form (CRF). Otherwise, the concomitant medication changes throughout the study will be recorded in the subject's chart as needed for general medical records but will not be recorded in the study database.

4.5. Dose Escalation Plan

There are two dose escalation reviews in this study. The activation of additional dose groups will proceed in a staged manner that is governed by the outcome of these planned interim PSRT data reviews. The PSRT must assess the data as showing no significant safety concerns before proceeding with group activation.

Part A of the study will begin with direct enrollment of subjects into Group 1. Following the first product administration in Group 1, the study team will wait at least 3 days before administering CIS43LS to additional subjects within that group.

The first dose escalation review (from 5 mg/kg IV to 5 mg/kg SC and 20 mg/kg IV) will occur when 3 subjects who received the 5 mg/kg dose by IV have completed the Day 7 safety follow-up visit. The PSRT review will determine whether enrollment into Groups 2 and 3 may begin.

The second dose escalation review (from 20 mg/kg IV to 40 mg/kg IV) will occur when 3 subjects who received the 20 mg/kg IV dose have completed the Day 7 safety follow-up visit. The PSRT review will determine whether enrollment into Groups 4A and 4B may begin.

If there are discontinuations from the study before there are sufficient data to conduct the dose escalation review for a specific group, then additional subjects may be enrolled to complete the dose evaluation. Additionally, AEs assessed as related to study product at the time of a dose escalation review may warrant enrollment of additional subjects into a dose group to reassess safety before proceeding to a higher dose.

Consultation with the IRB or FDA, if needed, as per study pause criteria (Section 4.5) will occur if indicated. One outcome of a dose escalation review may be to recommend evaluation of additional subjects at the current dose level and reassess for safety before proceeding to a higher dose level.

4.6. Criteria for Discontinuation of Product Administration or Protocol Participation

Decisions by the PI or designee to discontinue a subject from receiving additional product administrations or from protocol participation for a subject will be made with the following criteria.

4.6.1. Discontinuation of Study Product Administration

Part A subjects will not be permitted to receive additional study product in Part B if any of the following apply:

1. Pregnancy;
2. Malaria infection;
3. Grade 3 AE assessed as related to study product (except Grade 3 solicited reactogenicity lasting less than 48 hours);
4. Grade 4 AE assessed as related to study product;
5. An SAE of any grade assessed as related to study product;
6. Immediate hypersensitivity reaction associated with study product;
7. Intercurrent illness that is not expected to resolve prior to the next scheduled product administration (if applicable);
8. Diagnosis of a new chronic or clinically significant medical condition that in the opinion of the investigator would jeopardize the safety or rights of the subject;
9. Repeated failure to comply with protocol requirements;
10. The IND Sponsor or the study PI decide to terminate the study; or
11. The IRB, Office for Human Research Protections (OHRP) or the FDA halt the study.

4.6.2. Discontinuation from Protocol Participation

A subject will be discontinued from protocol participation for the following reasons:

1. Subject voluntarily withdraws;
2. Subject develops a medical condition that is a contraindication to continuing study participation;
3. The IND Sponsor or regulatory authority stops the protocol; or
4. The IND Sponsor or PI assesses that is it not in the best interest of the subject to continue participation in the study or that the subject's compliance with the study is not sufficient.

For subjects who wish to discontinue protocol participation, the following rules apply with the exception that research sample collections will be discontinued for pregnant women or others in which it is contraindicated:

- Subjects who have received at least one dose of CIS43LS but do not participate in the CHMI are expected to continue with safety follow-up through at least Visit A9 or B9.

- Subjects who have completed the CHMI are expected to continue with safety follow-up through Visit C20 or C40 as applicable.

4.7. Criteria for Pausing and Resuming the Study

The study team will closely monitor and review study data as they become available to make determinations regarding the presence, severity and attribution of AEs. Study product administrations and new enrollments will be paused if any of the following criteria are met:

- **One** (or more) subject experiences a **SAE** that is assessed as related (possibly, probably or definitely) to the study product, or
- **Two** (or more) subjects experience the same **Grade 3 or higher AE** that is assessed as related (possibly, probably or definitely) to the study product (other than self-limited Grade 3 solicited reactogenicity).

4.7.1. Plan for Review of Pauses and Resuming Rules

In the event of a pause, the IND Sponsor Medical Officer (MO) and the PSRT will be promptly notified.

The IND Sponsor MO and PI, in consultation with the PSRT, will conduct a review of available information, including the events that lead to the pause, and will make the decision to resume, amend or close the study. As part of the pause review, the reviewers may also advise on whether the study needs to be paused again for any subsequent events of the same type.

Study product administrations and new enrollments would resume only if review of the AEs that caused the pause results in a recommendation to permit further study product administrations and study enrollments. Safety data reports and changes in study status will be submitted to relevant regulatory authorities in accordance with Section 5 and institutional policy.

5. SAFETY AND ADVERSE EVENTS

5.1. Adverse Events

An adverse event (AE) is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research. In the context of FDA-required reporting, an AE means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

- Solicited AEs (i.e., reactogenicity parameters as defined in Section 4.3.6) will be recorded without attribution assessments by the subject on paper or an electronic diary for 7 days after each product administration.
- Unsolicited AEs will be recorded in the study database with attribution assessments during the following periods:

  1) from product administration through the Day 28 post-product administration visit; and

  2) from CHMI through the Day 28 post-CHMI visit.

After and between the indicated time periods, only SAEs (as detailed in Section 5.3) and new chronic medical conditions will be recorded as AEs through the last expected study visit or contact.

Malaria and the associated signs and symptoms of parasitemia events occurring at any time during the study will be recorded on a Malaria Endpoint CRF and will not be recorded as an AE.

APPENDIX II describes how attribution assessments, the relationship between an AE and the study product, CHMI or both, will be determined. Also available in APPENDIX II is the link to the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1 [July 2017], which will be used to determine the severity grades of AEs in this protocol with several modifications as noted.

5.2. Serious Adverse Events

The term "Serious Adverse Event" (SAE) is defined in 21 CFR 312.32 as follows: "An adverse event or suspected adverse reaction is considered serious if, in the view of either the investigator or the sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse."

"Life threatening" refers to an AE or suspected adverse reaction that represents an immediate risk of death to the subject. It does not include an AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. Similarly, a hospital admission for an elective procedure is not considered a SAE.

5.3. Adverse Event Reporting to the IND Sponsor

AEs that meet SAE criteria must be reported and submitted by the clinical site on an expedited basis to the IND Sponsor, VRC/NIAID/NIH, according to sponsor guidelines as follows:

- Results in death;
- Is life threatening (places the subject at immediate risk of death from the event as it occurred);
- Results in inpatient hospitalization or prolongation of existing hospitalization;
- Results in a persistent or significant disability/incapacity;
- Results in a congenital anomaly/birth defect in the offspring of a study subject; OR
- Based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

In addition, any event, regardless of severity, which in the judgment of an investigator represents a SAE, may be reported on an expedited basis.

An investigator will communicate an initial SAE report within 24 hours of site awareness of occurrence to the IND Sponsor by data entry into the database, which triggers an alert to the IND Sponsor MO. Within 3 working days, a written summary by the investigator should be submitted to the IND Sponsor.

In order for the IND Sponsor to comply with regulations mandating sponsor notification of specified SAEs to the FDA within 7 and/or 15 calendar days, the investigator must submit additional information as soon as it is available.

5.4. IND Sponsor Reporting to the FDA

The IND Sponsor is responsible for making the determination of which SAEs are "serious and unexpected suspected adverse reactions" (SUSARs) as defined in 21 CFR 312.32.

- *Suspected Adverse Reaction* means any AE for which there is a reasonable possibility that the drug caused the AE.
- *Unexpected Adverse Event* means an AE that is not listed at the specificity or severity that has been observed.

All SUSARs (as determined by the IND Sponsor) will be reported to the FDA as IND Safety Reports per 21 CFR 312.32 as soon as possible but not exceeding 7 calendar days for unexpected

fatal or life-threatening events, and not exceeding 15 calendar days for other qualifying events. IND Safety Reports will also be provided to the IRB.

The IND Sponsor will also submit an IND Annual Report of the progress of the investigation to the FDA as defined in 21 CFR 312.33.

5.5. Reporting to the Institutional Review Board

The following information is consistent with NIH IRB Policy 801: Reporting Research Events.

Reportable Event - An event that occurs during the course of human subject research that requires notification to the IRB.

For the purposes of this policy, reportable events include the following:

- Unanticipated Problems (UPs) involving risks to subjects or others
- Non-compliance (including major protocol deviations and noncompliance that is not related to a protocol deviation)
- Deaths related or possibly related to research activities
- New information that might affect the willingness of subjects to enroll or continue participation in the study

5.5.1. Unanticipated Problem

An Unanticipated Problem (UP) is defined as any incident, experience, or outcome that meets **all** the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied; **and**
- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); **and**
- Suggests that the research places subjects, or others (which may include research staff, family members or other individuals not directly participating in the research) at a greater risk of harm (including physical, psychological, economic, or social harm) related to the research than was previously known or expected.

A UP must be reported within 7 calendar days of an investigator becoming aware of the actual or suspected UP.

5.5.2. Non-Compliance

Non-compliance is the failure of investigator(s) to follow the applicable laws, regulations, or institutional policies governing the protection of human subjects in research, or the requirements or determinations of the IRB, whether intentional or not.

Non-compliance may be unintentional (e.g. due to lack of understanding, knowledge, or commitment), or intentional (e.g. due to deliberate choice to ignore or compromise the requirements of any applicable regulation, organizational policy, or determination of the IRB).

Non-compliance is further characterized as serious or continuing as follows:

- Serious non-compliance – Non-compliance, whether intentional or not, that results in harm or otherwise materially compromises the rights, welfare and/or safety of the subject. Non-compliance that materially effects the scientific integrity or validity of the research may be considered serious non-compliance, even if it does not result in direct harm to research subjects.
- Continuing non-compliance – A pattern of recurring non-compliance that either has resulted, or, if continued, may result in harm to subjects or otherwise materially compromise the rights, welfare and/or safety of subjects, affect the scientific integrity of the study or validity of the results. The pattern may comprise repetition of the same non-compliant action(s), or different noncompliant events.

Any actual or suspected non-compliance by any investigator or entity associated with the protocol must be reported to the IRB by the PI/designee within 7 calendar days of any investigator or individual associated with the protocol first becoming aware.

5.5.3. Protocol Deviation

A Protocol Deviation (PD) is defined as any change, divergence, or departure from the IRB-approved research protocol and are further characterized as major and minor as follows:

- Major Deviations – Deviations from the IRB approved protocol that have, or may have the potential to, negatively impact, the rights, welfare or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.
- Minor Deviations – Deviations that do not have the potential to negatively impact the rights, safety, or welfare of subjects or others, or the scientific integrity or validity of the study.

For the reporting purposes, failure of subjects to comply with the research protocol does not represent non-compliance unless that failure is due to an action or omission of a member of the research team, for example, the failure to give adequate instruction to the subject.

A major deviation must be reported within 7 calendar days of an investigator becoming aware of an actual or suspected deviation. Although PDs are also non-compliance, these should only be reported once as deviations. Major deviations resulting in death must be reported within 24 hours of the occurrence of the event or of any member of the study team becoming aware of the death.

Researchers are responsible for monitoring their studies throughout the year for adherence to the IRB approved protocol. The purpose of this monitoring is to identify major deviations and to look for trends in minor deviations that may indicate a systemic issue in how the study is being conducted that could potentially negatively impact the rights, safety, or welfare of participants or the study's ability to produce scientifically valid results. A series of minor deviations pointing toward a more global issue that could affect the rights, safety or welfare of the participant or

affect the validity of the study should be reported as a major deviation. In all other instances, a summary of minor deviations should be provided to the IRB at the time of continuing review.

5.5.4. Death

Any death of a research subject that is possibly, probably or definitely related to the research must be reported within 24 hours of an investigator becoming aware of the death.

5.5.5. New Information

New information that might affect the willingness of a subject to enroll or remain in the study should be reported within 7 calendar days of an investigator first becoming aware.

5.5.6. Suspension or Termination of Research Activities

Any suspension or termination of research activities, including holds on new enrollment, placed upon the research by the study sponsor, NIH or IC leadership, or any regulatory agency must be reported within 7 calendar days of an investigator becoming aware.

5.5.7. Expedited Reporting to the IRB

Death related to research must be reported within **24 hours**.

