Convenience and cost-aspects of a new 1-step reconstitution injectable artesunate compared to conventional 2-step injectable artesunate for the treatment of severe falciparum malaria: a multi-centre study

Short title: Study to compare feasibility of new artesunate antimalarial injection versus conventional artesunate antimalarial injection

Protocol version: version 2.0 dated 18 August 2021

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ACRONYM: 1STEP-AS

Protocol no.: MAL21006

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Corresponding to the SPIRIT checklist https://www.bmj.com/content/bmj/346/bmj.e7586.full.pdf, the roles and responsibilities of the funder are as follows. Consultation during study design, no role in the conduct, data analysis and interpretation, manuscript writing, and dissemination of results. The funder does not control the final decision regarding any of these aspects of the trial.

Investigators and protocol development team:

AM Dondorp, L von Seidlein and TJ Peto conceived of the study. S Gesase, M Onyamboko, T Cope, C Fanello, LvS, and TJP designed and will conduct the study. Y Lubell designed and will conduct the costing analysis. M Mukaka designed and will conduct the primary statistical analysis. B Adhikari designed and will conduct the social science data analysis. N Waithira designed and will conduct the data management. All authors contributed to refinement of the study protocol and approved the final version.

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, host institution, relevant ethics committees and regulatory authorities.

Investigator Agreement and Conflict of Interest

"I have read this protocol and:

- agree to abide by all provisions set forth therein.
- agree to comply with the principles of the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice.
- and declare no conflict of interest, according to the current version of the Declaration of Helsinki"

Prof. Arjen M. Dondorp		
Principal Investigator	Principal Investigator's signature	Date:
Prof. M. Onyamboko (DRC)		
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Dr. S. Gesase (Tanzania)		
Site Principal Investigator	Site Principal Investigator's signature	Date:

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1. LIST OF ABBREVIATIONS

ACT	Artemisinin-based combination therapy
AE	Adverse event
A/L	Artemether-Lumefantrine
ALT	Alanine Aminotransferase
AS	Artesunate
AST	Aspartate aminotransferase
CRF	Case Report Forms
CTCAE	Common terminology criteria for adverse events
CTSG	Clinical trials support group (MORU)
DHA	Dihydroartemisinin
DNA	Deoxyribonucleic Acid
DOT	Directly Observed Therapy
DRC	Democratic Republic of the Congo
DSMB	Data and Safety Monitoring Board
EDC	Electronic data capture
EDTA	Ethylene-diamine-tetra-acetic acid
GCP	Good Clinical Practice
GCS	Glasgow Coma Scale
Hb	Haemoglobin
Hct	Haematocrit
IM	Intramuscular
IRB	Institutional Review Board
IV	Intravenous
KIMORU	Kinshasa Medical-Oxford Research Unit
LDH	Lactate Dehydrogenase
MORU	Mahidol-Oxford Tropical Medicine Research Unit
NIMR	National Institute for Medical Research (Tanzania)
NMCP	National Malaria Control Programme
PI	Principal Investigator
PCR	Polymerase Chain Reaction
PCT	Parasite Clearance Time
QA	Quality Assurance
QC	Quality Control
RDT	Rapid Diagnostic Tests
SAE	Serious Adverse Event
SmPC	Summary of product characteristics
SOP	Standard Operating Procedure
SSIs	Semi-structured Interviews
SUSAR	Suspected unexpected severe adverse reaction
WHO	World Health Organisation

2. SYNOPSIS

Study Title	Convenience and cost-aspects of a new 1-step reconstitution injectable artesunate compared to conventional 2-step injectable artesunate for the treatment of severe falciparum malaria: a multi-centre study
ACRONYM	1STEP-AS
Trial Design	Individually-randomised controlled non-inferiority trial comparing 1-step parenteral artesunate vs conventional 2-step parenteral artesunate, with ancillary qualitative and cost-evaluation components
Trial Participants	Part 1: • Children with severe *falciparum* malaria admitted to hospital Part 2: • Semi-structured Interviews (SSIs) with study staff, and other health staff who treat severe malaria patients • SSIs with malaria policy makers and stakeholders
Sample size	Part 1: Total 200 participants. Estimated 100 per site. • Korogwe District Hospital, Tanga, Tanzania (East Africa) • Kinshasa Medical Oxford Research Unit, D.R. Congo (Central Africa) Part 2: For both sites in total 40 SSIs with study staff, health staff, policy makers and stake holders; and survey/questionnaires with 150 health staff.
Inclusion Criteria	Part 1: • Male and female children aged >3months and <16 years. • Clinical diagnosis of severe *P. falciparum* malaria; or parasitological confirmed *P. falciparum* hyperparasitaemia >350,000/ uL. • Positive malaria test result, by rapid diagnostic test (RDT). • Weight of 5 kg or greater. • Written informed consent by the parent or guardian. Part 2: Study staff and health staff • Study staff who prepare and administer artesunate injection to patients in the study or health staff who have not administer the artesunate injection to patients in the study by themselves but are either aware of malaria treatment, artesunate injection, or have observed the treatment provided to severe malaria patients. • Written informed consent by the study staff and health staff Study staff for a short video-record • Study staff who would like to participate in the video record of a procedure to show how 1-step and 2-step artesunate injections are prepared and administered. • Written informed consent by the study staff

	Policymakers and stakeholders • Those who are working in the National Malaria Control Program (NMCP) or relevant organizations (WHO, INGOs/NGOs) within the country. • Written informed consent by the potential participant
Exclusion Criteria	Part 1: • Participation in other intervention studies • Known allergy to artemisinin derivatives. • Known history of parenteral treatment for severe malaria for the current episode of illness before admission. Treatment before admission with an oral antimalarial drug (used for the treatment of uncomplicated malaria) or a single dose of pre-referral rectal artesunate are not exclusion criteria. Part 2: Study staff and health staff; Policymakers and stakeholders; Study staff and health staff for a short video-record • Unwilling to participate in the study • Unable to communicate
Planned Trial Period	18 months, start in Q4 2021. (Estimated patient recruitment November 2021 – October 2022. Patient duration of participation 1 month.)
Co-Primary Objectives	Assessment of: 1.Convenience and rapidness of administration of 1-step vs. conventional 2-step parenteral artesunate formulations and 2. Costs of administration of 1-step vs. conventional 2-step parenteral artesunate formulations
Secondary Objective	1. To assess the acceptability, policy and implementation perspectives, and health care worker satisfaction regarding the use of 1-step formulation of parenteral artesunate..
Exploratory Objectives	1. To assess and compare *P*. *falciparum* parasite clearance rates of 1-step vs. conventional 2-step parenteral artesunate formulations. 2. To assess and compare time from intravenous treatment to follow up treatment with oral drugs with 1-step vs. conventional 2-step parenteral artesunate formulations. 3. To assess and compare disease outcome parameters between 1-step vs. conventional parenteral artesunate formulations. 4. To assess and compare adverse events between 1-step vs. conventional 2-step parenteral artesunate formulations.
Co-primary endpoints	1. Time to administration of treatment comparing 1-step vs. conventional 2-step parenteral artesunate formulations (by time-and-motion methods). and 2. Costs of administration of 1-step vs. conventional 2-step parenteral artesunate formulations at health facility, and at health system level.

Secondary endpoint	1. A mixed method social science study will assess feasibility, practicability, and satisfaction of 1-step formulation of parenteral artesunate. (To include study staff, health staff, and policymakers and stakeholders.)
Exploratory endpoints	1. Parasite clearance half-life and other parasite clearance parameters (PC50, PC90, 12-hour parasite reduction ratio) compared between 1-step vs. conventional 2-step parenteral artesunate formulations. 2. Time from start parenteral treatment to follow up treatment with an oral ACT (recovery to per os treatment) of 1-step vs. conventional 2-step parenteral artesunate formulations. 3. Fever clearance time (i.e. the time taken for the tympanic temperature to fall below 37.5˚C and remain there for at least 24 hours) of 1-step vs. conventional 2-step parenteral artesunate formulations. 4. Incidence of adverse events and serious adverse events by study arms within the first 28 days.
Drugs	Artesunate (1-step parenteral) VERSUS Artesunate (conventional 2-step parenteral) Then oral artemisinin combination therapy as follow-on treatment from parenteral treatment: artemether-lumefantrine (A/L), if available and no contraindication, otherwise other ACT. Rescue treatment: quinine.

3. BACKGROUND AND RATIONALE

3.1 Background

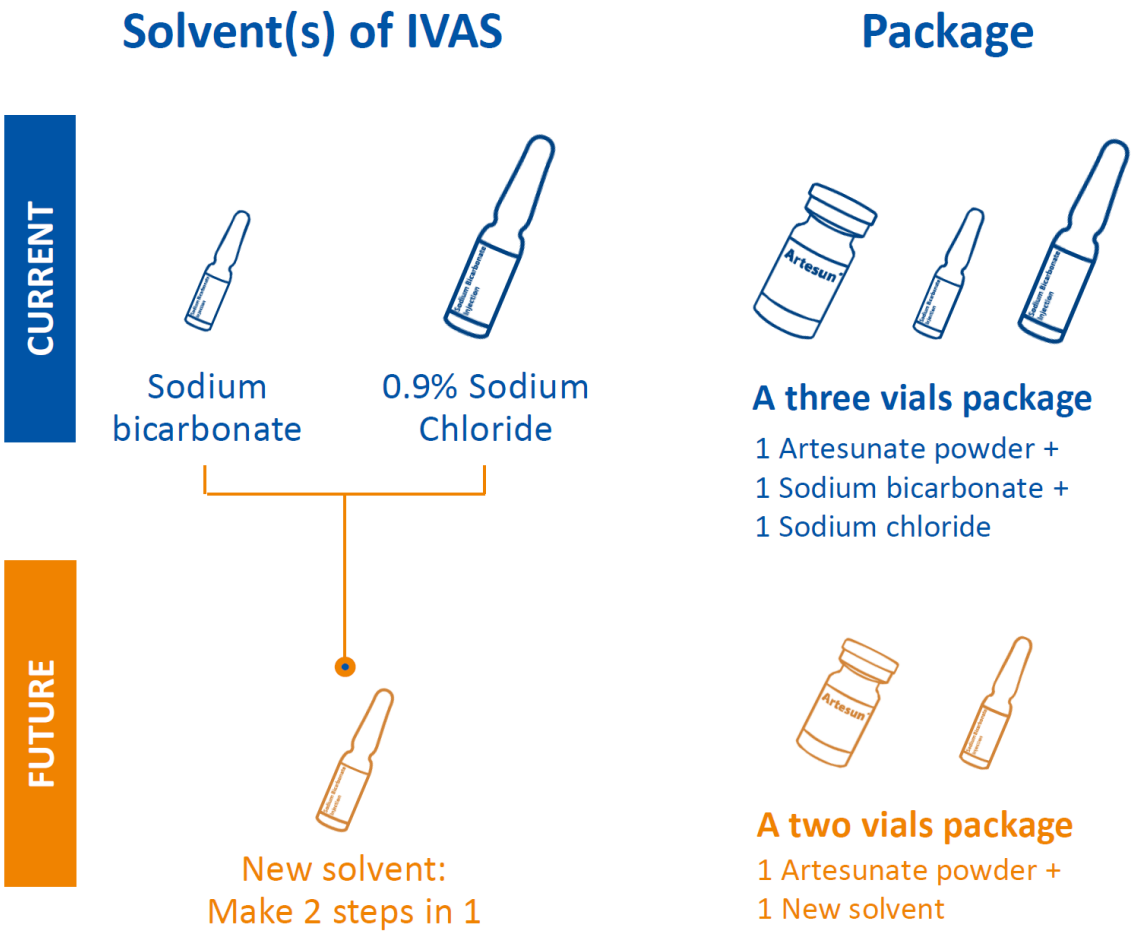
Parenteral artesunate is the first-line treatment for severe malaria.[1,2] The two largest ever treatment trials conducted in patients hospitalised with severe malaria, which compared parenteral artesunate (produced by Guilin/ Fosun) with quinine: SEAQUAMAT in Asia in 1,461 patients[3], and AQUAMAT in Africa in 5,425 children[4]. In both trials artesunate was associated with a significant and substantial reduction in mortality compared to quinine (a 34.7% relative reduction in deaths in SEAQUAMAT, 22.5% in AQUAMAT), which was not at the expense of an increase in severe sequelae. These findings resulted in a change of the WHO recommended first line antimalarial therapy for severe malaria in all endemic settings, and it has been estimated by the Medicines for Malaria Venture that since 2011 this has saved well over 500,000 young lives in Africa (https://www.mmv.org/our-impact/achievements/168-million-vials-artesun-delivered-treat-children-severe-malaria).

