Exposure-Response Evaluation of IV Artesunate in Children with Severe Malaria

DMID Protocol Number: 19-0007

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STATEMENT OF COMPLIANCE

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following *(use applicable regulations depending on study location and sponsor requirements; samples follow):*

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46; 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 312)
- International Council for Harmonisation (ICH) E6; 62 Federal Register 25691 (1997)
- National Institutes of Health (NIH) Clinical Terms of Award
- Uganda National Council for Science and Technology (UNCST) Guidelines

All key personnel (all individuals responsible for the design and conduct of this study) have completed Human Subjects Protection Training.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Site Principal Investigator:

Signed: _____ Date: _____

Pauline Byakika-Kibwika, MBChB, MSc, MMed, PhD
Professor of Medicine

Principal Investigator:

Signed: _____ Date: _____

Matthew B. Laurens, MD, MPH
Professor of Pediatrics and of Medicine

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LIST OF ABBREVIATIONS

ACT	Artemisinin Combination Therapy
AE	Adverse Event/Adverse Experience
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUC	Area Under the Curve
BCS	Blantyre Coma Score
BQL	Below Quantification Limit
BUN	Blood urea nitrogen
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CI	Confidence Interval
C_{max}	Maximum Concentration
CMS	Clinical Materials Services
COI	Conflict of Interest
CRF	Case Report Form
CROMS	Clinical Research Operations and Management Support
DAIDS	Division of AIDS
DHA	Dihydroartemisinin
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
DNA	Deoxyribonucleic acid
eCRF	Electronic Case Report Form
E_{max}	Maximal Effect at High Drug Concentrations
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HPLC	High-Performance Liquid Chromatography
IATA	International Air Transport Association
ICF	Informed Consent Form
ICH	International Council for Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDES	Internet Data Entry System
IDI	Infectious Diseases Institute
IND	Investigational New Drug
IRB	Institutional Review Board

ISM	Independent Safety Monitor
IV	Intravenous
JHU	Johns Hopkins University
LC-MS/MS	Liquid Chromatography and Tandem Mass Spectrometry
LLC	Limited Liability Company
MedDRA®	Medical Dictionary for Regulatory Activities
mmHG	Millimeter(s) of Mercury
MOP	Manual of Procedures
N	Number (typically refers to subjects)
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
NONMEM	Nonlinear Mixed Effects Modeling
OCRA	Office of Clinical Research Affairs, DMID, NIAID, NIH, DHHS
PCE	Parasite Clearance Estimator
PCT_{50}	Parasite Clearance Half-Life
PCT_{90}	Parasite Clearance Time to 90% Reduction
PD	Pharmacodynamic
PI	Principal Investigator
PK	Pharmacokinetics
QA	Quality Assurance
QC	Quality Control
REC	Research Ethics Committee
SAE	Serious Adverse Event/Serious Adverse Experience
SAP	Statistical Analysis Plan
SDCC	Statistical and Data Coordinating Center
SDW	Source Document Workbooks
SOCS	Safety Oversight Committee Support
SOP	Standard Operating Procedure
$t_{1/2}$	Half-Life
T_{max}	Time to Reach C_{max}
UMB	University of Maryland, Baltimore
UNCST	Uganda National Council for Science and Technology
US	United States
WBC	White Blood Cell
WHO	World Health Organization
WWARN	WorldWide Antimalarial Resistance Network

PROTOCOL SUMMARY

Title:	Exposure-Response Evaluation of IV Artesunate in Children with Severe Malaria
Phase:	4
Population:	Children with severe malaria who are 6 months to 14 years of age living in or near Tororo District, Uganda
Number of Sites:	1
Study Duration:	1 year
Subject Participation Duration:	6 months
Description of Agent or Intervention:	Intravenous (IV) artesunate
Objectives:	Primary: • To determine the relationship between dihydroartemisinin (DHA) exposures following IV dosing and markers of physiologic dysfunction associated with severe malaria Secondary: • To determine the relationship between DHA exposures and time to hospital discharge • To determine the relationship between DHA exposures and parasite clearance associated with treatment of severe malaria. Exploratory: • To determine the relationship between DHA exposures and neurodevelopmental outcomes associated with treatment of severe malaria outcomes and explore predictors that may affect this relationship

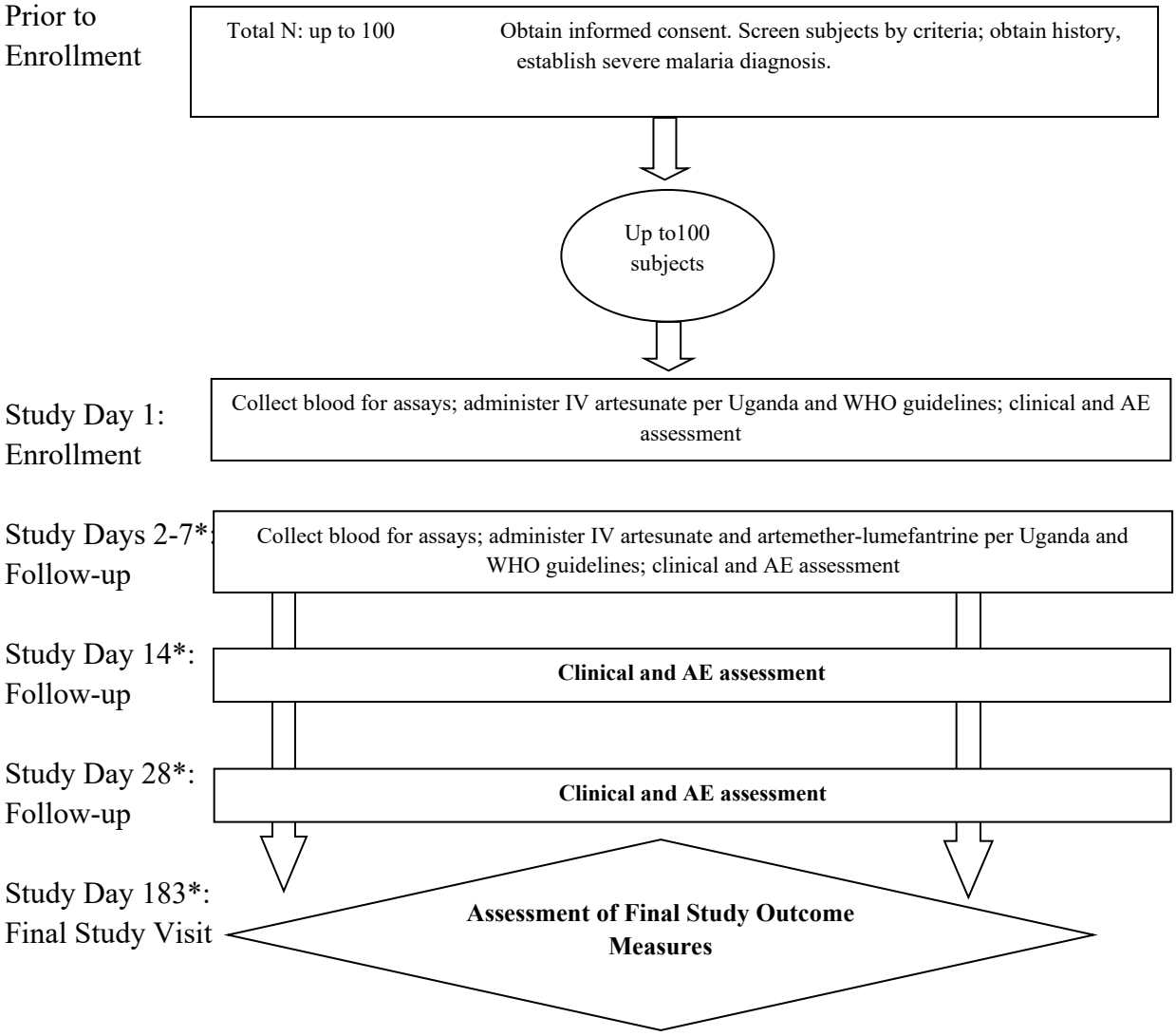
- To evaluate the role of parasite clearance as a mediator of the relationship between DHA exposures and markers of physiologic dysfunction associated with severe malaria
- To develop a score comprised of markers of physiologic dysfunction and describe its relationship to clinical outcomes
- To assess *Plasmodium falciparum* infections for artemisinin resistance
- To store blood for future use, such as identification and characterization of parasite gene expression.

Description of Study Design: This clinical study is a phase 4, single-site, open-label pharmacokinetic (PK) study of IV artesunate in up to 100 Ugandan children 6 months-14 years of age who are diagnosed with severe malaria according to standardized World Health Organization (WHO) criteria (any *P. falciparum* parasitemia and the presence of danger signs). Participants will receive the standard of care IV artesunate for initial treatment of severe malaria per WHO guidelines: children weighing <20 kg should receive 3.0 mg/kg/dose compared to children weighing ≥20 kg who should receive 2.4 mg/kg/dose, at times 0, 12, 24, 48 and 72 hours (WHO 2015). Parenteral treatment will be administered for a minimum of 24 hours (irrespective of the patient's ability to tolerate oral medication earlier), after which patients will be evaluated clinically and assessed for ability for oral intake of antimalarials. Children who are able to transition to oral antimalarial therapy will initiate a 3-day course of artemisinin-combination oral therapy per national guidelines. Biomarkers of physiologic dysfunction will be quantified at regular intervals, including serum lactate, serum glucose, total and direct bilirubin, bicarbonate levels, Blantyre Coma Score (BCS), creatinine and hemoglobin. These biomarkers will be considered both independently and together as a weighted score to relate to the PK of the active metabolite of IV artesunate, DHA and to efficacy markers that more accurately reflect clinical outcomes. We will also quantify *P. falciparum* parasitemia using standardized thick blood smear and relate this outcome to DHA dose and exposure for comparison with historical studies.

	Children 6 months to 14 years of age living in or near Tororo District, Uganda, who are diagnosed with severe malaria and who meet inclusion and exclusion criteria will be enrolled.
Estimated Time to Complete Enrollment:	6 months

Schematic of Study Design:

Figure 1.1: Study Schematic



*Study day windows:
Day 7 (±2 days)
Day 14 (±2 days)
Day 28 (±4 days)
Day183 (±14 days)

1 KEY ROLES

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Despite recent advances in malaria control, *Plasmodium falciparum* continues to kill almost half a million persons annually, most of whom are children living in sub-Saharan Africa (WHO 2019).

Plasmodium falciparum is the most prevalent malaria parasite specie and the cause of the most severe form of disease and death. It accounted for 99.7% of all malaria cases in the WHO African Region in 2018.

Children younger than 5 years of age and pregnant women are most at risk of malaria and death. They accounted for 67% of the estimated 405,000 malaria deaths worldwide, with 94% of these in the African Region. Although there was an overall reduction in malaria deaths in 2018 compared with 2010, the malaria mortality reduction rate slowed since 2016 and this has malaria researchers questioning if current malaria treatment regimens are optimal and based on sound scientific evidence.