The following will be reported within **7 calendar days** of investigator awareness:

- Actual or suspected UPs;
- Actual or suspected non-compliance;
- Actual or suspected Major PDs;
- SAEs that are actual or suspected UPs;
- New information that might affect the willingness of a subject to enroll or remain in the study;
- Suspension or termination of research activities, including holds on new enrollment, placed upon the research by the study sponsor, NIH or IC leadership, or any regulatory agency.

5.5.8. Annual Reporting to the IRB

The following will be reported to the IRB in summary at the time of Continuing Review:

- Summary of UPs and non-compliance;
- AEs, including SAEs, that are not UPs, as a narrative summary statement indicating whether these events were within the expected range;
- Minor PDs (aggregate summary);
- Any trends or events which in the opinion of the investigator should be reported.

6. STATISTICAL CONSIDERATIONS

6.1. Overview

This study is a Phase 1, dose-escalation study in healthy adults to assess the safety, PK and protective efficacy of CIS43LS, an investigational human antimalarial mAb.

6.2. Sample Size and Accrual

Trial recruitment will target about 92 healthy adults, ages 18 to 50 years, as shown in Table 1. The permitted accrual is 100 subjects in total to allow for additional enrollments in the event that an enrolled subject does not complete the minimum evaluations needed to meet the protocol criteria for a dose-escalation evaluation. Dose escalation rules are described in Section 4.5. If a subject enrolls into a study product group but does not receive a product administration or withdraws before the challenge for reasons that are not safety related, or a control group subject withdraws before the malaria challenge occurs, then additional subjects may be enrolled to achieve the accrual target. Following a withdrawal from a study product group, additional enrollments may be made if there is sufficient time to complete product administration prior to the scheduled CHMI.

The primary goal of this study is to identify safety concerns associated with CIS43LS at different doses. Primary sample size considerations are expressed in terms of the ability to detect serious adverse experiences. The ability of the study to identify SAEs will be expressed in terms of the probability of observing a certain number of serious adverse events. With sample size n=6, there is over 90% chance to observe at least 1 SAE if the true rate is at least 0.319 and over 90% chance to observe no SAE if the true rate is no more than 0.017. With sample size n=4, there is over 90% chance to observe at least 1 SAE if the true rate is no less than 0.438 and over 90% chance of observing no SAE if the true rate is no more than 0.025. Probabilities of observing 0 or more than 1 SAE within a group are presented in Table 2 for a range of possible true event rates.

Table 2: Probability of Events for Different Safety Scenarios within a Group (n=6 or 4)

True Event Rate	n=6		n=4	
	Pr(0)	Pr(>1)	Pr(0)	Pr(>1)
0.005	0.970	0.000	0.980	0.000
0.01	0.941	0.001	0.961	0.001
0.02	0.886	0.006	0.922	0.002
0.035	0.808	0.017	0.867	0.007
0.05	0.735	0.033	0.815	0.014
0.1	0.531	0.114	0.656	0.052
0.15	0.377	0.224	0.522	0.110
0.2	0.262	0.345	0.410	0.181
0.3	0.118	0.580	0.240	0.348

Table 3 gives the upper and lower bounds for 95% exact binomial confidence intervals of the true SAE rate at possible numbers of events within a group. Within a group of size n=6, if none experience an SAE, the 95% exact confidence interval has upper bound 0.459. Within a group of size n=4, if none experience an SAE, the exact 95% confidence interval has an upper bound 0.602.

Table 3: 95% Confidence Intervals for the True Rate at Possible Observed Number of Events in a Group (n=6 or n=4) or across Groups (n=24)

Observed Number of Events	95% Confidence Interval (n=6)		95% Confidence Interval (n=4)		95% Confidence Interval (n=24)	
	Lower Bound	Upper Bound	Lower Bound	Upper Bound	Lower Bound	Upper Bound
0	0.000	0.459	0.000	0.000	0.000	0.142
1	0.004	0.641	0.001	0.001	0.001	0.211
2	0.043	0.777	0.010	0.010	0.010	0.270
3	0.118	0.882	0.027	0.027	0.027	0.324
4	0.223	0.957	0.047	0.047	0.047	0.374
5	0.359	0.996	0.071	0.071	0.071	0.422
6	0.541	1.000	0.098	0.098	0.098	0.467

6.3. Statistical Analysis

Enrollment of CIS43LS recipients may occur on the same day as product administration at Visit 02 (Day 0) or in advance of product administration at Visit A1R or B1R (Day -28 to Day 0), except in the case of controls. All subjects who receive product administration will provide safety data.

Three analysis cohorts are involved in statistical analysis. The Intent-to-treat (ITT) cohort will include all enrolled subjects who receive product administration and will be analyzed according to the assigned group. The Per-Protocol (PP) cohort will include all enrolled subjects who receive product administration and will be analyzed according to the actual dose they receive. The Modified intent-to-treat (mITT) cohort will include all enrolled subjects who receive product administration followed by CHMI challenge and will be analyzed according to the assigned group.

All statistical analyses will be performed using SAS and R statistical software.

6.3.1. Analysis Variables

The analysis variables consist of baseline, safety parameters, PK, and presence or absence of malaria infection after CHMI. Descriptive statistics will be used to summarize baseline characteristics, inclusive of demographics and safety laboratory measurements.

6.3.2. Safety Analysis

Safety evaluation will be performed over the ITT cohort. The number and percentage of subjects with one or more AEs will be summarized by dose group along with the exact 95% confidence intervals of the AE rate. For subjects experiencing more than one AE, they will be counted once under the event of highest severity. In addition, a complete listing of AEs for each subject will provide details such as severity, duration, and relationship to study product. Summaries will be provided for any solicited or unsolicited AEs.

a. Solicited Reactogenicity

Solicited AE data will be collected after product administration. The number and percentage of subjects experiencing each type of solicited sign or symptom will be tabulated by severity and by dose group and overall (i.e., pooled IV dose groups and pooled SC and IV dose groups). Subjects with multiple occurrences of the same event will be counted once using the event of highest severity.

b. Adverse Events

All reportable AEs will be recorded and coded by Medical Dictionary for Regulatory Activities (MedDRA) system organ class (SOC) and preferred term (PT). The number and percentages of subjects with each unsolicited AE will be tabulated by severity and relationship to the study product, and by dose group and overall (i.e., pooled IV dose groups and pooled SC and IV dose groups). Subjects with multiple occurrences of the same event will be counted once using the event highest severity or strongest relationship to the study treatment.

A by-subject listing of all unsolicited AEs will provide details including severity, relationship to treatment type, seriousness, new medical condition status, onset and end date, duration, and outcome.

c. Local Laboratory Values

Boxplots of local laboratory values will be generated for baseline values and for values measured during the course of the study. Each boxplot will show the 1st quartile, the median, and the 3rd quartile. Outliers, or values outside the boxplot, will also be plotted. If appropriate, horizontal lines representing boundaries for abnormal values will be plotted.

6.3.3. Pharmacokinetics Analysis

PK analysis will be performed over the PP cohort. Blood samples for PK evaluations will be collected at defined time-points as listed in APPENDIX II.

Individual Subject Pharmacokinetic Analysis: A non-compartmental (NC) PK analysis will be performed using Phoenix 7.0 (Certara^{R}), PKPlus or a similar program on the CIS43LS concentration data generated from each subject. Individual subject and dosing group concentration vs time profiles will be constructed in linear and semi-log scales. In the NC analysis the maximum concentration (Cmax) and time of maximal concentration (Tmax) will be taken directly from the observed data. The area under the concentrations vs. time curve (AUC) will be calculated using the trapezoidal method and determined out to the final concentration collected. If a subjects CIS43LS concentration falls below the quantitative limit (QL) of the assay before the final of PK sample collection, the sample with concentration below the QL will be assigned a CIS43LS concentration value of "0" for AUC calculations. Later PK samples after the initial concentration below the QL sample will be ignored in AUC calculations because partial AUC is not contributory to the total AUC. In addition to calculation of the total AUC from Time=0 to the last CIS43LS concentration ($AUC_{0\text{-last}}$), partial AUCs from Time=0 to Week 16 ($AUC_{0\text{-16WK}}$), Time= 0 to the CHMI challenge ($AUC_{0\text{-CHMI}}$) and Time of CHMI to Week 16 ($AUC_{CHMI\text{-16WK}}$) will be determined. The time weighted average concentrations (Cave) during these intervals will be calculated as the AUC divided by the AUC collection interval, e.g. $Cave_{0\text{-16WK}} = (AUC_{0\text{-16WK}}) / 16$ weeks. The terminal slope, λ_z will be determined by regression of the terminal, log-linear portion of the concentration vs. time profile. If the final PK sample has

measurable CIS43LS concentrations greater than the assay QL, the AUC post final PK collection ($AUC_{last\text{-}infinity}$) will be estimated as C_{last}/λ_z and $AUC_{0\text{-}infinity}$ will be calculated as sum $AUC_{0\text{-}last}$ + $AUC_{last\text{-}infinity}$.

Population Pharmacokinetic Analyses: Population PK analyses will be performed on the PK data following IV and SC administration to determine compartmental PK parameters with the PK program NONMEM 7.3 or later (ICON^R). Based on preclinical PK results for CIS43LS and known PK behavior studies of mAbs, two-compartment model will be used (subroutine ADVAN4 TRANS4). Both zero order and first order absorption following SC administration will be evaluated and a lag time included if a delay is seen in the raw data figures. The First Order Conditional Estimation Method with Interaction (FOCEI) will be used. The population analysis will generate estimates for clearance (CL) central and peripheral volumes of distribution (Vd_1 and Vd_2), inter-compartmental clearance (Q), CL and SC bioavailability (F). Total volume of distribution at steady-state (Vdss), will be calculated as the sum of Vd_1 + Vd_2. Alpha and beta half-lives will be calculated from CL, Q, Vd_1 and Vd_2 using standard equations (M. Gilbaldi and D Perrier, Pharmacokinetics Marcel Dekker,1975). While the number of subjects is expected to be sufficient to characterize the typical PK parameters and their between-subject variabilities (BSVs), the sample number is too small for a robust broad population PK covariate analysis. Therefore, the impact of subjects' size will be accounted for using allometric scaling normalized to 70kg with dose level and CHMI response being the only covariates explored. Individual subjects' empiric Bayesian PK parameter estimates will be generated using the posthoc subroutine.

Final model selection will be based on changes in the objective function, a goodness of fit statistic generated by NONMEM, and graphically by goodness of fit plots. The final population PK model will be assessed using bootstrap analysis. Dosing strategies and their ability to achieve and maintain target CIS43LS concentrations will be performed using the final population PK model and Monte Carlo simulations with at least 5000 replicates.

6.3.4. Efficacy Analysis

Efficacy analysis will be performed over the mITT cohort. The primary efficacy analysis will be based on a two-sided Barnard test on the proportion of infection among those who receive CIS43LS versus the controls who undergo CHMI concurrently. The secondary efficacy analyses will be based on time to parasitemia, where CIS43LS recipients will be compared with the controls via a log-rank test. Kaplan-Meier curves will also be provided for each group. Efficacy analyses will be performed for Part B and Part C separately. An efficacy analysis over all participants in Part B and Part C combined will also be performed.

Due to variable timing of challenge among study participants, a logistic regression will be performed to estimate the infection risk as a function of the mAb concentration at the time of challenge. For the same reason, a Cox proportional hazards regression will be performed to estimate the hazard function with the mAb concentration at the time of challenge as a regressor. Both regressions will be carried out over the subjects in Part B and Part C combined, as well as in each Part separately, provided that there are cases of infection among CIS43LS recipients.

6.3.5. Interim analyses

Safety and PK data will be analyzed at the completion of each dose group in Parts A and B. Preliminary PK analyses may be done once per dose level as the data for each dose level is obtained. This may be performed before a dose group's PK data is complete and may only generate a subset of the final PK parameters. The interim analyses will be used to inform decisions about dose levels, timing of CHMI, and subject population for Parts B and C.

7. PHARMACY PROCEDURES

The study groups are shown in Table 1.

7.1. Study Product

VRC-MALMAB0100-00-AB (CIS43LS) is a sterile buffered solution that is filled into single-dose vials. Each vial contains 5.5 ± 0.10 mL of CIS43LS at a concentration of 100 ± 10 mg/mL in formulation buffer composed of 10 mM Acetate-Phosphate, 25 mM Sodium Chloride, 10mM Sodium Citrate, 100 mM Arginine Hydrochloride, and 0.02% (w/v) Polysorbate-68 at pH 6.0.