The conventional formulation of injectable artesunate requires a 2-step reconstitution and dilution of the artesunate hemisuccinate powder, including reconstitution in a sodium bicarbonate solution followed by further dilution in 5% dextrose or normal saline. The 2-step procedure takes time and is error prone, dissolving the artesunate powder in the bicarbonate solution is sometimes difficult, and the 2-step procedure requires additional consumables, like sterile syringes (https://www.severemalaria.org/toolkits-training/injectable-artesunate-tools-training). Preparation of conventional injectable artesunate (Artesun SmPC) requires shaking the vial for 3 to 5 minutes to mix well until the powder is completely dissolved and the solution is clear. Another disadvantage of the conventional artesunate is the two steps needed to reconstitute artesunate powder by sodium bicarbonate first then to dilute the solution by sodium chloride should not be exchanged. Otherwise, artesunate will not be dissolved. Such malpractice can cause a waste of medicine and put critical ill severe malaria patient under risk. 1-step artesunate can avoid such mistakes.[5]

For treatment of severe malaria, a medical emergency, this poses important disadvantages, in particular in under-resourced healthcare settings in Sub-Saharan Africa. A new formulation of injectable artesunate has been developed by Fosun Pharma requiring a simpler 1-step reconstitution. Bio-equivalence of the new formulation to the conventional formulation was shown in healthy volunteers led by Prof. Joel Tarning, University of Oxford (report attached in Appendix 6). The summary of the results for artesunate and dihydroartemisinin were *"Parenteral administration of test and reference formulations showed matching exposures to the combined exposure to artesunate and dihydroartemisinin, and bioequivalence of the test formulation could be demonstrated both when administered IV (Table 3) and IM (Table 6)".* The new formulation is currently awaiting WHO prequalification and is expected to be launched in 2022, "Argesun" WHO prequalification reference number MA168 (Michelle Xiong, Fosun Pharma, personal communication).[6]

The new 1-step artesunate parenteral formulation is thus expected to be equally effective, but likely more convenient and faster to use then conventional formulation. And to avoid reconstitution mistakes in field practice. Also, for the conventional formulation, the volumes of 0.9% sodium chloride used for IV route and IM route are different, for IV route: After reconstitution and dilution, one ml of solution for injection contains 10 mg artesunate. IM route: After reconstitution and dilution, one ml of solution for injection contains 10 mg artesunate. This can cause confusion among health workers and lead to mistakes. The 1-step formulation uses the same volume of solvent for IV and IM. In addition, the 1-step reconstitution is expected to be cheaper to administer, because it will use less of the health professional's time and will use less consumables. We propose a study to compare and quantify convenience and costs of the new 1-step artesunate parenteral formulation versus the conventional formulation in a randomised study. This study will also integrate social science studies to explore and compare acceptability of 1 step artesunate with the conventional formulation using

SSIs and surveys among study staff, health staff and policymakers. In addition, efficacy data and adverse events will be captured.



3.2 Study Rationale

We seek to evaluate the practical benefits of the new formulation in a clinical trial supported by an ancillary social sciences study, and a cost analysis informed by the first two activities. The randomised study will formally compare between the two artesunate formulations: the speed and convenience of preparation and use, the costs of drug administration, basic pharmacodynamic measures and adverse events. A mixed method social science study will explore the opinion of healthcare workers regarding the use of the new artesunate formulation, including the policy and implementation perspectives from policymakers. Previous comparisons of artesunate suppositories and parenteral artesunate have reported parasite clearance times[7] (https://www.severemalaria.org), and although both arms of the trial contain an identical active drug (artesunate) in the same dosages, we will also collect this information as a precaution to exclude the possibility of an unexpected, substantial difference in drug activity in patients with severe malaria.[8]

A feasibility assessment of injectable AS is important.[9] Previous analyses have shown the mortality benefit and cost-effectiveness of reduced delays to effective treatment for severe malaria.[10,11] Studies to evaluate new treatments for severe malaria should not only rely on efficacy (mortality) outcomes, but also include factors determining effectiveness under field conditions.[12] An important factor is the convenience to use the antimalarial drug. An objective measure of this is the time needed to correctly prepare the artesunate solution for injection, which will be evaluated in this study. In

addition, social science studies will explore the practicalities such as convenience, policy and implementation related issues and perceived benefits of using the new 1-step formulation compared to the conventional 2-step formulation.

Cost effectiveness of drug treatment for severe malaria is important and needs to be based on reliable evidence in the context of the high mortality caused by the disease.[13] Previous assessments have shown that compared to parenteral quinine, AS is highly cost effective for the treatment for severe malaria in Asia and Africa.[14,15] In the current study we will focus on the costs of the two different artesunate formulations and the consumables needed for their administration. These direct costs are important at the hospital level, but also for deployment in the wider health system. For example, for the public health system: the shipment cost for the 1-step artesunate will be cheaper than the cost for the conventional 2-step artesunate because the unit volume per pack (box) is smaller for the 1-step artesunate, and lighter.

The new formulation contains the same dose of active compound, artesunate, as the conventional formulation, and has shown bioequivalence in a comparative study in healthy subjects (Prof Joel Tarning, University of Oxford, personal communication). However, it has not been deployed, yet, for the treatment of severe malaria patients. Although not the primary goal of the study, we will accurately document efficacy, safety and tolerability outcomes using the new formulation. As an exploratory endpoint, these outcomes will be compared with the conventional artesunate formulation, since the study is not powered to compare these formally. Efficacy, safety and tolerability will be assessed through in-hospital monitoring of vital signs, physical examinations, blood smears, biochemistry assays and full blood counts. Adverse events will be recorded.[16] As an important pharmacodynamic measure of the drug, parasite clearance dynamics will be assessed by repeated assessments of the parasite counts after the start of parenteral antimalarial treatment.[17]

The sites have been chosen based on their ability to recruit patients with severe malaria, presence of established clinical research programme, and the feasibility to perform the proposed research activities. They also represent large African countries, one French-speaking in Central Africa, and one English-speaking in East Africa, and both with a substantial burden of severe falciparum malaria. Moreover, advanced marketing authorisation has been approved in Tanzania and the DRC, and in two other countries, Malawi and The Gambia (Michelle Xiong, Fosun Pharma, personal communication).

All the organisations in this collaboration will work closely with local counterparts including the National Malaria Control Programmes (NMCPs), non-governmental and other relevant organisations. The sites have extensive experience of conducting trials of severe malaria.[18] If 1-step parenteral AS is more feasible than the existing formulation, the study will facilitate the decision of countries whether to switch to the new formulation within a reasonable time frame.[19]

3.3 Proposed activities and outcome

This individually randomised study will compare the two artesunate formulations: the speed and convenience of preparation and use, the costs of drug administration, and basic pharmacodynamic measures as well as adverse events. A mixed method social science study will explore the opinion of healthcare workers and policymakers regarding the use of the new compared to the old artesunate formulation. To ensure policymakers and stakeholders are familiar with the topic for the SSIs, i.e. 1-step artesunate injection versus 2-step artesunate injection (conventional treatment), a brief video documenting how the artesunate injection is prepared and administered (1-step versus 2-step artesunate injection) will be recorded. The video will only serve to familiarize the topic for

SSIs with the policymakers.

The main activity is an objective assessment of the convenience to use the artesunate formulations, as well as an *in vivo* clinical and parasitological assessment in 200 participants with severe falciparum malaria at two sites: 1 in DRC and 1 in Tanzania. The subjects will be randomized between the 1-step parenteral AS and conventional 2-step parenteral AS (currently standard of care at both sites).

The secondary activity proposed is a cost analysis of 1-step AS vs conventional 2-step parenteral AS.

The third activity is a feasibility and acceptability survey using mixed methods social science study with health workers and policymakers to explore their experience and opinions related to using 1-step AS compared to the old formulation.

All research-related activities, from study design, planning, implementation through to analysis and writing of reports will be performed jointly with local counterparts. Both on-the-job training and formal training will be provided when needed, including Good Clinical Practice (GCP) skills. The close interaction between WHO and its regional offices will ensure that new knowledge is disseminated efficiently and effectively. The trial will be overseen by a Data and Safety Monitoring Board (DSMB).

4. OBJECTIVES

4.1. Co-primary Objectives

Assessment of:

(1) Convenience and rapidness of administration of 1-step vs. conventional 2-step parenteral artesunate formulations

and

(2) Costs of administration of 1-step vs. conventional 2-step parenteral artesunate formulations

4.2. Secondary Objective

(1) To assess the acceptability, policy and implementation perspectives, and health care worker satisfaction regarding the use of 1-step formulation of parenteral artesunate.

4.3. Exploratory Objectives

(1) To assess and compare *P*. *falciparum* parasite clearance rates of 1-step vs. conventional 2-step parenteral artesunate formulations.

(2) To assess and compare time from intravenous treatment to follow up treatment with oral drugs with 1-step vs. conventional 2-step parenteral artesunate formulations.

(3) To assess and compare disease outcome parameters between 1-step vs. conventional 2-step parenteral artesunate formulations.

(4) To assess and compare adverse events between 1-step vs. conventional 2-step parenteral artesunate formulations.

5. TRIAL DESIGN

5.1 Study sites

The study will take place at 1 site in the DRC and 1 site in Tanzania.

- Magunga District Hospital, National Institute for Medical Research (NIMR) Korogwe Research Laboratory, Korogwe, Tanga Region, Tanzania.
- The Kinshasa Mahidol Oxford Research Unit (KIMORU), Kinshasa, DRC.

Malaria transmission at both sites is endemic and both sites estimate numbers of pediatric patients attending with suspected severe malaria is greatly in excess of the number of patients necessary for screening and enrollment into the present study.

5.2 Summary of trial design

Part 1

A comparison of 1-step parenteral artesunate vs. conventional 2-step parenteral artesunate in patients with severe malaria to assess the feasibility of administration, parasite and fever clearance times of 1-step parenteral AS to conventional AS in 2 countries. Patients with severe malaria will be enrolled and randomly allocated to treatment with conventional injectable artesunate or 1-step injectable artesunate. A Time and Motion study will record the time to prepare the artesunate solution for injection and administer treatment, number of actions performed to prepare treatment, and consumables used. Clinical, parasitological, and laboratory assessments will be recorded while patients are admitted in hospital and then weekly up to day 28 to assess recovery and determine final status.

Part 2

A mixed method social science study that entails SSIs and survey with study staff (involved in part 1), health workers (who treat severe malaria) and policymakers and stakeholders (who are involved in devising national malaria policy and its implementation), to assess the acceptability, feasibility, pros and cons, costs, and logistics and training implications of 1-step AS vs conventional AS. For both sites, SSIs will be conducted with minimum of a total of 40 respondents consisting of study staff, health staff and policy makers and stakeholders. The ultimate sample for SSI will be dependent on the data saturation [20]. For survey/questionnaire, a maximum number of relevant participants (study staff and health care staff) will be attempted to be recruited from two sites. A minimum of 150 study staff and health care staff will be recruited in the survey.

Part 3

A cost analysis of 1-step parenteral AS using data from Part 1 & Part 2. This will assess health facility-level costs, and also health system costs to encompass all costs of a potential change from conventional to 1-step artesunate, including re-training, materials, drug replacement.[20]

5.3 Study duration

The recruitment phase of the study is expected to last 12 months once a site starts to recruit. The sites intend to start recruiting patients during Q4 2021. Training will precede study execution by up to 1 month. The mixed method social science study and costing analysis will take place during Q1 and Q2 2022 and run in parallel with the main study. SSIs with policy stakeholders will take place in Q2 and Q3 2022 to allow findings from the study and experiences and opinions of health workers to be discussed. Data management and analysis, and report writing and submit for publication in peer-reviewed medical journal are expected to take about 6 months per site. Therefore, the total time to complete the study will be about 18 months.

5.4 Primary and secondary endpoints

5.4.1. Primary Endpoint

(1) Time to administration of treatment comparing 1-step vs. conventional 2-step parenteral artesunate formulations (by time-and-motion methods).

and

(2) Costs of administration of 1-step vs. conventional 2-step parenteral artesunate formulations at health facility, and at health system level.

5.4.2. Secondary Endpoints

(1) A mixed method social science study will assess feasibility, practicability, and satisfaction of 1-step for-mulation of parenteral artesunate. (To include study staff, health staff, and policy-makers and stakeholders)

5.4.3. Exploratory endpoints

(1) Parasite clearance half-life and other parasite clearance parameters (PC50, PC90, 12-hour parasite reduction ratio) compared between 1-step vs. conventional 2-step parenteral artesunate formulations.

(2) Time from start parenteral treatment to follow up treatment with an oral ACT (recovery to per os treatment) of 1-step vs. conventional 2-step parenteral artesunate formulations.

(3) Fever clearance time (i.e. the time taken for the tympanic temperature to fall below 37.5˚C and remain there for at least 24 hours) of 1-step vs. conventional 2-step parenteral artesunate formulations.

(4) Incidence of adverse events and serious adverse events by study arms within the first 28 days.

5.5 Trial Participants

5.5.1. Overall Description of Trial Participants

This will be an open-label, randomised trial. All study patients must meet the applicable inclusion and exclusion criteria. The treating physician will decide whether the patient is severely ill and eligible for inclusion.