Severe malaria is defined as presence or history of fever plus a positive blood film for *P. falciparum* malaria with at least one of the following symptoms and signs:

- Impaired consciousness: A Glasgow coma score < 11 in adults or a Blantyre coma score < 3 in children
- Prostration: Generalized weakness so that the person is unable to sit, stand or walk without assistance
- Multiple convulsions: More than two episodes within 24 h
- Acidosis: A base deficit of > 8 mEq/L or, if not available, a plasma bicarbonate level of < 15 mmol/L or venous plasma lactate ≥ 5 mmol/L. Severe acidosis manifests clinically as respiratory distress (rapid, deep, laboured breathing).
- Hypoglycaemia: Blood or plasma glucose < 2.2 mmol/L (< 40 mg/dL)
- Severe malarial anaemia: Haemoglobin concentration ≤ 5 g/dL or a haematocrit of ≤ 15% in children < 12 years of age (< 7 g/dL and < 20%, respectively, in adults) with a parasite count > 10 000/μL
- Renal impairment: Plasma or serum creatinine > 265 μmol/L (3 mg/dL) or blood urea > 20 mmol/L

- Jaundice: Plasma or serum bilirubin > 50 µmol/L (3 mg/ dL) with a parasite count > 100 000/ µL
- Pulmonary oedema: Radiologically confirmed or oxygen saturation < 92% on room air with a respiratory rate > 30/ min, often with chest indrawing and crepitations on auscultation
- Significant bleeding: Including recurrent or prolonged bleeding from the nose, gums or venepuncture sites; haematemesis or melaena
- Shock: Compensated shock is defined as capillary refill ≥ 3 s or temperature gradient on leg (mid to proximal limb), but no hypotension. Decompensated shock is defined as systolic blood pressure < 70 mm Hg in children or < 80 mmHg in adults, with evidence of impaired perfusion (cool peripheries or prolonged capillary refill).
- Hyperparasitaemia: *P. falciparum* parasitaemia > 10%

Severe malaria is a medical emergency with an immediate threat to life and so requires prompt treatment with effective therapy. The priority requirement for successful diagnosis and treatment is early recognition of the signs and symptoms of severe malaria that should lead to emergency care in an in-patient setting. Artemisinin derivatives are highly effective and rapidly schizonticidal drugs, devoid of major side-affects and their use does not warrant intensive monitoring. Large randomized clinical trials and systematic reviews demonstrated superiority of artesunate to quinine for treatment of severe malaria, with significant reduction in mortality and complications associated with malaria as well as less adverse effects. These trials provided support for parenteral artesunate to replace parenteral quinine as drug of first choice in treating severe malaria. Currently, the WHO recommends use of IV artesunate as first line treatment for severe malaria, administered for at least 24 hours, until the patient can tolerate oral medication and to complete treatment with a full course of an artemisinin combination therapy (ACT). In case of unavailability of artesunate, to use intramuscular artemether in preference to quinine for treatment of severe malaria.

Uganda adopted this policy for severe malaria treatment, however; parenteral artesunate is not widely available and effort to improve accessibility should be reinforced.

Since 1991 and before April 2019, the only Food and Drug Administration (FDA)-approved treatment for severe malaria in the US was IV quinidine gluconate. Availability of quinidine gluconate in the US has declined over the past 20 years, such that the last lot manufactured reached expiry in March 2019. A replacement essential medicine is needed in the US for treatment of severe malaria. IV artesunate is now the only option available for treatment in the US and is uniquely available through the Centers for Disease Control and Prevention (CDC) under an expanded access investigational new drug (IND) protocol via scattered distribution

centers located near transportation hubs. Unfortunately, severe malaria can progress rapidly, and delays in delivery of IV artesunate from treatment distribution centers to patients with severe malaria may lead to complications, including treatment failure and death. To mitigate risk of severe malaria complications, pharmacologic treatment for severe malaria treatment should be optimized for patients with severe malaria.

Malaria treatment and Drug Pharmacokinetics

Severe malaria is a medical emergency which if not immediately treated results in 100% mortality. It is fundamental that plasma concentrations of a highly effective antimalarial drug are achieved as rapidly as possible. Artesunate is a water soluble hemisuccinate artemisinin derivative; available as sodium hemisuccinate salt for injection (Guilin Pharmaceutical Factory, Guangxi, People's Republic of China). Artesunate's excellent antimalarial property demonstrated by rapid parasite clearance, is enhanced by its high initial maximum concentration (C_{max}) and rapid hydrolysis to its active metabolite DHA. High plasma concentrations of artesunate and DHA are achieved within 5 minutes of drug administration and artesunate is rapidly cleared from circulation. The C_{max} for both artesunate and DHA are observed rapidly post dose administration indicating rapid conversion of artesunate to DHA. Artesunate is cleared from circulation rapidly while DHA has a longer elimination half-life ($t_{1/2}$). Accurate measurement of plasma concentrations of artesunate and DHA should guide adequate dosing as well as facilitate differentiation of treatment failure due to inadequate drug exposure from failure due to drug resistance.

2.2 Rationale

Current dosing regimens for IV artesunate are based on time to parasite clearance and not clinical endpoints related to physiologic changes that occur with parasitemia. Other measures of physiologic dysfunction can be used as study endpoints for a malaria treatment outcome analysis of current regimens for IV artesunate for severe malaria treatment. Improved dosing regimens will lead to fewer complications and likely fewer deaths. Such a strategy is needed to further reduce public health gains against malaria mortality that seem to have recently stalled.

For pediatric patients, IV artesunate dosing is weight-based to adjust for artesunate clearance and has been developed based on time to parasitemia clearance times, which may not completely reflect clinical cure. The most recent WHO 2015 guidelines state that children weighing <20 kg should receive IV artesunate as 3.0 mg/kg/dose compared to children weighing ≥20 kg and adults who should receive 2.4 mg/kg/dose at times 0, 12, 24, 48 and 72 hours (WHO 2015), Parenteral treatment should be given for a minimum of 24 hours (irrespective of the patient's ability to tolerate oral medication earlier), after which patients should be evaluated clinically and assessed ability to take medications orally. Children who are able to transition to oral

antimalarial therapy will initiate a 3-day course of oral artemisinin-combination therapy per national guidelines. The December 2016 CDC guidelines recommended 2.4 mg/kg/dose for all pediatric and adult patients, regardless of weight (CDC 2016), while the recently updated October 2019 CDC guideline recommends the same weight-based dosing scheme as the WHO (CDC 2019). A recent study of severe malaria in sub-Saharan African children documented equivalent time to parasite clearance using 2.4 mg/kg/dose at 0, 12, 24, 48 and 72 hours; and a larger 4.0 mg/kg/dose at 0, 24 and 48 hours.

P. falciparum parasites are known for their capacity to infect red blood cells that then display variant surface antigens that adhere to endothelial vasculature in target organs. For this reason, measurement of parasite clearance in the peripheral blood may not reflect physiologic effects of severe malaria that result from end organ damage, whether through direct impairment of blood flow and decreased delivery of oxygen, a local inflammatory response directed against infected red blood cells attached to vascular endothelium, or rosetting of infected and uninfected red blood cells at the vasculature. Other measures of physiologic dysfunction when quantified alone or in an algorithmic fashion such as a score, may more accurately reflect successful treatment, including serum lactate, serum glucose, bicarbonate levels, BCS, creatinine and hemoglobin. These measures, compared to the standard parasite clearance times from peripheral blood, may be more important to quantify and relate to pharmacologic dosing of antimalarial therapy.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

No significant additional risks are anticipated as participants will be receiving standard of care antimalarial therapy per national guidelines. Frequent blood draws are also common for patients with severe malaria. The amount of blood drawn for research purposes will be minimized to reduce added risk.

2.3.1.1 Risks from blood drawing

Risks associated with blood drawing include small risks of bleeding, hematoma and infection. To minimize this risk, the skin is cleaned with alcohol before puncture; sterile, single-use needles and lancets will always be used; and pressure will be held at the puncture site after removal of the needle or lancet. Trained technicians and medical staff will perform the procedure to reduce potential risk. Clinicians will be available for evaluation if there is any untoward effect.

2.3.1.2 Potential risk of loss of confidentiality

There is a potential risk of loss of confidentiality to study participants. Private health information recorded on participant case report forms (CRFs) could theoretically become available to non-

study personnel. To protect against this potential risk, the Principal Investigator (PI) will carefully monitor study procedures to protect the safety of participants and the quality of the data. Participant samples will be labeled with a participant ID number. The key to this number, and all private health information will be kept in a locked cabinet in a locked room that is accessible only to study personnel.

2.3.1.3 Risks to study personnel

The main risks to study personnel are from accidental exposure to blood and body fluid-borne infections. Standard Operating Procedures (SOPs) for staff safety are used in clinical and laboratory areas, including sharps management, hazardous waste management, etc. Universal precautions are used for handling all body fluids.

2.3.2 Known Potential Benefits

No direct benefit is anticipated for study participants. If this research is successful and an improved IV artesunate dosing regimen for treatment of severe malaria in children is developed, the community of Tororo District, Uganda and the rest of the *P. falciparum*-endemic world will benefit.

3 OBJECTIVES

3.1 Study Objectives & Outcome Measures

3.1.1 Primary Study Objective

- To determine the relationship between DHA exposures following IV artesunate dosing and markers of physiologic dysfunction associated with severe malaria in Ugandan children

3.1.1.1 Primary Outcome Measure

- DHA PK following the first dose of IV artesunate after enrollment, including C_{max}, area under the curve over hours 0-12 (AUC_{0-12}) and $t_{1/2}$ and time to C_{max} (T_{max})
- Physiologic measures such as temperature, blood pressure, venous serum lactate and bicarbonate levels, serum glucose, BCS, total and direct bilirubin, hemoglobin and creatinine

3.1.2 Secondary Study Objectives

- To determine the relationship between DHA exposures and time to hospital discharge
- To determine the relationship between DHA exposures and parasite clearance associated with treatment of severe malaria

3.1.2.1 Secondary Outcome Measures

- Time to hospital discharge
- Measures of parasite clearance calculated from parasite density, as measured by thick blood smear such as parasite clearance $t_{1/2}$, parasite clearance by Day 2, and time to 90% reduction in parasitemia

3.1.3 Exploratory Study Objectives

- To determine the relationship between DHA exposures and neurodevelopmental outcomes associated with treatment of severe malaria outcomes and explore predictors that may affect this relationship
- To evaluate the role of parasite clearance as a mediator of the relationship between DHA exposures and markers of physiologic dysfunction associated with severe malaria
- To develop a score comprised of markers of physiologic dysfunction and describe its relationship to clinical outcomes
- To assess *P. falciparum* infections for markers of artemisinin resistance

- To store blood for future use, such as identification and characterization of parasite gene expression.

3.1.3.1 Exploratory Outcome Measures

- Neurodevelopmental outcomes at 1 and 6 months after severe malaria diagnosis
- The proportion of the effect of DHA exposure on markers of physiologic dysfunction attributable to parasite clearance
- The association between the physiologic dysfunction score, resolution of symptoms, time to hospital discharge, and return to normal range in markers of physiologic dysfunction
- Presence of known K13 mutations associated with artemisinin resistance

4 STUDY DESIGN

This clinical study is a phase 4, single-site, open-label PK and pharmacodynamic (PD) study of IV artesunate in up to 100 Ugandan children 6 months-14 years of age who are diagnosed with severe malaria according to standardized WHO criteria (any *P. falciparum* parasitemia and the presence of danger signs).

Treatment
Participants will receive the standard of care with IV artesunate (Guilin Pharmaceutical Factory, Guangxi, People's Republic of China), for treatment of severe malaria per WHO guidelines. Artesunate will be reconstituted as follows:

- 30 mg vial of artesunate powder will be dissolved in 0.5 mL sodium bicarbonate and provided in the pack to form sodium artesunate and then mixed with 2.5 mL of sodium chloride (9mg/ml for injection).