7.2. Storage and Temperature Excursions

The site pharmacist must promptly report any storage temperature excursions outside of the normal allowance to the IND Sponsor. The affected product must be quarantined in a separate area under protocol-specific temperature ranges until further notice from the Sponsor. If the excursion results in thawed material, DO NOT REFREEZE; store the thawed, vialed material at 2°C to 8°C.

When a storage/shipping/handling excursion occurs, the IND Sponsor designee must send a notification of the occurrence of an excursion to [redacted] An automatic email reply will be sent to the notifier, including (as an attachment) the Clinical Excursion Reporting Form, which can be filled electronically (or manually and scanned, if needed). The completed form and relevant attachments (e.g. temperature charts) must be emailed to the VRC via the same email address [redacted] using the "reply" function. The IND Sponsor will notify the site pharmacist if continued clinical use of the product is acceptable or will provide further instructions.

7.3. Labeling of Study Product Vial

Vials of study product will be individually labeled with the name of the material, volume, lot number, concentration, storage instructions, Investigational Use Statement ("Limited by Federal Law to Investigational Use"), and manufacturer information.

7.4. Preparation of Study Product for Administration

This section describes how to prepare study injections and will be updated when information is available.

7.4.1. Preparation for IV Administration

For each IV infusion order, the subject's weight, dose level, and study group code will be included in the pharmacy order. To prepare an IV infusion, the pharmacist will: 1) calculate the total milligrams of CIS43LS needed, 2) retrieve the minimum number of thawed vials required to prepare the full dose, 3) withdraw the necessary amount of CIS43LS, and 4) add this volume to a 150 mL capacity, partial fill, 100 mL bag of normal saline using sterile compounding techniques to maintain sterility.

Thaw and equilibrate vials for a minimum of 1 hour and 30 minutes at ambient temperature (15°C to 32°C). If thawed vials are removed from 2°C to 8°C, equilibrate at ambient

temperature for a minimum of 30 minutes. Prior to preparation for administration in the IV bag, vials should be gently swirled for approximately 30 seconds while avoiding foaming. DO NOT SHAKE THE VIAL.

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

The study product solution will typically be administered IV over about 15-30 minutes using a volumetric pump. The total time needed to administer the dose may be longer than 30 minutes based on factors such as subject tolerance. The rate of infusion may range from 10-20 mg/kg/hr at the lowest dose level to 80-160 mg/kg/hr at the highest dose level. The mL/hr infusion rate may vary based on the total volume needed to administer a full dose.

At the end of product administration, the IV administration set must be flushed with about 30 mL (or appropriate volume) of normal saline.

7.4.2. Preparation for SC Administration

For each SC administration order, the subject's weight, dose level, and study group code will be included in the pharmacy order. To prepare a SC administration dose, the pharmacist will calculate the total mg needed and retrieve the minimum number of vials needed to prepare the full dose. Thaw and equilibrate vials for a minimum of 1 hour and 30 minutes at ambient temperature (15°C to 32°C). If thawed vials are removed from 2°C to 8°C, equilibrate at ambient temperature for a minimum of 30 minutes. Prior to preparation for administration, vials should be gently swirled for approximately 30 seconds while avoiding foaming. DO NOT SHAKE THE VIALS.

The needed volume of CIS43LS must be withdrawn from the vial into 1 to 6 syringes using a 5-micron filter needle. A new filter needle must be used for each syringe. The filter needle must be discarded prior to dispensing and replaced with a needle suitable for SC injection at the time of administration. The product may be administered by direct SC injection with needle and syringe. The clinician will use proper SC technique to ensure administration into SC fatty layer and a slow push to minimize discomfort or the excessive distention of overlying skin.

7.4.3. Handling of Prepared Product for IV or SC Administration

After product preparation in an IV bag, the prepared CIS43LS may be stored at 2°C to 8°C for a maximum of 24 hours and/or at ambient temperature (15°C to 32°C) for a maximum of 4 hours total including the infusion time. Product may not be stored in direct sunlight.

After preparation in syringes for SC administration, the prepared CIS43LS may be stored at 2°C to 8°C up to 24 hours and/or at ambient temperature (15°C to 32°C) up to 4 hours. Product may not be stored in direct sunlight.

7.5. Study Product Accountability

A study pharmacist or designee will be responsible for maintaining an accurate record of the study group codes, inventory, and an accountability record of study agent supplies. Electronic documentation as well as paper copies may be used.

7.6. Study Product Disposition

The empty vials and the unused portion of a vial will be discarded in a biohazard containment bag and incinerated or autoclaved in accordance with the institutional or pharmacy policy. Any unopened vials that remain at the end of the study will be returned to the production facility or discarded at the discretion of the sponsor in accordance with policies that apply to investigational agents. Partially used vials will not be administered to other subjects or used for *in vitro* experimental studies. These vials will be disposed of in accordance with institutional or pharmacy policy.

8. HUMAN SUBJECT PROTECTIONS AND ETHICAL OBLIGATIONS

This research will be conducted in compliance with the protocol, International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) guidelines and all applicable regulatory requirements.

8.1. Institutional Review Board

A Reliance Agreement will be established with the collaborating UMB site and WRAIR CHMI facility such that the NIH IRB is the IRB of Record for the conduct of the VRC 612 protocol. The protocol, proposed informed consent form, other written subject information, and any proposed advertising material will be submitted by the Coordinating Site (VRC) to the IRB for review and written approval.

The Protocol Chair must submit and, where necessary, obtain approval from the IRB for all subsequent protocol amendments and changes to the informed consent document. The Protocol Chair will notify the NIH IRB of research events that occur on study as described in Section 5.5.

The Protocol Chair will be responsible for obtaining IRB approval of the annual Continuing Review throughout the duration of the study.

8.2. Informed Consent

The study informed consent form (ICF) is provided as a separate document and describes the investigational product to be used and all aspects involved in protocol participation.

The site PI or designee is responsible for obtaining written informed consent from the subject after adequate explanation of the aims, methods, anticipated risks and benefits of the study and before any protocol-specific procedures or study product is administered. The AoU will be completed before the study ICF is signed.

The acquisition of informed consent will be documented in the subject's medical records, as required by 21 CFR 312.62, and the ICF will be signed and personally dated by the subject and the person who conducted the informed consent discussion. The signed ICF will be retained in the medical chart and a copy of the ICF will be provided to the subject.

8.3. Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, the investigators, the Investigational New Drug (IND) and regulatory authorities as appropriate. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and Sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants

- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

The study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the Sponsor, IRB and/or FDA.

8.4. Confidentiality and Privacy

Participant confidentiality and privacy are strictly held in trust by the participating investigators, their staff, and the Sponsor and their representatives. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the Sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or Sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored by [redacted] the Data Coordinating Center. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical site and by Emmes research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

8.5. Risk/Benefit Assessment

8.5.1. Potential Risks

Risk of CIS43LS: While CIS43LS has been well-tolerated to date in this study, only preliminary safety data in humans exists and risks are not fully known. See Section 2.3 for a summary of available interim data collected in VRC 612.

Risks of mAb Administration: Administration of mAbs may cause immune reactions such as acute anaphylaxis, serum sickness and the generation of antibodies. However, these reactions are rare and more often associated with mAbs targeted to human proteins or with the use of mouse mAbs that would have a risk of human anti-mouse antibodies [29]. In this regard, because CIS43LS is targeted to a parasite antigen and is a human mAb, it is expected to have a low risk of such side effects.

Typically, the side effects of mAbs are mild to moderate and may include local reactions at the injection site (including pain, redness, bruising, swelling) and systemic reactions such as fever, chills, rigors, nausea, vomiting, pain, headache, dizziness, shortness of breath, bronchospasm, hypotension, hypertension, pruritus, rash, urticaria, angioedema, diarrhea, tachycardia or chest pain. Clinical use of mAbs that are targeted to cytokines or antigens associated with human cells may be associated with an increased risk of infections [29]; however, this is not expected to be a risk for a mAb targeted to a parasite antigen.

Severe reactions, such as anaphylaxis, angioedema, bronchospasm, hypotension and hypoxia, are infrequent and more often associated with mAbs targeted to human proteins or with non-human mAb, such as a mouse mAb [29]. Most infusion-related events occur within the first 24 hours after initiation of mAb administration.

Published experience with human mAbs directed against cell surface targets on lymphocytes shows that infusion of a mAb may be associated with cytokine release, causing a reaction known as cytokine release syndrome (CRS) [30]. CRS reactions commonly occur within the first few hours of infusion start and with the first mAb infusion received. This is because the cytokine release is associated with lysis of the cells targeted by the mAb and the burden of target cells is greatest at the time of the first mAb treatment. With licensed therapeutic mAbs, CRS is managed by temporarily stopping the infusion, administering histamine blockers and restarting the infusion at a slower rate [31]. Supportive treatment may also be indicated for some signs and symptoms.

Delayed allergic reactions that include a serum sickness type of reaction characterized by urticaria, fever, lymph node enlargement, and joint pains, typically occur several days after mAb exposure and are more commonly associated with chimeric types of mAbs [29]. In general, and with due consideration of the needs dictated by individual subject symptoms and treating clinician discretion, immediate and delayed reactions to study product would be managed according to the principles of the American Academy of Allergy, Asthma, and Immunology guidelines established in the Drug Allergy: Practice Parameters (2010).

Participation in this study may limit a subject's eligibility for future mAb studies.

Risks of Blood Drawing: Blood drawing may cause pain, bruising, and a feeling of lightheadedness or fainting. Rarely, it may cause infection at the site where blood is taken. In this

study, an IV line that can be used for blood collection may be placed in the arm and left in place for several hours on days when there is product administration for frequent PK blood draws. Problems from use of an IV for blood drawing are generally mild and may include pain, bruising, minor swelling or bleeding at the IV site and rarely, infection, vein irritation (phlebitis), or blood clot.

Risks of Mosquito Bites for CHMI: Risks associated with CHMI include local inflammatory reactions, lymphadenitis, persistent local pruritus and larger local reactions involving the whole forearm, allergic reactions to mosquito bites. Another remotely possible risk includes a systemic allergic reaction to the mosquitoes.

Risks of Acquiring Malaria Infection: There is also the possibility of complications of malaria, which are seen during naturally acquired malaria when diagnosis and treatment are delayed and high levels of parasitemia develop. Under the carefully controlled conditions of this study that supports early diagnosis and treatment, the chance of such complications is highly unlikely and the risk of death from malaria infection is very small. Transient abnormalities, e.g. fever, headache, myalgia, shaking chills, abdominal discomfort, nausea, vomiting, mild anemia, leukopenia, thrombocytopenia, hepatosplenomegaly, hepatic tenderness and fatigue, are expected consequences of malaria, but are nearly absent with early RT-qPCR detection [32]. In uncontrolled circumstances, malaria infections can lead to kidney, liver or brain injury (seizures, coma) and death.

Risks of Antimalarial Medication: Additional risks include possible side effects of the antimalarial medication taken (chloroquine) following challenge with chloroquine-sensitive Pf. These side effects include nausea, vomiting, diarrhea, abdominal pain, dizziness, headaches, sleep disturbances, blurred vision, pruritus, skin rash, exacerbation of psoriasis or porphyria, tinnitus, and photosensitivity. Rarely, there may be changes in electrocardiograms and hypotension. Side effects of atovaquone/proguanil (Malarone®) include nausea, vomiting, abdominal pain, anorexia, diarrhea, headache, cough and rarely, anemia, oral ulcerations, insomnia, fever, edema, rash and alopecia. Another remotely possible risk includes a systemic allergic reaction to chloroquine (or Malarone®). The study team will discuss these medications and their possible side effects in detail both as part of the informed consent process, prior to CHMI, and prior to initiation of treatment for diagnosed malaria infections.

8.5.2. Potential Benefits

Study subjects will not receive direct health benefit from study participation. Others may benefit from knowledge gained in this study that may aid in the development of malaria prevention.

8.5.3. Assessment of Potential Risks and Benefits

This research study will be conducted in compliance with the protocol, GCP guidance, and all applicable regulatory requirements.