5.5.2. Inclusion criteria

Part 1:

- Male and female children aged >3months and <16 years.
- Clinical diagnosis of severe *P. falciparum* malaria; or parasitological confirmed *P. falciparum* hyperparasitaemia >350,000/ uL.
- Positive malaria test result, by rapid diagnostic test RDT.
- Weight of 5 kg or greater.
- Written informed consent by the parent or guardian.

Part 2:

Study staff and health staff

- Study staff who prepare and administer Artesunate injection to patients in the study or health staff who have not administer the Artesunate injection to patients in the study by themselves

but are either aware of malaria treatment, Artesunate injection, or have observed the treatment provided to severe malaria patients.

- Written informed consent by the study staff and health staff

Study staff for a short video-record

- Study staff who would like to participate in the video record of a procedure to show how 1-step and 2-step Artesunate injections are prepared and administered.
- Written informed consent by the study staff

Policymakers and stakeholders

- Those who are working in the National Malaria Control Program (NMCP) or relevant organizations (WHO, INGOs/NGOs) within the country.
- Written informed consent by the potential participant

5.5.3. Exclusion criteria

Part 1:

- Participation in other intervention studies
- Known allergy to artemisinin derivatives.
- Known history of parenteral treatment for severe malaria for the current episode of illness before admission. Treatment before admission with an oral antimalarial drug (used for the treatment of uncomplicated malaria) or a single dose of pre-referral rectal artesunate are not exclusion criteria.

Part 2:

Study staff and health staff; Policymakers and stakeholders; Study staff and health staff for a short video-record

- Unwilling to participate in the study
- Unable to communicate

6. PROCEDURES

Part 1

A trial nurse will receive all children who could have severe malaria and are potentially eligible to participate in the study and call the attending physician. The diagnosis of *falciparum* malaria will be made by a PfHRP2-based rapid malaria test. If the test is positive, and there is a clinical judgement that the patient has severe disease (according to a check list provided in the Case Report Forms [CRFs]), the patient can be enrolled, provided written informed consent has been obtained and all eligibility confirmed. The screening sequence will differ for patients with hyperparasitaemia only with no symptoms of severe malaria, as their eligibility will be determined when microscopy results are available, if performed.

Once the patient is enrolled, the randomisation deciding the drug allocation will be carried out. This is done in the following way: The next in a series of numerically sequenced envelopes is opened. The envelope will contain the unique participant number. From the moment the envelope is opened the patient is considered in the study whether or not the protocol is followed correctly thereafter. The envelope contains a slip of paper with a treatment box code number. The treatment box with this code number is then opened.

With the help of the study nurse, the attending physician will take the patient's history, and do a physical exam, where the list in the Case Report Forms (CRFs) serves as a guideline. Parameters include basic demographic parameters, questions regarding the previous history and current history, vital signs, coma score, and others. Patient monitoring during admission: to assure the best possible treatment of each study patient the following variables will be monitored based on WHO guidelines[21]

A detailed monitoring sheet will outline the planned observations and their frequency. In case of abnormal findings staff will be trained to repeat the test and to inform the key decision makers regarding the need to change the management of the patient. To help the treating physician, a practical guideline on the treatment of severe malaria (appendix 4) will be provided to all involved physicians.

When the patient is well enough to take and retain oral medication the attending clinician will be free to prescribe first line antimalarial therapy, according to national guidelines ensuring that a complete course of treatment is provided. In the case of Tanzania and DRC this will be an ACT (to be artemether-lumefantrine if available and not contraindicated, otherwise or other ACT and will be prescribed according to weight. The patient will be encouraged to stay on the ward until the complete course of ACT is taken. If the patient is discharged from hospital before the full course of ACT is administered, the remaining doses will be given to the attending caregiver with clear instructions.

A time and motion design will be employed to record ease of administration of 1-step vs conventional artesunate. Each nurse administering the IV artesunate will be paired with an additional study team member acting as an observer. The observer starts the stopwatch when the nurse breaks the seal of the IV artesunate package and stops the stopwatch when preparation of the artesunate solution for injection is completed. The time, in minutes and seconds is recorded by the observer in the CRF. This methodological approach is considered appropriate for short tasks and provides detailed field data, and typically requires a 1:1 observer to subject ratio.[22]

6.1. Informed Consent

Part 1:

Informed consent will be sought from the parent/guardian of the patient before any study specific procedures are performed according to standard Good Clinical Practice regulations. Written participant information and informed consent in the local language will be presented to the parent/guardian of the participants detailing no less than: the exact nature of the study; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that participation is voluntary and that the participant or their parent/guardian is free to withdraw consent for participation from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal.

The parent/guardian will be allowed as much time as possible to consider the information and take the opportunity to question the Investigator, or other independent parties to decide whether they will (or allow his/her child to) participate in the study. However, the study team will try to ensure that no more than one hour elapses between presentation and treatment either on or off the study.

Written informed consent will then be obtained by means of the parent/guardian's dated signature and dated signature of the person who presented and obtained the informed consent. If the parent/guardian is illiterate and unable to read and write, a witness is required to be present during the entire informed consent process. The parent/guardian provides his/her thumbprint, and the witness provides his/her signature and date on the consent form. A copy of the signed informed consent document(s) will be given to the parent/guardian of the patient.

Assent is not required for either of the countries where the study will recruit.

Part 2:

A total of three various types of written informed consents will be used.

The first written informed consent will be used to study staff and health staff. Both SSIs and survey will be conducted among study staff and health staff.

The second informed consent for video record will be conducted among study staff to record the procedure of preparing the 1-step versus 2-step artesunate injections and administering it to patients. This video will be used to familiarize the policymakers before conducting the SSIs with them.

The third written informed consent will be collected from policymakers and stakeholder who will be interviewed using SSI-guide.

Part 3:

No specific consent process is required for this sub-study.

6.2. Screening, Eligibility and Baseline Assessments

Part 1:

Patients who present at the participating sites will be screened to assess eligibility. Full consent will be obtained before any enrolment procedures are conducted. A screening log will be kept.

6.2.1. Screening tests

These will be EDTA-anticoagulated blood or a finger prick for:

- Malaria RDT

If no signs of severe diseases and RDT-positive, then where microscopy is available promptly:

- A parasite count from Giemsa or Field stained thick and thin blood films
- Haematocrit

6.2.2. Demographics and Medical History

Basic demographic data (e.g., sex, age, address, prior treatment and previous participation in this or previous studies), and a full medical history will be recorded by the study staff. To reduce the waiting time to drug administration, all information that are not immediately relevant for the inclusion and clinical care of the patient (such as epidemiological data regarding malaria risk factors) might be collected after the patient is stabilised and parents/caregivers feel comfortable.

6.2.3. Physical Examination and Vital Signs

Physical examination will be conducted by a qualified study team member. Weight, height, pulse rate, blood pressure, respiratory rate, temperature, and spleen and liver size will be recorded if palpable. Non-invasive neurological exams will be performed to monitor recovery and neurological sequalae. This will be done before discharge from the study ward, on day 28 of follow up. And, for participants who have sequalae at day 28, neurological exams will be done at month 3, month 6, and month 12.

6.2.4. Drug history

All prescribed or over-the-counter and traditional medications used within the last 7 days will be recorded. Any drug allergies will be recorded.

6.3. Randomisation and blinding

Patients will be randomised to treatment with either conventional 2-step parenteral artesunate (1) or 1-step parenteral artesunate (*2*). Patients who fulfil all the inclusion criteria and have none of the exclusion criteria will be randomised 1:1 to one of the two treatment arms according to a randomisation schedule. Randomisation will be in blocks of 8-12. Allocation will be done by drawing the next sequential numbered opaque envelope, which contains the study number and treatment allocation.

The patients will be assigned a study arm through a computer-generated randomisation schedule by the study statistician in MORU. Individual, sealed and sequentially numbered envelopes will be provided for each trial site with one envelope per patient, indicating the treatment allocation. This is an open-label study so the blinding of investigators and patients is not applicable. However, the randomisation procedure allows for adequate drug allocation concealment before envelopes are

opened. All laboratory investigations will be performed without knowledge of the treatment allocation.

6.4. Procedures during hospitalisation

A physical examination will be performed daily and every six hours on indication. Vital signs recorded hourly for unstable patients, then 6 hourly for stabilised patients, and at each FU visit. A symptom questionnaire will be taken daily to help identify adverse events. All studies procedure will follow appendix 3.

6.5. Follow-up visits

Patients will be asked to come back for a follow-up visit at day 7, 14, 21 and 28. Patients will not be followed up longer. In case of unresolved important sequelae at day 28, the patient will be followed and treated longer in order to provide the appropriate clinical care. All follow up studies procedure will follow appendix 3.

Additional unscheduled visits

Patients presenting to the clinic with a fever or other symptoms on unscheduled days will be assessed by the study physician. Their temperature will be recorded and blood smear will be made for any patient with a documented fever (tympanic temperature ≥ 37.5˚C) or a history of fever. Patients will be treated as clinically indicated.

The monitoring of the patients is not strictly defined in the protocol and should be done according to good medical practice. An exception is monitoring of blood glucose. Blood glucose will be measured on admission and between 4 and 6 hourly thereafter for at least 24 hours or until the patient recovers consciousness and can eat if longer than 24hrs. Blood glucose can be checked at any other time as clinically indicated. The parameters that will be monitored during admission will vary slightly between the study sites, according to different requirements expressed by the IRBs of the different countries. If a patient's clinical situation deteriorates the doctor on call has to be informed immediately. Appropriate steps for the treatment of (complications of) severe malaria are outlined appendix 4.

Time window for follow up visits

The time-window for the visit on Day 7, 14, 21 is +/-3 days and for the visits on Days 28 is -3/+7 days

6.6. Blood sampling

6.6.1. On admission (time 0)

Patients will have an intravenous catheter inserted for at least the first 24 hours. An SOP will be provided instructing how this is to be done and how to take blood for protocol tests.

On study admission, immediately before drug administration, blood will be collected according to appendix 3 for the following:

- Parasite count (thick and thin films) and Hct.
- Haemoglobin
- Full blood count
- Reticulocyte count (EDTA-anticoagulated)
- Biochemistry assessments including bilirubin, ALT, AST, Alkaline Phosphatase, and creatinine levels, LDH and any other tests that might be deemed relevant by the treating physician.

Blood glucoseIn this study patients will be enrolled based on clinical signs of severity and the results of the Rapid Diagnostic Test for reasons of administering the treatment for severe malaria as soon as possible, as the microscopy results are not available before 1 hour. In the case the slides from screening and admission are negative the treating physician will review the diagnosis and change the patient management accordingly. The patient will however be retained in the study (intention to treat).

6.6.2. During hospitalisation

During hospitalisation, patients will have blood taken for malaria films and haematocrit at 4h, 8h and 12h and thereafter every 6 hours until parasite clearance (when two consecutive malaria slides are negative). Haematocrits will be done whenever a malaria blood film is made.

During hospitalisation (24 hr, 48 hr 72 hr and 96 hr and follow-up visits (day 7, 14, 21, 28), blood will also be taken for:

- 1 mL EDTA: Full blood count, , and Hb
- Reticulocyte count
- 1 mL heparinised blood: biochemistry assessments including bilirubin, ALT, AST, Alkaline Phosphatase, and creatinine levels, and LDH.

Other tests that might be deemed relevant by the treating physician for the care of the patients can be performed.

Note: Once parasitemia has been cleared and the patient tolerates oral antimalarial medications. The patients can be discharged according to the judgement of treating physician.

6.6.3. Blood volumes

The blood volumes for the protocol mandated tests are detailed in the study schedule. Maximum blood volumes are presented below for children. The maximum blood volume will be approximately **14 ml.** For children of 5 kg and more, this is less than 4% of the total blood volume during the whole study period and well below the 10% of total blood volume taken over 8 weeks as recommended by WHO *Bulletin of the World Health Organization 2011:89:46-53* (On average the total blood volume is 80 ml/kg body weight]*)*.

Test	n. test	mL blood for one sample	N.	Total	Time points
RDT	1	0.02	1	0.02	Admission
Malaria film	1	0.06	25	1.5	Admission and H0,4,8,12, then +6 hourly until H48, then 12 hourly until 2 consecutive negative slides, day 7, 14, 21, 28 and unscheduled (n) (max expected 25 blood slides)
Biochemistry tests (ALT, AST, BIL, AKP, Cr, LDH)	5	1	9	9	H0, 24, 48, 72 then day 7, 14, 21, 28, n
Haemoglobin	1	0.08	9	0.72	H0, 24, 48, 72 then day 7, 14, 21, 28, n
Haematocrit	1	0.05	25	1.25	Same time points as slides
Full Blood Count	1	0.1	9	0.9	H0, 24, 48, 72 Days,7,14,21,28,n
Reticulocytes	1	0.05	9	0.45	H0, 24, 48, 72 Days,7,14,21,28,n
Total				13.84	

Part 2:

A mixed method social science study that entails SSIs and survey with study staff, health workers and policymakers and stakeholders, to assess the acceptability, feasibility, pros and cons, costs, and logistics and training implications of 1-step AS vs 2-step conventional AS. Each interview will take around 30 - 45 minutes. The interview will be audio-recorded, if participant allowed. Topic guide will be used during the interview.