- 60 mg vial of artesunate powder will be dissolved in 1 mL sodium bicarbonate and provided in the pack to form sodium artesunate and then mixed with 5 mL of sodium chloride (9mg/ml for injection).

- 120 mg vial of artesunate powder will be dissolved in 2 mL sodium bicarbonate and provided in the pack to form sodium artesunate and then mixed with 10 mL of sodium chloride (9mg/ml for injection).

This will be injected as a bolus into an indwelling IV cannula.

Dosage of Artesunate
Children weighing <20 kg will receive IV artesunate at a dose of 3.0 mg/kg/dose compared to older children weighing ≥ 20kg, who will receive 2.4 mg/kg/dose, at times 0, 12, 24, After the initial 24 hours of IV artesunate, children will be assessed clinically and for ability to take oral medications. If unable to take oral medication, IV artesunate will continue at 48 and 72 hours (WHO 2015). Children who recover and are able to transition to oral antimalarial therapy after a minimum of 24 hours or later, will initiate a 3-day course of oral artemisinin-combination therapy per national guidelines. Participants will be given standardized instructions by a nurse on how to take the artemether-lumefantrine regimen according to standard practice and told that all doses should be taken 30 minutes to 1 hour after food, preferably containing fat or oil.

Variables

Biomarkers of physiologic dysfunction will be quantified at regular intervals, including serum lactate, bicarbonate, serum glucose, total and direct bilirubin, creatinine, BCS and hemoglobin. These biomarkers will be considered both independently and together as a weighted score to relate to the PK of the active metabolite of IV artesunate, DHA and to efficacy markers that more accurately reflect clinical outcomes. We will also quantify *P. falciparum* parasite density using standardized thick blood smear and determine the presence of known artemisinin resistance K13 mutations and relate these outcomes to DHA dose and exposure for comparison with historical studies.

Study Population

Children 6 months-14 years of age living in or near Tororo District, Uganda, who are diagnosed with severe malaria and who meet inclusion and exclusion criteria will be enrolled.

Additional study design elements:

- Study monitoring delegated to IDI internal monitors and DMID or DMID representative
- Screening will be done within 24 hours before enrollment
- Data collection forms will serve as source documents. Only information that cannot be collected initially onto data collection forms (namely, clinical laboratory test results and adverse event (AE) medical records) will first be collected onto separate source documents before transcription into data collection forms. The information in the data collection form will then be entered directly into the Internet data entry system (IDES).
- Clinical laboratory evaluations will occur at the clinical laboratory onsite at the Lancet Laboratory
- Blood samples for PK assays will be processed and stored temporarily at the onsite laboratory at the Infectious Diseases Research Collaboration Laboratory at Tororo Hospital, before shipment to the Translation Laboratory, at the Infectious Diseases Institute, Mulago, Kampala (IDI Mulago). Frozen samples will be transported to the IDI Translation lab at the IDI in Kampala or the Clinical Pharmacology Analytical Laboratory at JHU, Baltimore, Maryland, USA, and stored there in temperature-monitored freezers.
- *P. falciparum* parasitemia will be quantified at the IDRC laboratory according to SOP.
- A dried blood spot will be obtained at enrollment for assessment of known K13 artemisinin resistance mutations. Specimens will be shipped to the University of Maryland, Baltimore (UMB) for parasite deoxyribonucleic acid (DNA) extraction and sequencing.
- PK samples for DHA will be quantified by liquid chromatography and tandem mass spectrometry (LC-MS/MS) (See Section 8.3.2).

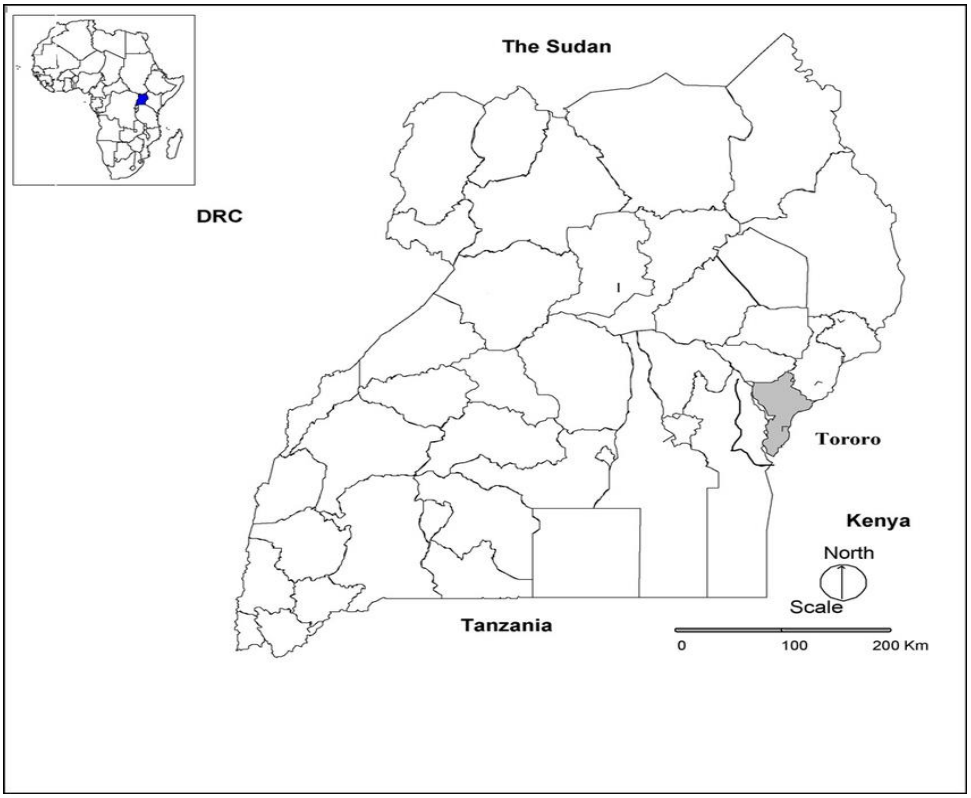
- Study duration will be 6 months per participant, and the entire clinical trial will last approximately 12 months
- 7-day surveillance (day of treatment initiation and 6 days thereafter) is planned for initial PK and biophysical parameter measurements
- Follow-up of serious adverse events (SAEs) until resolution or stability; follow-up of AEs until resolution or stability or until the end of follow-up.
- Once the last participant has completed the final visit, the clinical database will be cleaned and locked. The analysis of the primary and secondary outcome measures will be included in the Clinical Study Report (CSR) following database lock. Any available data from the exploratory endpoints may also be included. Additional exploratory endpoint data not available at the time of CSR preparation may be included in one or more addenda to the CSR.

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 Site Description

Participants for this study will be drawn from the population of children aged 6 months to 14 years residing in Tororo District, Uganda (***Figure 5.1***).

Figure 5.1: Map of Uganda showing Tororo district



Tororo District is a district in Eastern Uganda of 487,900 inhabitants. The town of Tororo serves as the district's headquarters and has a population of about 41,906 inhabitants.

Tororo has two rainy seasons in the year from March to May and from October to December. The primary economic activity in the district is agriculture. The predominant ethnic groups are the Itesot and Japhadhola. A number of languages are spoken including; Ateso, Japadhola, Swahili, Lugisu, and Lugwere . English is the official language of Uganda.

Tororo Hospital is a 200-bed public hospital funded by the Uganda Ministry of health. General care in the hospital is free of charge.

5.2 Subject Inclusion Criteria

1. Children ages 06 months-14 years at the time of severe malaria diagnosis, inclusive
2. Meet the case definition for severe malaria, per WHO standardized guidelines
3. Parent/guardian willing to provide informed consent
4. Assent for children between 8 and 14 years who are conscious and otherwise able to provide assent, inclusive

5.3 Subject Exclusion Criteria

1. Receipt of >24 hours of artemisinin therapy

5.4 Treatment Procedures

Participants will receive IV artesunate for severe malaria according to national guidelines. After a minimum of 24 hours and when they can tolerate oral therapy, they will transition to oral ACT, also per national guidelines. No blinding of study participants, their parent/guardian, or study staff will be done as this is an open label PK study of standard of care IV artesunate.

5.4.1 Reasons for Withdrawal

The following criteria will be checked before IV artesunate administration. If any become applicable before completion of a study product administration regimen, further IV artesunate will not be administered but the participant will be followed for the duration of the study. If any become applicable during the study but after an IV artesunate administration regimen is completed, the participant will not be required to discontinue the study.

- Grade 3 hypersensitivity to IV artesunate
- Severe side effects following IV artesunate administration

A child between 8-14 years may withdraw or a parent/guardian of a study participant may withdraw a participant voluntarily from continuing study follow-up upon request for any reason.

5.4.2 Handling of Withdrawals

Every effort will be made to collect data on any participant discontinued from receipt of additional IV artesunate for any reason. If possible, participants who leave the study area will be traced and visited by clinical investigators to collect safety follow-up data.

5.4.3 Termination of Study

The trial may be suspended or terminated by DMID or by the PI due to any major safety concern identified by the independent safety monitor (ISM). The trial may also be suspended by the IRBs if deemed necessary.

6 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

6.1 Study Product Description

Artesunate for Injection

Artesunate for injection that is WHO-prequalified, procured by the Infectious Diseases Institute (IDI), and kept under the proper storage conditions at the clinical sites will be used. This is currently the first-line drug used by the Ministry of Health Uganda for treatment of severe malaria, and no potential safety concerns exist as we plan to use the WHO-recommended dosing regimen.

6.1.1 Acquisition

Study drug will be procured by the IDI.

6.1.2 Formulation, Packaging, and Labeling

Artesunate for injection appears as a white, crystalline powder.
Solvent (sodium bicarbonate injection) appears as a clear, colorless liquid.

Each 30 mg box contains:

- 1 vial of 30 mg artesunate powder for solution for injection,
- 1 ampoule of 0.5 mL sodium bicarbonate 50 mg/mL solution for injection, and
- 1 ampoule of 2.5 mL sodium chloride 9 mg/mL for injection;

Each 60 mg box contains:

- 1 vial of 60 mg artesunate powder for solution for injection,
- 1 ampoule of 1 mL sodium bicarbonate 50 mg/mL solution for injection, and
- 1 ampoule of 5 mL sodium chloride 9 mg/mL for injection;

Each 120 mg box contains:

- 1 vial of 120 mg artesunate powder for solution for injection,
- 1 ampoule of 2 mL sodium bicarbonate 50 mg/mL solution for injection, and
- 1 ampoule of 10 mL sodium chloride 9 mg/mL for injection;

6.1.3 Product Storage and Stability

Artesunate for injection should be stored at <30 degrees Celsius and protected from light. The reconstituted solution should be stored at <30 degrees Celsius and should be used within 1 hour. Artesunate will be procured by IDI, transported under monitored temperature conditions to Tororo site and stored in the Infectious Diseases Research Collaboration (IDRC) Pharmacy which has adequate 24-hour temperature monitoring. It will be dispensed by the study nurse.

6.2 Dosage, Preparation and Administration of Study Intervention/Investigational Product

Artesunate for injection will be administered by study nursing staff per Ministry of Health Uganda guidelines.

6.3 Modification of Study Intervention/Investigational Product for a Participant

Dosing of IV artesunate will not be adjusted due to toxicity or other reasons. If a participant experiences toxicity related to IV artesunate, then the investigators will follow Ministry of Health Uganda guidelines for alternative treatment of severe malaria.