The plan for reduction of known and unknown risks to participants includes appropriate training of study personnel; education of study subjects for participation in care throughout the study; monitoring of study subject's health status and experiences; withdrawal from study procedures upon evidence of difficulty, contraindication, or a significant adverse event; and referral for treatment, counseling or other necessary follow-up. The VRC CTP Risk Management Plan

guides the reduction and mitigation strategies applied to the known and unknown risks associated with study participation and trial management/operations.

As study subjects will not receive direct health benefit from study participation or product administration, no alternative procedures are planned. The alternative course of action is to choose not to participate.

8.6. Plan for Use and Storage of Biological Samples

The plan for use and storage of biological samples from this protocol is outlined in the following sections.

8.6.1. Use of Samples, Specimens and Data

Samples, specimens and data collected under this protocol may be used to conduct protocol-related safety, parasitology and immunogenicity evaluations, exploratory laboratory evaluations related to malaria, exploratory laboratory evaluations related to mAb, vaccine or infectious disease research in general and for research assay validation.

8.6.2. Storage and Tracking of Blood Samples and Other Specimens

All research samples use coded labels that only the site staff can link to the subject. Samples are stored at VIP, VRC laboratories in [redacted] Bethesda, MD, or at UMB laboratories, which are all secure facilities with limited access. Data will be kept in password-protected computers. Only investigators or their designees will have access to the samples and data. Samples will be tracked in the Laboratory Information Management System (LIMS) database or using another software designed for this purpose (e.g., Freezerworks).

8.6.3. Disposition of Samples, Specimens and Data at Completion of the Protocol

In the future, other investigators (both at NIH and outside) may wish to study these samples and/or data. IRB approval must be sought prior to any sharing of samples with non-NIH investigators and any clinical information shared about those samples would similarly require prior IRB approval. The research use of stored, unlinked or unidentified samples will be exempt from the need for prospective IRB review and approval. Exemption requests will be submitted in writing to the NIH Office of Human Subjects Research, which is authorized to determine whether a research activity is exempt.

At the time of protocol termination, samples will remain in the VIP facility, VRC laboratories, or UMB Laboratories or, after IRB approval, will be transferred to another repository. Regulatory oversight of the stored samples and data may be transferred to a stored samples protocol as part of the IRB approved termination plan. Data will be archived by the VRC in compliance with requirements for retention of research records, or after IRB and study sponsor approval, it may be either destroyed or transferred to another repository.

8.6.4. Loss or Destruction of Samples, Specimens or Data

Any loss or unanticipated destruction of samples (for example, due to freezer malfunction) or data (for example, misplacing a printout of data with identifiers) will be reported to the IRB.

The Protocol Chair or site PI will also notify the IRB if the decision is made to destroy the remaining samples.

8.7. Safety Oversight

Close cooperation between the designated members of the Protocol Team will occur to evaluate and respond to individual AEs in a timely manner. The VRC clinic designated Safety Officer (SO) for the day conducts a daily safety review of clinical data per VRC Standard Operating Procedures.

The Protocol Safety Review Team (PSRT) will be comprised of the Protocol Chair, Site Principal Investigator (PI), Associate Investigators, Study Coordinator, Protocol Specialists, other study clinicians, the IND MO, and external (non-VRC) collaborators. The PSRT will review the summary study safety data reports on a weekly basis through 4 weeks after the last subject receives the last product administration. The PSRT will also perform weekly safety review through 4 weeks post CHMI. Otherwise, if there have been no product administrations or CHMI in the prior 4 weeks, the PSRT will monitor the study safety data reports on a monthly basis through completion of the last study visit. The PSRT will evaluate and respond to safety concerns in a timely manner.

9. ADMINISTRATIVE AND OPERATIONAL OBLIGATIONS

9.1. Protocol Amendments and Study Termination

Protocol amendments must be made only with prior approval of the IND Sponsor and with agreement from the Protocol Chair and IND MO. All study amendments will be submitted to the central IRB for approval.

The IND Sponsor, the IRB, OHRP, the site PIs, Protocol Chairs, and/or the FDA reserve the right to terminate the study. The Protocol Chair will notify the IRB in writing of the study's completion or early termination.

9.2. Study Documentation and Storage

The Protocol Chair will delegate the study responsibilities to the study team, and a list of appropriately qualified persons to whom trial duties have been delegated will be maintained.

Source documents are original documents, data, and records from which the subject's data are obtained. These include but are not limited to hospital records, clinical and office charts, laboratory and pharmacy records, and correspondence. Long-term storage of source documents may be in the form of electronic files.

The Protocol Chair and staff are responsible for maintaining a comprehensive and centralized filing system of all study-related (essential) documentation, suitable for inspection at any time by representatives from the IND Sponsor, VRC/NIAID/NIH, IRB, NIH, FDA, and/or applicable regulatory authorities. Elements include:

- Subject files containing completed informed consent forms, and supporting copies of source documentation.
- Study files containing the protocol with all amendments, IBs, copies of all correspondence with the IRB.

In addition, all original source documentation must be maintained and be readily available.

All essential documentation should be retained by the institution for the same period of time required for medical records retention. The FDA requires study records to be retained for up to two years after marketing approval or refusal (21 CFR 312.62). If no marketing application is filed, or if the application is not approved, the records will be retained for two years after the investigation is discontinued and the FDA is notified. The HHS protection of human subjects' regulations require that institutions retain records of IRB/EC activities and documentation of informed consent of subjects for at least 3 years after study completion (45 CFR 46).

No study document should be destroyed without prior written agreement between the VRC and the investigator. Should the investigator wish to assign the study records to another party or move them to another location, they must notify the VRC in writing of the new responsible person and/or the new location.

9.3. Clinical Monitoring

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the

conduct of the trial is in compliance with the currently approved protocol/amendment(s), with ICH GCP, and with applicable regulatory requirement(s).

Monitoring for this study will be performed by a designated contract research organization (CRO), [redacted] Details of clinical site monitoring are documented in a Clinical Monitoring Plan. The Clinical Monitoring Plan describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.

9.4. Data Collection and Sharing

9.4.1. Data Collection

Clinical research data will be collected in a secure electronic web-based clinical data management system (CDMS) through a CRO, Emmes (Rockville, MD). Extracted, anonymized data will be sent to the PSRT for safety review and to Protocol Statistician for statistical analysis.

9.4.2. Source Documents

The site will maintain appropriate medical and research records for this trial, in compliance with ICH E6(R2) GCP, applicable regulations, and institutional requirements for the protection of confidentiality of subjects. Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Examples of these original documents and data records include, but are not limited to, medical records, laboratory reports, pharmacy records and other research records maintained for the clinical trial.

9.4.3. Data Sharing

Data generated in this study will be shared as de-identified data in the government-funded public repository, www.clinicaltrials.gov. Data may be shared prior to publication at approved public presentations or for collaborative development and will be shared at the time of publication or within one year of the primary completion date.

9.5. Quality Assurance and Quality Control

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. The VEC's Quality Management Plan will be used to perform quality management for this trial.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site for clarification/resolution.

The monitors will verify that the clinical trial is conducted, and data are generated, and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, ICH GCP, and applicable regulatory requirements.

The site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

9.6. Language

All written information and other material to be used by subjects and investigative staff must use vocabulary and language that are clearly understood.

9.7. Research-Related Injuries

9.7.1. NIH

The NIH Clinical Center will provide short-term medical care for any injury resulting from participation in research here. In general, no long-term medical care or financial compensation for research-related injuries will be provided by the NIH, the NIH Clinical Center, or the Federal Government. However, subjects have the right to pursue legal remedy if they believe that their injury justifies such action.

9.7.2. WRAIR

If subjects are injured because of participation in this research during the CHMI and they are a DOD healthcare beneficiary (e.g., active duty in the military, military spouse or dependent, retiree), they are entitled to medical care for their injury within the DOD healthcare system, as long as they remain a DOD healthcare beneficiary.

If subjects are injured because of participation in this research during the CHMI and they are not a DOD healthcare beneficiary, they are entitled to medical care for their injury at a DOD hospital or clinic, but such care for the injury at DOD hospitals or clinics may be time-limited, and the subject's insurance may be billed. It cannot be determined in advance which DOD hospital or clinic will provide care. If subjects obtain care for research-related injuries outside of a DOD hospital or clinic, they or their insurance will be responsible for medical expenses.

For DOD healthcare beneficiaries and non-DOD healthcare beneficiaries: Transportation to and from hospitals or clinics will not be provided. No reimbursement is available if subjects incur medical expenses to treat research-related injuries. No compensation is available for research-related injuries. Subjects are not waiving any legal rights. If subjects believe they have sustained a research-related injury, they may contact the PI. If subjects have any questions, they can contact the PI.

9.7.3. UMB

The Center for Vaccine Development and Global Health and the University of Maryland will provide short-term medical care or assessment for any injury resulting in participating in research. If an injury is directly related to participation in this project, and requires intervention, UMB and/or one of its affiliated institutions or health care groups will help the subject obtain medical treatment for the specific injury and provide referrals to other health care facilities, as appropriate. UMB and/or its affiliated institutions or health care groups will not provide subjects with financial compensation or reimbursement for the cost of care provided to treat a research-related injury or for other expenses arising from a research-related injury. The institution or group providing medical treatment will charge the subject's insurance carrier. Subjects do not waive their right to pursue legal compensation or damages to recover losses related to injury.

9.8. CHMI Facilities and Management

The VRC, NIAID, NIH is the coordinating center for this protocol. Dr. Martin Gaudinski, VRC, NIAID, NIH, is the Protocol Chair and NIH site PI overseeing the management and monitoring of the protocol conduct overall.

UMB will be a clinical site and also the CHMI administrator for Part C of the protocol. Dr. Kirsten Lyke will be the UMB site PI. UMB investigators and staff will be responsible for completion of all clinical and post-CHMI visits as well as administration of the CHMI (with WRAIR staff support) for participants in Part C.

Investigators from WRAIR, the facility that administered the Part B CHMI, will have limited interaction with study subjects during the administration of the CHMI. In Part C, WRAIR investigators will provide entomologic support to UMB during CHMI. Certain immediate pre- and post-CHMI assessments of subjects will take place in the CHMI facility with some participation by CHMI facility personnel and NIH staff. All other post-CHMI clinical visits for subjects will take place at the NIH clinical site.

A Reliance Agreement will be established with the collaborating CHMI facilities such that the NIH IRB is the IRB of Record for the conduct of the VRC 612 protocol. Referring to Section 5 of the VRC 612 protocol, any Serious Adverse Events, Unanticipated Problems or Protocol Deviation which are reportable to the NIH IRB which are in relation to the conduct of the CHMI will also be communicated to the collaborating CHMI facility within the same reporting period specified in the protocol.

9.8.1. Roles and Responsibilities for the CHMI

The Protocol Chair and site PI will delegate responsibility for overseeing the administration of the CHMI at the collaborating CHMI facilities (i.e., application of and assessment of the mosquito bites) to designated Associate Investigators from those facilities. Follow-up and medical care of the study subjects after the CHMI is administered remains the responsibility of the site PI and the designated personnel at the clinical site.

The Authorized Representative from the CHMI facility may review research records related to the CHMI conducted at their own facility.

The roles and responsibilities for this collaborating institution and facility personnel for the conduct of CHMI as described above are delineated in the corresponding VRC 612 Study Personnel Page.

9.9. WRAIR Protocol Review and Reporting Requirements

Initial Protocol Review

WRAIR will defer their IRB review to the NIH IRB once a reliance agreement is in place. WRAIR Human Subjects Protection Branch (HSBP) will still perform an administrative review of the protocol to ensure that the WRAIR reporting requirements are met. WRAIR Commander Approval Authorization will be issued once the NIH IRB approval has been submitted to the WRAIR HSPB and the administrative WRAIR comments have been adequately addressed. Headquarters level review will be conducted as appropriate.

Protocol Modifications/Amendments

All amendments/modifications to the protocol and supporting documents (informed consent, recruitment materials, etc.) must be reviewed by the WRAIR HSPB and a WRAIR Commander Authorization Approval issued prior to WRAIR participation on the amended/modified protocol.

Continuing Reviews and Closeout Report

The WRAIR Point of Contact will be responsible for preparing and submitting continuing review reports as per UWZ-C-618 and a closeout report as per WRAIR Policy 12-12. The WRAIR HSPB will review and acknowledge the reports in order for WRAIR personnel to continue their participation on the study. Once all study activities have been completed, to include data analysis, a closeout report will need to be submitted to the WRAIR HSPB to close the study.

