16 September 2021
EMA/584728/2021
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Artesunate Amivas

International non-proprietary name: artesunate

Procedure No. EMEA/H/C/005550/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

AS	artesunate
ARDS	acute respiratory distress syndrome
AST	aspartate transaminase
AUC	area under the curve
AQUAMAT	African Quinine Artesunate Malaria Trial
BUN	blood urea nitrogen
CAS	Chemical Abstracts Service
CDC	Centers for Disease Control and Prevention
CI	confidence interval
Cmax	maximum concentration
CSF	cerebrospinal fluid
CYP	cytochrome P450 enzyme
DDI	drug-drug interaction
DHA	dihydroartemisinin
dL	deciliter
ECG	electrocardiogram
eGFR	estimated glomerular filtration rate
EU	European Union
FDA	Food and Drug Administration
g	gram
HRP-2	histidine rich protein 2
IV	intravenous(ly)
Kg	kilograms
L	liter
µL	microliter
µmol	micromole
MELD	model for end-stage liver disease
mEq	milliequivalent
mg	milligram
mmol	millimol
mOsm	milliosmoles
NDA	New Drug Application

P	Plasmodium
PADH	post-artesunate delayed hemolysis
Pf	Plasmodium falciparum
Pk	Plasmodium knowlesi
PK	pharmacokinetics
Pm	Plasmodium malariae
Po	Plasmodium ovale
Pv	Plasmodium vivax
RBC	red blood cells
SAE	serious adverse event
SEAQUAMAT	South East Asian Quinine Artesunate Malaria Trial
TropNet	European Network for Tropical Medicine and Travel Health
US	United States
USAMMDA	United States Army Medical Materiel Development Activity
WHO	World Health Organization

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Amivas Ireland Ltd submitted on 14 September 2020 an application for marketing authorisation to the European Medicines Agency (EMA) for Artesunate Amivas, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 27 February 2020.

Artesunate Amivas, was designated as an orphan medicinal product EU/3/20/2251 on 28 February 2020 in the following condition: Treatment of malaria.

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Artesunate Amivas as an orphan medicinal product in the approved indication. More information on the COMP's review can be found in the orphan maintenance assessment report published under the 'Assessment history' tab on the Agency's website: https://www.ema.europa.eu/en/medicines/human/EPAR/artesunate-amivas

The applicant applied for the following indication:

Artesunate Amivas is indicated for the initial treatment of severe malaria in adults and children (See sections 4.2 and 5.1).

Consideration should be given to official guidance on the appropriate use of antimalarial agents.

1.2. Legal basis, dossier content

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain tests or studies.

1.3. Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0328/2020 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0328/2020 was not yet completed as some measures were deferred.

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

1.4.2. Derogation(s) from market exclusivity

Not applicable

1.5. Applicant's request for consideration

1.5.1. New active Substance status

The applicant requested the active substance artesunate contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

1.6. Protocol assistance

The applicant did not seek Protocol assistance from the CHMP.

1.7. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Jayne Crowe Co-Rapporteur: Johann Lodewijk Hillege

The application was received by the EMA on	14 September 2020
The procedure started on	1 October 2020
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	18 December 2020
The CHMP Co-Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	21 December 2020
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	4 January 2021
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	28 January 2021
The applicant submitted the responses to the CHMP consolidated List of Questions on	21 April 2021
The following GLP inspection(s) were requested by the CHMP and their outcome taken into consideration as part of the Quality/Safety/Efficacy assessment of the product:	
A GLP inspection took place at the CRO site in the USA between 22/02/2021 and 3/03/2021. The outcome of the inspection carried out was issued on:	18 March 2021
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	31 May 2021

The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	10 June 2021
The CHMP agreed on a list of outstanding issues in writing to be sent to the applicant on	24 June 2021
The applicant submitted the responses to the CHMP List of Outstanding Issues on	17 August 2021
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	01 September 2021
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Artesunate Amivas on	16 September 2021
Furthermore, the CHMP adopted a report on New Active Substance (NAS) status of the active substance contained in the medicinal product (see Appendix on NAS)	16 September 2021

2. Scientific discussion

2.1. Problem statement

Artesunate Amivas is a semi-synthetic artemisinin derivative intended for the initial intravenous treatment of severe malaria in adults and paediatric patients. When the patient is able to take oral medications, a complete course of an appropriate oral antimalarial regimen should follow.

2.1.1. Disease or condition

Malaria is a potentially fatal illness caused by protozoal infection of red blood cells (RBC) with parasites belonging to the genus Plasmodium, transmitted to humans by the bite of a Plasmodium-infected female anopheline mosquito usually between dusk and dawn.

2.1.2. Epidemiology

Malaria transmission occurs in five WHO regions. Globally, an estimated 3.4 billion people in 92 countries are at risk of being infected with malaria and developing disease and 1.1 billion are at high risk (>1 in 1000 chance of getting malaria in a year). According to the World Malaria Report 2018, there were 219 million cases of malaria globally in 2017 (uncertainty range 203–262 million) and 435,000 malaria deaths, representing a decrease in malaria cases and deaths rates of 18% and 28% since 2010, respectively. The burden was heaviest in Africa, where an estimated 93% of all malaria deaths occurred, and in children aged under 5 years, who accounted for 61% of all global deaths. In the EU and in the US, malaria occurs in returning travelers or very recent immigrants from malaria endemic areas. Severe malaria occurred in 293, 259 and 306 US residents in 2014, 2015 and 2016, respectively. In Europe in 2018, the total number of all malaria cases (uncomplicated and severe combined) was 8,349 (ECDC-2020a). However, as in the US, the proportion of severe cases in Europe was very low, typically 10% of the total.

2.1.3. Aetiology and pathogenesis

Five species of Plasmodium infect humans: Plasmodium falciparum (Pf), P vivax (Pv), P ovale (Po), P malariae (Pm) and P knowlesi (Pk). Most severe malaria is due to Pf, although severe malaria due to Pv, Po and Pk has been recognised. In Europe in 2018, among 4,516 confirmed cases for which the Plasmodium species was reported, 3,793 (84.0%) had Pf, 339 (7.5%) had Pv, 236 (5.2%) had Po, 135 (3.0%) had Pm and 3 (0.1%) had Pk. In addition, one case had P. cynomolgi (a Plasmodium species that typically infects monkeys) and 9 (0.2%) were mixed infections with various Plasmodium species.

The severity of Pf reflects sequestration of infected erythrocytes within the microvasculature of various organs, including the brain. Several mechanisms have been proposed for cerebral malaria including mechanical microvascular obstruction by sequestered infected erythrocytes, activation of immune cells and release of pro-inflammatory cytokines, endothelial dysfunction, dysregulation of coagulation pathways, blood–brain barrier permeability disruption and brain swelling.

2.1.4. Clinical presentation, diagnosis

The signs and symptoms of malaria illness commonly include fever, headache, back pain, chills, increased sweating, myalgia, nausea, vomiting, diarrhea and cough. Untreated infections in malaria-naïve patients due to *Pf* can rapidly progress to coma, renal failure, respiratory distress and death. The WHO defines severe falciparum malaria according to one or more clinical features occurring in the presence of *Pf* asexual parasitaemia:

Impaired consciousness: Glasgow coma score <11 in adults or Blantyre coma score <3 in children.

Multiple convulsions: More than 2 episodes in 24 hours.

Prostration: Generalised weakness - unable to sit, stand or walk without assistance.

Significant bleeding: Including recurrent or prolonged bleeding from the nose, gums or venipuncture sites, hematemesis or melena.

Shock: Compensated shock (defined as capillary refill ≥3 seconds or temperature gradient on leg but no hypotension or decompensated shock (defined as systolic blood pressure <80 mmHg in adults or < 70 mmHg in children, with evidence of impaired perfusion).

Pulmonary edema: Pulmonary edema radiologically confirmed or oxygen saturation <92% on room air with respiratory rate >30/min, often with chest in drawing and crepitations on auscultation.

These clinical features are accompanied by one or more laboratory findings that may include:

- *Hypoglycemia*: Blood or plasma glucose <2.2 mmol/L (40 mg/dL).
- *Acidosis:* A base deficit of >8 mEq/L or plasma bicarbonate <15 mmol/L or venous plasma lactate ≥5 mmol/L. Severe acidosis manifests as respiratory distress (rapid, deep, labored breathing).
- *Severe malarial anemia:* Hemoglobin ≤5 g/dL or hematocrit ≤15% in children <12 years (≤7 g/dL and <20%, respectively, in adults) with parasitemia > 10,000/µL.
- *Hyperparasitemia: Pf* parasitemia >5%.
- *Renal impairment:* Blood creatinine >265µmol/L (3 mg/dL) or BUN >20 mmol/L.
- *Jaundice:* Bilirubin >50 µmol/L (3 mg/dL) with >100,000 parasites/µL.

Severe vivax malaria is defined as for Pf malaria but with no parasite density thresholds. Severe knowlesi malaria is defined as for Pf malaria, but with 2 density thresholds: either hyperparasitemia >100,000/µL or ~2% or jaundice with parasite density >20,000/µL.

Complicated malaria is defined as malaria complicated by conditions precluding oral treatment thus making parenteral therapy preferable. Complicated malaria does not equate with severe malaria since the term encompasses malaria infections not meeting the WHO (2015) criteria for severe malaria but in which the patient may benefit from parenteral treatment.