6.4 Accountability Procedures for the Study Supply of IV Artesunate

The study team will procure a supply of artesunate for injection and will maintain this study stock in a temperature-controlled area with daily temperature monitoring at the IDRC Pharmacy. The Site PI is responsible for the distribution and disposition of the study stock of artesunate for injection, and has ultimate responsibility for accountability. The Site PI may delegate to the study coordinator responsibility for study product accountability. The study coordinator will be responsible for maintaining complete records and documentation of study product receipt, accountability, dispensation, temperature monitoring, storage conditions, and final disposition of artesunate for injection. All artesunate for injection, whether administered or not, must be documented on the appropriate study product accountability record or dispensing log. Unused study product will be retained as per DMID requirements.

Upon completion of the study and after the final monitoring visit, any remaining unused study product will be donated to the hospital, or in accordance with disposition plans. Further details regarding final accountability and disposition of used and unused study product are included in the protocol-specific Manual of Procedures (MOP).

6.5 Concomitant Medications/Treatments

At enrollment and at subsequent study visits, investigators will question the participant's parent/guardian about any medication taken, including traditional, herbal, supplements and over-the-counter medicines. Concomitant medication, including any administered during the period starting from 7 days before enrollment and ending at the end of the study follow-up period will be recorded with trade name and/or generic name of the medication, medical indication, start and end dates of treatment.

The study team will procure a supply of medications for treatment of common concurrent ailments that patients with severe malaria may develop such as paracetamol for fever, and antibiotics for sepsis but these should not have any antimalarial effects. Antibiotics such as cotrimoxazole which are known to have some antimalarial effects will not be administered for such ailments; however, participants who are taking concomitant medications that have some antimalarial effect for conditions in which the medication is standard of care may be continued, including but not limited to Septrin (cotrimoxazole) in HIV coinfected children. This stock of concomitant medications will ensure that study participants do not buy or receive additional medications outside the study or without the knowledge of the study physicians. The site PI will ensure that this additional study stock of concomitant medications is stored in a temperature-controlled area at the IDRC Pharmacy, with daily temperature monitoring. The Site PI is responsible for the distribution and disposition of the study stock of concomitant medications and has ultimate responsibility for accountability. The Site PI may delegate to the study coordinator responsibility for study concomitant drug accountability. The study coordinator will be responsible for maintaining complete records and documentation of study concomitant drug receipt, accountability, dispensation, temperature monitoring, storage conditions, and final disposition of drugs. All drugs whether administered or not, must be documented on the appropriate study concomitant drug accountability record or dispensing log. Unused study drug will be retained as per DMID requirements.

7 STUDY SCHEDULE

7.1 Screening

Starting at the emergency department of the hospital, participants diagnosed by the attending physician and have received a prescription of artesunate for severe malaria will be screened for study eligibility and enrolled.

Recruitment will be progressive over a single malaria transmission season so that up to 100 children of either sex who fulfill the inclusion criteria are enrolled. Participants will be recruited by non-coercive methods among children aged 6 months-14 years residing near the study site. A parent/guardian of a child diagnosed with severe malaria will be informed of the study to determine if they are interested to have their child participate. Children who are of the age of assent (age 8 in Uganda) and above and who are conscious will also be informed of the study to determine if they are interested. Informed consent of a parent/guardian and assent for children $\geq$8 and <18 years of age who are conscious at enrollment and otherwise able to provide assent is required for enrollment. After the study has been explained to a potential participant's parent/guardian, and to a potential participant in the case of a conscious child aged 8-14 years old, they will be provided with a copy of the consent form and assent form if applicable. The consent/assent process will be conducted in a semi-private area to ensure confidentiality, to reduce the likelihood of other participants influencing their decision, and to allow further time to make a final decision. A single informed consent document, and a single assent document when applicable, will be used for screening and other study procedures. Only participants who receive a first dose of IV artesunate are considered enrolled in the study. Administration of artesunate therapy will not be delayed for enrollment procedures. Participants may still be enrolled up to 24 hours after first dose of IV artesunate. In the case of unconscious children who are the age of assent, only informed consent of a parent/guardian is required for enrollment. Once the child becomes conscious, assent will be obtained from the child to determine if their participation in the study will continue.

All screening tests, medical history and examinations will be performed only after study consent/assent where applicable is obtained. If an individual's medical chart or results diagnostic tests performed as part of an individual's medical care are going to be used for screening, written informed consent must be obtained before review of that information.

An eligibility checklist will be prepared for each participant and will later become part of the source document for participants enrolled in the trial. A unique identification number will be assigned to each study participant. A medical history will be taken. Concomitant medications will be documented. Physical examination (including Blantyre coma score (BCS)) and laboratory

screening tests will include: hemoglobin, platelets, white blood cell (WBC) count, neutrophil count; serum lactate, bicarbonate, blood urea nitrogen (BUN), calcium, creatinine, glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total and direct bilirubin, sodium, and potassium; and urinalysis. Laboratory testing will be performed at the Lancet laboratory at the study site and at a local reference laboratory if needed. Results obtained within 24 hours prior to enrollment can be included as baseline labs and would satisfy both screening and enrollment/baseline labs. A thick and thin blood smear will be performed for estimation of parasite density. A dried blood spot specimen will be collected for artemisinin resistance assessment. A blood specimen will be collected in a PAXgene tube for *P. falciparum* gene expression analysis. Recruitment will continue over a single malaria transmission season until up to 100 eligible participants have fulfilled all the inclusion criteria and none of the exclusion criteria.

7.2 Enrollment/Baseline

On study day 1, study staff will collect baseline blood and urine samples, conduct a physical exam and document vital signs (axillary temperature, respiratory rate, blood pressure, pulse), and record medication and medical history. Results of baseline blood and urine samples are not necessarily resulted and reviewed before IV artesunate. After they receive their first dose of IV artesunate, participants are considered enrolled into the study.

A complete physical examination, including neurological assessment and determination of the BCS, will be performed and documented at least daily for each patient while hospitalized. Vital signs (pulse, respiratory rate, blood pressure, and axillary temperature will be measured approximately every 6 h until 6 h post parasite clearance.

Additional details of the schedule of events are included in the Study Schedule (Table 19.1).

7.3 Follow-up

Details of the schedule of events, including laboratory analyses, are included in the summary of study procedures (Section 8.1) and Schedule of Events (Table 19.1). If a subject is discharged before study day 7, the required follow-up visits are study day 7, 14, 28, and 183.

7.4 Final Study Visit

The final study visit should occur six months after study treatment administration initiation (study day 183±14 days).

Evaluations to be done during the final study visit are listed in the daily study procedures and in the summary of study procedures. Procedures include the following:

- Medical history including concomitant medication review
- Physical exam, including vital signs, height, and weight
- Venous blood collection for hemoglobin, platelets, WBC count, and neutrophil count
- Neurodevelopmental assessments

7.5 Early Termination Visit

If a participant wishes to end their participation early and is willing to have evaluations performed, a targeted physical examination should be done if needed. If termination occurs before or on study day 7, then study day 7 blood collections should be obtained. If termination occurs between study days 8-14, 3 mL venous blood may be drawn for serum bicarbonate, hemoglobin, platelets, WBC count, and neutrophil count. If termination occurs after study day 14, 1 mL venous blood may be drawn for hemoglobin, platelets, WBC count, and neutrophil count.

Investigators will make every effort to continue follow-up visits for any participant who has received one or more doses of IV artesunate for the duration of the study even if it is determined that subsequent IV artesunate should not be administered.

7.6 Unscheduled Visit

Unscheduled visits may occur at any time during the trial and may prompt a history and targeted physical examination when indicated, clinical laboratory tests if indicated, documentation of any AEs, and any other medically indicated diagnostic or therapeutic procedures. These visits will be recorded as observations in the participant's study record.

8 STUDY PROCEDURES/EVALUATIONS

8.1 Daily Study Procedures

Screening (may take place over more than one visit) up to 24 hours before enrollment

- Written assent for children ≥ 8 years as applicable
- Informed consent obtained from the parent
- Check of inclusion and exclusion criteria
- Participant identification number assigned
- Demographics and medical history of participant
- Concomitant medication documentation
- Physical examination and vital signs (pulse, respiratory rate, blood pressure, axillary temperature)
- Document height and weight
- BCS
- Urinalysis
- Collect 8.25 mL venous blood sample for:
  - Hematology: hemoglobin, platelets, WBC count, neutrophil count
  - Biochemistry: serum creatinine, AST, ALT, total and direct bilirubin, bicarbonate, lactate, sodium, potassium, glucose, BUN, calcium
  - *P. falciparum* parasite quantification
  - Artemisinin resistance
  - Population PK study per sampling plan specified in Section 8.3.1
  - *P. falciparum* mRNA collection using PAXgene tube

Day 1 Enrollment

- Physical examination and vital signs (pulse, respiratory rate, blood pressure, axillary temperature every 6 hours until 6 hours post-parasite clearance)
- Urinalysis

- An IV cannula will be inserted for administration of study drugs and for PK blood sampling and blood draws.
    - Collect venous blood samples for population PK study per sampling plan specified in Section 8.3.1
    - Administration of IV artesunate dose in accordance with WHO and Uganda guidelines
- Collect 11.5 mL venous blood sample for:
    - Hematology: hemoglobin, platelets, WBC count, neutrophil count (at least 12 hours after initial screening collection)
    - Biochemistry: serum creatinine, AST, ALT, total and direct bilirubin, serum bicarbonate, lactate, sodium, potassium, glucose, BUN, calcium (at least 12 hours after initial screening collection)
    - Population PK study per sampling plan specified in Section 8.3.1
    - *P. falciparum* parasite quantification
- Concomitant medication review
- BCS

Day 2 Follow-up

- Physical examination and vital signs (pulse, respiratory rate, blood pressure, axillary temperature)
- Administration of IV artesunate dose or ACT in accordance with WHO and Uganda guidelines
- Urinalysis
- Collect 3.5 mL venous blood sample for:
    - Hematology: hemoglobin, platelets, WBC count, neutrophil count
    - Biochemistry: serum creatinine, AST, ALT, total and direct bilirubin, serum bicarbonate, lactate, sodium, potassium, glucose, BUN, calcium
    - *P. falciparum* parasite quantification
- Concomitant medication review
- BCS

Day 3 Follow-up

- Physical examination and vital signs (pulse, respiratory rate, blood pressure, axillary temperature)
- Administration of IV artesunate dose or ACT in accordance with WHO and Uganda guidelines
- Urinalysis
- Collect 3.25 mL venous blood sample for:
  - Hematology: hemoglobin, platelets, WBC count, neutrophil count
  - Biochemistry: serum creatinine, AST, ALT, total and direct bilirubin, serum bicarbonate, lactate, sodium, potassium, glucose, BUN, calcium
  - *P. falciparum* parasite quantification
- Concomitant medication review
- BCS

Day 4 Follow-up

- Physical examination and vital signs (pulse, respiratory rate, blood pressure, axillary temperature)
- Administration of IV artesunate dose or AL in accordance with WHO and Uganda guidelines
- Urinalysis
- Collect 3.25 mL venous blood sample for:
  - Hematology: hemoglobin, platelets, WBC count, neutrophil count
  - Biochemistry: serum creatinine, AST, ALT, total and direct bilirubin, serum bicarbonate, lactate, sodium, potassium, glucose, BUN, calcium
  - *P. falciparum* parasite quantification
- Concomitant medication review
- BCS

Day 5 Follow-up

- Physical examination and vital signs (pulse, respiratory rate, blood pressure, axillary temperature)