The following reporting requirements apply:

Unanticipated Problems Involving Risks to Subjects or Others

Unanticipated problems, as defined in Section 5.5.1, should be promptly reported (48 hours) by telephone, email, or fax to the WRAIR HSPB. A complete written report should follow the initial notification within 10 working days. All unanticipated problems occurring within the reporting period should also be summarized in the continuing review reports submitted to the WRAIR HSPB (contact information is below at end of section).

Serious Adverse Events

All related SAEs and deaths, as defined in Section 5.2, should be reported to the WRAIR HSPB within 48 hours by telephone, email, or fax. A complete written report should follow the initial notification within 10 working days. All SAEs occurring within the reporting period should also be summarized in the continuing review reports submitted to the WRAIR HSPB.

Protocol Deviations

All major protocol deviations that adversely affect the safety or rights of a subject or scientific integrity of the study, as defined in Section 5.5.3, will be reported to the WRAIR HSPB within 48 hours and a written report should be submitted within 10 working days. All protocol deviations occurring within the reporting period should be summarized in the continuing review reports that are submitted to the WRAIR HSPB.

Pending Inspections/Issuance of Reports

The knowledge of any pending compliance inspection/visit by the FDA, Office for Human Research Protections (Department of Health and Human Services), or other government agency concerning clinical investigation or research, the issuance of Inspection Reports, FDA Form 483, warning letters, or actions taken by any regulatory agency including legal or medical actions and any instances of serious or continuing noncompliance with the regulations or requirements will be reported immediately to the WRAIR HSPB.

WRAIR HSPB Contact Information

Director, Human Subjects Protection Branch

[redacted]

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APPENDIX I: SCHEDULE OF EVALUATIONS

There is a system incompatibility between the study clinical database software and the LIMS sample tracking system used by the VIP. The incompatibility creates a discrepancy of visit number nomenclature between the approved protocol and the database. The VRC clinic used the protocol-based convention for source documents.

In addition, the visit number nomenclature in the clinical study database managed by Emmes has been modified from the visit number nomenclature in the approved protocol due to an inability to match the protocol within its limitations. This is because the Emmes database requires visit numbers to have a leading zero to assure proper sorting. The protocol visit numbers do not contain a leading zero because such a convention would be out of specifications required by the LIMS system. These limitations will not interfere with the ability to identify and track study samples.

Refer to the following table for an example of the differences between the Emmes database and the protocol Schedule of Evaluations, source documents and LIMS:

Protocol Schedule of Evaluations	Source Documentation	LIMS	Emmes Database
Visit A1R	Visit A1R	Visit A1R	Visit A**0**1R
Visit A2	Visit A2	Visit A02	Visit A**0**2
Visit B1R	Visit B1R	Visit B1R	Visit B**0**1R
Visit B2	Visit B2	Visit B02	Visit B**0**2
Visit C2	Visit C2	Visit C02	Visit C**0**2
Visit C2A	Visit C2A	Visit C2A	Visit C**0**2A

PART A SCHEDULES

Schedule of Evaluations: Part A IV Groups (Groups 1, 3, 4A, 4B)																			
Visit Number		01	[1] A1R	[1] A2	A2A	A2B	A2C	A2D	A3	A4	A6	A7	A8	A9	A10	A11	A12	A13	A14
Time After Infusion				Pre	EOI	1hr	3h	6h	24hr	48hr	Wk1	Wk2	Wk3	Wk4	Wk8	Wk12	Wk16	Wk20	Wk24
Day of Study		-56 to -1	-28 to 0	D0	D0	D0	D0	D0	D1	D2	D7	D14	D21	D28	D56	D84	D112	D140	D168
Study Procedures	**Tube**	**Screen**	**Enroll**	**Day of infusion**															
VRC 500 Screening Consent		X																	
VRC 612 Informed Consent, AoU			X																
[2] Physical Exam		X	X	X	X				X	X	X	X	X	X	X	X	X	X	X
[3] Medical History		X	X	X					X	X	X	X	X	X	X	X	X	X	X
EKG		X																	
Concomitant Medications		X	X	X					X	X	X	X	X	X	X	X	X	X	X
Product Administration				**X**															
Begin 7-day Diary Card				X															
[4] Pregnancy Prevention Counseling		X	X	X								X					X		X
Clinical Evaluations																			
[4] Pregnancy Test (urine or serum)		X	X	X								X					X		X
CBC with differential	EDTA	3		3					3		3	3		3		3			
ALT, creatinine	GLT	X		X					4		4	4		4		4			
[5] CMP	GLT	4		4															
HIV Ag/Ab Combo	EDTA	3																	
Sickle Cell Test	EDTA	3																	
Troponin	GLT	4																	
Research Samples																			
[6] PK	SST			4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
PBMC	EDTA	20										40							
Serum	SST	8		16†						8	8†	8	8	8†	8	8†	8	8	8†
Daily Volume (mL)		**45**	**0**	**27**	**4**	**4**	**4**	**4**	**11**	**12**	**19**	**59**	**12**	**19**	**12**	**19**	**12**	**12**	**12**
Cumulative Volume (mL)		**45**	**45**	**72**	**76**	**80**	**84**	**88**	**99**	**111**	**130**	**189**	**201**	**220**	**232**	**251**	**263**	**275**	**287**

Visit windows: Schedule Visits A2A–A14 with respect to Visit A2. Visit A2A (within 10 min of EOI); Visits A2B, A2C (±10 min); Visit A2D (-2 hrs); Visits A3, A4 (±6 hrs); Visits A6, A7, A8, A9 (±2 days); Visits A10, A11, A12, A13, A14 (±7 days). Visit A5 is not applicable to this schedule.

[1] Visit A1R is the day of enrollment and may be done on the same day as Day 0. VA2/Day 0 is day of product administration and preferably scheduled within 14 days after enrollment at Visit A1R but may be scheduled up to 28 days after Visit A1R.

[2] Screening includes physical exam, vital signs (blood pressure (BP), temperature, pulse, respiratory rate (RR)), height, weight. Day 0 includes vital signs and weight (for ordering study product dose). At other visits, perform a targeted exam if medically indicated, otherwise only vital signs are required.

[3] Perform full medical history at screening. At other visits, perform interim medical history.

[4] Pregnancy test results must be negative for women of reproductive potential before product administration. Complete Pregnancy Prevention Counseling Form when pregnancy test is done.

[5] CMP includes sodium, potassium, chloride, total CO2, creatinine, glucose, urea nitrogen, albumin, alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, total bilirubin, calcium, total protein

[6] PK blood draws, defined by time after an infusion, are relative to the exact time of the end of infusion (EOI). Record the exact start / end times of product administration and of blood draw to ensure accurate PK analysis.

† Anti-drug antibodies (ADA) assessed from serum samples at timepoints as indicated.

Schedule of Evaluations: Part A SC Group (Group 2)																	
Visit Number		01	[1] A1R	[1] A2	A2A	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14
Time after Infusion				Pre	EOI	24hr	48hr	72hr	Wk1	Wk2	Wk3	Wk4	Wk8	Wk12	Wk16	Wk20	Wk24
Study Day		-56 to -1	-28 to 0	D0	D0	D1	D2	D3	D7	D14	D21	D28	D56	D84	D112	D140	D168
Study Procedures	**Tube**	**Screen**	**Enroll**	**Day of injection**													
VRC 500 Screening Consent		X															
VRC 612 Informed Consent, AoU			X														
[2] Physical Exam		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
[3] Medical History		X	X	X		X	X	X	X	X	X	X	X	X	X	X	X
EKG		X															
Concomitant Medications		X	X	X		X	X	X	X	X	X	X	X	X	X	X	X
Product Administration				**X**													
Begin 7-day Diary Card				X													
[4] Pregnancy Prevention Counseling		X	X	X						X					X		X
Clinical Evaluations																	
[4] Pregnancy Test (urine or serum)		X	X	X						X					X		X
CBC with differential	EDTA	3		3		3			3	3		3		3			
ALT, creatinine	GLT	X		X		4			4	4		4		4			
[5] CMP	GLT	4		4													
HIV Ag/Ab Combo	EDTA	3															
Sickle Cell Test	EDTA	3															
Troponin	GLT	4															
Research Samples																	
[6] PK	SST			4		4	4	4	4	4	4	4	4	4	4	4	4
PBMC	EDTA	20								40							
Serum	SST	8		16†			8	8	8†	8	8	8†	8	8†	8	8	8†
Daily Volume (mL)		**45**	**0**	**27**	**0**	**11**	**12**	**12**	**19**	**59**	**12**	**19**	**12**	**19**	**12**	**12**	**12**
Cumulative Volume (mL)		**45**	**45**	**72**	**72**	**83**	**95**	**107**	**126**	**185**	**197**	**216**	**228**	**247**	**259**	**271**	**283**

Visit windows: Schedule Visits A2A – A14 with respect to Visit A2. Visit A2A (within 10 min of EOI); Visits A3, A4, A5 (±6 hrs); Visits A6, A7, A8, A9 (±2 days); Visits A10, A11, A12, A13, A14 (±7 days).

[1] Visit A1R is the day of enrollment and may be done on the same day as Day 0. VA2/Day 0 is day of product administration and preferably scheduled within 14 days after enrollment at Visit A1R but may be scheduled up to 28 days after Visit A1R.

[2] Screening includes physical exam, vital signs (BP, temperature, pulse, RR), height, weight. Day 0 includes vital signs and weight (for ordering study product dose). At other visits, perform a targeted exam if medically indicated, otherwise only vital signs are required.

[3] Perform full medical history at screening. At other visits, perform interim medical history.

[4] Pregnancy test results must be negative for women of reproductive potential before product administration. Complete Pregnancy Prevention Counseling Form when pregnancy test is done.

[5] CMP includes sodium, potassium, chloride, total CO2, creatinine, glucose, urea nitrogen, albumin, alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, total bilirubin, calcium, total protein

[6] PK blood draws, defined by time after infusion, are relative to the exact time of the end of infusion (EOI). Record exact start / end times of product administration and of blood draw to ensure accurate analysis.

† ADA assessed from serum samples at timepoints as indicated.

PART B SCHEDULES

Schedule of Evaluations: Part B IV Groups (Groups 7 and 9) Initial or Repeat Dose																			
Visit Number		01	[1] B1R	[1] B2	B2A	B2B	B2C	B2D	B3	B4	B6	B7	B8	B9	B10	B11	B12	B13	B14
Time After Infusion				Pre	EOI	1hr	3h	6h	24hr	48hr	Wk1	Wk2	Wk3	Wk4	Wk8	Wk12	Wk16	Wk20	Wk24
Day of Study		-56 to -1	-28 to 0	D0	D0	D0	D0	D0	D1	D2	D7	D14	D21	D28	D56	D84	D112	D140	D168
Study Procedures	**Tube**	**Screen**	**Enroll**	**Day of infusion**															
VRC 500 Screening Consent		X																	
VRC 612 Informed Consent, AoU[7]			X																
[2] Physical Exam		X	X	X	X				X	X	X	X	X	X	X	X	X	X	X
[3] Medical History		X	X	X					X	X	X	X	X	X	X	X	X	X	X
EKG		X	[X]																
Concomitant Medications		X	[X]	X					X	X	X	X	X	X	X	X	X	X	X
Product Administration				**X**															
Begin 7-day Diary Card				X															
[4] Pregnancy Prevention Counseling		X	X	X								X					X		X
Clinical Evaluations																			
[4] Pregnancy Test (urine or serum)		X	X	X								X					X		X
CBC with differential	EDTA	3	[X]	3					3		3	3		3		3			
ALT, creatinine	GLT	X	[X]	X					4		4	4		4		4			
[5] CMP	GLT	4	[X]	4															
HIV Ag/Ab Combo	EDTA	3	[X]																
Sickle Cell Test	EDTA	3[7]																	
Troponin	GLT	4	[X]																
SARS-CoV-2 (PCR)	Swab	X	[X]																
Research Samples																			
[6] PK	SST			4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
PBMC	EDTA	20										40							
Serum	SST	8		16†						8	8†	8	8	8†	8	8†	8	8	8†
Daily Volume (mL)		**45**	**0**	**27**	**4**	**4**	**4**	**4**	**11**	**12**	**19**	**59**	**12**	**19**	**12**	**19**	**12**	**12**	**12**
Cumulative Volume (mL)		**45**	**45**	**72**	**76**	**80**	**84**	**88**	**99**	**111**	**130**	**189**	**201**	**220**	**232**	**251**	**263**	**275**	**287**

Visit windows: Schedule Visits B2A – B14 with respect to Visit B2. Visit B2A (within 10 min of EOI); Visits B2B, B2C (±10 min); Visit B2D (-2 hrs); Visits B3, B4 (±6 hrs); Visits B6, B7, B8, B9 (±2 days); Visits B10, B11, B12, B13, B14 (±7 days). Visit B5 is not applicable.