For survey/questionnaire, a maximum number of relevant participants (150 study staffs and health care staffs) will be attempted to be recruited from two sites.

Part 3:

1. Gather all necessary data from multiple sources.
2. Summarize relevant findings related to real-life practice from Part 1 & Part 2 of study.
3. Calculate cost types: consumables, training, time savings, shipping, wastage, storage.
4. Calculate costs: fixed, one-time, variable costs
5. Calculate unit costs
6. Compare costs and over period of time under different scenarios
7. Rank best options over selected time periods
8. Assessment of uncertainties and externalities

The costing analysis will occur during the second half of the trial as data from Part 1 and Part 2 become available.

A cost analysis of 1-step parenteral AS using data from Part 1 & Part 2. This will assess health facility-level costs, and health system costs to encompass all costs of a potential change from conventional to 1-step artesunate, including re-training, materials, drug replacement. Unit costs will be assigned to healthcare worker time spent on managing patients with severe malaria (derived from the time and motion surveys). Micro costing will be carried out in each of the sites to estimate the costs of equipment and consumables associated with provision of artesunate with each of the formulations. The resulting labour and consumable mean costs will be compared in patients treated with 1-step vs conventional artesunate formulation.

In addition to time measurement, the observer will also record the resources used for the administration of artesunate. This will include the vials themselves, as well as other consumables required for the administration (syringes, needles, catheters, etc.).

Unit costs for these resources will be obtained online and this does not require data collection on site. Costs can be obtained by communication with representatives of companies, hospital administrators and malaria control programme officers. This will be sufficient to capture the specific costs for the administration of the treatments, and assumes that other care and length of stay will not be affected (consistent with the working assumption that there will be no difference in clinical outcomes).

6.7. Study drug regimens

Overview of drug regimens	
1-step AS-arm	**2-step AS-arm**
1-step parenteral AS **New injectable artesunate (Argesun) 60mg** **Each vial of powder contains 60mg artesunate.** **Each ampoule of 6 ml solvent contains: sodium bicarbonate 8.4mg/ml; arginine 20mg/ml.** **Guilin Pharma, Guangxi, China** **Artesunate 2.4 mg/kg STAT on admission, 12hrs, then daily. <20 kg children will get artesunate 3.0 mg/kg. Minimal 3 doses.** **FOLLOWED BY** **Oral artemisinin combination therapy x 3 days**	**Conventional 2-step parenteral AS** **Conventional injectable artesunate (Artesun) 60mg** **Each vial of powder contains 60 mg artesunate.** **Each ampoule of 1 ml solvent contains 50 mg sodium bicarbonate.** **Each ampoule of 5 ml diluent contains 45 mg sodium chloride.** **Guilin Pharma, Guangxi, China** **Artesunate 2.4 mg/kg STAT on admission, 12hrs, then daily. <20 kg children will get artesunate 3.0 mg/kg. Minimal 3 doses.** **FOLLOWED BY** **Oral artemisinin combination therapy x 3 days**

Patients will be treated with weight-based doses according to the schedule in Appendix 1.

The ACT used will be artemether-lumefantrine if available, given according to manufacturer's instructions. The ACT will be administered by study medical or nursing staff while the patients are hospitalised. Directly Observed Therapy (DOT) will be done if possible, but in all cases the first dose of ACT will be by DOT. During DOT, if the patient vomits within half an hour after intake of ACT, the full dose will be repeated. If vomiting occurs between half and one hour, half of the dose will be repeated. If vomiting occurs more than one hour after drug administration, no repeat dosing will be done. Repeat doses will be recorded on the CRF. If vomiting within 1 hour occurs more than one time, no repeat dosing is allowed. The patient will be treated at the discretion of the investigator.

6.8. Discontinuation/ Withdrawal of Participants from the Study

Each participant (or the responsible caregiver) has the right to discontinue the study drug or the study participation at any time without giving a reason.

The investigators may as well decide to discontinue participation in the study of a participant if necessary.

The reason for withdrawal or discontinuation, if available, will be recorded in the CRF. If the study drug or participation in the study is discontinued due to an adverse event, the investigator will arrange for follow-up visits at least until the adverse event has resolved or stabilised.

6.9. Source Data

Part 1 and 2

Source documents are original documents, data, and records from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and CRFs.

CRF entries will be considered source data if the CRF is the site of the original recording (e.g., there is no other written or electronic record of data). In this study, the CRF will be used as the source document for most of the data points.

All documents including audio-records, survey questionnaire forms will be stored safely in confidential conditions. On all study-specific documents, other than the signed consent form, the participant will be referred to by the participant number, not by name.

6.10. Definition of end of the trial

The end of the trial will be the date of the last visit of the last participant.

7. STUDY DRUGS

7.1 Storage of Study Drugs

All efforts will be made to store the study drugs in accordance with the manufacturers' recommendations in a secure area.

7.2 Compliance with Study Drugs

Parenteral study drugs will be administered by study medical staff (either a doctor or nurse). The first dose of ACT will be administered by DOTs and if the patient vomits ACT, and is re-dosed; this will be recorded in the CRF. If vomiting within 1 hour occurs again after retreatment, no repeat dosing is allowed. In this case the patient will be treated at the discretion of the Investigator. The patient may be discharged if recovered in the opinion of the study physician even if the full 3-day course of ACT is not complete. All drug doses will be recorded in the CRF.

7.3 Accountability of the Study Treatment

All movements of study medication will be recorded. Both study medication of individual patient and overall drug accountability records will be kept up to date by the study staff.

7.4 Concomitant Medication

Throughout the study, investigators may prescribe concomitant medications or treatments deemed necessary. Any medication, other than the study medication taken during the study will be recorded in the CRF.

8. SAFETY REPORTING

This trial will use parenteral artesunate followed by an ACT (expected to be artemether-lumefantrine, or other ACT), drugs that are registered and have been evaluated extensively.

To allow for comparison of safety and tolerability of 1-step injectable artesunate compared to conventional injectable artesunate we will record and review all Adverse Events (AEs) and Serious Adverse Events, (SAEs) that occur in the study.

A symptom questionnaire will be performed daily during hospitalization and at each subsequent visit to the health care facility, to aid in the identification of adverse events.

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an adverse event (AE), or serious adverse event (SAE), as provided in this protocol.

All SAEs, and AEs will be promptly documented from the moment of randomization in the study to discontinuation of the patient from study participation. Any events occurring between screening and randomization will be considered as baseline, pre-existing conditions. Any conditions that were present at baseline will not be considered adverse events unless the grade of the condition deteriorates.

Each adverse event will be graded according Common Terminology Criteria for Adverse Events (CTCAE) version 5.0:

https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50

All adverse events must be recorded in the AE /SAE CRF. To avoid colloquial expressions, the adverse event should be reported in standard medical terminology. Whenever possible, the adverse event should be evaluated and reported as a diagnosis rather than as individual signs or symptoms. If a definitive diagnosis is not possible, then the individual symptoms and signs should be recorded. Whenever possible, the aetiology of the abnormal findings will be documented on the CRF. Any additional relevant laboratory results obtained by the Investigator during the course of this study will be recorded on the CRF.

8.1 Definitions

8.1.1. Adverse Event (AE)

An AE is any undesirable event or clinical deterioration that occurs to a study participant during the course of the study; that is, from the time of administration of study drugs until study ends (i.e., until the follow up visit) whether or not that event is considered related to the study drugs, or to a concomitant drug or procedure, e.g.

- any unfavourable and unintended symptom
- physical sign
- abnormal laboratory result
- an illness
- any pre-existing condition that has worsened in grade

Any new clinical sign or clinical deterioration that occurs between signing the consent form and the administration of study drugs is not an AE. This information will be recorded in the medical records, as a pre-existing condition.

Condition associated with severe malaria

Common conditions resulting from a deterioration of severe malaria as per list should not be considered or reported as Adverse Events. They are recorded in the specific section of the CRF.

A list of the most common conditions in severe malaria includes,

- Deterioration of BCS or GCS
- Coma not present at admission
- Convulsions not present at admission
- Posturing not present at admission
- Decompensated shock (children systolic BP <70 mm Hg)
- Compensated shock (capillary refill >3sec /temperature gradient)
- Respiratory distress
- Hypoglycaemia (glucose <3 mmol/L)
- Severe anaemia
- Severe bleeding (requiring blood transfusion)
- Hemoglobinuria
- Severe jaundice
- Renal failure (urine output <0.5 mL/kg/hr >24 hours)

8.1.2. Serious Adverse Event

A serious adverse event is an AE that:

- results in death
- is life-threatening i.e. the patient was at risk of death at the time of the AE
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapacity
- Any other significant medical condition

More than one of the above criteria can be applicable to the one event. Other significant medical conditions may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the patient or require medical or surgical intervention to prevent one of the outcomes listed in the definition above.

All AEs must be followed until resolution, or until the AE is deemed permanent or leads to death.

8.2 Reporting Procedures for Serious Adverse Events (SAEs)/ SUSARs

All SAEs are recorded in a CRF. They need are to be reported as follows:

Safety team:
All SAEs are to be reported to Safety team (PI, Sponsor representative (CTSG), project coordinator, and medical monitor) by e-mail to 1stepsafetyteam@tropmedres.ac within 24 hours of site awareness. If further data is required, additional documentation can be submitted, but an initial report should be submitted within the 24-hour timeframe.

SAE, including death, resulting from a deterioration of severe malaria will not follow expedited reporting (24-hour timeframe), but reported monthly.

DSMB:
All SAEs are reported to DSMB on a monthly basis

Ethics Committee and the Regulatory Authorities:
All SAEs must also be reported to the ECs and the Regulatory Authorities as per required by local guidelines

8.3 Evaluating Adverse Events and Serious Adverse Events

8.3.1. Assessment of Intensity AE or SAE

Each adverse event will be graded according Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. This will be included in the safety monitoring plan.

If an adverse event is not listed in the CTCAE table, the Investigator will assess the severity using the following guidelines:

1 = Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

2 = Moderate; minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental ADL*

3 = Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**

4 = Life-Threatening consequences; urgent intervention indicated

5 = Death related to AE

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden. According to age- & culture-appropriateness.

8.3.2. Clarification of the difference in meaning between ‘SEVERE’ and ‘SERIOUS’

The term “severe” is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as “serious”, which is based on the outcome or criteria defined under the serious adverse event definition and the defined study specific SAEs. An event can be considered serious without being severe if it conforms to the seriousness criteria, similarly severe events that do not conform to the criteria are not necessarily serious. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

8.3.3. Assessment OF RELATEDNESS OF AE OR SAE TO STUDY DRUGS

The investigator is obligated to assess the relationship between study drug and the occurrence of each AE/SAE using the following categories of relatedness:

- Definite: clear-cut temporal association

- Probable: clear-cut temporal association, with improvement upon drug withdrawal, and not reasonably explained by the patient's known clinical state or other aetiology.
- Possible: less clear temporal association; other aetiologies are possible. (Other possible aetiologies should be recorded on the CRF).
- Not related: no temporal association with the study drug; assessed as related to other aetiologies such as concomitant medications or conditions, or patient's known clinical state.

The investigator will provide the assessment of causality as per the AE/SAE case record form.

8.3.4. Outcome

The investigator will follow-up the AE and SAE until resolution, death, deemed permanent and no further medically relevant information can be expected. AE and SAE outcome will be classified as follows:

- Continuing/ongoing
- Resolved
- Resolved with sequelae
- Permanent
- Fatal

9. STATISTICAL CONSIDERATIONS

9.1. Sample size justification

Part 1:

Sample size calculations for non-inferiority study is based on dissolution test data from Guilin Pharmaceutical Co. Ltd. ("Study report on the solubility of artesunate for injection (60mg), Doc. RR-QC-ZSYQHHZ (60mg)RJX-2021 30th April 2021).[23] This is supported by unpublished in-house experience (from a bioequivalence study in healthy volunteers, personal communication, Dr Podjanee Jittmala, Mahidol University) that the conventional formulation takes about 4 minutes with a standard deviation of 2 minutes to administer injectable artesunate. Our non-inferiority margin is 1 minute of drug re-constitution for the new simpler artesunate formulation i.e. if participants in the 1-step artesunate formulation will have a mean of the time of 5 minutes or less of drug re-constitution, with a standard deviation of 2 minutes, then the new formulation will be considered to be non-inferior to the old formulation. With this non-inferiority margin, detecting non-inferiority with 90% power and with a one sided alpha of 0.025, we will need to recruit 85 participants in the new formulation and 85 in the old formulation arm giving a total of 170 participants. In order to compensate for a 15% loss to follow-up or withdrawal, we will need 100 participants in each arm giving a total of 200 participants in the two arms combined. These calculations were performed in Stata 16 (StataCorp LLC Software).