- Administration of IV artesunate dose or ACT in accordance with WHO and Uganda guidelines
- Urinalysis
- Collect 3.25 mL venous blood sample for:
  - Hematology: hemoglobin, platelets, WBC count, neutrophil count
  - Biochemistry: serum creatinine, AST, ALT, total and direct bilirubin, serum bicarbonate, lactate, sodium, potassium, glucose, BUN, calcium
  - *P. falciparum* parasite quantification
- Concomitant medication review
- BCS

Day 6 Follow-up

- Physical examination and vital signs (pulse, respiratory rate, blood pressure, axillary temperature)
- Urinalysis
- Collect 3 mL venous blood sample for:
  - Hematology: hemoglobin, platelets, WBC count, neutrophil count
  - Biochemistry: serum creatinine, AST, ALT, total and direct bilirubin, serum bicarbonate, lactate, sodium, potassium, glucose, BUN, calcium
- Concomitant medication review
- BCS

Day 7 (±2 days) Follow-up

- Physical examination and vital signs (pulse, respiratory rate, blood pressure, axillary temperature)
- Urinalysis
- Collect 3 mL venous blood sample for:
  - Hematology: hemoglobin, platelets, WBC count, neutrophil count
  - Biochemistry: serum creatinine, AST, ALT, total and direct bilirubin, serum bicarbonate, lactate, sodium, potassium, glucose, BUN, calcium

- Concomitant medication review
- BCS
- Neurodevelopmental assessments

Day 14 (±2 days) Follow-up

- Concomitant medication review
- Record blood pressure, pulse, respiratory rate and axillary temperature
- Targeted physical examination if needed
- BCS
- Neurodevelopmental assessments
- Collect 3 mL venous blood sample for hemoglobin and serum bicarbonate

Day 28 (±4 days) Follow-up

- Concomitant medication documentation
- Record blood pressure, pulse, respiratory rate, axillary temperature, height and weight
- Targeted physical examination if needed
- BCS
- Neurodevelopmental assessments
- Collect 1 mL venous blood sample for hemoglobin

Day 183 (±14 days) Final Study Visit

- Concomitant medication review
- Physical exam, including blood pressure, pulse, axillary temperature, height, and weight
- Collect 1 mL venous blood for hemoglobin, platelets, WBC count, and neutrophil count
- BCS
- Neurodevelopmental assessments

8.2 Clinical Evaluations

8.2.1 Neurodevelopmental Assessments

8.2.1.1 Children 6 months to 6 years of age at time of assessment will undergo testing using the Malawi Developmental Assessment Testing. Details are provided in a separate SOP.

8.2.1.2 Children >6 years of age will not undergo neurodevelopmental assessment

8.3 Laboratory Evaluations

8.3.1 Clinical Laboratory Evaluations

Malaria Diagnostics

Samples for malaria diagnostics will be collected according to the following schedule in hours: 0 (0-2 hour acceptable window), 6 (4-8 hour acceptable window), 12 (10-14 hour acceptable window), 24 (22-26 hour acceptable window), 36 (34-38 hour acceptable window) and 48 (46-50 hour acceptable window), and then parasitemia should be measured at least every 24 hours until clearance. Thick and thin blood smears for malaria diagnosis will be stained with Giemsa stain and read at 100X power light microscope. Parasites will be quantitated by counting the number of asexual *P. falciparum* parasites per 200 WBC using the patient's WBC count per μL of blood. Thick blood smears will be used for diagnosis and thin blood smears for typing the species of malaria parasites. Thin and thick blood smears will be repeated on all follow up days because the different species of malaria parasites have different incubation periods.

Collection of blood for Artesunate Pharmacokinetic assays

Approximately 10mLs (~2 mL/timepoint) of venous blood will be collected into fluoride-oxalate tubes from the arm opposite of that used for drug administration at 0 (pre-dosing), and 4 times following the first dose in the hospital during different sampling windows. Sampling windows are defined for post-dose collections: 0-1 hour, 1-2.5, 2.5-4, 4-6 and 6-24 hours post-dose. Participants will be randomly assigned to either the 4-6 hour or the 6-24 hour collection timepoint via the Screening and Enrollment Log. All blood samples will be chilled immediately to prevent artesunate degradation by plasma esterases. Samples will be centrifuged within 30 minutes to minimize hemolysis, and aliquots of plasma will be stored at -80°C (-60°C or colder acceptable). Plasma aliquots will be shipped intermittently to the IDI Core Laboratory for storage prior to quantification. Plasma samples will be assayed by a validated high-performance liquid chromatography (HPLC) method.

Diagnosis of Severe Malaria

Severe malaria is defined as presence or history of fever plus a positive blood film for *P. falciparum* malaria with at least one of the following symptoms and signs:

- Impaired consciousness: A Glasgow coma score < 11 in adults or a Blantyre coma score < 3 in children
- Prostration: Generalized weakness so that the person is unable to sit, stand or walk without assistance
- Multiple convulsions: More than two episodes within 24 h
- Acidosis: A base deficit of > 8 mEq/L or, if not available, a plasma bicarbonate level of < 15 mmol/L or venous plasma lactate ≥ 5 mmol/L. Severe acidosis manifests clinically as respiratory distress (rapid, deep, laboured breathing).
- Hypoglycaemia: Blood or plasma glucose < 2.2 mmol/L (< 40 mg/dL)
- Severe malarial anaemia: Haemoglobin concentration ≤ 5 g/dL or a haematocrit of ≤ 15% in children < 12 years of age (< 7 g/dL and < 20%, respectively, in adults) with a parasite count > 10 000/μL
- Renal impairment: Plasma or serum creatinine > 265 μmol/L (3 mg/dL) or blood urea > 20 mmol/L
- Jaundice: Plasma or serum bilirubin > 50 μmol/L (3 mg/ dL) with a parasite count > 100 000/ μL
- Pulmonary oedema: Radiologically confirmed or oxygen saturation < 92% on room air with a respiratory rate > 30/ min, often with chest indrawing and crepitations on auscultation
- Significant bleeding: Including recurrent or prolonged bleeding from the nose, gums or venepuncture sites; haematemesis or melaena
- Shock: Compensated shock is defined as capillary refill ≥ 3 s or temperature gradient on leg (mid to proximal limb), but no hypotension. Decompensated shock is defined as systolic blood pressure < 70 mm Hg in children or < 80 mmHg in adults, with evidence of impaired perfusion (cool peripheries or prolonged capillary refill).
- Hyperparasitaemia: *P. falciparum* parasitaemia > 10%

Blood samples for hematologic and biochemical assays

Serum biochemistries will be examined on site using a COBAS C111 Analyzer. Full blood count will be measured with a Haematology - Mindray Shenzhen analyzer.

Urinalysis

Urine will be collected into a clean container. A dipstick will be used that measures a variety of analytes, including pH, urobilinogen, bilirubin, protein, glucose, hemolyzed and nonhemolyzed blood, nitrites, ketones, and leukocyte esterase.

8.3.2 Special Assays or Procedures

DHA analyte

Blood Samples for IV artesunate/DHA will be collected in fluoride-oxalate tubes. Blood samples will be delivered within 15 min of collection to the Infectious Diseases Research Collaboration laboratory in Tororo Hospital for plasma separation. Aliquots of plasma will be stored at -80°C. Plasma aliquots will be shipped intermittently to the IDI Core Laboratory for storage prior to quantification. Aliquots will be tested at either at the IDI Core Laboratory or at the Clinical Pharmacology Analytical Laboratory at JHU, Baltimore, Maryland, USA.

Dried blood spot for known K13 artemisinin resistance mutations

A dried blood spot will be obtained at enrollment for assessment of known K13 artemisinin resistance mutations. Specimens will be shipped to the University of Maryland, Baltimore (UMB) for parasite deoxyribonucleic acid (DNA) extraction and sequencing. The presence of K13 mutations known to be associated with artemisinin resistance will be determined for each specimen.

RNA preservation for parasite variant surface antigen expression analysis

Venous blood will be collected in PAXgene tubes at screening for future analysis of preserved parasite RNA. RNA will be analyzed to determine the predominant variant surface antigens expressed by malaria parasites, allowing for classification of severe malaria cases in this regard.

Other special procedures including neurodevelopmental assessments and BCS will be included in the study MOP and/or SOPs.

8.3.3 Specimen Preparation, Handling, and Shipping

Samples for DHA will be processed using solid-phase extraction and DHA will be quantified by LC MS/MS with an assay validated according to FDA guidelines.

8.3.3.1 Instructions for Specimen Preparation, Handling, and Storage

Detailed SOPs are maintained for these activities. Briefly, blood samples are obtained and processed in the sample processing laboratory according to SOPs. Plasma is frozen in freezers according to SOPs. Dried blood spot specimens will be shipped to the UMB. Frozen samples will be transported to the IDI Translation lab at the IDI in Kampala, and stored there in temperature-monitored freezers or shipped to the DMID clinical material services (DMID-CMS). International Air Transport Association (IATA) guidelines will be followed for specimen handling, transport and shipping.

8.3.3.2 Specimen Shipment

The Investigators will maintain detailed SOPs for specimen transport and storage. All staff and investigators will be trained in the SOPs relevant to their duties and will sign copies of the SOPs to document this training. Copies of SOPs will be available for inspection and review by DMID and study monitors. During the study, SOPs may be modified to improve them, and new SOPs may be developed as needed to improve operations and ensure adherence with the protocol.

9 ASSESSMENT OF SAFETY

9.1 Specification of Safety Parameters

No safety outcome measures are planned for this trial.

9.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

9.2.1 Adverse Events

An AE includes any noxious, pathological or unintended change in anatomical, physiological or metabolic functions as indicated by physical signs, symptoms and/or laboratory-detected changes occurring in any phase of the clinical study whether associated with the study product and whether or not considered related to the intervention. This definition includes an exacerbation of pre-existing conditions or events, intercurrent illnesses, or drug interaction. Anticipated day-to-day fluctuations of pre-existing conditions that do not represent a clinically significant exacerbation need not be considered AEs. Discrete exacerbations of chronic conditions that are deemed to be different than regularly sustained day-to-day fluctuations, occurring during a study period will be reported as AEs in order to assess changes in frequency or severity.

AEs will be documented in terms of a medical diagnosis. When this is not possible, the AE will be documented in terms of signs and/or symptoms observed by the investigator or reported by the subject at each study visit. Abnormalities in clinical findings and in clinical laboratory testing will only be captured as AEs after the baseline visit. Pre-existing conditions or signs and/or symptoms (including any which are not recognized at study entry but are recognized during the study period) present in a participant before the start of the study will be recorded on the participant's CRF and will be recorded as an AE if deterioration or exacerbation in the condition occurs during the study. Any inpatient overnight hospitalization other than the inpatient stay for severe malaria treatment will be considered an SAE. Information to be collected include event description, date of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), relationship to study procedures and date of resolution/stabilization of the event. All AEs will be followed to adequate resolution or stabilization.

All AEs must be graded for severity.

FDA defines AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Severity of Event: All AEs will be assessed by the PI or appropriate sub-investigator using the Division of AIDS (DAIDS) Table for Grading of Severity of Adults and Pediatric Adverse Events. For unsolicited events not included in the DAIDS Table, the following guidelines will be used to quantify intensity:

- Mild (Grade 1)- events require minimal or no treatment and do not interfere with the participant's daily activities.
- Moderate (Grade 2)- events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning and daily activities.
- Severe (Grade 3)– events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

If an AE changes in severity, it is not a new event, and each AE is documented at its highest severity grading. AEs characterized as intermittent require documentation of onset and duration of each episode.