[1] Visit B1R is the day of enrollment or re-assessment for Group 7 and may be done on the same day as Day 0. VB2/Day 0 is day of product administration and preferably scheduled within 14 days after enrollment at Visit B1R but may be scheduled up to 28 days after Visit B1R. [X] is only required for Group 7.

[2] Screening includes physical exam, vital signs (blood pressure (BP), temperature, pulse, respiratory rate (RR)), height, weight. Day 0 includes vital signs and weight (for ordering study product dose). At other visits, perform a targeted exam if medically indicated, otherwise only vital signs are required.

[3] Perform full medical history at screening. At other visits, perform interim medical history.

[4] Pregnancy test results must be negative for women of reproductive potential before product administration. Use Preg Prevention Counseling Form when pregnancy test is done.

[5] CMP includes sodium, potassium, chloride, total CO2, creatinine, glucose, urea nitrogen, albumin, alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, total bilirubin, calcium, total protein.

[6] PK blood draws, defined by time after an infusion, are relative to the exact time of the end of infusion (EOI). Record the exact start / end times of product administration and of blood draw to ensure accurate PK analysis.

[7] Not required for Part A veterans in Part B † Anti-drug antibodies (ADA) assessed from serum samples at timepoints as indicated.

Schedule of Evaluations: Part B SC Group (Group 6)																	
Visit Number		01	[1] B1R	[1] B2	B2A	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14
Time after Infusion				Pre	EOI	24hr	48hr	72hr	Wk1	Wk2	Wk3	Wk4	Wk8	Wk12	Wk16	Wk20	Wk24
Day of Study		-56 to -1	-28 to 0	D0	D0	D1	D2	D3	D7	D14	D21	D28	D56	D84	D112	D140	D168
Study Procedures	**Tube**	**Screen**	**Enroll**	**Day of injection**													
VRC 500 Screening Consent		X															
VRC 612 Informed Consent, AoU			X														
[2] Physical Exam		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
[3] Medical History		X	X	X		X	X	X	X	X	X	X	X	X	X	X	X
EKG		X															
Concomitant Medications		X	X	X		X	X	X	X	X	X	X	X	X	X	X	X
Product Administration				**X**													
Begin 7-day Diary Card				X													
[4] Pregnancy Prevention Counseling		X	X	X						X					X		X
Clinical Evaluations																	
[4] Pregnancy Test (urine or serum)		X	X	X						X					X		X
CBC with differential	EDTA	3		3		3			3	3		3		3			
ALT, creatinine	GLT	X		X		4			4	4		4		4			
[5] CMP	GLT	4		4													
HIV Ag/Ab Combo	EDTA	3															
Sickle Cell Test	EDTA	3															
Troponin	GLT	4															
SARS-CoV-2 (PCR)	Swab	X															
Research Samples																	
[6] PK	SST			4		4	4	4	4	4	4	4	4	4	4	4	4
PBMC	EDTA	20								40							
Serum	SST	8		16†			8	8	8†	8	8	8†	8	8†	8	8	8†
Daily Volume (mL)		**45**	**0**	**27**	**0**	**11**	**12**	**12**	**19**	**59**	**12**	**19**	**12**	**19**	**12**	**12**	**12**
Cumulative Volume (mL)		**45**	**45**	**72**	**72**	**83**	**95**	**107**	**126**	**185**	**197**	**216**	**228**	**247**	**259**	**271**	**283**

Visit windows: Schedule Visits B2A – B14 with respect to Visit B2. Visit B2A (within 10 min of EOI); Visits B3, B4, B5 (±6 hrs); Visits B6, B7, B8, B9 (±2 days); Visits B10, B11, B12, B13, B14 (±7 days).

[1] Visit B1R is the day of enrollment and may be done on the same day as Day 0. VB2/Day 0 is day of product administration and preferably scheduled within 14 days after enrollment at Visit B1R but may be scheduled up to 28 days after Visit B1R.

[2] Screening includes physical exam, vital signs (BP, temperature, pulse, RR), height, weight. Day 0 includes vital signs and weight (for ordering study product dose). At other visits, perform a targeted exam if medically indicated, otherwise only vital signs are required.

[3] Perform full medical history at screening. At other visits, perform interim medical history.

[4] Pregnancy test results must be negative for women of reproductive potential before product administration. Complete a Pregnancy Prevention Counseling Form when pregnancy test is done.

[5] CMP includes sodium, potassium, chloride, total CO2, creatinine, glucose, urea nitrogen, albumin, alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, total bilirubin, calcium, total protein.

[6] PK blood draws, defined by time after an infusion, are relative to the exact time of the end of infusion (EOI). Record exact start / end times of product administration and of blood draw to ensure accurate analysis. † ADA assessed from serum samples at timepoints as indicated.

Schedule of Evaluations: Part B CHMI for Groups 8 and 10																
Visit Number		01	[1] B1R	B1S	C1	C2	[2]C2A	[2] C2B	C3	C4	C5	C6	C7	C8	C9	C10
Time After CHMI							24hr	72hr	Wk1	Wk1	Wk1	Wk1	Wk1	Wk1	Wk1	Wk2
CHMI Day		-56 to -1	-56 to -1	-5 to -1	-1	D0	D1	D3	D7	D8	D9	D10	D11	D12	D13	D14
Study Procedures	**Tube**	**Screen**	**Enroll**			**CHMI**			**Parasitemia Check** (daily until treatment criteria met)							
VRC 500 Screening Consent		X														
VRC 612 Informed Consent, AoU[9]			X													
[3] Physical Exam, Vital Signs		X	X	X	X	X			X	X	X	X	X	X	X	X
[4] Medical History		X	X	X	X	X			X	X	X	X	X	X	X	X
EKG		X	[X]													
CHMI						**X**										
Phone Contact							X	X								
[5] Pregnancy Prevention Counseling		X	X		X											
Clinical Evaluations																
[5] Pregnancy Test (urine or serum)		X	X		X											
CBC with differential	EDTA	3	[X]		3				3							
ALT, creatinine	GLT	X	[X]		4											
[6] CMP	GLT	4	[X]													
HIV Ab/Ag Combo	EDTA	3	[X]													
Sickle Cell Test	EDTA	3[9]														
Troponin	GLT	4	[X]													
SARS-CoV-2 (PCR)	Swab	X		X												
[7] Parasitemia Evaluation (PCR)	EDTA								3	[3]	[3]	[3]	[3]	[3]	[3]	[3]
Research Samples																
[8] PK	SST				4				4							4
PBMC	EDTA	20							40							**60
Serum	SST	8							16							**16
Daily Volume (mL)		**45**	**0**	**0**	**11**	**0**	**0**	**0**	**66**	**3**	**3**	**3**	**3**	**3**	**3**	**83**
Cumulative Volume (mL)		**45**	**45**	**45**	**56**	**56**	**56**	**56**	**122**	**125**	**128**	**131**	**134**	**137**	**140**	**223**

[1] Visit B1R is the day of re-assessment for Group 8, and for Group 10 in the case of Part A Group 5 veteran subjects. [X] is only required for Group 8 and veteran Group 10
[2] After CHMI, schedule Visit C2A on Day 1 or 2, Visit C2B on Day 3 or 4.
[3] Screening includes physical exam, vital signs (BP, temperature, pulse, RR), height, weight. On D0, perform a targeted physical exam if medically indicated, otherwise only vital signs are required.
[4] Perform full medical history at screening. At other visits, perform interim medical history.
[5] Pregnancy test results must be negative for women of reproductive potential within 2 days prior to CHMI. Use Preg Prevention Counseling Form when testing for pregnancy.
[6] CMP includes sodium, potassium, chloride, total CO2, creatinine, glucose, urea nitrogen, albumin, alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, total bilirubin, calcium, total protein.
[7] Parasitemia evaluations should continue daily until a subject has one positive PCR or blood smear; brackets [3] indicate optional draw as needed. A positive result prior to Day 21 is required for initiation of antimalarial treatment.
[8] PK draw is for Group 8 only; does not apply to Group 10.
[9] Not required for Part A veterans in Part B
** Research blood will be drawn at 14 days post-CHMI (Visit C10) from ALL subjects.

Schedule of Evaluations: Part B CHMI for Groups 8 and 10 (continued)									
Visit Number		C11	C12	C13	C14	[1] C15	C16	C17	[2] C20
Time after CHMI		Wk2	Wk2	Wk2	Wk2	Wk3	Wk3	Wk4	Wk7
CHMI Day		D15	D16	D17	D18	D21	D23	D28	D49
Study Procedures	**Tube**	**Parasitemia Check** (daily until treatment criteria met)							
[3] Physical Exam, Vital Signs		X	X	X	X	X	X		[X]
Interim Medical History		X	X	X	X	X	X		[X]
Phone Contact								X	[X]
Clinical Evaluations									
CBC with differential	EDTA					*3	*3		[3]
ALT, creatinine	SST					*4	*4		[4]
[4] Parasitemia Evaluation (PCR)	EDTA	[3]	[3]	[3]	[3]	[3]			[3]
Anti-Malarial Treatment						[X]	[X]		
Research Samples									
[5] PK	SST					4			[4]
PBMC	EDTA								[60]
Serum	SST					8			[16]
Daily Volume (mL)		**3**	**3**	**3**	**3**	**22**	**7**	**0**	**90**
Cumulative Volume (mL)		**226**	**229**	**232**	**235**	**257**	**264**	**264**	**354**

[1] Visit C15 is only required for CHMI participants not previously diagnosed with malaria parasitemia to rule out a delayed case. At Visit C15 (Day 21), any subject not already on antimalarial treatment will be given DOT. Subjects must return for DOT for 2 more days after initiation. Brackets [X] indicate optional as needed per subject treatment status.

[2] Visit C20 is only required for subjects who had a positive PCR or blood smear to document test of cure. It is scheduled to occur 26±5 days after DOT completion and is shown at Day 49 for convenience. Visit C20 may be completed via phone for subjects who remain malaria negative, brackets [X] indicate optional as needed per subject infection status.

[3] Perform a targeted physical exam if medically indicated, otherwise only vital signs (BP, temperature, pulse, RR) are required.

[4] Parasitemia evaluations should continue daily until a subject has one positive PCR or blood smear; brackets [3] indicate optional draw as needed. A positive result prior to Day 21 is required for initiation of antimalarial DOT. Otherwise, initiate DOT at Day 21.

[5] PK draw is for Group 8 only; does not apply to Group 10.

* Blood for CBC, ALT and creatinine will be drawn at onset of DOT and at about 2 days later; shown at visits C15 and C16 to account for blood draw. Parasitemia evaluations are not performed during treatment through to test of cure.

Schedule of Evaluations: Part B CHMI for Groups 6, 7, 9														
Visit Number		B1S	C1	C2	[1] C2A	[1] C2B	C3	C4	C5	C6	C7	C8	C9	C10
Time after CHMI					24hr	72hr	Wk1	Wk1	Wk1	Wk1	Wk1	Wk1	Wk1	Wk2
CHMI Day		-5 to -1	-1	D0	D1	D3	D7	D8	D9	D10	D11	D12	D13	D14
Study Procedures	Tube			**CHMI**			**Parasitemia Check** (daily checks until treatment criteria met)							
[2] Physical Exam, Vital Signs		X	X	X			X	X	X	X	X	X	X	X
Interim Medical History		X	X	X			X	X	X	X	X	X	X	X
CHMI				**X**										
Phone Contact					X	X								
Pregnancy Prevention Counseling			X											
Clinical Evaluations														
[3] Pregnancy Test (urine or serum)			X											
CBC with differential	EDTA		3				3							
ALT, creatinine	GLT		4											
SARS-CoV-2 (PCR)	Swab	X												
[4] Parasitemia Evaluation (PCR)	EDTA						3	3	[3]	[3]	[3]	[3]	[3]	[3]
Research Samples														
PK	SST		4				4							4
PBMC	EDTA						40							**60
Serum	SST						16†							**16
Daily Volume (mL)		**0**	**11**	**0**	**0**	**0**	**66**	**3**	**3**	**3**	**3**	**3**	**3**	**83**
Cumulative Volume (mL)		**0**	**11**	**11**	**11**	**11**	**77**	**80**	**83**	**86**	**89**	**92**	**95**	**178**

[1] After CHMI, schedule Visit C2A on Day 1 or 2, Visit C2B on Day 3 or 4
[2] Perform a targeted physical exam if medically indicated, otherwise only vital signs (BP, temperature, pulse, RR) are required.
[3] Pregnancy test results must be negative for women of reproductive potential within 2 days prior to CHMI. Complete a Pregnancy Prevention Counseling Form when pregnancy test is done.
[4] Parasitemia evaluations should continue daily until a subject has one positive PCR or blood smear; brackets [3] indicate optional draw as needed. A positive result prior to Day 21 is required for initiation of antimalarial directly observed therapy (DOT).
** Research blood will be drawn at 14 days post-CHMI (Visit C10) from ALL subjects.
† ADA assessed from serum samples at timepoints as indicated.