The sample size calculation dovetails with pragmatic needs for the other study activities the 200 participants provides sufficient variability of participant characteristics to allow staff to gain experience of using 1-step artesunate and be able to provide an informed opinion of its pros and cons. Recruitment of 100 patients per site is feasible over 12 months based on the expected numbers of cases of severe malaria (Dr Fanello and Dr Gesase, personal communication April 2021). The sample size calculation is based only on the time-to-treatment of participants and not on the costing analysis.

Part 2:

Number of respondents (sample size) for this study will be based on the principles of 'data saturation', that is data are collected until no new data/themes emerge from further interviews. A minimum of 40 respondents in total will be recruited for the SSIs, and at least 150 health workers will be interviewed with the survey questionnaire.

9.2. Analysis approach

Part 1:

The time and motion study will record the number of steps and time taken to administer the correct dosage of artesunate. We will compare median (IQR, ranges) times for 1-step artesunate and conventional artesunate. Analysis of other endpoints will be described in a Statistical Analysis Plan. We assume 1-step parenteral artesunate is bioequivalent to conventional parenteral artesunate. A brief overview is given below.

9.2.1 General Analysis Strategy

The principle of intention to treat (ITT) will be the main strategy of analysis for the primary outcome. These analyses will be conducted on all patients assigned to the treatment groups as randomised. Any patients subsequently found not to be eligible during the trial or that did not receive a full course of study drug will be included in these analyses. Per Protocol (PP) analysis will be conducted to examine robustness of ITT results. This will be a form of sensitivity analysis to the intention to treatment analysis principle. In the PP analyses, all patients wrongly included in the study based on the inclusion/exclusion criteria will be excluded from analyses.

The cost analysis of 1-step parenteral AS will assess health facility-level costs, and health system costs to encompass all costs of a potential change from conventional to 1-step artesunate, including re-training, materials, drug replacement. Unit costs will be assigned to healthcare worker time spent on managing patients with severe malaria (derived from the time and motion surveys). Micro costing will be carried out in each of the sites to estimate the costs of equipment and consumables associated with provision of artesunate with each of the formulations. The resulting labour and consumable mean costs will be compared in patients treated with 1-step vs conventional artesunate formulation.

9.2.2 Proportions

These will be compared using chi squared or Fisher's exact test, as appropriate. Crude proportions will be calculated with the exact 95% confidence intervals (CI), where relevant.

9.2.3 Continuous data

These will be summarised by medians (IQR, ranges) and means (standard deviations, 95% CIs), as appropriate, and will include the parasite counts and laboratory parameters. Comparisons of continuous data will be assessed using the paired/unpaired t tests or the sign rank/Mann Whitney U tests, as appropriate. Analyses of the parasite clearance data will be conducted.

9.2.4 Safety analysis

This is not a study designed chiefly to determine the safety of 1-step artesunate vs conventional 2-step artesunate, as both contain identical drugs. Moreover, this is a small study, and thus the patient populations may vary by chance or by another factor. However, clinical outcome data will be recorded. Safety analyses will be based on the whole population that get administered the study drug. Safety data will be presented in tabular and/or graphical format and summarized descriptively. Any clinically relevant abnormalities or values of potential clinically concern will be described.

Part 2:

All transcripts and the interviewer's notes will be first cross-checked with the audio-recordings. Transcripts and the notes will be coded line by line in qualitative data analysis soft-ware NVivo 12 (QSR International, Doncaster, Australia). Coding of data will be based on the initial codebook prepared based on the interview guide (deductive approach) and will be supplemented by revision of codes based on the emerging themes (inductive approach) as the analysis progresses. Two investigators will independently check the coded data against the transcripts. Regular de-briefings will be held between the team members concerning the final themes and their interpretation to reach a consensus. Themes and the supporting quotes will be presented as the findings of this study to address the research question. Survey data will compare the findings between the study staff, and health staff. Differences in acceptability and satisfaction among these two groups of respondents will be evaluated using relevant statistical tests.

Part 3:

In the current study we will focus on the costs of the two different artesunate formulations and the consumables needed for their administration. These direct costs are important at the hospital level, but also for deployment in the wider health system. For example, for the public health system: the shipment cost for the 1-step artesunate will be cheaper than the cost for the conventional 2-step artesunate because the unit volume per pack (box) is smaller for the 1-step artesunate, and lighter.

Finally, using data on the incidence of severe malaria admissions at the sites (primary data) and nationally (national and international annual malaria reports) the total per annum costs of treating patients with the different formulations at the facility and national levels will be estimated.

In the unlikely event that there is an indication of a clinical benefit for the new formulation as compared with the 2-step artesunate, and should it be more costly, we will also perform a cost-effectiveness analysis to determine whether the apparent clinical benefit justifies the incremental cost.

10. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

Direct access will be granted to authorised representatives from the sponsor and host institutions and the regulatory authorities, if applicable, to permit trial-related monitoring and inspections.

11. QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

The study will be conducted in accordance with the current approved protocol, ICH GCP, any national regulations that may apply to this study and standard operating procedures. Regular external monitoring of all sites will be coordinated by the MORU CTSG according to ICH GCP and a Monitoring Plan. Their role will include but not be limited to monitoring adherence to SOPs for collection of clinical data and laboratory specimens and quality checks (curation) of clinical and laboratory data according to standard methodologies.

An online site initiation visit will cover GCP, and study-specific procedures, including: CRF completion, screening and enrolment procedures, data management, follow up scheduling.

11.1. Monitoring

Study sites may have in place a system for internal monitoring. In addition, regular external monitoring of all sites will be performed by the MORU CTSG according to ICH GCP and a Monitoring Plan. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. The monitors will check whether the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

11.2. DSMB

An independent Data Safety and Monitoring Board (DSMB) will be set up consisting of qualified volunteers with the necessary knowledge of clinical trials. The DSMB will receive summary reports, prior to each meeting. All data reviewed by the DSMB will be in the strictest confidence. A DSMB charter will outline its responsibilities and how it will operate.

The DSMB will meet formally at the following timepoints:

- Before the study starts
- At the end of the study (i.e. after the last patient has finished follow up)
- Unscheduled meetings can be held on the initiative of the Medical Monitor (Dr Jacqueline Deen, deen.jacqueline@gmail.com), Principal Investigator, or DSMB to consider e.g. serious adverse events as they are reported on monthly basis OR if an earlier safety review is thought to be indicated.

12. ETHICS

12.1. Declaration of Helsinki

The Investigator will ensure that this study is conducted in compliance with the current revision of the Declaration of Helsinki (Fortaleza 2013).

12.2. ICH Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted according to any National Regulations and that it will follow the principles of the ICH Guidelines for Good Clinical Practice.

12.3. Approvals

The study protocol and its associated documents will be submitted to the Oxford Tropical Research Ethics Committee (OxTREC) and the appropriate local ethics committees for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

12.4. Risks

Part 1:

This study will use drugs that have been studied thoroughly and their toxicities are well described. In general, they are all well tolerated.

12.4.1. Risks of parenteral artesunate

One of the concerns about the artemisinin derivatives is neurotoxicity, in particular involving the brain stem[24]. Nevertheless, a discrepancy seems to prevail with regard to the toxicity and safety of the artemisinin family of antimalarials. While these compounds have been found to be virtually void of any serious side effects in humans, their neurotoxicity in animal models has raised concerns about their use[25,26]. The prolonged presence of artemisinins upon slow release from oil-based intramuscular formulations at several orders of magnitude above the therapeutic level appear to be the main cause of the observed toxicity in laboratory animals.

Artesunate is rapidly and extensively converted to dihydroartemisinin (DHA), the active metabolite, primarily by plasma and erythrocyte esterases. DHA elimination from the blood compartment is also rapid (half-life approximately 45 min). The potential for drug-drug interactions with other medicinal products is limited. *In vitro* drug-interaction studies have demonstrated minimal effects of artesunate on cytochrome P450 isoenzymes. In the limited number of clinical drug-drug interaction studies that have been performed, however, no clinically significant drug-drug interactions have been identified.

Delayed haemolytic anaemia following treatment with injectable artesunate has been observed in children in malaria endemic areas and in non-immune travellers presenting with severe falciparum malaria.[27] The risk was most pronounced in patients with hyper-parasitaemia and in younger children. Some cases have been severe and required blood transfusion. The risk of post-artesunate delayed haemolytic anaemia has been reported however to be very low in children in highly endemic settings (Mozambique and DRC) and statistically not different from intravenous quinine[18]. Vigilance for delayed onset anaemia is therefore advised, particularly in hyper-parasitaemic patients and younger children, and prolonged follow-up should be considered (e.g. 14-28 days). As the overall benefit-risk ratio remains highly favourable for injectable artesunate in the treatment of severe malaria, WHO strongly recommends its continued use.

The most important reported side effect of artesunate is a rare severe allergic reaction (estimated risk approximately 1 in 3000 patients), which can present as an urticarial rash or more severe allergic symptoms, including hypotension, pruritus, oedema, and/or dyspnoea. More common minor side effects described in association with parenteral administration of artesunate include dizziness, light-headedness, rash, and taste alteration (metallic/ bitter taste). Nausea, vomiting, anorexia and diarrhoea have also been reported, however it is uncertain whether these are attributable to the drug or to the disease severe malaria.

12.4.2. Risk of phlebotomy, venepuncture, finger stick

The primary risks of phlebotomy include local discomfort, occasional bleeding or bruising of the skin at the site of needle puncture, and rarely haematoma or infection.

Part 2:

12.4.3. Risks to participation in social science studies

Some participants may feel uncomfortable by the details they have to think through during the interview. If the interviewer notices any inconvenience or distress, can divert the topic and ask if participant is comfortable for further discussion. However, we will ensure that participants are informed of their freedom not to answer questions. In addition, we will provide mechanisms to ensure responses are confidential when requested or needed. If in case interview goes longer than planned (e.g. more than 45 minutes), interviewer will remind the respondent and ask if he/she is comfortable to continue beyond the designated time.

12.5. Benefits

Part 1:

Severe malaria is a disease that needs to be treated promptly. All patients will benefit from receiving efficacious treatment at no cost. They will be followed up closely and will be given rescue treatment if clinically indicated.

Part 2:

There are no direct benefits to the participants other than their perspectives will contribute to the evidence-generation, specifically about acceptability, and practicalities related to the treatment of sever malaria using 1-step versus 2-step artesunate injections. The feasibility, acceptability and practicalities will help inform the treatment guidelines for severe malaria.

12.6. Alternatives to Study Participation

Patients or their parent/guardian can freely decline participation in this study. If so, they will receive standard care for their malaria.

12.7. Incentives & Compensation

Part 1:

The study will pay for food during hospitalization for the patients and the caregivers, and small benefits such as insecticide treated nets and soap, plus local transport costs for the follow-up visits. Any additional reimbursement and the amount in monetary terms (e.g. for the time lost from work as a result of hospitalisation) will be at the instruction of the national ethics committees. We anticipate that these are not allowed in the DRC as they are considered coercive. The study will pay locally appropriate costs for treatment of drug-related SAEs or other research-related injuries. Although the study cannot pay for long term care for disability after hospital discharge resulting from complications

of the illness the study will pay the reasonable costs for treatment of such sequelae during the follow up assessments for 12 months.

Part 2:

Appropriate reimbursement will be provided to all the participants based on the valid invoices of their travel costs.

12.8. Confidentiality

The trial staff will ensure that the participants' anonymity is maintained. The participants will be identified only a unique participant number on the CRF and the MACRO EDC database. All documents will be stored securely and be accessible to trial staff and authorised personnel only. The study will comply with the Data Protection Act 2018, which requires that personal data must not be kept as identifiable data for longer than necessary for the purposes concerned.

13. SAMPLE MANAGEMENT

Samples collected will be used for the purpose of this study as stated in the protocol.

14. DATA HANDLING AND RECORD KEEPING

Part 1:

Study data will be recorded on standard Case Report Forms (CRF) and entered to MACRO EDC©, a GCP-compliant data management system. The database is password-protected and includes internal quality checks to identify data that appear inconsistent, incomplete, or inaccurate.

Study participants will be identified by a unique participant number in the database. The study data management plan outlines all activities that will be carried out to ensure security and quality of the data.

Subject records at site will, taking into account the ability of the sites, be stored in binders or scanned and stored electronically. The records will be retained until the youngest child participating in the trial reaches 21 or five years following completion of the study, whichever is longer. The study database will be retained indefinitely.

Participant's data and results from blood analyses stored in the database may be shared according to the terms defined in the MORU data sharing policy with data repositories or other researchers to use in the future. All personal information will be de-identified so that no individual can be identified from their treatment records, through interviews.