9.2.2 Serious Adverse Events

An AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death,
- A life-threatening AE*,
- 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 hospitalizations 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 AE. An AE is considered “life-threatening” if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an AE, had it occurred in a more severe form, might have caused death.

All SAEs will be:

- recorded on the appropriate SAE form and electronic case report form (eCRF)
- reviewed and evaluated by an ISM, DMID, and the IRB, if indicated

9.3 Reporting Procedures

AEs and SAEs will be documented from the first study intervention, through the end of the follow-up period.

All AEs and SAEs will be captured on the appropriate data collection form. Information to be collected includes event description, date of onset, investigator assessment of severity, relationship to study product, date of resolution of the event, and outcome.

9.3.1 Serious Adverse Events

AEs will be followed to adequate resolution or stabilization for participants enrolled in the study. After termination of the study, participants will be referred to appropriate care for follow-up of ongoing AEs. Any AE that is associated with the study drug will be followed to resolution or stabilization with the expectation that it will remain chronic. Any AE that meets a protocol-defined serious criterion must be submitted to DMID within 24 hours of site awareness via the IDES or a DMID SAE form. SAE information is reported via the Adverse Event eCRF in Advantage eClinical. At the time of submission of the AE eCRF in Advantage eClinical, an automatic email will be sent to the DMID Medical Monitor, DMID, and the study PI, alerting them to the occurrence of an SAE. No further steps are necessary to notify the DMID Medical Monitor. Updates to the SAE should be reported via the AE eCRF.

In addition to the reporting to DMID, the investigator must also report to the ethics committee and IRB. Any AE that meets a protocol-defined serious criterion and is considered related to the investigational product must be submitted, unless otherwise requested by the ISM, sponsor or the investigators:

- Within 48 hours to the Uganda IRB
- Within 5 days to the University of Maryland IRB

For other AEs that meet a protocol-defined serious criterion and are considered not related to the investigational product, this information can be submitted, unless otherwise requested by the ISM, sponsor or the investigators:

- In the annual summary to the Uganda IRB
- In the annual summary to the University of Maryland IRB

9.4 Type and Duration of Follow-up of Subjects after Adverse Events

Investigators will follow up subjects with AEs and SAEs until the event has resolved, or, if a chronic condition has developed, until the end of the study follow-up period. Outcome will be assessed as Recovered/Resolved, Recovered/Resolved with Sequelae, Recovering/Resolving, Not recovered/Not resolved, or Fatal.

9.5 Halting Rules

Decisions to halt or pause the study will be made by the host REC and DMID.

Subsequent review of serious, unexpected, and related AEs by the Medical Monitor, ethics review committee, the sponsor, or relevant local regulatory authorities may also result in suspension of further trial interventions/administration of study product at a site. The host REC and study sponsor retain authority to suspend additional enrollment and study interventions/administration of study product for the entire study, as applicable.

9.6 Safety Oversight (ISM)

A Safety Monitoring Committee is not required for this protocol.

An ISM is a physician with relevant expertise whose primary responsibility is to provide to DMID an independent safety assessment in a timely fashion. Participation is for the duration of the DMID study and is a voluntary position that does not receive payment.

The ISM:

- Is near the study site and has the authority and ability to readily access study participant records in real time.
- May be a member of the participating institution’s staff but preferably be from a different organizational group within the institution.
- Should not be in a direct supervisory relationship with the investigator.
- Should have no direct involvement in the conduct of the study.

The ISM will:

- Sign a Conflict of Interest (COI) certification at the time they are asked to participate and provide updates to this information as needed.
- Receive reports of SAEs from the site investigator and will be notified by email when the REC and DMID are notified of the SAE.
- Evaluate the SAE and report their clinical assessment to the REC and DMID, through DMID-Clinical Research Operations and Management Support (CROMS) Safety Oversight Committee Support (SOCS) in a timely manner and email the report to REC and DMID-CROMS SOCS.
- Communicate with the investigator at the participating site as needed.
- Review additional safety related events at the request of the REC and DMID.
- Provide additional information to REC and DMID by teleconference as requested.

10 CLINICAL MONITORING

10.1 Site Monitoring Plan

Study site monitoring will be conducted by the internal IDI monitor and DMID clinical monitoring contractor to ensure that the conduct of the trial is in compliance with the currently approved protocol, amendment(s), ICH, sponsor requirements GCP standards, and regulatory guidelines are being followed. Clinical monitoring will also verify that any critical study procedures are completed following specific instructions in the protocol-specified MOP. A pre-trial monitoring visit will be made to the study site, including the clinical laboratory. All records will be made available to monitors, including regulatory files, CRFs and source documents, quality assurance (QA)/quality control (QC) documentation, SOPs, etc. Additional study site visits may be made during the trial and at the end of the surveillance period.

In addition, monitors from the University of Maryland may make study site visits, in coordination with the primary monitoring group designated by DMID.

In conjunction with the clinical monitoring contractor designated by the sponsor, a detailed monitoring plan will be developed. This document describes who will conduct the monitoring, at what frequency monitoring will be done, and what level of detail monitoring will be conducted. The clinical monitoring plan will be written by DMID and the DMID clinical monitoring contractor. This separate monitoring plan will be agreed upon with the Office of Clinical Research Affairs (OCRA) and will describe protocol-specific items to be monitored. The monitoring plan will include the number of participant charts to be reviewed, which/what proportion of data fields and what will be monitored, who will be responsible for conducting the monitoring visits, and who will be responsible for ensuring that monitoring findings are addressed. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, electronic case report forms (eCRFs), ICFs, medical and laboratory reports, site study intervention storage records, training records, and protocol and GCP compliance. Site monitors will have access to each participating site, study staff and all study documentation according to the DMID-approved CMP. Study monitors will meet with all participating site PIs to discuss any problems and outstanding issues and will document site visit findings and discussions.

11 STATISTICAL CONSIDERATIONS

11.1 Study Hypothesis

The primary objective of this study is to determine the relationship between DHA exposures following IV dosing and markers of physiologic dysfunction associated with severe malaria. This objective can be framed as a series of statistical hypothesis tests, each evaluating the effect of the DHA exposure parameter on the marker of physiologic dysfunction, measured at a specific timepoint, while adjusting for covariates. The null hypothesis is that there is no effect of exposure on response, and the alternative hypothesis is that there is an effect. The secondary objectives can be framed in an analogous manner, with time to hospital discharge and measures of parasite clearance as the response variables.

11.2 Sample Size Considerations

A maximum of 100 subjects would provide sufficient sample size to achieve precision on PK parameters following the FDA pediatric precision criteria for conducting PK studies (achieving a 95% confidence interval (CI) within 60% and 140% of the geometric mean).

Simulations in R version 3.4.2 were used to explore power to detect simple linear relationships between exposure and response in the analysis of the primary objective with the planned sample size. The primary objective of this study encompasses multiple exposure and response variables, and the response variables are measured at multiple timepoints. Thus, there are multiple exposure-response relationships that are of interest (one for each unique combination of exposure variable, response variable, and timepoint), and there is a need to control type I error. As this study is exploratory (hypothesis-generating) rather than confirmatory, Benjamini-Hochberg procedure is used to control the false discovery rate at 0.05 (Benjamini 1995).

For a multiple hypothesis test, average power is defined as the proportion of false null hypotheses that were correctly rejected (Benjamini 1999). Table 11.1 provides the average power computed from 5000 simulations, varying the following quantities:

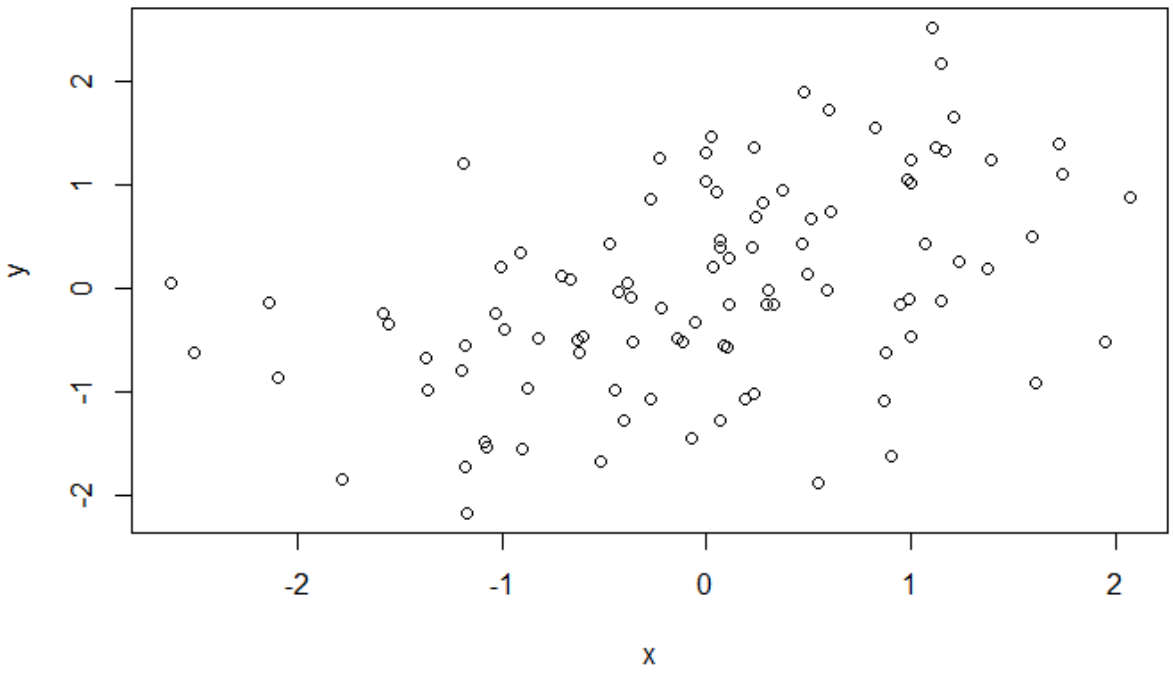
- Number of total statistical tests, representing the number of combinations of exposure variable, response variable, and timepoint to be tested in support of the primary objective
- Value of R^2 for the true, non-null exposure-response relationships
- Proportion of tests with true, non-null exposure-response relationships with the specified R^2
- Sample size

Table 11.1: Average Power for Identifying Simple Linear Exposure-Response Relationships

Number of Tests	R^2	Proportion of Tests with Specified R^2	Power for N=100 Subjects	Power for N=80 Subjects
24	0.2	0.05	0.95	0.88
48	0.2	0.05	0.95	0.88
24	0.2	0.10	0.97	0.92
48	0.2	0.10	0.98	0.92

Simulation results indicate that the target sample size of 100 subjects provides >90% power for detecting simple linear relationships with an underlying R^2 of at least 0.2, if the proportion of non-null relationships among those tested is at least 5%. If only 80 subjects complete follow-up for the primary endpoint, power for the same scenario is approximately 88%.

Figure 11.1: Example Data with $R^2 = 0.2$ and N=100 Observations



11.3 Final Analysis Plan

Further details on the analyses discussed below will be provided in the Statistical Analysis Plan (SAP), which will be finalized prior to data lock. In case the language in this section differs from the language in the SAP, the SAP will take precedence.