Schedule of Evaluations: Part B CHMI for Groups 6, 7, 9 (continued)									
[1] Visit Number		C11	C12	C13	C14	[1] C15	C16	C17	[2] C20
Time after CHMI		Wk2	Wk2	Wk2	Wk2	Wk3	Wk3	Wk4	Wk7
CHMI Day		D15	D16	D17	D18	D21	D23	D28	D49
Study Procedures	**Tube**	**Parasitemia Check** (daily until treatment criteria met)							
[3] Physical Exam, Vital Signs		X	X	X	X	X	X		[X]
Interim Medical History		X	X	X	X	X	X		[X]
Phone Contact								X	[X]
Clinical Evaluations									
CBC with differential	EDTA					*3	*3		[3]
ALT, creatinine	SST					*4	*4		[4]
[4] Parasitemia Evaluation (PCR)	EDTA	[3]	[3]	[3]	[3]	[3]			[3]
Anti-Malarial Treatment						[X]	[X]		
Research Samples									
PK	SST					4			[4]
PBMC	EDTA								[60]
Serum	SST					8†			[16]
Daily Volume (mL)		**3**	**3**	**3**	**3**	**22**	**7**	**0**	**90**
Cumulative Volume (mL)		**181**	**184**	**187**	**190**	**212**	**219**	**219**	**309**

[1] Visit C15 is only required for CHMI participants not previously diagnosed with malaria parasitemia to rule out a delayed case. At Visit C15 (Day 21), any subject who has not already started antimalarial treatment will be given definitive DOT. Subjects must return for DOT for 2 more days after initiation. Brackets [X] indicate optional as needed per subject treatment status.

[2] Visit C20 is only required for subjects who had a positive PCR or blood smear to document test of cure. It is scheduled to occur 26±5 days after DOT completion and is shown at Day 49 for convenience. Visit C20 may be completed via phone for subjects who remain malaria negative, brackets [X] indicate optional as needed per subject infection status.

[3] Perform a targeted physical exam if medically indicated, otherwise only vital signs (BP, temperature, pulse, RR) are required.

[4] Parasitemia evaluations should continue daily until a subject has one positive PCR or blood smear; brackets [3] indicate optional draw as needed. A positive result prior to Day 21 is required for initiation of antimalarial DOT. Otherwise, initiate DOT at C15.

* Blood for CBC, ALT, and creatinine will be drawn at onset of DOT and about 2 days later; shown at visits C15 and C16 to account for blood draw. Parasitemia evaluations are not performed during treatment through to test of cure.

† ADA assessed from serum samples at timepoints as indicated.

PART C SCHEDULES

Schedule of Evaluations: Part C IV Groups (Groups 11, 12, 14)																			
Visit Number		01	[1] D1R	[1] D2	D2A	D2B	D2C	D2D	D3	D4	D6	D7	D8	D9	D10	D11	D12	D13	D14
Time After Infusion				Pre	EOI	1hr	3h	6h	24hr	48hr	Wk1	Wk2	Wk3	Wk4	Wk8	Wk12	Wk16	Wk20	Wk24
Day of Study		-56 to -1	-28 to 0	D0	D0	D0	D0	D0	D1	D2	D7	D14	D21	D28	D56	D84	D112	D140	D168
Study Procedures	**Tube***	**Screen**	**Enroll**	**Day of infusion**															
VRC 500 Screening Consent		X																	
VRC 612 Informed Consent, AoU			X																
[2] Physical Exam		X	X	X	X				X	X	X	X	X	X	X	X	X	X	X
[3] Medical History		X	X	X					X	X	X	X	X	X	X	X	X	X	X
EKG		X																	
Concomitant Medications		X	X	X					X	X	X	X	X	X	X	X	X	X	X
Product Administration				**X**															
Begin 7-day Diary Card				X															
[4] Pregnancy Prevention Counseling		X	X	X								X					X		X
Clinical Evaluations																			
[4] Pregnancy Test (urine or serum)		X	X	X								X					X		X
CBC with differential	EDTA	3		3					3		3	3		3		3			
ALT, creatinine	GLT	X		X					4		4	4		4		4			
[5] CMP	GLT	4		4															
HIV Ag/Ab Combo	EDTA	3																	
Sickle Cell Test	EDTA	3																	
Troponin	GLT	4																	
Research Samples																			
[6] PK	SST			4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
PBMC	EDTA	20										40							
Serum	SST	8		16†						8	8†	8	8	8†	8	8†	8	8	8†
Daily Volume (mL)		**45**	**0**	**27**	**4**	**4**	**4**	**4**	**11**	**12**	**19**	**59**	**12**	**19**	**12**	**19**	**12**	**12**	**12**
Cumulative Volume (mL)		**45**	**45**	**72**	**76**	**80**	**84**	**88**	**99**	**111**	**130**	**189**	**201**	**220**	**232**	**251**	**263**	**275**	**287**

Visit windows: Schedule Visits D2A–D14 with respect to Visit D2. Visit D2A (within 10 min of EOI); Visits D2B, D2C (±10 min); Visit D2D (-2 hrs); Visits D3, D4 (±6 hrs); Visits D6, D7, D8, D9 (±2 days); Visits D10, D11, D12, D13, D14 (±7 days). Visit D5 is not applicable to this schedule.

Footnotes (continue to next page):

[1] Visit D1R is the day of enrollment and may be done on the same day as Day 0. Visit D2/Day 0 is day of product administration and preferably scheduled within 14 days after enrollment at Visit D1R but may be scheduled up to 28 days after Visit D1R.

[2] Screening includes physical exam, vital signs (blood pressure (BP), temperature, pulse, respiratory rate (RR)), height, weight. Day 0 includes vital signs and weight (for ordering study product dose). At other visits, perform a targeted exam if medically indicated, otherwise only vital signs are required.

[3] Perform full medical history at screening. At other visits, perform interim medical history.

[4] Pregnancy test results must be negative for women of reproductive potential before product administration. Complete Pregnancy Prevention Counseling Form when pregnancy test is done.

[5] CMP includes sodium, potassium, chloride, total CO2, creatinine, glucose, urea nitrogen, albumin, alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, total bilirubin, calcium, total protein

[6] PK blood draws, defined by time after an infusion, are relative to the exact time of the end of infusion (EOI). Record the exact start / end times of product administration and of blood draw to ensure accurate PK analysis.

[†] Anti-drug antibodies (ADA) assessed from serum samples at timepoints as indicated.

*Tube types/volumes are shown to estimate blood volumes in mL. Different tubes for clinical evaluations may be used to meet site requirements but research sample tubes must be used as shown or as instructed by the IND Sponsor.

Schedule of Evaluations: Part C SC Group (Groups 13 and 15)																	
Visit Number		01	[1] D1R	[1] D2	D2A	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
Time after Infusion				Pre	EOI	24hr	48hr	72hr	Wk1	Wk2	Wk3	Wk4	Wk8	Wk12	Wk16	Wk20	Wk24
Study Day		-56 to -1	-28 to 0	D0	D0	D1	D2	D3	D7	D14	D21	D28	D56	D84	D112	D140	D168
Study Procedures	**Tube***	**Screen**	**Enroll**	**Day of injection**													
VRC 500 Screening Consent		X															
VRC 612 Informed Consent, AoU			X														
[2] Physical Exam		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
[3] Medical History		X	X	X		X	X	X	X	X	X	X	X	X	X	X	X
EKG		X															
Concomitant Medications		X	X	X		X	X	X	X	X	X	X	X	X	X	X	X
Product Administration				**X**													
Begin 7-day Diary Card				X													
[4] Pregnancy Prevention Counseling		X	X	X						X					X		X
Clinical Evaluations																	
[4] Pregnancy Test (urine or serum)		X	X	X						X					X		X
CBC with differential	EDTA	3		3		3			3	3		3		3			
ALT, creatinine	GLT	X		X		4			4	4		4		4			
[5] CMP	GLT	4		4													
HIV Ag/Ab Combo	EDTA	3															
Sickle Cell Test	EDTA	3															
Troponin	GLT	4															
Research Samples																	
[6] PK	SST			4		4	4	4	4	4	4	4	4	4	4	4	4
PBMC	EDTA	20								40							
Serum	SST	8		16†			8	8	8†	8	8	8†	8	8†	8	8	8†
Daily Volume (mL)		**45**	**0**	**27**	**0**	**11**	**12**	**12**	**19**	**59**	**12**	**19**	**12**	**19**	**12**	**12**	**12**
Cumulative Volume (mL)		**45**	**45**	**72**	**72**	**83**	**95**	**107**	**126**	**185**	**197**	**216**	**228**	**247**	**259**	**271**	**283**

Visit windows: Schedule Visits D2A – D14 with respect to Visit D2. Visit D2A (within 10 min of EOI); Visits D3, D4, D5 (±6 hrs); Visits D6, D7, D8, D9 (±2 days); Visits D10 – D14 (±7 days).

Footnotes (continue to next page):

[1] Visit D1R is the day of enrollment and may be done on the same day as Day 0. Visit D2/Day 0 is day of product administration and preferably scheduled within 14 days after enrollment at Visit D1R but may be scheduled up to 28 days after Visit D1R.

[2] Screening includes physical exam, vital signs (BP, temperature, pulse, RR), height, weight. Day 0 includes vital signs and weight (for ordering study product dose). At other visits, perform a targeted exam if medically indicated, otherwise only vital signs are required.

[3] Perform full medical history at screening. At other visits, perform interim medical history.

[4] Pregnancy test results must be negative for women of reproductive potential before product administration. Complete Pregnancy Prevention Counseling Form when pregnancy test is done.

[5] CMP includes sodium, potassium, chloride, total CO2, creatinine, glucose, urea nitrogen, albumin, alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, total bilirubin, calcium, total protein

[6] PK blood draws, defined by time after infusion, are relative to the exact time of the end of infusion (EOI). Record exact start / end times of product administration and of blood draw to ensure accurate analysis.

[†] ADA assessed from serum samples at timepoints as indicated.

*Tube types/volumes are shown to estimate blood volumes in mL. Different tubes for clinical evaluations may be used to meet site requirements but research sample tubes must be used as shown or as instructed by the IND Sponsor.