The proportion of suspected malaria cases receiving a malaria diagnostic test has increased markedly since 2010, especially in Africa, mainly due to an increase in the use of rapid diagnostic tests. Thus, rather than relying on microscopy of thin and thick films, which requires trained personnel and appropriate laboratory equipment, cases may be diagnosed using finger prick samples using tests that pick-up antigens of plasmodial species. Some of these tests can be used in remote areas and lead to rapid institution of treatment. The sensitivity and specificity of commercially available tests is variable but has been improving in recent years and WHO has issued guidance of criteria for test selection

2.1.5. Management

Quinine was the mainstay of treatment of severe malaria since the introduction of Cinchona Bark to European medicine in the 1630s until the rediscovery of artemisinin in China in 1972 and the subsequent synthesis of artemether and artesunate, which provided highly effective alternatives to quinine. Artemisinin derivatives are now widely recognised to be the most rapidly acting of all the antimalarial drugs. Prior to 2005, the largest clinical trials performed in severe malaria had compared artemether to quinine but overall survival was not significantly different. A pilot comparison of intravenous (IV) artesunate (AS) and IV quinine then showed that mortality was 22% in the quinine group and 12% in the AS group.

The South East Asian Quinine Artesunate Malaria Trial (SEAQUAMAT) Consortium compared IV AS to IV quinine for severe malaria in 1461 (mostly adult) Asian patients and found that AS was statistically superior to quinine in preventing death. This was followed by a study of similar design in 5,425 African children in 2010 (African Quinine Artesunate Malaria Trial [AQUAMAT]), which also showed that AS was superior to quinine in preventing mortality.

These trials led to the adoption of IV AS for primary therapy for this disease worldwide. Based on high-quality evidence, the WHO issued a strong recommendation for the use of IV or IM AS in patients with severe malaria for at least 24 h and until oral medication is possible. The recommended oral follow-on treatment consists of 3 days of an artemisinin-based combination therapy.

In Europe, there are no medicinal products specifically authorised for the initial treatment of severe malaria. There is one antimalarial available for IV administration, but it is licensed only in France as SURQUINA (IV quinine). While many EU centres have moved to use of IV artesunate for initial treatment of severe malaria, the available products are either the WHO-prequalified (2011) Guilin formulation or artesunate formulations manufactured or approved in other jurisdictions.

2.2. About the product

Artemisinin itself is a sesquiterpene lactone produced by the Chinese medicinal herb Artemisia annua. The artemisinins in the broader sense are endoperoxides derived from artemisinin for which activation of the endoperoxide bridge is essential for antimalarial activity. The endoperoxide bridge is activated by haem iron inside Plasmodium-parasitised erythrocytes. This leads to oxidative stress, inhibition of protein and nucleic acid synthesis, ultrastructural changes and a decrease in parasite growth and

survival. Artemisinins act very rapidly against intra-erythrocytic asexual blood-stage malaria parasites, affecting up to 10,000-fold reductions in parasite burden every 48 h. Parasitemia is usually cleared within 48 to 72 h.

Semisynthetic lactol derivatives such as dihydroartemisinin (DHA) have higher bioavailability and activity compared to artemisinin. After oral or parenteral administration of agents in the artemisinin group, including IV artesunate, DHA forms in vivo. Artesunate and DHA are active against the blood-stage asexual parasites and gametocytes of Plasmodium species, including chloroquine-resistant strains, but they are not active against the hypnozoite liver stage forms of *P. vivax* and *P. ovale.*

2.3. Aspects on development

Since it is not practical to conduct a randomised study of IV AS vs IV quinine in the US or EU in returning travelers, the applicant's intravenous artesunate preparation was used under a CDC protocol to treat returning US travelers. This retrospective uncontrolled study supplements the pivotal comparative trials conducted using Guilin artesunate (SEAQUAMAT and AQUAMAT).

2.4. Quality aspects

2.4.1. Introduction

The finished product is presented as powder and solvent for solution for injection containing 110 mg of artesunate as active substance.

Each vial of powder contains 110 mg of artesunate.

Each vial of solvent for reconstitution contains 12 mL of 0.3 M sodium phosphate buffer.

After reconstitution, the solution for injection contains 10 mg of artesunate per mL.

Other ingredients are:

Solvent: monosodium phosphate monohydrate, disodium phosphate dihydrate, phosphoric acid concentrated (for pH adjustment), sodium hydroxide (for pH adjustment), and water for injection.

The powder is supplied in a Type I glass vial capped with a latex-free bromobutyl rubber stopper and aluminium seal as described in section 6.5 of the SmPC.

The solvent is supplied in a Type I glass vial capped with a latex-free bromobutyl rubber stopper and aluminium seal as described in section 6.5 of the SmPC.

2.4.2. Active Substance

General information

The chemical name of artesunate is butanedioic acid, mono[(3*R*,5a*S*,6*R*,8a*S*,9*R*,10*S*,12*R*,12a*R*)-decahydro-3,6,9-trimethyl-3,12-epoxy-12*H*-pyrano[4,3-j]-1,2-benzodioxepin-10-yl] ester corresponding to the molecular formula $C_{19}H_{28}O_8$. It has a relative molecular mass of 384.43 and the following structure:

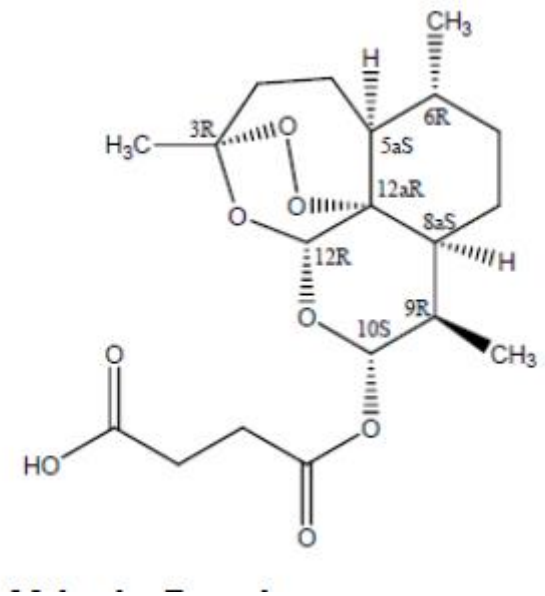


Figure 1: active substance structure

The chemical structure of active substance was elucidated by a combination of methods. The physicochemical properties were also determined by a combination of methods.

The active substance is a non-hygroscopic fine, white to almost white, crystalline powder, very slightly soluble in water, very soluble in dichloromethane, freely soluble in ethanol and acetone.

Artesunate contains eight chiral centres, of which seven are derived from the starting material artemisinin. A new chiral centre is formed at C-10 with the possible formation of two epimers: 10α and 10β. However, the substance formed is a single isomer and it is stated only the 10α-epimer is present. Up to now, only one polymorphic form is known (Form A).

Manufacture, characterisation and process controls

The active substance is manufactured by one manufacturing site.

Detailed information on the manufacturing process of the active substance has been provided in the restricted part of the ASMF and it was considered satisfactory.

Artesunate is a sesquiterpene endoperoxide produced from the starting material artemisinin and succinic acid anhydride**.** The first starting material of artesunate is artemisinin. The information provided for this application refers exclusively to the active substance produced from artemisinin obtained from the semi-synthetic process. The starting materials were controlled by acceptable specification. The chemical synthesis of artesunate involves several stages of synthesis using artemisinin as a well-defined starting material with acceptable specifications. The desired stereochemistry is ensured by the process conditions under substrate control

Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances.

Potential and actual impurities were discussed with regards to their origin and characterisation.

From an ICH M7(R1) perspective, artesunate does not contain any structural alert features and, was negative in all GLP genotoxicity studies. It is also well established in the literature that DHA is the main

active metabolite of both artesunate and artemisinin and it does not exhibit genotoxic activity in the AMES test. Based on this and the structural similarities between artesunate and the impurities, they are all considered as ICH M7(R1) Class 4-5 non-mutagenic impurities.

The solvents used in the semi-synthetic artesunate synthesis are all ICH Q3C(R6) Class 2 or 3 solvents and are controlled in artesunate batches at or below the corresponding ICH Q3C(R6) limits.

After the synthesis, micronisation of the active substance under nitrogen is performed at a manufacturing site. The unmilled material is fed into a jet mill and the milled material is transferred into the container closure system. The only raw material used in the micronisation of the active substance was presented with an adequate specification.

A full (2 x 2) factorial study using the current jet mill at a specific feed rate and mill pressure was performed. The results all exhibited nearly identical particle size distributions, and on this basis, the operational feed rate and mill pressure ranges with only one of these parameters changed at a time. However, because material is continuously fed into, and discharged from the mill, during a production run, the system dynamics change with time, and therefore multiple adjustments within a production run are required. Out of necessity, one or both of these parameters may be adjusted more than once during a commercial run. This is justified since the micronized material is continuously discharged and collected from the microniser, and therefore any single adjustment of either of these parameters will be independent of any already processed material, i.e., there is no space-dependence associated with a single parameter adjustment. CPPs are mill pressure and feed rate.

The design space has been verified at commercial scale.

Sterilisation of the active substance is performed by ethylene oxide (EO). Because EO sterilisation is the least reliable method of sterilisation, there were significant concerns with this approach. A MO was raised, requiring a comprehensive justification for use of the EO as mode of sterilisation, as per EMA/CHMP/CVMP/QWP/850374/2015 decision tree. The applicant confirmed that standard Ph. Eur steam sterilisation, gamma irradiation and UV irradiation all induced significant decomposition in the active substance. Sterilisation of an artesunate solution using sterilising filter was also not successful. The provided discussion and updated justification of the selected sterilisation method was considered acceptable; thus, EO sterilization was confirmed as the proposed manufacturing process of sterile artesunate. Particle size is a critical parameter to prevent entrapment of microbes in crystals and acceptable justification of the particle size has been provided. The controls in place to ensure the sterility of the active substance have been provided and considered satisfactory.