Part 2:

All data collected during the interviews in addition to the written informed consent will be stored at password protected principal interviewer's official computer (laptop) and an external hard drive until the end of the study. All data will be provided with a unique code that will link the personal data with the research data and will be kept separately from the research data. Research data will not have personal identifiers (e.g. name). Personal data such as their age, profession and workplace will be anonymized from the transcripts and immediately transferred and backed-up to a secure server at MORU. The audio-recordings (with personal identifiers) will be deleted after the end of the study. Duration of storage of transcripts will be guided by the MORU's data access policy. The study will comply with the General Data Protection Regulation (GDPR), which requires that personal data must not be kept as identifiable data for longer than necessary for the purposes concerned which is approximately 5 years after study completion.

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15. SPONSORSHIP AND INSURANCE

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London).

16. FUNDER

The funder, Shanghai Fosun Pharmaceutical Industrial Development Co., Ltd, had a role in the design of the study. The funder will not have any role in its execution, analyses, and interpretation of data or decision to submit results.

17. PUBLICATION POLICY

Any data published in the peer-reviewed medical literature will protect the identity of the patients. This trial will be registered in a web-based protocol registration scheme. All those who have made a substantial contribution will be co-authors on publications. The sites have the right to publish their data individually and to include members of the sponsor's team who have made a significant contribution. There will also publications of pooled data which will be coordinated by the MORU group. All sites will have the opportunity to contribute to these publications.

The research findings disseminated to policy makers, National Malaria Control Programmes (NMCPs) and other researchers.

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APPENDIX 1. DOSING SCHEDULES

The dosing scheme for parenteral artesunate is 2.4 mg/kg at hour 0, hour 12, hour 24, and each 24 hours until the patient can take oral medication. Children <20 kg will get 3.0 mg/kg. At least 3 parenteral doses should be given before switching to oral therapy can be considered i.e.: stop injectable artesunate and give oral antimalarial. When the patient is improving and able to take oral tablets a switch to oral ACT should be considered.

2-step conventional injectable artesunate. Refer to Artesun SmPC page 2&3.

One unit box of Artesun (conventional injectable artesunate) contains:
Each vial contains: artesunate powder 60 mg
Each ampoule of solvent contains: sodium bicarbonate 50 mg/ml, 1 ml
Each ampoule of diluentcontains: sodium chloride 9 mg/ml, 5ml (There is no need to use additional 5% dextrose for dilution.)

For intravenous (IV) injection (10 mg/ml)
After reconstitution and dilution one ml of solution for injection contains 10 mg artesunate.
Using a syringe, add 5 ml of sodium chloride 0.9% for injection to the vial containing the reconstituted artesunate solution. This will yield 6 ml of a solution containing artesunate 10 mg/ml. Shake to mix well, ensuring that the resulting solution is still clear. If the solution appears cloudy or a precipitate is present, it should be discarded.
The volume of the solution required (ml) will be:
Volume (ml) = [dose (mg)] ÷ 10

For intramuscular (IM) injection (20 mg/ml)
After reconstitution and dilution one ml of solution for injection contains 20 mg artesunate.
Using a syringe, add 2 ml of sodium chloride 0.9% for injection to the vial containing the reconstituted artesunate solution. This will yield 3 ml of a solution containing artesunate 20 mg/ml. Shake to mix well, ensuring that the resulting solution is still clear. If the solution appears cloudy or a precipitate is present, it should be discarded.
The volume of the solution required (ml) will be:
Volume (ml) = [dose (mg)] ÷ 20

1-step injectable artesunate. Refer to Argesun SmPC page 2&3.

Dose: Adults and children weighing more 20 kg or more: 60mg is administered at a dose of 2.4mg of artesunate / kg body weight, by intravenous (IV) or intramuscular (IM) injection, at 0, 12 and 24 hours, then once daily until oral treatment can be substituted. Children weighing less than 20 kg: 60mg is administered at a dose of 3 mg of artesunate/kg body weight, by intravenous (IV) or intramuscular (IM) injection, at 0, 12 and 24 hours, then once daily until oral treatment can be substituted (see section 5.1).

New injectable artesunate (Argesun®) 60mg should be administered for a minimum of 24 hours (3 doses), regardless of the patient's ability to tolerate oral medication earlier. After at least 24 hours of Argesun® 60mg and when able to tolerate oral medication, the patient should be switched to a complete treatment course of an oral antimalarial regimen.

Preparation

Because of the instability of artesunate in aqueous solutions, the reconstituted solution must be used within one hour of preparation. Therefore, the required dose of artesunate should be calculated (dose in mg = patient's weight in kg x 2.4 for patients weighing more than 20 kg; or dose in mg = patient's weight in kg x 3 for children weighing less than 20 kg) and the number of vials of artesunate needed
should be determined prior to reconstituting the artesunate powder.

Reconstitution of the artesunate solution Following reconstitution the solution must be diluted according to the method of injection, as described below.

For intravenous (IV) injection & intramuscular (IM) injection (20 mg/ml), using a syringe, withdraw 3 ml of the sodium bicarbonate and arginine solvent, which is half the volume of the ampoule, and inject this into the vial containing the artesunate powder. Gently shake the vial for up to 3-5 minutes or until the powder is completely dissolved and the solution is clear. If the solution appears cloudy or a precipitate is present, it should be discarded. The reconstituted artesunate solution should always be used immediately, and discarded if not used within one hour. The end concentration of the solution will be 20 mg artesunate per ml solvent. Thus, the volume in ml for administration to the patient will be equal to: (desired dose in mg)/20

Withdraw the required volume of artesunate solution from the vial with a syringe and then administer to the patient by slow intravenous or intramuscular injection over 1-2 minutes. New injectable artesunate (Argesun®) 60mg should NOT be administered as an intravenous drip. Reconstituted vials of Artesunate for Injection and ampoules of the solvent Sodium bicarbonate and Arginine injection are for single use only. Discard unused portions. Do not use water for injection for reconstitution of the artesunate powder or for dilution of the resulting solution prior to injection.

APPENDIX 2. LIST OF STUDY SITES & PRINCIPAL INVESTIGATORS

TANZANIA

Site PI: Dr Samwel Gesase

Site 1: **Magunga District Hospital,** NIMR Korogwe Research Laboratory, Korogwe, Tanga Region

DRC

Site Co-PIs: Prof Marie Onyamboko & Dr Caterina Fanello

Site 1: **KIMORU,** Kinshasa

APPENDIX 3. TIME AND EVENT SCHEDULE

TEST/APPLICATION	SAMPLE	SCR	Day 0					Day 1				Day 2				Day 3				Dischage day	Day 7 ***** ***	Day 14 ***** ***	Day 21 ***** ***	Day 28 ***** ***	F/U for sequeale*********		
			H0	H4	H8	H12	H18	H24	H30	H36	H42	H48	H54	H60	H66	H72	H78	H84	H90						Month 3	Month 6	Month 12
Informed Consent		X																									
Demogrphic/ drug history		X																									
Medical history		X																							X	X	X
Temperature*		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X
Vital signs**		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X
Weight/Height		X																									
Physical examination		X						X				X				X					X	X	X	X	X	X	X
Neurological exam																				X				X	X	X	X
Symptom questionnaire			X					X				X				X					X	X	X	X	X	X	X
Randomisation			X																								
Injectable artesunate***			X			X		X																			
Oral ACT****									(X)		(X)				(X)				(X)								
SAMPLE COLELCTION																											
Malaria RDT		X																									
Malaria blood film*****	EDTA	X	X	X	X	X	X	X	X	X	X	X		X		X		X			X	X	X	X			
Haematocrit*****	EDTA	X	X	X	X	X	X	X	X	X	X	X		X		X		X			X	X	X	X			
Haemoglobin	EDTA		X					X				X				X					X	X	X	X			
Blood glucose******			X	(X)	(X)	(X)	(X)	(X)																			
Full Blood Count	EDTA		X					X				X				X					X	X	X	X			
Biochemistry*******	EDTA		X					X				X				X					X	X	X	X			
Reticulocytes	EDTA		X					X				X				X					X	X	X	X			

*Temperature measured at time of malria blood film until 2 consecutive measurements below 37.5°C

**Vital signs recorded hourly for unstable patients, then 6 hourly for stabilised patients, and at each F/U visit.

***Injectable artesunate at 0, 12, 24 hrs and until able to tolerate oral medication

**** Oral ACT starts 8 hours after the last dose of iv artesunate. ACT for 3 days, first dose directly observed and then DOTs on study wards if patient agrees, or patient can be discharged to complete treartment at home.

*****Malaria blood film and HCT taken at admission and 0h, 4h, 8h and 12h, then +6 hourly until H48, then 12 hourly until 2 consecutive negative slides, day 7, 14, 21, 28, and unscheduled (n).

******Blood glucose measured on admission and between 4 and 6 hourly thereafter for at least 24 hours or until the patient recovers consciousness and can eat if longer than 24 hrs.

*******Biochemistry assessments including bilirubin, ALT, AST, Alkaline Phosphatase, and creatinine levels, LDH and any other tests that might be deemed relevant by the treating physician.

********The time-window for the visit on Day 7, 14, 21 is +/-3 days and for the visits on Days 28 is -3/+7 days.

*********Longer term follow up only of patients who are not fully recovered by D29

APPENDIX 4. TREATMENT GUIDELINES FOR SEVERE MALARIA

Introduction

Severe *falciparum* malaria is a medical emergency. Mortality is high and most deaths occur within 24 hours after admission to the hospital. Cerebral malaria, the most prominent manifestation of severe malaria carries a 16-20% mortality, but once multiple vital organ dysfunction occurs, the figure increases to as high as 30% (WHO 1990, 2000). The clinical manifestations depend on age. Hypoglycaemia, convulsions, and severe anaemia are relatively more common in children; acute renal failure, jaundice, and the acute respiratory distress syndrome (ARDS) are more common in adults. Cerebral malaria (with coma) and a severe metabolic (lactate) acidosis may occur at any age, and the severity of both features is a strong prognostic factor for a fatal outcome.

Cerebral malaria is characterized by coma of varying depth, usually with no focal signs. Convulsions occur in up to 80% of cases in children, but in less than 10% of adults. If the patient survives, there are usually (>90%) no neurological sequelae. Unlike in septicaemia, circulatory shock ('algid malaria') is not common in severe malaria. If hypotension is present, one should consider concomitant (gram negative) septicaemia or meningitis. Also, leucocytosis is not common in malaria, although it can be present in very severe disease. Leucocytosis with a shift to the left, should trigger the possibility of concomitant bacterial infection. Thrombocytopenia is always present in severe malaria, but this is seldom accompanied by bleeding problems. Diffuse intravascular coagulation (DIC) is rare (less than 5% of severe cases), and can cause serious bleeding. Black water fever is a rare complication of *falciparum* malaria, with severe intravascular haemolysis and haemoglobinuria. It is associated with the use of quinine and G-6-PD deficiency. About 10% of these patients have oliguric renal failure (Tran 1996). Table 1 gives a brief summary of the manifestations of severe malaria.

Diagnosis and differential diagnosis

In patients presenting with symptoms compatible with severe malaria, the diagnosis should be confirmed by a peripheral blood slide, or by rapid test based on the detection of the malaria antigen HRP2 (e.g., Paracheck-F test). The sensitivity of these rapid antigen test is high (97%), meaning that a negative test almost excludes the diagnosis *falciparum* malaria. However, the specificity depends on the endemicity of the disease, since the test can remain positive for a few weeks after a malaria attack. Especially in highly endemic areas a positive test can thus be caused by a recent malaria episode, whereas the cause of the presenting fever can be a different one. The specificity of the test in non-endemic countries can be as high as 100% but drops to 85% in malaria endemic areas. A positive malaria slide, especially in the adult patient, does not prove that the presenting symptoms are caused by severe malaria, because of the high background prevalence of peripheral blood parasitaemia. Other causes of coma have thus also to be considered, including: hypoglycaemia, meningitis, septic shock and post-ictal coma (after febrile convulsion). Moreover, sedative drugs, severe anaemia, severe acidosis or severe hyponatraemia can contribute. All these factors (except meningitis and sedative drugs) can occur as a complication of severe malaria. Consequently, blood glucose and Haematocrit should be checked in every patient (i-STAT or glucose-stick), a lumbar puncture should be performed, and the blood pressure has to be measured. If available electrolytes and acid-base status should be checked. The treatment of these conditions is described below

Antimalarial treatment

The mainstay of the treatment of severe malaria is the immediate start of parenteral antimalarial treatment. The available antimalarial drugs to treat severe malaria are parenteral quinine and the parenteral artemisinin derivatives (artesunate).

Artesunate belongs to the most rapidly acting and potent group of antimalarial drugs (artemisinin derivatives). They are safer and easier to administer compared to quinine. Artesunate and artemether are used widely in SE Asia, and increasingly in sub-Saharan Africa, for the treatment of uncomplicated and severe malaria as alternatives to quinine (Van Agtmael 1999). Injectable artesunate, which is water soluble, can be given intravenously and gives very reliable plasma levels. The dose of artesunate is 2.4 mg/kg i.v. or i.m. on admission, 12 hours, 24 hours, and then daily. Children <20 kg will get 3.0 mg/kg. If possible, to take oral medication, the patient can switch to an oral course of Coartem (dosing see above). The artemisinin derivatives have remarkably few side effects. Allergic skin reactions and anaphylaxis have been reported.