Schedule of Evaluations: Part C CHMI CIS43LS Groups (Groups 11, 12, 13, 14, 15)														
Visit Number			C1	C2	[1] C2A	[1] C2B	C3	C4	C5	C6	C7	C8	C9	C10
Time after CHMI					24hr	72hr	Wk1	Wk1	Wk1	Wk1	Wk1	Wk1	Wk1	Wk2
CHMI Day			-1	D0	D1	D3	D7	D8	D9	D10	D11	D12	D13	D14
Study Procedures	Tube*			**CHMI**			**Parasitemia Check** (daily checks until treatment criteria met)							
[2] Physical Exam, Vital Signs			X	X			X	X	X	X	X	X	X	X
Interim Medical History			X	X			X	X	X	X	X	X	X	X
CHMI				**X**										
Phone Contact					X	X								
Pregnancy Prevention Counseling			X											
Clinical Evaluations														
[3] Pregnancy Test (urine or serum)			X											
CBC with differential	EDTA		3				3							
ALT, creatinine	GLT		4											
[4] Parasitemia Evaluation (PCR)	EDTA						3	3	[3]	[3]	[3]	[3]	[3]	[3]
Research Samples														
PK	SST		4				[5]4							[5]4
PBMC	EDTA						[5]40							[5]**60
Serum	SST						[5]16†							[5]**16
Daily Volume (mL)			**11**	**0**	**0**	**0**	**66**	**3**	**3**	**3**	**3**	**3**	**3**	**83**
Cumulative Volume (mL)			**11**	**11**	**11**	**11**	**77**	**80**	**83**	**86**	**89**	**92**	**95**	**178**

[1] After CHMI, schedule Visit C2A on Day 1 or 2, Visit C2B on Day 3 or 4
[2] Perform a targeted physical exam if medically indicated, otherwise only vital signs (BP, temperature, pulse, RR) are required.
[3] Pregnancy test results must be negative for women of reproductive potential within 2 days prior to CHMI. Complete a Pregnancy Prevention Counseling Form when pregnancy test is done.
[4] Parasitemia evaluations should continue daily until a subject has one positive PCR or blood smear; brackets [3] indicate optional draw as needed. A positive result prior to Day 21 is required for initiation of antimalarial directly observed therapy (DOT).
[5] Research samples for Visits C3 and C10 may be collected at either Visit C3 or C4, or at Visit C9, C10, or C11, respectively.
*Tube types/volumes are shown to estimate blood volumes in mL. Different tubes for clinical evaluations may be used to meet site requirements but research sample tubes must be used as shown or as instructed by the IND Sponsor.
** Research blood will be drawn at 14 days post-CHMI (Visit C10) from ALL subjects.
† ADA assessed from serum samples at timepoints as indicated.

Schedule of Evaluations: Part C CHMI CIS43LS Groups (Groups 11, 12, 13, 14, 15)									
[1] Visit Number		C11	C12	C13	C14	[1] C15	C16	C17	[2] C20
Time after CHMI		Wk2	Wk2	Wk2	Wk2	Wk3	Wk3	Wk4	Wk7
CHMI Day		D15	D16	D17	D18	D21	D23	D28	D49
Study Procedures	**Tube***	**Parasitemia Check** (daily until treatment criteria met)							
[3] Physical Exam, Vital Signs		X	X	X	X	X	X		[X]
Interim Medical History		X	X	X	X	X	X		[X]
Phone Contact								X	[X]
Clinical Evaluations									
CBC with differential	EDTA					**3	**3		[3]
ALT, creatinine	SST					**4	**4		[4]
[4] Parasitemia Evaluation (PCR)	EDTA	[3]	[3]	[3]	[3]	[3]			[3]
Anti-Malarial Treatment						[X]	[X]		
Research Samples									
PK	SST					4			[4]
PBMC	EDTA								[60]
Serum	SST					8†			[16]
Daily Volume (mL)		**3**	**3**	**3**	**3**	**22**	**7**	**0**	**90**
Cumulative Volume (mL)		**181**	**184**	**187**	**190**	**212**	**219**	**219**	**309**

[1] Visit C15 is only required for CHMI participants not previously diagnosed with malaria parasitemia to rule out a delayed case. At Visit C15 (Day 21), any subject who has not already started antimalarial treatment will be given definitive DOT. Subjects must return for DOT for 2 more days after initiation; Day 2 of DOT (Day 22) visit will be assigned "C15S". Brackets [X] indicate optional as needed per subject treatment status.

[2] Visit C20 is only required for subjects who had a positive PCR or blood smear to document test of cure. It is scheduled to occur 26±5 days after DOT completion and is shown at Day 49 for convenience. Visit C20 may be completed via phone for subjects who remain malaria negative, brackets [X] indicate optional as needed per subject infection status.

[3] Perform a targeted physical exam if medically indicated, otherwise only vital signs (BP, temperature, pulse, RR) are required.

[4] Parasitemia evaluations should continue daily until a subject has one positive PCR or blood smear; brackets [3] indicate optional draw as needed. A positive result prior to Day 21 is required for initiation of antimalarial DOT. Otherwise, initiate DOT at C15.

*Tube types/volumes are shown to estimate blood volumes in mL. Different tubes for clinical evaluations may be used to meet site requirements but research sample tubes must be used as shown or as instructed by the IND Sponsor.

** Blood for CBC, ALT, and creatinine will be drawn at onset of DOT and about 2 days later; shown at visits C15 and C16 to account for blood draw. Parasitemia evaluations are not performed during treatment through to test of cure.

† ADA assessed from serum samples at timepoints as indicated.

Schedule of Evaluations: Part C CHMI for Control Group 16																
Visit Number		01	[1] D1R		C1	C2	[2] C2A	[2] C2B	C3	C4	C5	C6	C7	C8	C9	C10
Time After CHMI							24hr	72hr	Wk1	Wk1	Wk1	Wk1	Wk1	Wk1	Wk1	Wk2
CHMI Day		-56 to -1	-56 to -1		-1	D0	D1	D3	D7	D8	D9	D10	D11	D12	D13	D14
Study Procedures	**Tube***	**Screen**	**Enroll**			**CHMI**			**Parasitemia Check** (daily until treatment criteria met)							
VRC 500 Screening Consent		X														
VRC 612 Informed Consent, AoU			X													
[3] Physical Exam, Vital Signs		X	X		X	X			X	X	X	X	X	X	X	X
[4] Medical History		X	X		X	X			X	X	X	X	X	X	X	X
EKG		X														
CHMI						**X**										
Phone Contact							X	X								
[5] Pregnancy Prevention Counseling		X	X		X											
Clinical Evaluations																
[5] Pregnancy Test (urine or serum)		X	X		X											
CBC with differential	EDTA	3			3				3							
ALT, creatinine	GLT	X			4											
[6] CMP	GLT	4														
HIV Ab/Ag Combo	EDTA	3														
Sickle Cell Test	EDTA	3														
Troponin	GLT	4														
[7] Parasitemia Evaluation (PCR)	EDTA								3	[3]	[3]	[3]	[3]	[3]	[3]	[3]
Research Samples																
PK	SST				4											
PBMC	EDTA	20							[8]40							[8]**60
Serum	SST	8							[8]16							[8]**16
Daily Volume (mL)		**45**	**0**		**11**	**0**	**0**	**0**	**66**	**3**	**3**	**3**	**3**	**3**	**3**	**83**
Cumulative Volume (mL)		**45**	**45**		**56**	**56**	**56**	**56**	**122**	**125**	**128**	**131**	**134**	**137**	**140**	**223**

Footnotes (continue to next page):

[1] Visit D1R is the day of enrollment and may be done on the same day as Day C1.

[2] After CHMI, schedule Visit C2A on Day 1 or 2, Visit C2B on Day 3 or 4.

[3] Screening includes physical exam, vital signs (BP, temperature, pulse, RR), height, weight. On D0, perform a targeted physical exam if medically indicated, otherwise only vital signs are required.

[4] Perform full medical history at screening. At other visits, perform interim medical history.

[5] Pregnancy test results must be negative for women of reproductive potential within 2 days prior to CHMI. Use Preg Prevention Counseling Form when testing for pregnancy.

[6] CMP includes sodium, potassium, chloride, total CO2, creatinine, glucose, urea nitrogen, albumin, alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, total bilirubin, calcium, total protein.

[7] Parasitemia evaluations should continue daily until a subject has one positive PCR or blood smear; brackets [3] indicate optional draw as needed. A positive result prior to Day 21 is required for initiation of antimalarial treatment.
[8] Research samples for Visits C3 and C10 may be collected at either Visit C3 or C4, or at Visit C9, C10, or C11, respectively.
*Tube types/volumes are shown to estimate blood volumes in mL. Different tubes for clinical evaluations may be used to meet site requirements but research sample tubes must be used as shown or as instructed by the IND Sponsor.
** Research blood will be drawn at 14 days post-CHMI (Visit C10) from ALL subjects.

Schedule of Evaluations: Part C CHMI for Control Group 16									
Visit Number		C11	C12	C13	C14	[1] C15	C16	C17	[2] C20
Time after CHMI		Wk2	Wk2	Wk2	Wk2	Wk3	Wk3	Wk4	Wk7
CHMI Day		D15	D16	D17	D18	D21	D23	D28	D49
Study Procedures	**Tube***	**Parasitemia Check** (daily until treatment criteria met)							
[3] Physical Exam, Vital Signs		X	X	X	X	X	X		[X]
Interim Medical History		X	X	X	X	X	X		[X]
Phone Contact								X	[X]
Clinical Evaluations									
CBC with differential	EDTA					**3	**3		[3]
ALT, creatinine	SST					**4	**4		[4]
[4] Parasitemia Evaluation (PCR)	EDTA	[3]	[3]	[3]	[3]	[3]			[3]
Anti-Malarial Treatment						[X]	[X]		
Research Samples									
PK	SST								
PBMC	EDTA								[60]
Serum	SST					8			[16]
Daily Volume (mL)		**3**	**3**	**3**	**3**	**22**	**7**	**0**	**90**
Cumulative Volume (mL)		**226**	**229**	**232**	**235**	**257**	**264**	**264**	**354**

[1] Visit C15 is only required for CHMI participants not previously diagnosed with malaria parasitemia to rule out a delayed case. At Visit C15 (Day 21), any subject not already on antimalarial treatment will be given DOT; Day 2 of DOT (Day 22) visit will be assigned "C15S". Subjects must return for DOT for 2 more days after initiation. Brackets [X] indicate optional as needed per subject treatment status.

[2] Visit C20 is only required for subjects who had a positive PCR or blood smear to document test of cure. It is scheduled to occur 26±5 days after DOT completion and is shown at Day 49 for convenience. Visit C20 may be completed via phone for subjects who remain malaria negative, brackets [X] indicate optional as needed per subject infection status.

[3] Perform a targeted physical exam if medically indicated, otherwise only vital signs (BP, temperature, pulse, RR) are required.

[4] Parasitemia evaluations should continue daily until a subject has one positive PCR or blood smear; brackets [3] indicate optional draw as needed. A positive result prior to Day 21 is required for initiation of antimalarial DOT. Otherwise, initiate DOT at Day 21.

*Tube types/volumes are shown to estimate blood volumes in mL. Different tubes for clinical evaluations may be used to meet site requirements but research sample tubes must be used as shown or as instructed by the IND Sponsor.

** Blood for CBC, ALT and creatinine will be drawn at onset of DOT and at about 2 days later; shown at visits C15 and C16 to account for blood draw. Parasitemia evaluations are not performed during treatment through to test of cure.

APPENDIX II: ASSESSMENT OF AE RELATIONSHIP AND SEVERITY GRADING

Assessment of Relationship of an Adverse Event

The relationship between an AE and the study product or CHMI will be assessed by the investigator on the basis of clinical judgment and the definitions below.

- **Definitely Related**: The AE and administration of study product and/or CHMI are related in time, and a direct association can be demonstrated.
- **Probably Related**: The AE and administration of study product and/or CHMI are reasonably related in time, and the AE is more likely explained by study agent or CHMI than other causes.
- **Possibly Related**: The AE and administration of study product and/or CHMI are reasonably related in time, but the AE can be explained equally well by causes other than study agent or CHMI.
- **Not Related**: The AE is clearly explained by another cause not related to the study product or CHMI.

For purposes of preparing summary data reports in which AE attributions are simplified to "Related" or "Not Related", in this protocol, the "Definitely, Probably and Possibly" attributions above will be mapped to the "Related" category, while the "Unlikely/Probably Not Related" and "Not Related" attributions above will be mapped to the "Not Related" category. The definitions that apply when these two attribution categories alone are used are as follows:

- **Related**: There is a reasonable possibility that the AE may be related to the study product or CHMI.
- **Not Related**: There is not a reasonable possibility that the AE is related to the study product or CHMI.

Grading the Severity of an Adverse Event

The Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1 [July 2017] will be used to determine the severity grades of AEs in this protocol and is available from: https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables.

Several modifications were made to the table as follows:

- Weight loss will be recorded as an AE only if it is considered deleterious to the participant's health.
- For severity grading of the solicited bruising parameter at the product administration site, the definitions based on size of the largest diameter and listed for the "Injection Site Erythema or Redness" will be used. The severity grade definition for "Bruising" provided under the Dermatologic Clinical Conditions will be used only for unsolicited AEs involving bruising at other body locations.
- Creatinine changes will be graded on the basis of the upper limit of normal provided by the grading table and not change from baseline.
- Creatinine clearance changes will be graded according to ml/min provided by the grading table and not change from baseline.
- Subclinical CMP results for sodium, potassium, chloride, bicarbonate, BUN, and glucose will not be considered an AE unless Grade 2 or higher.