During the evaluation, the CHMP considered that a more detailed process narrative and schematic of the manufacturing process of the active substance should have been provided, divided into the following phases – packaging, (pre)conditioning, sterilisation, aeration and routine controls. This was requested as Major Objection (MO). In response to the MO, adequate information was provided and considered satisfactory.

For manufacturing process development, sufficient discussion has been provided and the approach taken has been acceptably justified.

The active substance is packaged to comply with the EC directive 2002/72/EC and EC 10/2011 as amended.

Specification

The final active substance (artesunate after micronisation and sterilization) specifications shown in include tests for appearance (visual), identification, (IR, HPLC), pH of solution (Ph. Eur.), related

substances (HPLC), assay (HPLC), related substances (HPLC), sulphated ash (Ph. Eur.), water content (KF), residual ethylene oxide (GC), bacterial endotoxins (Ph. Eur.), sterility (Ph. Eur.), and container closure integrity (microscopic evaluation).

Testing for impurities structurally related to artesunate is performed as a release and stability.

The individual related substances levels after micronisation/sterilization are very low and are due to carryover from the active substance synthesis. Accordingly, the acceptance criteria for the individual specified and unspecified related substances have been set at the current CH Q3A qualification and identification thresholds, respectively and the acceptance criterion for total related substances has been set to NMT 0.5%. Testing for the exclusively synthesis process impurity is performed on artesunate prior to micronisation.

The particle size is currently controlled exclusively at the micronized stage

The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for identification, assay and related substances tests has been presented.

Batch analysis data on 9 commercial scale batches of the active substance were provided. The results are within the specifications and consistent from batch to batch.

Stability

Stability data from a number of commercial scale batches of artesunate after synthesis and before micronisation/sterilization from the proposed manufacturer stored in the intended commercial package covering up to 60 months' storage at 25°C/60% RH and/or 30°C/65% RH and 6 months' storage at 40°C/75% RH according to the ICH guidelines were provided.

Stability data from three commercial scale batches of the final (micronized and sterilized) active substance, generated in accordance with ICH Q1A(R2) guidelines, are provided after storage at 25°C/60% RH for up to 24 months.

Since micronized artesunate is only held for a short period prior to sterilization, stability data for micronized artesunate alone has not been generated.

The following parameters were tested: appearance, pH of solution, assay, related substances, water content, and sterility. The analytical methods used were the same as for release and are stability indicating.

All stability data remained within specifications and generally unchanged within normal analytical variability.

In addition, one batch was exposed to light as defined in the ICH Q1B. The results demonstrate that the active substance is not sensitive to light.

The stability results indicate that the active substance manufactured by the proposed supplier is sufficiently stable. The stability results justify the proposed retest period of 24 months when stored at or below 25 °C in the proposed container.

2.4.3. Finished Medicinal Product

Description of the product and Pharmaceutical development

The finished product consists of a sterile active substance powder and a sterile solution for reconstitution.

Artesunate Powder

It is supplied as a sterile powder for reconstitution and injection, consisting exclusively of the active substance (sterile micronized artesunate) as a white or almost white, fine crystalline powder, practically free from particles of foreign matter, aseptically filled into a clear 20 mL Type 1 glass vial with a nominal fill of 110 mg/vial, capped with a 20 mm, blowback, grey bromobutyl rubber stopper and crimped with an aluminium seal containing a royal blue, flip off 20 mm cap. It does not contain excipients.

In accordance with ICH Q8(R2) guidelines, a prospective Quality Target Product Profile (QTPP) is provided in

, along with the quality attributes deemed important to ensure the QTPP is met.

Since initial development, the finished product has been prepared by aseptic filling of artesunate into vials. This is due to the necessary intravenous route of administration and the well-known degradation of artesunate to slowly form dihydroartemisinin (DHA) in aqueous solution, requiring administration shortly after reconstitution. While the formation of DHA occurs *in vivo* and is not considered a toxicity issue, it has limited aqueous solubility and so will eventually form a turbid solution, possibly with visible particulates, which render the solution unsuitable for intravenous administration.

The active substance is not hygroscopic and the solid-state characteristics (polymorphic form, particle size distribution after micronisation) are consistent between batches and are not a consideration once the product is brought into solution prior to administration. The active substance solubility in aqueous media is pH dependent and there have been no solubilization issues, with reconstitution generally complete within 1-3 minutes, and the pH of the selected diluent (0.3 M phosphate buffer solution) tightly controlled at 7.9-8.1.

The bulk/tapped density of the active substance is very low and as a consequence, the flowability is poor. The dosing heads for filling of the active substance into vials have been evaluated to optimize the filling rate.

There have been two synthesis pathways for the artesunate precursor artemisinin; the vegetal (non-commercial) and semi-synthetic (proposed commercial) route. Although artesunate production by the vegetal route was discontinued in 2018, additional vegetal finished product batches for non-commercial use have been prepared in 2019 using existing vegetal artesunate active substance inventory. Comparative batch data were provided, demonstrating that artesunate produced by the vegetal and semi-synthetic routes is essentially indistinguishable from a quality perspective.

Since there is only one ingredient in the finished product, there has been no formulation development.

Manufacture of the finished product involves filling of the sterile active substance into the vials which is performed under aseptic conditions. The only process development has been a transition from manual filling to semi-automated filling with 100% fill weight checks for all vegetal and semi-synthetic finished product batches filled at the manufacturing site.

Sufficient information regarding the development of the product has been provided. An extractable and leachables study did not identify any safety issues. The integrity of the drug product container closure

system has been demonstrated, and microbial considerations are adequately discussed. Compatibility with the diluent has been demonstrated

The most relevant physicochemical properties of the finished product involve the reconstitution characteristics, specifically the permissible storage time after reconstitution with the phosphate buffer solution. The reconstitution time has been relatively consistent at release and on stability, with values typically between 1-3 minutes and a proposed limit of NMT 6 minutes. Particle size distribution in the finished product is not considered an issue since the particle size is controlled in the micronized active substance and has been shown not to change significantly upon finished product manufacture and storage. Furthermore, reconstitution time is not affected even if the artesunate is not micronized. There is no significant increase in subvisible particulates upon storage of the finished product reconstituted in the requisite amount of phosphate buffer solution for up to at least 1.5 hours at 25°C. Accordingly, a permissible waiting time of NMT 1.5 hours after reconstitution in phosphate buffer solution is stated in the labelling.

The primary packaging is Type I glass vial capped with a latex-free bromobutyl rubber stopper and aluminium seal. The material complies with Ph. Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

Diluent

The diluent for reconstitution of the Artesunate Powder is provided in a vial containing 12 mL sterile 0.3 M sodium phosphate, pH 8.0 ± 0.1 buffer solution as a clear, colorless solution, practically free from particles of foreign matter.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. standards. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SmPC and in paragraph 2.1.1 of this report.

Phosphate buffer solutions covering a wide range of pH values and ionic strengths have been extensively studied and are commercially available for numerous medicinal product and biologics applications. Accordingly, very little development was required once the pH and ionic strength ranges were confirmed.

The most relevant physicochemical property of the phosphate buffer solution is the ability to fully solubilize the finished product upon reconstitution, which is controlled by the appearance (after reconstitution) and the reconstitution time in the finished product specifications The reconstitution time has been relatively consistent at release and on stability, with values typically between 1-3 minutes and a proposed limit of NMT 6 minutes. The appearance/turbidity measured during development consistently results in complete solubilization of artesunate. For the amount of the administered dose, there are no biological properties of the phosphate buffer solution that are relevant.

Very little manufacturing process development was required as there are numerous preparations in the literature for phosphate buffer solutions of varying pH values and ionic strengths.

The primary packaging is Type I glass vial capped with a latex-free bromobutyl rubber stopper and aluminium seal. The material complies with Ph. Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

An extractable and leachables study did not identify any safety issues. The integrity of the finished product container closure system has been demonstrated, and microbial considerations are adequately discussed. Compatibility with the diluent has been demonstrated.

Manufacture of the product and process controls

Artesunate Powder

The powder is manufactured by one manufacturing site

The manufacturing process consists of 3 main steps: aseptic filling of the micronized, sterilized artesunate active substance into the 20 mL vials followed by capping and sealing. All staging and filling operations are performed in a Class 100 (ISO 5) room, with filling performed in restricted access barrier technology. The process is considered to be a non-standard manufacturing process.

During the assessment, the CHMP requested as a MO that the process needed to be validated before opinion. It was confirmed that finished product process validation for both artesunate finished product and sodium phosphate buffer solution have been completed successfully on 3 consecutive production scale batches, and the validation reports were provided and considered satisfactory. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate for this type of manufacturing process.

Diluent

The diluent is manufactured by one manufacturing site.

The manufacturing process consists of 8 main steps: addition of sterilised water for injections, addition and stirring of monobasic sodium phosphate monohydrate, addition and stirring of disodium phosphate dihydrate, cooing, adjusting of pH, sterilizing, filling into vials, and terminal sterilization. All steps are performed under aseptically controlled conditions. All areas are supplied with HEPA filtered air at the required air change volumes and pressurizations. The process is considered to be a non-standard manufacturing process.