Treatment of specific complications.

Generally, all patients who are in a critical condition (i.e., their survival is questionable) on admission should be transferred immediately to the intensive care unit. Patients who have been admitted to the ward and who show signs of deterioration which endangers their survival should be transferred from the ward to the ICU.

Fluid management

Severe malaria is a multi-organ disease, and supportive treatment for all kinds of organ failure can be indicated. *Fluid management* can be difficult. The patient is usually dehydrated on admission, and should be rehydrated to support the already compromised microcirculation. Hyponatraemia is common, and mainly a consequence of dehydration. Dextrose-Saline can be used for rehydration, and not only dextrose solutions. (Saline without dextrose can be used in adults but should never be used in paediatric patients.) Dextrose solutions without any NaCl will further lower the plasma sodium concentration, and will inadequately rehydrate the patient. However, there is a strong tendency to develop pulmonary oedema, so care should be taken to avoid too aggressive fluid infusion.

Hypotension is not a common feature of severe malaria and in case of circulatory shock, concurrent septicaemia should be considered. Blood cultures should be taken and the patient should be treated empirically with antibiotics, covering gram-negative bacteria (e.g., the combination of cefotaxime and gentamicin). In case of hypotension, the patient should receive fluid resuscitation. Systemic salmonella infection may develop, presumably because of increased translocation of bacteria in the gut (Mabey 1987). If the patient has been admitted for several days in the ICU, hospital acquired infections should also be considered. Secondary pneumonia is common.

Acidosis in severe malaria can be due to acute renal failure, but usually is a lactic acidosis resulting from tissue hypoxia, generally in the absence of hypotension. Treatment should therefore be directed to optimize the microcirculation, but we have only limited tools to achieve this. Prompt treatment of the parasite causing sequestration and obstruction in small vessels, optimal fluid management and treatment of severe anaemia can all contribute. Using iSTAT results can help in the management of acidosis. If the arterial pH drops below 7.2, 100 ml 8.4% HCO_3 over 30 minutes can be given.

Anaemia is a frequent feature, especially in young children with severe malaria. Transfusion of packed red cells can be restricted to those with haematocrit falling below 20%. It is essential that blood products are negative for transmissible diseases like HIV, hepatitis B, hepatitis C and syphilis.

Disseminated Intravascular Coagulation (DIC)

For those rare patients who develop DIC with bleeding, fresh frozen plasma can be given. In addition, vitamin K should be administered, in a daily dose of 10 mg slowly i.v., for 3 days.

Coma

Hypoglycaemia as a cause for convulsions should be ruled out. A lumbar puncture should be considered. The usual nursing care for the unconscious patient should be applied. If available a nasogastric tube should be inserted and the stomach contents let out. To decrease the chances of aspiration, enteral feeding should not be started on the first day of admission. The patient should be turned every 2 hours to prevent bedsores. If possible, it is preferable to insert a urethral ('Foley') catheter to prevent maceration of the skin and for monitoring fluid management. Eyes should be kept irrigated with saline or artificial tears and the lids kept closed with eye pads. The severity of coma should be graded using the Glasgow coma scale for adults and the Blantyre coma scale for children. Deterioration in the coma score should be reported immediately to the physician on call.

Convulsions are very common in children with cerebral malaria. As for coma, hypoglycaemia as a cause for convulsions should be ruled out. A lumbar puncture should be considered. In adults, convulsions are relatively rare, so that prophylaxis is not generally recommended. If seizures occur, they should be treated promptly with intravenous benzodiazepines (adults 10 mg iv, children 0.3 mg/kg). Since children are prone to febrile convulsions, treatment to reduce fever (paracetamol, cold sponging, etc.) should be given if the rectal temperature is above 39°C.

Hypoglycaemia is particularly common in children and pregnant women with malaria, and, as mentioned above, with the use of quinine. It can be treated with 1 ml/kg of a 50% glucose solution.

Oliguric renal failure is a common complication of severe malaria in adults. However, occasionally the renal failure is non-oliguric. If the adult patient is oliguric (< 20 ml per hour), this can be caused by dehydration or acute tubular necrosis. Dehydration requires rehydration with normal saline, whereas acute tubular necrosis (ATN) requires dialysis and carries the danger for fluid overloading since the patients is not likely restore diuresis with appropriate clearance during the following 2 to 5 days. The clinical assessment of the hydration status (skin turgor, dry lips and mouth, tears, jugular venous pressure). If there is still no diuresis after adequate rehydration, furosemide can be given in increasing doses 40mg-100mg-200mg-400mg at half hour intervals. This will not improve renal function, but if diuresis is still absent after this procedure, it is likely that the patient has ATN.

Acute respiratory distress syndrome (ARDS) is a feared complication of mainly adult severe malaria. The chest X-ray will generally show diffuse shadowing of the lung fields. In case of respiratory distress, the patient should be propped upright and receive oxygen therapy (nasal catheter or face mask). Furosemide should be tried to lower the venous pressure, but this will not often relief symptoms, since the pathogenesis is capillary leakage and not fluid overload *per se*.

Blackwater fever with severe intravascular haemolysis can cause severe anaemia requiring transfusion. On theoretical grounds alkalinisation of the urine can be recommended in this condition, although no clinical studies are available.

Conclusions.
A patient with severe *falciparum* malaria with multiple organ failure is a challenge for the treating physician. If the patient, often a young individual, survives, there are usually no sequelae. Prompt diagnosis and start of antimalarial treatment are essential. But good supportive treatment, realizing the common complications of the disease, is equally important.

Table 1 Brief summary of the manifestations of severe malaria.

Manifestations of severe malaria	Pathophysiology	Treatment
Coma	Compromised microcirculation, other factors like local nitric oxide overproduction?	Good nursing care. Lumbar puncture to rule out meningitis. Rule out hypoglycaemia.
Convulsions	Compromised microcirculation, Hypoglycemia.	Diazepam, phenobarbitone, phenytoin Lumbar puncture to rule out meningitis. Rule out hypoglycaemia.
Severe anaemia	Clearance of parasitized cells and rigid uninfected red cells.	Transfusion if in distress, or <Hct 20%
Hyperpyrexia	TNFα and other cytokines	Paracetamol, sponging, etc.
Hyperparasitaemia	Host and strain dependant	Anti-malarial drugs.
Hypoglycaemia	Increased use, decreased production, quinine related hyperinsulinism	Glucose 10%, 50%
Renal failure	ATN	dialysis
Hepatic dysfunction, jaundice		No specific treatment, check plasma glucose.
Fluid, electrolyte imbalance and metabolic acidosis	Dehydration, slight capillary leakage, lactic acidosis (compromised microcirculation), renal failure, severe anaemia	Careful fluid resuscitation, HCO_3 if pH<7.20, packed cell transfusion if Hct<20%
ARDS	Unknown (cytokines, compromised microcirculation).	Careful fluid management.
Black water fever	Unknown, related to quinine use and G6PD deficiency.	Transfusion if needed, alkalinisation of the urine.
Circulatory collapse	Uncommon in malaria (NO binding?), consider concurrent septicaemia	Fluids, vasopressor drugs, antibiotics.

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APPENDIX 5: PHARMACOKINETIC REPORT

MORU Tropical Health Network

Pharmacokinetic Report

Report No.: PKR_102 v.2

Internal Project No.: 18002

Protocol No.: PHARMA1702

Protocol title:
Randomised open-label, single dose, cross-over study to evaluate the bioequivalence of a new parental formulation of artesunate with the currently used formulation in healthy adult Thai subjects.

Sponsor:
Guilin Pharmaceutical Co., Ltd.

This report supersedes PKR_102, issued 25 February 2019.

REPORT REVIEWED AND APPROVED BY:

Dr. Richard Hoglund _____
(Head of Phamacometrics) Date

Prof. Dr. Joel Tarning _____
(Head of Department) Date

COMPLIANCE STATEMENT

Activities performed at the Department of Clinical Pharmacology for study protocol No. PHARMA1702 (Sponsor: Guilin Pharmaceutical Co., Ltd.) were conducted in compliance with the statistical analysis plan and study protocol. There were no deviations from the applicable procedures that would affect the integrity of the study or the interpretation of the results.

QUALITY ASSURANCE STATEMENT

The Department of Clinical Pharmacology Project Report No. PKR_102 v.2, which contains the final pharmacokinetic analysis of artesunate (ARS) and its metabolite dihydroartemisinin (DHA) from study protocol No. PHARMA1702 (Sponsor: Guilin Pharmaceutical Co., Ltd.), has been reviewed by a senior scientist, not directly involved in the analysis. The review focused on raw data transfer and data analysis accuracy.

PERSONNEL AND RESPONSIBILITIES

Pharmacokinetic analysis was performed from 4 Frb 2019 to 22 Feb 2019 by:

Dr. Richard Hoglund	Head of Pharmacometrics	_____

Pharmacokinetic report generated by:

Dr. Richard Hoglund	Head of Pharmacometrics	_____

Pharmacokinetic report reviewed by:

Dr. Junjie Ding	Senior Scientist	_____

Pharmacokinetic report approved by:

Dr. Richard Hoglund	Head of Pharmacometrics	_____
Prof. Dr. Joel Tarning	Head of Department	_____

TABLE OF CONTENT

METHODOLOGY

STUDY DESIGN

This was a pharmacokinetic cross-over study evaluating a new parenteral formulation of intravenous (IV) and intramuscular (IM) artesunate, and the currently used parenteral formulation of artesunate.

A total of 72 male and female healthy subjects were enrolled and completed all drug administration arms and sampling according to the protocol. The study comprised of 39 male (54.2%) and 33 female (45.8%) volunteers, with a median (range) age of 35 (21- 53) years and body weight of 58.55 (44.3-82.3) kg. All participants received 2.4 mg/kg of the new parenteral formulation of artesunate (test formulation, T) and the currently used parenteral formulation of artesunate (reference formulation, R) both IV and IM, in a randomised four-period cross-over design.

Blood samples for plasma artesunate and dihydroartemisinin quantification were collected at: pre-dose, 5 min, 15 min, 30 min, 45 min, 1 hr, 1.5 hr, 2 hr, 3 hr, 4 hr, 6 hr, 8 hr, 12 hr and 24 hr post dose in all patients, in all drug administration arms.

DRUG ANALYSIS

The drug analysis was performed at the department of clinical pharmacology, Mahidol-Oxford Tropical Medicine Research Unit, Thailand (see bioanalytical drug analysis report AR03_18002_ARS v.2 for more information). The laboratory is ISO-accredited for competency (ISO 15189:2012) and safety (ISO 15190:2003). All quality control samples were within regulatory acceptance limits (±15%CV).

PHARMACOKINETIC ANALYSIS

Individual concentration-time data were evaluated using non-compartmental analysis in Phoenix 64 version 8.1 (Certara, Princeton, New Jersey, USA). Total exposure up to the last measured concentration ($AUC_{0\text{-}LAST}$) was calculated using the linear trapezoidal method for ascending concentrations and the logarithmic trapezoidal method for descending concentrations. Drug exposure was extrapolated from the last observed concentration to time infinity by C_{LAST}/λ_Z for each individual subject to compute total drug exposure ($AUC_{0\text{-}\infty}$). The terminal elimination half-life ($t_{1/2}$) was estimated by log-linear regression (best fit option) of the observed concentrations in the terminal elimination phase. Maximum concentration (C_{MAX}) and time to maximum concentration (T_{MAX}) were taken directly from the observed data. Apparent volume of distribution (V) and elimination clearance (CL) were computed individually according to standard procedures in Phoenix. Artesunate and dihydroartemisinin were evaluated separately, and also combined by adding the molar concentrations of artesunate and dihydroartemisinin (at each time point) for each individual before pharmacokinetic evaluation.

As a secondary analysis the intramuscular bioequivalence of artesunate and dihydroartemisinin exposures were evaluated comparing the IM test formulation and the IV test formulation.

STATISTICAL ANALYSIS

Ln-transformed pharmacokinetic exposure parameters (C_{MAX}, $AUC_{0\text{-}LAST}$ and $AUC_{0\text{-}\infty}$) were evaluated with the bioequivalence function in Phoenix 64 to assess the bioequivalence of drug formulations. Study arm was used as "*formulation input*", subject ID was used as "*subject input*", individual sequence of the four administrations was used as "*sequence input*" (e.g. TIM RIV TIV RIM), and individual study occasion visit as "*period input*" (ranging from 1 to 4) in the bioequivalence function. Bioequivalence was assumed if the 90% confidence intervals of the ratio (test formulation/reference formulation) of C_{MAX}, $AUC_{0\text{-}LAST}$ and $AUC_{0\text{-}\infty}$ fell within 80% to 125%. The 90% confidence interval of this ratio was calculated according to the equation below. If the administered doses of the test and reference formulation deviated with more than 5% within a subject, the above analysis was carried out with dose normalised parameter values.