11.3.1 Analysis of Demographics and Baseline Characteristics

Demographic characteristics (age, sex, and town/village of residence) of each participant will be tabulated. Weight-for-age and height-for-age z-scores will be determined based on WHO Child Growth Standards (WHO, 2006). Summary statistics (e.g. mean, range, and standard deviation) of age, weight, height, weight z-score, and height z-score will be tabulated by sex.

11.3.2 Analysis of Safety

The number of subjects experiencing unsolicited AEs, classified using the Medical Dictionary for Regulatory Activities (MedDRA®) System Organ Classes and Preferred Terms, reported throughout the study after study drug administration will be tabulated by severity, and relationship to study product. SAEs will be described. A complete listing of AEs for each subject will provide details including severity, relationship to study drug, onset, duration, and outcome.

11.3.3 Clinical Laboratory Parameters

Hematological and biochemical laboratory parameters will be measured at study days 1, 2, 3, 4, 5, 6 and 7. Clinically relevant abnormal values based on reference intervals determined in a similar population will be tabulated and a trend analysis performed if deemed necessary.

11.3.4 Analysis of Pharmacokinetics of Dihydroartemisinin

Population PK models for DHA have been previously published but have not described the population PK of DHA in critically ill malaria patients and related it to the PD and clinical outcomes in the same analysis. This planned analysis involves use of the data to characterize the PK of DHA in these critically ill pediatric patients, and for the assessment of selected covariates on intersubject variability in the PK of DHA. Overall, the information gained from the planned analyses will support the understanding of exposure-response relationship to support dose regimen selection.

Population PK analyses will be performed using Nonlinear Mixed Effects Modelling (NONMEM) v7.3 or higher, Pumas, or similar software. The general procedure that will be followed for the development of the population PK model is outlined below:

1. Exploratory data analysis

The number of samples per subject and by timing relative to last dose will be summarized. Concentration outliers and other implausible values will be identified using scatterplots. Frequency of below quantification limit (BQL) concentrations will be determined. Outliers detected by exploratory data analysis may be queried prior to data lock and may be flagged for exclusion from analysis if problematic for fitting the model.

2. Base structural model development for PK

One- and two-compartment parent-metabolite models of artesunate and DHA will be considered, as well as one- and two-compartment models that do not explicitly model artesunate since artesunate concentrations will not be measured. Competing models will be distinguished by their objective function value, the plausibility of parameter estimates, and standard diagnostic plots, including observed vs. individual-fitted concentrations, observed vs. population-fitted concentrations and weighted residuals.

3. Evaluation of covariate effects

Allometric scaling will be evaluated using both standard values (0.75 for clearance and 1.0 for volume) and estimated coefficients. Sex and whether the subject had a dose of artesunate prior to enrollment will also be evaluated. Additional covariates for evaluation may be specified in the SAP. Inclusion of covariate relationships in the final model will be based on forward selection, with criteria mainly based on a statistically significant likelihood ratio test but also considering reduction of unexplained between subject variability and improved characteristics seen in diagnostic plots.

4. Final model refinement

Backwards elimination will be used to remove statistically or clinically insignificant covariate relationships. Distributions of individual random effect values will be used to assess outliers or possible subpopulations unaccounted for in the model.

5. Model evaluation

Predictive performance of the final model will be assessed using visual predictive checks and normal prediction distribution error.

11.3.5 Analysis of Pharmacodynamics of Dihydroartemisinin

Analysis Plan for Primary Outcome

The primary outcome response variables are: temperature, serum lactate, serum glucose, acidosis, BCS, total and direct bilirubin, anemia. For each subject, values of the DHA exposure

parameters $AUC_{0\text{-}12}$ and C_{max} values for each individual subject and treated as fixed in downstream PD analyses.

Regression methods will be used to characterize the relationship between exposure and responses, while controlling for covariates. For continuous response variables (temperature, serum lactate, serum glucose, acidosis, and anemia), linear, polynomial and E_{max} models with random effects will be considered. BCS is an ordinal scale designed to assess malarial coma in children, with possible values of 0, 1, 2, 3, 4, 5. Proportional odds mixed effects logistic regression will be used to model BCS.

Covariates will include exposure, timepoint, the interaction between exposure and timepoint, and potential confounding variables such as receipt of artesunate or artemisinin-based therapy prior to enrollment. Specific details of all models will be described in the SAP, and will include:

- The precise definition or derivation of each response variable, including potential transformations and the method for accounting for baseline values and/or associations between the baseline value and exposure
- The pre-specified covariates to be included in the model
- The procedure for choosing the covariance structure
- The alternative methods which may be considered, if model assumptions do not hold

For the analysis of the response variables included in the primary outcome, the set of p-values corresponding to tests of the effect of exposure on response at each timepoint will be adjusted using the Benjamini-Hochberg procedure to preserve the false discovery rate for the primary outcome at 0.05. The corresponding point estimates and CIs will be reported. Graphical displays of response by exposure at each timepoint will show individual measurements alongside model estimates. Individual trend plots of responses over time will also be generated for each response variable.

Analysis Plan for Secondary Outcomes

The secondary outcome response variables are time to hospital discharge, and measures of parasite clearance calculated from parasite density, as measured by thick blood smear such as parasite clearance half-life, total parasite clearance by Day 2, and time to 90% reduction in parasitemia. Parasite clearance half-life (PCT_{50}) and time to 90% reduction (PCT_{90}) will be estimated using the WorldWide Antimalarial Resistance Network (WWARN) parasite clearance estimator (PCE) algorithm (Flegg 2011). The relationship between $AUC_{0\text{-}12}$ and C_{max} and the time-to-event response variables will be analyzed using Cox proportional hazards regression.

The relationship between AUC_{0-12} and C_{max} and the binary variable of total parasite clearance by Day 2 will be analyzed using logistic regression. Covariates will include exposure and potential confounding variables. The full list of covariates to be considered and the variable selection strategy will be described in the SAP. Analyses of secondary outcome variables will not be adjusted for multiplicity.

11.3.6 Missing Data

Every effort will be made to minimize missing data and collect all endpoints specified in this protocol. Subjects who discontinue treatment will be followed after treatment discontinuation for collection of all scheduled follow-up data with their consent. Sensitivity analyses will be conducted to exclude the subset of subjects who received IV artesunate prior to enrollment. No adjustments for missing safety data will be performed for the secondary analyses. Handling of missing dosing, PK, covariate, and outcome data will be described in the SAP.

11.4 Study Cohorts/Datasets to be Evaluated

Safety Population

The safety population will include all subjects who received at least 1 dose of IV artesunate

Population PK Analysis Population

The Population PK Analysis Population will include all evaluable subjects who received at least 1 dose of IV artesunate and had at least 1 measurable DHA concentration.

Pharmacodynamic Analysis Population

The PD Analysis Population will include all subjects for which exposure parameters can be obtained from the final population PK model and for which post-baseline response data are available.

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The participating study site will maintain appropriate medical and research records for this trial, in compliance with ICH harmonised tripartite guideline E6(R2): GCP, Section 4.9 and regulatory 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, hospital records, clinical and office charts, laboratory notes, memoranda, subjects' memory aid or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and participant files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial.

eCRFs will be supplied by The Emmes Company, LLC under contract to the National Institute of Allergy and Infectious Diseases (NIAID), and a remote data entry system will be used. Data collection forms derived from the eCRFs will be made available on the project website as Source Document Workbooks (SDWs). The SDW for each participant will be maintained at the study site. All SDWs will be filled out completely and by appropriate study personnel. These data collection forms compiled as SDWs will serve as source documents.

The study site will permit authorized representatives of DMID, DMID designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of QA reviews, audits and evaluation of the study safety and progress. These representatives will be permitted access to all source data.

13 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted study site quality management plan, the study site will conduct routine QA and QC activities to internally monitor study progress and protocol compliance. The quality management plan is located in the University of Maryland Center for Vaccine Development Office of Regulatory Affairs and Quality Management. The protocol-specific quality management plan is in conjunction with the University of Maryland Center for Vaccine Development Office of Regulatory Affairs and Quality Management and describes the study site's internal quality management activities including how the data will be evaluated for compliance with the protocol, which documents will be reviewed, and methods of training staff.

SOPs will be used at all clinical and laboratory sites. Routine monitoring will be performed according to ICH/GCP (E6) (e.g., data monitoring). IDI and DMID-designated clinical monitors will verify that the clinical trial is conducted, and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements. The study site will provide direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing by DMID, and inspection by local and regulatory authorities.

Emmes and the study site data manager will implement QC procedures beginning with the data entry system and generate data QC checks that will be run on the database. Any missing data or data anomalies will be communicated to the study site for clarification/resolution.

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Ethical Standard

The IDI Research Ethics Committee (REC), the National Drug Authority and the Uganda National Council for Science and Technology (UNCST) will review and approve the protocol before study start. In addition, the study will be reviewed by DMID and the University of Maryland IRB. Documentation of the approval by these ethical review boards will be kept in the study site's regulatory file.

14.2 Institutional Review Board

All amendments will be submitted to the local REC, the University of Maryland, Baltimore (UMB) IRB, and to DMID. No amendments will go into effect without written approval from the local REC, the UMB IRB, and DMID except when necessary to eliminate immediate hazards to the participants. Protocol deviations will also be reported to each REC/IRB according to the policy of each IRB.

The investigators will inform all the REC/IRBs and DMID of the following:

- All subsequent protocol amendments, informed consent/assent form (ICF) changes or revisions of other documents originally submitted for review
- Serious and/or unexpected AEs occurring during the study, where required
- New information that may affect adversely the safety of the participants or the conduct of the study
- An annual update and/or continuing review reports, as required
- A final report including SAE outcomes will be provided when the study has been completed.

14.3 Informed Consent/Assent Process

The principles of informed consent/assent in the current edition of the Declaration of Helsinki will be implemented before any protocol-specified procedures or interventions are carried out.

Information will be given in both oral and written form whenever possible. The written consent/assent documents will embody the elements of informed consent/assent as described in the current edition of the Declaration of Helsinki, will adhere to the ICH Harmonised Tripartite Guideline for Good Clinical Practice and 45CFR46 and 21CFR50, and will also comply with applicable host country regulations. The oral consent/assent process will be consistent with 45CFR46, 46.117, 21CFR50.27 and ICH E6 (R1) Section 4.8. Independent witnesses will be

used to attest that illiterate persons have understood the contents of the informed consent/assent document before signing the written consent/assent document.

14.3.1 Screening and Study Informed Consent/Assent

A period of 24 hours is allotted for screening and recruitment to allow plenty of time for a participant or participant's parent/guardian to consider their decision about participating and to discuss their/their child's participation. At the times of screening and recruitment, the consent/assent forms are either provided in written English or local languages (Ateso, Lugwere, Kiswahili, Luganda and Japadhola) or read to illiterate participants who speak English or local languages (Ateso, Lugwere, Kiswahili, Luganda and Japadhola). For illiterate participants, informed consent will be administered by oral translation of the text in presence of a witness. In all cases, the investigator will give the participants ample opportunity to inquire about the details of the study and to ask any questions before dating and signing the consent/assent forms, including the opportunity to take a copy of the consent/assent form home to review with family members or others before returning on a later day with their decision. All illiterate individuals will have the study and consent/assent forms explained to them point by point by the interviewer in the presence of a witness who will sign the consent form. Witnesses will have no association with the conduct of the study and will not be related to the study participant. Witnesses receive training that emphasizes protection of confidentiality and assist with ensuring full and accurate oral translation of the information on the consent/assent forms. Individual consent/assent to participate in research studies is given freely and is not subject to approval by village elders or others. Investigators will carefully explain to potential participants that they may withdraw their participation from the study at any point in the future.