Major steps of the manufacturing process have been validated by a number of studies on three consecutive full-scale batches. It has been demonstrated that the manufacturing process is capable of producing the diluent of intended quality in a reproducible manner. The in-process controls are adequate for this type of manufacturing process.

Product specification

Artesunate Powder

The powder release and shelf-life specifications include appropriate tests for this kind of dosage form: appearance before reconstitution (visual), appearance after reconstitution (Ph. Eur.), identification (IR, HPLC), extractable volume after reconstitution (Ph. Eur.), pH of solution after reconstitution (Ph. Eur.), water content (KF), reconstitution time (visual), related substances (HPLC), assay vial content (gravimetry), assay (HPLC), mass variation (Ph. Eur.), subvisible particle after reconstitution (Ph. Eur.), bacterial endotoxins (Ph. Eur.), and sterility (Ph. Eur.).

The particle size is not routinely controlled in the finished product as it is controlled in the intermediate micronized active substance. Osmolality is not routinely controlled since it is a predictable colligative property of the formulation composition and has been confirmed as being isoosmotic from osmolality measurements of drug product reconstituted with phosphate buffer solution.

The CHMP requested (as a MO) that the applicant discuss the potential safety impact of the impurities present in the active substance that may be carried over to the finished product. Discussion on the potential safety impact of all possible impurities of artesunate was provided and no safety concerns were identified. Furthermore, it has been demonstrated that no other impurities except those already

included in the active substance specification are carried over to the final active substance. Therefore, it can be concluded that no new impurities have been found that would raise any safety concerns.

The individual acceptance criterion for each specified and unspecified impurity is set corresponding to the ICH Q3B(R2) qualification threshold.

The potential presence of elemental impurities in the finished product has been assessed following a risk-based approach in line with the ICH Q3D Guideline for Elemental Impurities. Batch analysis data on 3 batches using a validated ICP-MS method was provided, demonstrating that each relevant elemental impurity was not detected above 30% of the respective PDE. Based on the risk assessment and the presented batch data it can be concluded that it is not necessary to include any elemental impurity controls.

A risk evaluation concerning the presence of nitrosamine impurities in the finished product has been performed (as requested as MO) considering all suspected and actual root causes in line with the "Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products" (EMA/409815/2020) and the "Assessment report- Procedure under Article 5(3) of Regulation EC (No) 726/2004- Nitrosamine impurities in human medicinal products" (EMA/369136/2020). Based on the information provided it is accepted that no risk was identified in the active substance or the finished product. Therefore, no additional control measures are deemed necessary.

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. The same reference standards described for the active substance are currently being used for drug product identification/assay/related substances testing.

Batch analysis results are provided for 3 commercial scale batches confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

The powder is released onto the market based on the above release specifications, through traditional finished product release testing

Diluent

The diluent release and shelf life specifications include appropriate tests: appearance (visual), identification (Ph. Eur.), extractable volume (Ph. Eur.), pH (Ph. Eur.), assay for phosphate (potentiometric titration), subvisible particulates (Ph. Eur.), bacterial endotoxins (Ph. Eur.), and sterility (Ph. Eur.).

The phosphate buffer solution used for the finished product reconstitution is a common, well characterized diluent used at various concentrations and pH values for reconstitution of many finished products and is composed entirely of compendial substances, and as such has not been characterized further for impurities.

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. No reference standards are used for phosphate buffer solution analysis.

Batch analysis results are provided for 4 commercial scale batches confirming the consistency of the manufacturing process and its ability to manufacture to the intended diluent specification.

The diluent is released onto the market based on the above release specifications, through traditional final product release testing.

Stability of the product

Artesunate Powder

Stability data from 3 commercial batches of powder stored for up to 24 months under long term conditions (5°C) and for up to 6 months under accelerated conditions (40°C / 75% RH) according to the ICH guidelines were provided. The batches of the powder are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing.

Additional stability data from 3 commercial batches of powder stored for up to 12 months under long term conditions (25°C/60% RH) and for up to 6 months under accelerated conditions (40°C / 75% RH) according to the ICH guidelines were provided. The batches of powder are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing samples were tested for appearance (before and after reconstitution in phosphate buffer solution), pH of solution, water content, reconstitution time, assay, related substances, subvisible particulates, bacterial endotoxins and sterility. The analytical procedures used are stability indicating.

All measured parameters remained well within specifications and unchanged within normal analytical variability. Based on these data, a shelf life of 24 months at the long term storage condition is proposed when the finished product is stored in the proposed commercial container.

To evaluate permissible storage time after reconstitution with the diluent, two studies have been performed: a short term study in which the finished product was stored for up to 28 days with reconstitution, and an in-use study in which the finished product was reconstituted with phosphate buffer solution and analysed at intervals up to 2 hours after reconstitution. Taking into account the results of these studies an in-use shelf-life of the reconstitution solution time determined as stated in the SmPC (section 6.3).

Based on available stability data, the proposed shelf-life of 2 years as stated in the SmPC (section 6.3) are acceptable.

Diluent

Stability data from 3 commercial scale batches of diluent stored under long term conditions (25°C / 60% RH) and under accelerated conditions (40°C / 75% RH) for up to 24 months according to the ICH guidelines were provided. The batches of the medicinal product are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing.

Samples were tested for appearance, phosphate content, pH, subvisible particulates, bacterial endotoxins and sterility. To date, all stability data remained well within specifications and unchanged within normal analytical variability.

In addition, one batch was exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products. The results show that the phosphate buffer solution does not absorb light to any significant extent throughout the measured range compared to the water reference, demonstrating photostability since there is no mechanism for light absorption.

Based on available stability data, the proposed shelf-life for the phosphate buffer solution when stored at or below 30°C is acceptable.

However, since the powder and diluent are co-packaged, the shelf life of the finished product as a whole is that of the powder stated above.

Adventitious agents

No excipients derived from animal or human origin have been used.

2.4.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

During the assessment, a more detailed process narrative and schematic of the manufacturing of the active substance, a comprehensive justification for use of ethylene oxide as the sterilisant of the active substance, confirmation of the process validation has been completed successfully for the finished product, and a validation report, a discussion of the potential safety impact of the impurities present in the active substance that may be carried over to the final product and an expansion of the nitrosamine risk assessment were requested as major objections. The applicant provided satisfactory responses and all these issues which were considered solved.

The applicant has applied QbD principles in the development of the micronisation process, and a design space has been proposed for micronisation of the active substance.

2.4.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

2.4.6. Recommendation(s) for future quality development

Not applicable.

2.5. Non-clinical aspects

2.5.1. Introduction

GLP

Safety pharmacology study 1466-103 was performed in compliance with GLP. Studies SR01-1 and AQ0008_02_AF were not performed in compliance with GLP, but they were of sufficient quality. Considering the clinical experience with artesunate, this is not considered a problem.

Pivotal toxicology studies were in general, performed in compliance with GLP. However, two genotoxicity studies were performed in a laboratory without mutagenicity expertise (area 3). These two laboratories were inspected around the time of completion for the conduct of toxicology studies (area 2). The studies related to the GLP concern are the bacterial mutagenicity assay and in vitro chromosomal aberration assay with artesunate (studies G305-08 and G306-08 both performed at SRI International, Menlo Park, CA, USA), which both were assessed as negative. Based on the Guidance on Triggers for audits of GLP studies (EMA/89741/2015), the CHMP concluded that a GLP inspection was warranted, as regarding question 3 of the GLP checklist, the concerning laboratory was not known as an area 3 expertise testing facility. A study audit was performed by the US-FDA upon request of the

EMA. US-FDA concluded that the final reports completely and accurately reflect the raw data and that there were no findings associated with these studies.

2.5.2. Pharmacology

Primary pharmacodynamic studies for artesunate addressed the in vitro and in vivo efficacy in *P. falciparum*-infected human RBCs, *P. berghei*- infected rats and *P. coatneyi*- infected rhesus monkeys. No dedicated studies on the mechanism of action of artesunate were presented by the applicant. A literature review supports the well accepted concept that the anti-malarial activity of artesunate is conferred by the endoperoxide bridge that undergoes iron-mediated cleavage to generate an unstable organic free radical and subsequent alkylation to enable binding to malarial proteins.

The in-vitro efficacy studies indicated that IC_{50} values for artesunate are low, the potency of DHA is similar to that of artesunate and non-GMP Guilin and WRAIR formulations performed similarly and within historical controls for artesunate. The in-vivo efficacy in the *P. berghei*- infected rat showed that artesunate was well-tolerated and efficacious; the CD100 dose (inducing 100% clearance of parasitaemia, 60 mg/kg) provided a 4-fold safety margin to the MTD (240 mg/kg). Although a severe malaria model with *P. coatneyi*- infected rhesus monkeys was investigated, the definitive study in an uncomplicated malaria model provides a more robust indication of efficacy for humans. 90% parasite clearance was achieved in the initial 26-hour period however complete clearance was delayed and recrudescence occurred within 2-15 days. The applicant compared exposure to the proposed clinical posology and the 8 mg/kg dose employed in this monkey study is equivalent to 1.5 mg/kg in human and is thus lower than the clinical dose.