Bioequivalence is computed by Phoenix 64 as follow:

$$Mean\ ratio\ (\%) = 100 \times e^{Parameter_{Test} - Parameter_{Reference}}$$

where $Parameter_{Test}$ and $Parameter_{Reference}$ are the least square mean of the natural logarithm of parameters derived for the test and reference formulation, respectively. The upper and lower boundary of the 90% confidence interval are calculated as follow:

$$90\%\ Confidence\ Interval = 100 \times e^{(Parameter_{Test} - Parameter_{Reference}) \pm (t_{0.95} \times SE_D)}$$

where $t_{0.95}$ is taken from the student-t distribution, SE_D is the standard error of the difference of the test and reference least squares means.

PHARMACOKINETIC RESULTS SUMMARY

Dose normalisation was not performed because all administered doses of artesunate (test and reference formulations) were within ±5% for each patient. Table 1-9 present the bioequivalence results. All other results and associated data are presented in appendices 1, 2 and 3 below. Each appendix contain raw data, Phoenix 64 user settings for the analysis, result graphs, and tabulated pharmacokinetic parameter estimates. The appendices 1, 2, and 3 report "*IV test vs. IV reference*", "*IM test vs. IM reference*", and "*IV test vs. IM test*" results separately.

ARTESUNATE RESULTS

Bioequivalence could be concluded for the parent drug, artesunate, for total exposure but not for maximum concentration when administered IV (Table 1). Bioequivalence was demonstrated for all parameters for artesunate when administered IM (Table 4).

DIHYDROARTEMISININ RESULTS

Parenteral administration of test and reference formulations showed matching exposures to the active metabolite, dihydroartemisinin, and bioequivalence of the test formulation could be demonstrated both when administered IV (Table 2) and IM (Table 5).

COMBINED ARTESUNATE AND DIHYDROARTEMISININ RESULTS

Parenteral administration of test and reference formulations showed matching exposures to the combined exposure to artesunate and dihydroartemisinin, and bioequivalence of the test formulation could be demonstrated both when administered IV (Table 3) and IM (Table 6).

INTRAVENOUS VS. INTRAMUSCULAR RESULTS

As expected bioequivalence could not be concluded for the maximum concentration of artesunate and dihydroartemisinin when comparing IV and IM administration of the test formulation, due to systematically lower peak levels of both compounds after IM administration. Furthermore, total exposure to artesunate was consistently higher after IM administration, compared to IV administration, and bioequivalence could not be concluded for artesunate (Table 7). However, bioequivalence was demonstrated for the total exposure to dihydroartemisinin (Table 8). Bioequivalence was demonstrated for the combined exposure to artesunate and dihydroartemisinin for the IM formulation, but not for the maximum concentration (Table 9).

Table 1. Bioequivalence of artesunate when administered intravenously

Parameter	Ratio (%)	CI 90% Lower	CI 90% Upper
Ln(C_{MAX})	81.50	74.74	88.87
Ln($AUC_{0\text{-}LAST}$)*	85.85	80.09	92.02
Ln($AUC_{0\text{-}\infty}$)*	85.87	80.11	92.05

Test: IV test formulation; Reference: IV reference formulation.

Ratio is the geometric mean ratio between the test and reference formulation (Test/Reference); CI 90% Lower is the lower boundary of the geometric 90% confidence interval; CI 90% Upper is the upper boundary of the geometric 90% confidence interval; Ln is the natural logarithm; C_{MAX} is the maximum concentration; $AUC_{0\text{-}LAST}$ is the area under the concentration-time curve from time zero to the last observation; $AUC_{0\text{-}\infty}$ is the area under the concentration-time curve from time zero to infinity.

* Indicates that the criterion for bioequivalence is fulfilled, i.e. the CI 90% of the ratio (test/reference) is within 80-125%.

Table 2. Bioequivalence of dihydroartemisinin when administered intravenously

Parameter	Ratio (%)	CI 90% Lower	CI 90% Upper
Ln(C_{MAX})*	100.18	92.63	108.35
Ln($AUC_{0\text{-}LAST}$)*	98.56	95.44	101.77
Ln($AUC_{0\text{-}\infty}$)*	98.54	95.40	101.78

Test: IV test formulation; Reference: IV reference formulation.

Ratio is the geometric mean ratio between the test and reference formulation (Test/Reference); CI 90% Lower is the lower boundary of the geometric 90% confidence interval; CI 90% Upper is the upper boundary of the geometric 90% confidence interval; Ln is the natural logarithm; C_{MAX} is the maximum concentration; $AUC_{0\text{-}LAST}$ is the area under the concentration-time curve from time zero to the last observation; $AUC_{0\text{-}\infty}$ is the area under the concentration-time curve from time zero to infinity.

* Indicates that the criterion for bioequivalence is fulfilled, i.e. the CI 90% of the ratio (test/reference) is within 80-125%.

Table 3. Bioequivalence of combined artesunate and dihydroartemisinin when administered intravenously

Parameter	Ratio (%)	CI 90% Lower	CI 90% Upper
Ln(C_{MAX})*	86.71	81.43	92.33
Ln($AUC_{0\text{-}LAST}$)*	95.17	92.38	98.04
Ln($AUC_{0\text{-}\infty}$)*	95.18	92.39	98.05

Test: IV test formulation; Reference: IV reference formulation.

Ratio is the geometric mean ratio between the test and reference formulation (Test/Reference); CI 90% Lower is the lower boundary of the geometric 90% confidence interval; CI 90% Upper is the upper boundary of the geometric 90% confidence interval; Ln is the natural logarithm; C_{MAX} is the maximum concentration; $AUC_{0\text{-}LAST}$ is the area under the concentration-time curve from time zero to the last observation; $AUC_{0\text{-}\infty}$ is the area under the concentration-time curve from time zero to infinity.

* Indicates that the criterion for bioequivalence is fulfilled, i.e. the CI 90% of the ratio (test/reference) is within 80-125%.

Table 4. Bioequivalence of artesunate when administered intramuscularly

Parameter	Ratio (%)	CI 90% Lower	CI 90% Upper
Ln(C_{MAX})*	110.74	104.50	117.35
Ln($AUC_{0\text{-}LAST}$)*	106.47	103.84	109.16
Ln($AUC_{0\text{-}\infty}$)*	106.43	103.80	109.12

Test: IM test formulation; Reference: IM reference formulation.

Ratio is the geometric mean ratio between the test and reference formulation (Test/Reference); CI 90% Lower is the lower boundary of the geometric 90% confidence interval; CI 90% Upper is the upper boundary of the geometric 90% confidence interval; Ln is the natural logarithm; C_{MAX} is the maximum concentration; $AUC_{0\text{-}LAST}$ is the area under the concentration-time curve from time zero to the last observation; $AUC_{0\text{-}\infty}$ is the area under the concentration-time curve from time zero to infinity.

* Indicates that the criterion for bioequivalence is fulfilled, i.e. the CI 90% of the ratio (test/reference) is within 80-125%.

Table 5. Bioequivalence of dihydroartemisinin when administered intramuscularly

Parameter	Ratio (%)	CI 90% Lower	CI 90% Upper
Ln(C_{MAX})*	107.32	102.35	112.53
Ln(AUC_{0-LAST})*	105.12	102.20	108.13
Ln($AUC_{0-\infty}$)*	105.13	102.22	108.12

Test: IM test formulation; Reference: IM reference formulation.

Ratio is the geometric mean ratio between the test and reference formulation (Test/Reference); CI 90% Lower is the lower boundary of the geometric 90% confidence interval; CI 90% Upper is the upper boundary of the geometric 90% confidence interval; Ln is the natural logarithm; C_{MAX} is the maximum concentration; AUC_{0-LAST} is the area under the concentration-time curve from time zero to the last observation; $AUC_{0-\infty}$ is the area under the concentration-time curve from time zero to infinity.

* Indicates that the criterion for bioequivalence is fulfilled, i.e. the CI 90% of the ratio (test/reference) is within 80-125%.

Table 6. Bioequivalence of combined artesunate and dihydroartemisinin when administered intramuscularly

Parameter	Ratio (%)	CI 90% Lower	CI 90% Upper
Ln(C_{MAX})*	111.29	106.24	116.58
Ln(AUC_{0-LAST})*	105.38	102.98	107.82
Ln($AUC_{0-\infty}$)*	105.39	103.01	107.83

Test: IM test formulation; Reference: IM reference formulation.

Ratio is the geometric mean ratio between the test and reference formulation (Test/Reference); CI 90% Lower is the lower boundary of the geometric 90% confidence interval; CI 90% Upper is the upper boundary of the geometric 90% confidence interval; Ln is the natural logarithm; C_{MAX} is the maximum concentration; AUC_{0-LAST} is the area under the concentration-time curve from time zero to the last observation; $AUC_{0-\infty}$ is the area under the concentration-time curve from time zero to infinity.

* Indicates that the criterion for bioequivalence is fulfilled, i.e. the CI 90% of the ratio (test/reference) is within 80-125%.

Table 7. Bioequivalence of artesunate when administered intramuscularly and intravenously

Parameter	Ratio (%)	CI 90% Lower	CI 90% Upper
Ln(C_{MAX})	24.16	22.43	26.02
Ln(AUC_{0-LAST})	164.95	154.83	175.73
Ln($AUC_{0-\infty}$)	165.16	155.02	175.95

Test: IM test formulation; Reference: IV test formulation.

Ratio is the geometric mean ratio between the test and reference formulation (Test/Reference); CI 90% Lower is the lower boundary of the geometric 90% confidence interval; CI 90% Upper is the upper boundary of the geometric 90% confidence interval; Ln is the natural logarithm; C_{MAX} is the maximum concentration; AUC_{0-LAST} is the area under the concentration-time curve from time zero to the last observation; $AUC_{0-\infty}$ is the area under the concentration-time curve from time zero to infinity.

* Indicates that the criterion for bioequivalence is fulfilled, i.e. the CI 90% of the ratio (test/reference) is within 80-125%.

Table 8. Bioequivalence of dihydroartemisinin when administered intramuscularly and intravenously

Parameter	Ratio (%)	CI 90% Lower	CI 90% Upper
Ln(C_{MAX})	43.74	40.43	47.33
Ln(AUC_{0-LAST})*	91.55	88.40	94.81
Ln($AUC_{0-\infty}$)*	91.60	88.46	94.86

Test: IM test formulation; Reference: IV test formulation.
Ratio is the geometric mean ratio between the test and reference formulation (Test/Reference); CI 90% Lower is the lower boundary of the geometric 90% confidence interval; CI 90% Upper is the upper boundary of the geometric 90% confidence interval; Ln is the natural logarithm; C_{MAX} is the maximum concentration; AUC_{0-LAST} is the area under the concentration-time curve from time zero to the last observation; $AUC_{0-\infty}$ is the area under the concentration-time curve from time zero to infinity.
* Indicates that the criterion for bioequivalence is fulfilled, i.e. the CI 90% of the ratio (test/reference) is within 80-125%.

Table 9. Bioequivalence of combined artesunate and dihydroartemisinin when administered intramuscularly and intravenously

Parameter	Ratio (%)	CI 90% Lower	CI 90% Upper
Ln(C_{MAX})	26.62	25.05	28.29
Ln(AUC_{0-LAST})*	102.10	98.59	105.73
Ln($AUC_{0-\infty}$)*	102.06	98.55	105.69

Test: IM test formulation; Reference: IV test formulation.
Ratio is the geometric mean ratio between the test and reference formulation (Test/Reference); CI 90% Lower is the lower boundary of the geometric 90% confidence interval; CI 90% Upper is the upper boundary of the geometric 90% confidence interval; Ln is the natural logarithm; C_{MAX} is the maximum concentration; AUC_{0-LAST} is the area under the concentration-time curve from time zero to the last observation; $AUC_{0-\infty}$ is the area under the concentration-time curve from time zero to infinity.
* Indicates that the criterion for bioequivalence is fulfilled, i.e. the CI 90% of the ratio (test/reference) is within 80-125%.

ANALYTICAL ISSUES

Three subjects (ID 122, 224, and 321) discontinued to study before completion, and were replaced. Pharmacokinetic data for these three individuals are not reported here.

RECORD KEEPING

The Phoenix 64 projects will be saved and stored together with the raw data in electronic form on the MORU server (Bowmore).

APPENDICES

There are three (3) attachments to this report.

1. Appendix 1: Data and results for IV test *vs.* IV reference evaluations
2. Appendix 2: Data and results for IM test *vs.* IM reference evaluations
3. Appendix 3: Data and results for IV test *vs.* IM test evaluations

VERSION HISTORY:

- PKR_102 20190225
  First issue
- PKR_102 v.2 20190325
  Updated sponsor information

PK report Guilin

Final Audit Report 2019-03-25

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APPENDIX 6: AMENDMENT HISTORY

Amend-ment No.	Protocol Ver-sion No.	Date issued	Author(s) of changes	Details of Changes made
NA	NA	NA	NA	NA