Informed consent/assent will be documented using a written consent/assent form approved by the IRBs and signed or thumb printed and dated by the participant, and by the person who conducted the informed consent/assent discussion. Thumb printing will be used for illiterate persons, who are expected to constitute the majority of participants. The English consent/assent form will be translated into local languages spoken at the study site. For illiterate participants who do not speak English, consent will be administered by a study clinician who is fluent in local language, or the study clinician use a translator. A witness will assist during the procedure. After the participant clearly states that she/he has understood what was explained and agrees to participate in the study, the consent/assent forms will be completed. The participant will be asked if she/he prefers to thumbprint or to sign. In the case of the thumbprint option, the distal end of her/his left thumb will be applied to a stamp inker and then firmly applied to the space on the consent/assent forms reserved for thumbprints.

The signature/thumbprint confirms that the consent/assent is based on information that has been understood. Each participant's signed ICF/assent is kept on file by the investigator for possible inspection by regulatory authorities. The participant will receive a copy of the signed and dated written ICF/assents and any other written information provided by the investigator, and will receive copies of any signed and dated consent/assent form updates and any amendments to the written information.

Since most study participants do not use telephones, fax or mail, contact information is provided in terms of local study site staff who can be visited directly and who can themselves reach the investigators directly or by telephone.

14.3.2 Compensation and Reimbursement

To compensate the participant for time lost to income generating activities as a result of participating in the study, each participant will receive 50,000 Ugandan shillings per inpatient day and 20,000 Ugandan shillings per outpatient follow-up visit, according to local ethics standards. Additionally, participants will be provided transport reimbursement of 20,000 Ugandan shillings for transport costs on scheduled study visits.

14.4 Exclusion of Women, Minorities, and Children (Special Populations)

As most severe malaria illnesses occur in children, it is deemed ethical to perform this study first in children whose parent/guardian can give full, informed independent consent and assent where applicable. Persons will be screened, recruited, and enrolled into this study without regard for ethnicity or gender.

14.5 Subject Confidentiality

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participating subjects.

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.

The study monitors or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The study site will permit access to such records.

Participants will be assigned a unique participant ID number. All results will be keyed to this number. Study records will only be available to staff members and will be kept locked at the study site conforming to the investigators' SOPs. Following the conclusion of the study, all records will be maintained on site for a minimum of two years, after which they will be stored long-term in the data storage facilities at the IDI. Representatives of DMID may review these records.

14.6 Study Discontinuation

The study may be discontinued at any time by DMID, the IRB, or the investigators. If the study is discontinued before completion, all participants who received study product will be asked to follow up with the study team for debriefing. Study team personnel will be available for questions or follow-up should evaluation be needed.

When the study ends, participants will be instructed to seek routine medical care offered at the local health clinic. AEs that are ongoing at the time of study discontinuation will be followed by study staff to resolution, or, if a chronic condition has developed, until the end of the study follow-up period.

14.7 Future Use

14.7.1 PAXgene Collection for Transcriptomics Assessments

Whole blood in PAXgene tubes will be collected for future research for RNA-Seq. The goal of these analyses could include identification and characterization of parasite gene expression.

14.7.2 Use of Stored Specimens

Future use of specimens is planned for this protocol. Assent and Parent/guardian consent for future use of leftover specimen will be required for storage of specimens for future use. If assent/parent/guardian consent for future use of leftover specimen is not obtained, any leftover specimen will be destroyed according to institutional SOPs after assays are completed and results are verified.

15 DATA HANDLING AND RECORD KEEPING

The PI is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

CRFs will serve as source documents. Only information that cannot be collected initially onto CRFs (namely, clinical laboratory test results and AE medical records) will first be collected onto separate source documents before transcription into CRFs. The information in the CRF will then be entered directly into the Internet data system.

CRFs derived from the eCRF will be provided and maintained for recording data for each participant enrolled in the study. Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained.

DMID and/or its designee will provide guidance to investigators on making corrections to the source documents and eCRF.

A copy of the cleaned and locked database will be provided to DMID and the PI at the end of the study.

15.1 Data Management Responsibilities

All source documents and laboratory reports must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. AEs must be graded, assessed for severity and causality, and reviewed by the study site PI or designee.

Data collection is the responsibility of the clinical trial staff at the study site under the supervision of the PI. During the study, the investigator must maintain complete and accurate documentation for the study.

The Emmes Company, LLC will serve as the Statistical and Data Coordinating Center (SDCC) for this study and, in collaboration with the study data manager, will be responsible for data management, quality review, analysis, and reporting of the study data.

15.2 Data Capture Methods

Clinical data (including AEs and concomitant medications) and clinical laboratory data will be entered into a 21 CFR Part 11-compliant IDES provided by Emmes. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

15.3 Types of Data

Safety assessments will be based on reports collected from review of AE and SAE reports. All AEs will be MedDRA® coded for Preferred Term and System Organ Class. The rate of AEs in aggregate, and by MedDRA® codes, will be computed.

The number of SAEs is likely to be small in this study and will be reported by a detailed listing showing the type, MedDRA® coding, relevant dates (study drug administration and AE), severity, and outcome for each event.

An individual forms grid, which indicates the current status of forms submission for each participant is provided as part of Emmes' Advantage eClinical^{SM} IDES.

15.4 Timing/Reports

Primary data analysis will occur after the primary study endpoint is reached at study day 183. Results of this primary data analysis will be available for public release.

Coding of AEs will be according to the MedDRA classification and will be managed by the statistical data and coordinating center as AE data is entered into the online database.

15.5 Study Records Retention

Study documents should be retained for a minimum of 2 years. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of DMID. It is the responsibility of DMID to inform the investigator when these documents no longer need to be retained.

15.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or MOP requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the study site and implemented promptly.

These practices are consistent with ICH E6:

4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3

5.1 Quality Assurance and Quality Control, section 5.1.1

5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the study site to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All deviations must be promptly reported to DMID, via Emmes' IDES .

All deviations from the protocol must be addressed in study subject source documents. A completed copy of the DMID Protocol Deviation Form (IDES form) must be maintained in the regulatory file, as well as in the subject's source document. Protocol deviations must be sent to the local IRB/IEC per their guidelines. The study site PI/study staff is responsible for knowing and adhering to their IRB/IEC requirements.

16 PUBLICATION POLICY

Following completion of the study, the investigator is expected to publish the results of this research in a scientific journal. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. It is the responsibility of DMID to register this trial in an acceptable registry. Any clinical trial starting enrollment after 01 July 2005 must be registered on or before patient enrollment.

The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study PK or major toxicity (e.g., Phase I trials), would be exempt from this policy.

17 LITERATURE REFERENCES

1. World Health Organization. Guidelines for the treatment of malaria [Internet]. World Health Organization; 2015 Aug 13. 317 p. Available from: https://apps.who.int/iris/bitstream/handle/10665/162441/9789241549127_eng.pdf.
2. World Health Organization. World malaria report 2019 [Internet]. 2019. 232 p. Available from: https://www.who.int/publications/i/item/9789241565721.
3. World Health Organization. Guidelines for the treatment of malaria [Internet]. World Health Organization; 2015 Aug 13. 317 p. Available from: https://apps.who.int/iris/bitstream/handle/10665/162441/9789241549127_eng.pdf.
4. Centers for Disease Control and Prevention. Intravenous artesunate for treatment of severe malaria in the United States. IND Protocol [Internet]. 2007 [Updated 2016 Dec 19]. 50p. Available from: https://www.cdc.gov/malaria/resources/pdf/artesunate/Artesunate_protocol_12_19_2016.pdf.
5. Centers for Disease Control and Prevention. Guidelines for treatment of malaria in the United States [Internet]. Atlanta: Centers for Disease Control and Prevention. 2013. 4 p. Available from: https://www.cdc.gov/malaria/resources/pdf/Malaria_Treatment_Table_120419.pdf.
6. World Health Organization. Guidelines for the treatment of malaria [Internet]. World Health Organization; 2015 Aug 13. 317 p. Available from: https://apps.who.int/iris/bitstream/handle/10665/162441/9789241549127_eng.pdf.
7. Benjamini Y, & Hochberg, Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Series B Stat Methodol. 1995 Jan;57(1):289-300. doi: https://doi.org/10.1111/j.2517-6161.1995.tb02031.x.
8. Benjamini Y, Liu W. A step-down multiple hypotheses testing procedure that controls the false discovery rate under independence. J Stat Plan Inference. 1999;82(1):163-70. doi: https://doi.org/10.1016/S0378-3758(99)00040-3.
9. World Health Organization. WHO child growth standards: length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development. World Health Organization; 2006 Nov 11. 312 p. Available from: https://www.who.int/publications/i/item/924154693X.
10. Flegg JA, Guerin PJ, White NJ, Stepniewska K. Standardizing the measurement of parasite clearance in falciparum malaria: the parasite clearance estimator. Malar J. 2011;10:339. Epub 2011/11/15. doi: 10.1186/1475-2875-10-339. PubMed PMID: 22074219; PMCID: PMC3305913.

18 SUPPLEMENTS/APPENDICES

19 APPENDIX A: SCHEDULE OF EVENTS

Table 19.1: Schedule of Events

Procedures	*Screening Day -1 to 1*	*Enrollment Day 1*	*Day 2*	*Day 3*	*Day 4*	*Day 5*	*Day 6*	*Day 7 (±2 days)*	*Day 14 (±2 days)*	*Day 28 (±4 days)*	*Final Study Visit, Day 183 (±14*	*Early termination (ET) Visit*
Informed consent	X											
Demographics	X											
Inclusion/Exclusion criteria	X											
Enrollment		X										
Administer IV artesunate		X	X	X								
Administer oral ACT					X	X	X					
Concomitant medication review	X	X	X	X	X	X	X	X	X	X	X	X
Physical exam	X	X	X	X	X	X	X	X	X*	X*	X	X*
Medical History	X	X									X	
*Vital signs***	X	X	X	X	X	X	X	X	X	X	X	X
Height	X									X	X	
Weight	X									X	X	
Thick blood smear for Pf parasitemia (per parasitemia sampling plan)	X	X	X	X	X	X						
Dried blood spot for artemisinin resistance	X											
PAXgene tube for mRNA	X											
Hematology	X	X	X	X	X	X	X	X	X**	X**	X	X^
Serum bicarbonate	X	X	X	X	X	X	X	X	X			X^
Serum lactate	X	X	X	X	X	X	X	X				X^
Serum sodium	X	X	X	X	X	X	X	X				X^
Serum potassium	X	X	X	X	X	X	X	X				X^
Serum creatinine	X	X	X	X	X	X	X	X				X^
Serum glucose	X	X	X	X	X	X	X	X				X^
Serum bilirubin, ALT and AST	X	X	X	X	X	X	X	X				X^
Urinalysis	X	X	X	X	X	X	X	X				
Blantyre coma score	X	X	X	X	X	X	X	X	X	X	X	
Pharmacokinetic assessments (per PK sampling plan)	X	X										
Neurodevelopmental assessments (parent or caregiver interview, standardized developmental, cognitive & behavioral measures)								X	X	X	X	

* Targeted physical exam, if needed
**Only hemoglobin
*** approximately every 6 h until 6 h post parasite clearance
^ If ET is before D7, collect D7 blood samples; if D8 to D14 may collect serum bicarbonate, hemoglobin, platelets, WBC count, and neutrophil count. If >D14 blood for hemoglobin, platelets, WBC count, and neutrophil count.