No secondary pharmacodynamics studies have been performed with artesunate. Safety pharmacology studies were conducted to assess cardiovascular (CV), respiratory and central nervous system (CNS) toxicity. No hERG studies have been conducted by the applicant as the applicant considers there are no clinical concerns at the proposed dose. In the study with telemetered beagle dogs no changes in CV parameters were noted, providing a dose-based safety margin of 11-fold to the clinical dose and a 4-fold exposure-based margin relative to exposure at 2 mg/kg from the healthy volunteer study (Miller 2012). The applicant's position is also leveraged on the absence of a CV signal in healthy volunteers and infected patients in clinical trials. This is supported. No respiratory effects were noted in the telemetered beagle dog. CNS toxicity has been previously identified as a potential concern for artemisinin derivatives and was the subject of a WHO review in the early 2000s. The applicant has referred to literature that was included in the WHO review on potential for neurotoxicity. The review concluded that a number of factors may influence neurotoxic effects including long duration of activity, lipid solubility, parenteral administration and duration of treatments. Artesunate was not implicated in this review. The applicant also undertook an assessment of neurotoxicity as part of a toxicology study in rhesus monkeys, including an evaluation of brain pathology with a number of artemisinin derivatives including artesunate. Neurological signs including spontaneous motor activity, prostration and ataxia (128 mg/kg only) were noted in artesunate-treated animals at doses of 32 mg/kg and higher, however no pathological correlate was identified at any of the dose levels. The risk of neurological sequelae with the proposed posology for Artesunate Amivas is considered to be therefore considered to be low. The potential effect of artesunate on the gut (muscle contractility or transit time) was investigated and although no adverse effects were noted there was some evidence that artesunate may lower gastric pH in a dose-dependent fashion. No pharmacodynamic drug interaction studies were conducted with artesunate as part of this application.

2.5.3. Pharmacokinetics

Pharmacokinetic studies were conducted in rats, dogs and cynomolgus monkeys, following single or repeated IV administration with a number of artesunate formulations.

The bioanalytical methods used for the GLP studies in rats and dogs have been suitably validated in-line with the Guideline on Bioanalytical Method Validation (EMEA/CHMP/EWP/192217/2009 Rev. 1 Corr. 2) and are considered fit for purpose. Artesunate appears to be rapidly converted to DHA in nonclinical species. In the rat mass balance study, concentration of artesunate in plasma and whole blood peaked at 5 minutes after IV administration. A second peak occurring 3-4 hours after administration indicates enterohepatic recirculation occurs in rats. The DHA/artesunate ratio in the rat was approximately 1 at a dose of 5 mg/kg. The AUC ratio of DHA/artesunate was dose-dependent and reduced to 0.3-0.4 at the highest dose (50 mg/kg, single IV dose), notably lower than observed in humans (3.3-9.7). The elimination half-life was similar for both artesunate and DHA (0.32-0.52 hours). In repeat dose studies, peak concentrations of artesunate and DHA were achieved rapidly (less than 5 minutes) and artesunate was below the limit of quantification by 2 hours. DHA was measurable up to 3 hours at higher doses but generally speaking quantification of artesunate at lower doses (e.g. 3 or 10 mg/kg in rat; up to 40 mg/kg in dog) was not possible. No sex differences in absorption kinetics were apparent. No accumulation of artesunate was noted.

Tissue distribution in rats demonstrated 68% of C14-artesunate activity in the small intestine, rapidly decreasing in all tissues except for the spleen (with LSC method). High exposure was also observed in the kidney. In the 192-hour sampling period, brain concentrations of artesunate accounted for 1.07% of total radioactivity and were higher than plasma levels (70.7 vs. 29.4 µg equivalents.h/ml). Similar observations were produced using the QWBA method. Highest tissue concentrations were observed 0.5 hr after dosing, in the small intestine, spleen and bone marrow. CNS concentrations were low but persisted until the end of the study, similar to other tissues. A study in pregnant rats found measurable concentrations of (7.3% of AUC) in the ovary, placenta and uterus at 2-4-fold higher concentrations than plasma. Distribution in red blood cells (RBCs) was 4.3 times higher than plasma concentrations in vivo.

In metabolite profiling studies in rats, the majority of metabolites were from the total conjugation fractions (glucuronide and other conjugations): 87.7% and 89.6% of the administered dose in plasma and urine, respectively. Conjugation was the major metabolic pathway of artesunate in rat plasma, and the conjugation rate was time dependent. DHA was a major metabolite of artesunate and was metabolized almost immediately in the free fraction of plasma at 1 minute after dosing. Artesunate was rapid and extensively metabolized to more and less polar metabolites and unchanged artesunate was almost undetectable at 2 hours. Incubation of DHA in hepatocytes (including human) did not identify any unique human metabolites. It was noted after IV administration in rats that DHA was present as two tautomers – DHAα and DHAβ. No difference in activity is expected between these two tautomers. DHA undergoes rapid Phase 2 glucuronidation.

In-vitro metabolism studies identified CYP2A6, CYP2B6, CYP2E1, CYP1B1, and CYP4A11 as metabolizing enzymes of artesunate, CYP2A6 being the major isoform involved in artesunate metabolism. Glucuronidation is the major metabolic pathway for DHA, predominantly via UGT1A9. Enzyme induction/inhibition studies co-incubated artesunic acid with substrates of CYP1A2, CYP2C9, CYP2C19, CYP2D6 or CYP3A4 and no inference was noted. DHA moderately inhibited substrates for CYP1A2 (IC_{50} value of 10 µM) and CYP2C19 (IC_{50} value of 9 µM). However, this is not of great relevance considering that in clinical studies, no relevant CYP inhibition was found. An in-vivo interaction study in rhesus monkeys showed that small but significant changes were observed in the profile of markers for CYP3A4, CYP2D6 and CYP2C9 after IV artesunate administration. Uptake and efflux transporter studies were conducted. Artesunate and DHA did not demonstrate any potential to

inhibit or act as substrates at OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1 and MATE2K, P-gp, BCRP and BSEP at physiologically relevant concentrations.

In rats, artesunate was excreted in urine and faeces (56% and 38%, respectively), indicating the kidneys is the primary excretory organ. No nonclinical studies have evaluated the excretion of artesunate in milk.

Inhibition of CYP enzymes by artesunate was investigated only in a limited way, with few enzymes, other substrates used than recommended in the Guideline on the Investigation of Drug Interactions (CPMP/EWP/560/95/Rev. 1 Corr. 2) and lack of details in the study report (Melendez report). However, this is not considered of great importance since clinically no relevant CYP inhibition was observed. DHA was a poor inhibitor of CYP2B6, CYP2C8, CYP2C9, CYP2D6, CYP2E1, and CYP3A at 10 µM. DHA was a potent inhibitor in vitro of CYP1A2 and a moderate inhibitor of CYP2C19, both at 10 µM. The concentration used (10 µM) was much lower than 50-fold the mean unbound Cmax (here unbound Cmax of DHA was 0.70 µM) as recommended in the Guideline, for enzyme inhibition studies, however the clinical relevance of this is again, limited. As glucuronidation is the major elimination pathway of DHA UGT enzyme inhibition should be investigated as outlined in relevant guidance. Specifically, in vitro inhibition of UGT2B7 and UGT1A9 should be investigated, at concentrations as recommended in the Guideline on the Investigation of Drug Interactions. An *in vitro* study of the potential inhibition of UGT2B7 and UGT1A9 by DHA is ongoing and is scheduled for completion Sept 2021 and will be submitted post-approval (see post-authorisation measures).

2.5.4. Toxicology

The toxicology package for artesunate Amivas includes single and repeat-dose toxicity studies in rats, dogs and monkeys. Two pivotal, 28-day, GLP-compliant repeat-dose toxicity studies have been conducted with the SS API. All other studies were conducted with either Guilin or WRAIR formulations. IV administration was employed for single and repeat dose studies; oral administration was predominantly used in reproductive and developmental toxicity studies.

The most common observation in nonclinical species was haematological toxicity characterised by dose-dependent anaemia, reticulocytopenia and in some studies leukopenia and neutropenia were also observed. In the pivotal 28-day GLP study in rats, this was associated with mortality at doses of 10 mg/kg IV or higher, splenomegaly and erythroid hyperplasia which persisted in animal at highest dose. In the mid (females only) and high dose groups heart weight was increased, although this did not persist in the recovery phase and blood pressure was not measured in this study. In beagle dogs, haematological findings were minimal and resolved in the recovery phase of the study. The NOAELs in these pivotal studies were 3 mg/kg/day and 5 mg/kg/day, respectively. A 7-day non-GLP study in rhesus monkeys identified haematologic effects at 8mg/kg/day or higher. Gastrointestinal, renal & hepatic effects were noted at 16 mg/kg/day or greater. Transient neurological effects (with no pathological correlate) manifested in some animals treated at 32 and 128 mg/kg/day. The clinical relevance of the neurological effects is discussed in the Safety Pharmacology section, however in summary the potential for neurotoxicity is considered to be low with the proposed posology for IV artesunate Amivas.

Other findings in repeat-dose studies were as follows: hepatic and testicular effects at the highest dose in monkeys (128 mg/kg) associated with elevated ALP levels (a class effect reported in the literature), slight increases in transaminases, mild to marked hepatocyte vacuolation and moderate testicular spermatid degeneration. Dose-dependent haemolysis and haemoglobinuria were also observed in monkey studies. The dog also showed a dose-related increased incidence of vomiting at high doses particularly following 50 mg/kg upward.

The applicant considers the animal toxicity profile to be consistent with that seen in human clinical trials, identifying haematological effects as the primary adverse event of interest following IV artesunate dosing. Anaemia was the AE that was most consistently reported across the Phase 1 and 2 studies, occurring in >10% of healthy volunteers in the Phase 1 studies and in 23%-43% of patients with uncomplicated malaria in the Phase 2 studies. Similarly, among patients with severe or complicated malaria in Study R-CDC-060, anaemia was the most frequently reported AE overall, occurring in ~65% of patients in the safety analysis population. In the Phase 1 and 2 clinical trials, there were also modest decreases in reticulocyte count observed. Neutropenia was reported in up to 60% of patients with uncomplicated malaria in the Phase 2 studies; but it was primarily associated with serotherapeutic IV AS doses >2.4 mg/kg daily. Neutropenia occurred much less frequently in healthy volunteers (5.0%) in the Phase 1 studies and in patients with severe or complicated malaria treated with IV AS 2.4 mg/kg daily in Study R-CDC-060 (4.9%). Hepatobiliary AEs were not reported in the clinical Phase 1 studies in healthy volunteers. However, in the Phase 2 studies in patients with uncomplicated malaria, there were sporadic reports of jaundice, hepatomegaly, or ALT increased that affected <4% of patients at a given IV AS dose level. In human studies conducted with the Guilin artesunate/bicarbonate formulation, similar hematologic effects (e.g., decreases in RBC counts, haemoglobin, and/or reticulocytes) have been observed. Haemolysis and increases in bilirubin and ALT/AST have also been noted. Thus, it is considered that the nonclinical single and repeat-dose studies adequately characterise the toxicity of artesunate and DHA. Toxicokinetic data from nonclinical studies is limited, however it must be noted that exposure-based safety margins are narrow or non-existent to the reported clinical exposure.

The potential genotoxicity of artesunate was investigated by the Applicant (bacterial reverse mutagen assay, in vitro chromosome aberration assay and 2 in vivo micronucleus assays (mouse and rat) and was found to be negative for genotoxic or mutagenic potential. Studies were not performed with DHA, however with rapid conversion of artesunate to DHA in vitro and in vivo it is considered that the genotoxic potential of DHA will have been covered in artesunate studies. Carcinogenicity studies have not been conducted with artesunate. This is acceptable due to the short duration of treatment proposed with Artesunate Amivas, also taking into account the absence of tumour findings in repeat-dose studies conducted to date.

Fertility and early embryonic development studies were not conducted with artesunate. Male and female reproductive organs were examined in the 7-day monkey study. No apparent effects were noted in female organs, however moderate testicular spermatid degeneration was noted in males after daily IV dosing at 128 mg/kg. No such observations were evident in the 28-day pivotal repeat dose studies in rat and dog up to doses of 30 mg/kg and 40 mg/kg, respectively. A literature review presented by the applicant suggests that artesunate may induce effects of male spermatogenesis in rodents following oral administration. A comparison of IV and oral PK does not indicate prolonged exposure via the oral route in rats. The mechanism of toxicity thus remains unknown and the clinical relevance of this is unclear. However, the exposure to artesunate and DHA in the GLP IV studies conducted by the applicant (at the NOEL for organ pathology) is generally higher than reported clinical exposure in patients with uncomplicated or severe malaria. The risk to male fertility on the basis of the exposure-based safety margins could be low, however it cannot be ignored that artesunate induces adverse effects on epididymis, testis and sperm parameters in male animals and this is included in the appropriate sections of the SmPC. The Applicant provided a study protocol for "an intravenous injection fertility and early embryonic development (FEED) study of artesunate in SD rats" (3851-3), with final reporting due by Q4 2021.

Embryofetal development (EFD) was investigated in a number of studies in the rat, rabbit and cynomolgus monkey. Studies were conducted using oral administration of artesunate, and bioavailability by the oral route is expected to be low. Artesunate administration induced a pattern of

toxicity that was largely similar across nonclinical species and dose dependent. A classic spectrum of embyrotoxic effects were observed, including embryolethality, cardiovascular malformations (including ventral septal defects) and skeletal anomalies (e.g. ribs, long bones and/or scapulae). In rabbits, cranio-facial and CNS/brain malformations were observed at all doses (absent areas of brain, severely dilated brain ventricles, spina bifida, supraoccipital cleft, swelling of occipital region of skull 4/2 fetuses/litters at low dose and absent areas of brain 2/1 fetuses/litter and 1/1 fetuses/litter at mid and high dose, respectively). The applicant postulates that the difference in placentation and the presence of a visceral yolk sack in rats and rabbits versus humans may in part account for differential artesunate exposure in utero. Also, the duration of treatment in non-clinical studies accounts for a greater period of the gestational cycle in nonclinical species versus the human gestational period. Radiolabelled artesunate was also present at 2-4-fold higher concentrations in the placenta and uterus of pregnant rats versus blood exposure. Several studies in rats and monkeys also investigated critical or sensitive windows in the gestational period for artesunate-induced toxicity. GD 9-14 and GD 30-45 were identified as periods of higher susceptibility to artesunate-induced embryotoxicity in rats and monkeys, respectively. The embryotoxic mechanism of artesunate is thought to be in part due its effects on the haematological system and sensitivity of embryonic erythroblasts and maturation of RBCs in the gestational period. The exposures achieved in EFD studies is far lower than exposure achieved in pregnant humans following IV administration (4 mg/kg/day). These women were in their second or third trimesters of pregnancy outcomes and no adverse outcomes (including congenital abnormalities or developmental issues up to 1 year of age) were observed. Notably, erythroblasts are the primary form of circulating blood cells in weeks 6-13 of human gestation, thus it cannot be ruled out that a risk exists in early pregnancy. There are also insufficient data on the breastfeeding period in human (Schaeffer, 2015). Revisions have been made to section 4.6 of the SmPC accordingly. Pre- and postnatal developmental studies were not conducted. The applicant referenced a WHO report (2003) on the safety of artemisinin derivatives and no detrimental developmental effects were evident in the offspring of dams dosed with artesunate. Although supportive, the study design and exposure data was not presented, thus in light of the findings in studies conducted by the applicant it is possible that exposure of artesunate in these studies is lower than clinical exposure with IV artesunate. Overall, the clinical relevance of this data is unclear. A study in juvenile animals with a combination product (daily dose of artesunate: 0.235 mg/kg) was not associated with any adverse effects when administered from postnatal day 4-31 (orally). Local tolerance was evaluated in rat and dogs. Artesunate was considered non-irritant when administered up to 6 mg/ml and 50 mg/ml in rat and dog, respectively. Artesunate was compatible with human, rat, and monkey blood at concentrations up to 60 µM, while haemolysis was observed at 10 mg/mL. Blood compatibility of artesunate could not be evaluated in dogs due to incompatibility of the PBS vehicle in this species.

2.5.5. Ecotoxicity/environmental risk assessment

The Applicant should provide a complete Phase I ERA with an experimentally derived LogKow (and specific PBT assessment if necessary). The Applicant has committed to provide an updated Phase 1 assessment with the experimentally derived LogKow when the study is completed, and this will be submitted as a post-authorisation measure. As a result, there is insufficient data to definitively conclude on the potential risk of artesunate to the environment.

2.5.6. Discussion on non-clinical aspects

Nonclinical pharmacodynamic studies including in-vitro studies and in-vivo models of malaria demonstrate the anti-malarial efficacy of artesunate in rodent and non-rodent species.

No secondary pharmacology studies were conducted for artesunate. Safety pharmacology studies addressed cardiovascular, respiratory and central nervous system safety in addition to gastrointestinal safety. No respiratory effects were noted in the telemetered dog study. A hERG assay was not conducted with artesunate, however the applicant referenced a literature report on the hERG potential of DHA, which is clinically relevant given the rapid conversion of artesunate to DHA in vivo. hERG inhibition was evident *in vitro*, however the applicant considered that the clinical risk at the proposed dose of 2.4 mg/kg is low. On the basis of supporting evidence from clinical studies, demonstrating no direct effects on QT prolongation, it is agreed that the risk is low.

Central nervous system toxicity was evaluated with a review of literature and a neurotoxicity assessment in rhesus monkeys including a pathology assessment of brain tissue. Artemisinin derivatives have been associated with neurotoxicity in nonclinical species and the topic was the subject of a review by WHO.

Key findings of the report were that water-soluble artemisinins had less toxic potential and the vehicle or route of administration also altered the neurotoxic profile. Shorter duration of exposure was also associated with reduced toxicity. In the rhesus monkey study, transient neurological signs including prostration, drooling and spontaneous motor activity were noted at doses of 32 mg/kg or greater and resolved within 1 hour. No pathological correlates were noted in any brain region. Overall, the weight of evidence suggests the previously reported neurotoxicity in nonclinical species does not represent a likely risk to patients treated with IV artesunate at the proposed posology.

Pharmacokinetic studies were conducted in rats, dogs and monkeys. A bioequivalence study was conducted in rats and dogs to compare Guilin and WRAIR formulations.

Nonclinical PK studies demonstrated that artesunate is rapidly converted to DHA and has a biphasic pattern of distribution. Enterohepatic circulation of artesunate was identified by multiple peaks of radioactivity. The PK of artesunate was similar in rats and dogs. Conversion of artesunate to DHA was rapid in both species although the conversion rate in dogs was lower than humans. DHA was also rapidly cleared and there were no apparent sex differences. DHA was observed as two tautomers after rapid administration in rats. No difference in activity is expected between these two tautomers. IV administration with radiolabelled artesunate showed that the small intestine was the site for highest exposure (68%) and was rapidly cleared but concentrations were detected in the spleen at high levels after 192 hours. Exposure in the brain was also higher than plasma concentrations. In pregnant rats, there was prolonged exposure of reproductive tissues to artesunate.

Drug-drug interaction studies showed that DHA moderately inhibited substrates of CYP1A2 and CYP2C19. However, this is not of great relevance considering that in clinical studies, no relevant CYP inhibition was found. This is supported by the absence of changes in markers for these enzymes in an in-vivo DDI study in rhesus monkeys. It should be noted that inhibition studies were not conducted in line with guidance and there is outstanding information on UGT enzyme inhibition by DHA (Post-authorisation measure).

The toxicology of artesunate was characterised in a host of nonclinical species including Sprague Dawley rats, beagle dogs, rabbits and rhesus and cynomolgus monkeys. Some GLP-compliant studies were conducted with WRAIR formulation and 2 pivotal (GLP) repeat dose studies were conducted with the SS-API. Repeat-dose toxicology studies utilised IV administration and the predominant finding was haematological effects, characterized by dose-dependent anaemia and reticulocytopenia and sometimes transient leukopenia and neutropenia, consistently across all species. These findings correlated with enlarged spleens and erythroid hyperplasia and were observed at all doses in the pivotal studies, thus general toxicity NOAELs could not be defined. These haematological toxicities were reversible in all studies. Other toxicities identified included hepatic effects (monkeys), GI effects (monkey and dog) and haemolysis (monkeys), all of which were largely transient or reversible. As

discussed, the neurotoxic effects seen at high doses in monkeys were transient, without pathological correlate and unlikely to present a reasonable clinical risk with this product. A finding of increased heart rate in mid and high dose groups in the pivotal rat study were considered non-adverse, however in light of a pharmacodynamic mechanism (and increased blood pressure in cardiovascular safety pharmacology study in dogs), the Applicant was asked to discuss the clinical relevance of these observations. A thorough discussion of the cardiovascular findings in non-clinical and clinical studies by the Applicant concluded that based on the totality of the data there is no significant cardiovascular safety concern related to artesunate. This conclusion can be agreed. The toxicities identified in repeat dose studies captures those reported in clinical trials, most notably haematological toxicity. The absence of safety margins in nonclinical studies to the anticipated clinical exposure is considered acceptable as the risk are identified and can be monitored/managed and possibly acceptable in the context of the indication in severe malaria.

In relation to fertility, dedicated GLP studies in the rat and dog did not indicate any toxicity associated with IV artesunate although literature reports have suggested that oral artesunate induced testicular toxicity. This is not considered to be of concern for IV artesunate as there are sufficient margins at the top doses (30 mg/kg and 40 mg/kg, respectively) which represent NOELs for organ pathology. Embryofetal toxicity was observed in rats, rabbits and cynomolgus monkeys following oral administration and included embryolethality and malformations of the cardiovascular and skeletal systems. Exposures in these studies are significantly lower than clinical exposure. The applicant presented observational data from pregnant women treated with artesunate, which indicate that embryofetal toxicity is not a translatable risk in humans. The applicant postulates that differences in placentation/presence of a visceral yolk sack in rats/rabbits and humans and the duration of artesunate exposure during a 'window of sensitivity' to artesunate could explain the differential findings. The Applicant provided a study protocol for "an intravenous injection fertility and early embryonic development (FEED) study of artesunate in SD rats" (3851-3). (Post-authorisation measure)

A reduction in erythroblasts was one of the pathological findings in the monkey EFD study, in addition to heart defects. There is also evidence that foetal blood cells are more sensitive to artesunate than maternal cells. It is plausible that the toxicity observed in nonclinical species is related to the timing of erythroblast maturation in the developing embryo. Erythroblasts are the main form of circulating RBC in the human foetus between 6 - and 13 weeks gestation. On this basis it is considered that the risk in early pregnancy is greater and should be considered before administering to a pregnant woman (see Section 4.6 of the SmPC). Data on exposure via breastfeeding is not available and is clarified in said section of the SmPC.

GLP compliance of pivotal genotoxicity studies had been raised. The studies related to the GLP concern are the bacterial mutagenicity assay and in vitro chromosomal aberration assay with artesunate (studies G305-08 and G306-08 both performed at SRI International, Menlo Park, CA, USA), which both were assessed as negative. However, the FDA conducted an inspection of the 2 studies on behalf of the EMA in March 2021. This inspection concluded that the final reports completely and accurately reflect the raw data and that there were no findings associated with these studies. Carcinogenicity studies have not been conducted; this is acceptable. No other significant toxicities were observed.

Regarding the ERA, an experimentally derived Log Kow for artesunate is being undertaken and will be completed in Oct 2021. This will be included as part of the Phase 1 assessment and an updated ERA provided and submitted post-authorisation. As a result of the above considerations, the available data do not allow to conclude definitively on the potential risk of artesunate to the environment. The applicant commits to perform the following study as follow-up measure:

"Experimentally derived LoKow as per guidance is required to inform on potential risk to the environment. Due Q4 2021."

2.5.7. Conclusion on the non-clinical aspects

There are no outstanding preclinical issues to preclude the issue of a Marketing Authorisation. However, the CHMP considers the following measures necessary to be conducted:

- An *in vitro* study of the potential inhibition of UGT2B7 and UGT1A9 by DHA: Glucuronidation is the major elimination pathway of DHA and therefore UGT enzyme inhibition should be investigated as per guidance. *Due Q4 2021*.

- An Intravenous Injection Fertility and Early Embryonic Development Study of Artesunate in Sprague Dawley Rats: To assess the impact of IV artesunate on female fertility and early embryonic development. *Due Q4 2022.*

- Updated Phase 1 ERA with experimentally derived LogKow: Experimentally derived LoKow as per guidance is required to inform on potential risk to the environment. *Due Q4 2021.*

2.6. Clinical aspects

2.6.1. Introduction

GCP aspects

GCP inspections of the two pivotal efficacy studies are not feasible. The applicant proposes that the following three trials are pivotal:

- R-CDC-060 (non-comparative, retrospective; used a version of Artesunate Amivas)
- AQUAMAT (comparative but using Guilin artesunate)
- SEAQUAMAT (comparative but using Guilin artesunate)

The applicant has provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC, although they were not conducted fully in accordance with GCP.

CDC-060 was conducted by the US CDC under their IND. The CSR was prepared by the Department of the Army, in collaboration with the CDC. The principles of GCP were followed to the extent possible in both trial conduct and documentation procedures but the final study report is not fully compliant due to the nature of the study and various methods of data collection between involved sites.

The US Army obtained permissions from the Wellcome Trust to use the data from the SEAQUAMAT trial. It was not possible to employ traditional GCP inspection methods during the trial. The sponsor was unwilling to give permission for a study audit by the US Army or any subsequent Regulatory Authority. The same limitations applied to AQUAMAT but the applicant was also denied access to patient level data and permission to audit this study.

- **Tabular overview of clinical studies**

Three clinical studies with PK objectives involved IV administration of the US Army formulation that was the precursor to Artesunate Amivas.

Table 1: Summary of Individual Studies with Pharmacokinetic Objectives Using US Army IV AS

Study Reference	Study Title	Study Design	Phase	IV AS Source and Treatment Regimen	Subjects	Study Objectives
Study 1128	A Phase 1a randomized, double-blind, placebo-controlled dose-escalation study of the safety, tolerability, and pharmacokinetics of single intravenous doses of artesunate administered to healthy subjects	Randomized, double-blind, placebo-controlled, ascending, single dose Conducted in the United States	1	Single 2-minute IV infusion of AS or placebo at 0.5, 1.0, 2.0, 4.0, or 8.0 mg/kg	40 subjects; 32 males, 8 females. Mean age 39.8 years (range 19.0-55.0 years)	Safety, tolerability, pharmacokinetics of single dose of IV AS in sequentially escalating doses in healthy volunteers
Study 1142	A Phase 1 double-blind, placebo-controlled, randomized, multiple dose escalation study to evaluate the safety, tolerance, and pharmacokinetics/pharmacodynamics of a new GMP formulation of intravenous artesunate in healthy subjects	Phase 1 double-blind, placebo-controlled, randomized multiple dose Conducted in the United States	1	Single 2-minute IV infusion of AS or placebo at 0, 2.0, 4.0, or 8.0 mg/kg on 3 consecutive days	26 subjects; 24 males, 2 females. Mean age: 37.2 years (range 20-55 years)	Safety, tolerability, pharmacokinetics of multiple (3-day) administration of escalating doses in healthy volunteers bracketing anticipated compassionate use dose of 2.4 mg/kg
Study 1168	A Phase II, open label, study of the safety, tolerability, efficacy, and pharmacokinetics of intravenous artesunate in adults with uncomplicated malaria	Phase 2, open-label, nonrandomized Conducted in Kenya	2	2.4 mg/kg once daily for 3 consecutive days (Days 0, 1, 2) by IV push. On Days 2, 3, and 4 patients received follow-on atovaquone 250 mg-proguanil 100 mg (Malarone®).	30 patients; 10 males, 20 females. Mean age: 28 years (range 18-54 years)	PK profile, safety, tolerability, efficacy in adults with uncomplicated *Plasmodium falciparum* malaria

The demonstration of efficacy of IV artesunate is based on two prospective, randomised, controlled trials (RCTs) conducted between 2003 and 2010 funded by the Wellcome Trust.

- The South East Asian Quinine Artesunate Malaria Trial - **SEAQUAMAT** - conducted 2003-2005 in adults and children with severe falciparum malaria admitted to hospitals in Asia and SE Asia (*Lancet* 2005; 366: 717-25).
- The African Quinine Artesunate Malaria Trial - **AQUAMAT** - conducted 2005-2010 in children with severe falciparum malaria admitted to hospitals in Africa (*Lancet* 2010; 376: 1647-57).

- SEAQUAMAT and AQUAMAT used artesunate manufactured in Guilin, China.

Four efficacy studies used US Army artesunate to treat malaria, of which two concerned severe malaria.

Study	**Route of administration**	**Patient Population**
CDC-060	Intravenous	Returning US travellers of any age with severe/complicated malaria
Study 1168	Intravenous	Uncomplicated malaria
Study 1263ab	Intravenous 4 dose regimens	Uncomplicated malaria
EDCTP-MMV07-01	Intravenous 3 doses vs. 5 doses	Severe malaria in children

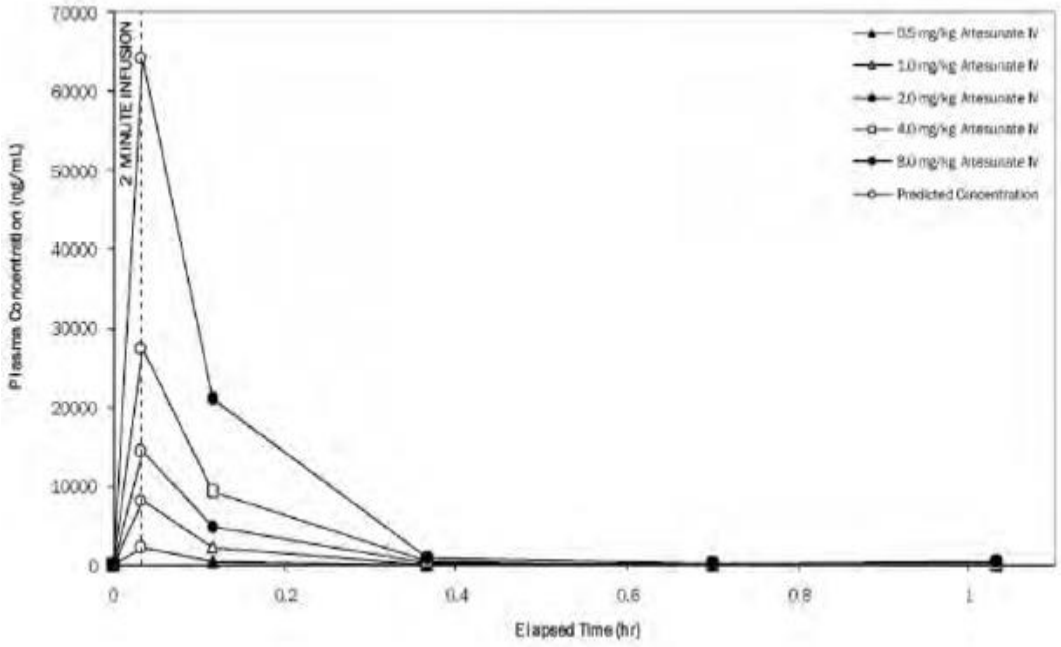
2.6.2. Clinical pharmacology

2.6.2.1. Pharmacokinetics

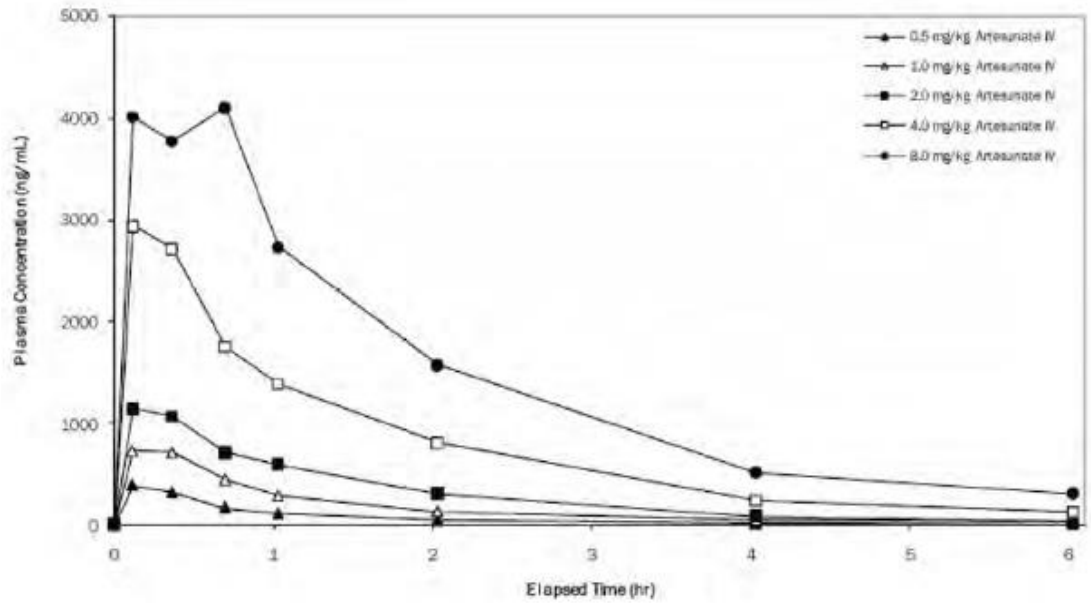
Artesunate Amivas is given intravenously as a bolus injection over 1-2 minutes.

Study 1128 - US Army 2005-2006

This was a placebo-controlled single ascending dose (SAD) study with doses from 0.5 to 8 mg/kg given by intravenous bolus over 2 min after an overnight fast. For artesunic acid, there were very few quantifiable levels reported beyond 1 h.



For DHA, the profiles were well defined at all dose levels for up to 6 h but not at 24 h.



The estimated mean concentrations of artesunic acid at the end of the infusion (C0.033) increased from 8241 ng/mL at a dose of 1.0 mg/kg to 64,336 ng/mL at the highest dose.

Thus, for an 8-fold increase in dose there was a 7.8-fold increase in the C0.033 value, suggesting linearity in this relationship. Similarly, there was a linear relationship between dose and AUC parameters; for an 8-fold increase in dose the mean $AUC_{0\text{-last}}$ values increased 8.6-fold and $AUC_{0\text{-}\infty}$ values increased 8.7 fold.

For DHA, across the 8-fold difference in dose from 1.0 to 8 mg/kg, the increment in mean $AUC_{0\text{-last}}$ was 8.8-fold and $AUC_{0\text{-}\infty}$ values increased 8.7-fold. However, linearity was not shown for the 0.5 mg/kg dose. Thus, the applicant concluded that artesunic acid and DHA exhibit linearity across the dose range of 1.0 mg/kg to 8.0 mg/kg.

Correspondingly, based on tabulated dose-normalised data, there were no significant differences between treatment groups for artesunate or across the 1-8 mg/kg dose groups for DHA.

Table 2: Statistical Summary for Artesunic Acid Pharmacokinetic Parameters

Parameter	Geometric Mean by Treatment Group[a]					
	0.5 mg/kg	1.0 mg/kg	2.0 mg/kg	4.0 mg/kg	8.0 mg/kg	P Value
$AUC_{0\text{-last}}$ (ng.hr/mL)	NC[b]	867	893	804	831	0.9752
$AUC_{0\text{-}\infty}$ (ng.hr/mL)	NC	889	900	806	854	0.9687
$C_{0.033}$ (ng/mL)	NC	7626	7098	6297	6292	0.8875

[a] Normalized to 1.0 mg/kg.
[b] Not calculated.

Table 3: Statistical Summary for Dihydroartimisinin Pharmacokinetic Parameters

Parameter	Geometric Mean by Treatment Group[a]					
	0.5 mg/kg	1.0 mg/kg	2.0 mg/kg	4.0 mg/kg	8.0 mg/kg	P Value
$AUC_{0\text{-last}}$ (ng.hr/mL)	375	535	445	567	607	0.0272
$AUC_{0\text{-}\infty}$ (ng.hr/mL)	382	566	457	597	631	0.0131
$C_{0.033}$ (ng/mL)	424	393	316	380	282	0.0504

[a] Normalized to 0.5 mg/kg.

Study 1142 – US Army 2006-2007

This was a multiple ascending dose (MAD) study in healthy subjects aged 18-55 years. Randomisation was to 2 mg/kg, 4 mg/kg or 8 mg/kg artesunate or placebo once daily for 3 days. At each dose level, plasma levels of artesunic acid declined very rapidly. DHA was quantifiable within 5 minutes of dosing and was detectable for at least 6 h. DHA Cmax was at ~20 min with a half-life of about 1 to 2 h. Comparison of AUCs indicated that DHA plasma exposure was approximately twice that of artesunate exposure. The ratios of DHA AUC_{24}^{TOTAL}/ AS_{last}^{TOTAL} were 6051/3027 = 2.0 for 2 mg/kg, 11370/5863 = 2.0 for 4 mg/kg and 25685/14733 = 1.74 for 8 mg/kg.

There were no significant differences for within dose group comparisons of Days 1, 2 and 3 data for artesunic acid AUC_{last} and C_0 and for DHA AUC_{24} and AUC_{last}. Similarly, there were no significant differences between the study days for DHA Cmax for the 4.0 mg/kg and 8.0 mg/kg treatments. A significantly higher DHA Cmax was observed on Day 3 vs. Days 1 or 2 in the 2.0 mg/kg dose group.

There were no significant differences between dose groups for dose-normalised artesunic acid AUC_{last}^{TOTAL} and C_0^3 and for dose-normalised DHA AUC_{24}^{TOTAL}. For DHA, the 2.0 mg/kg dose gave a significantly higher dose-normalised $Cmax^3$ than the 4.0 mg/kg and 8.0 mg/kg doses.

Table 4: Mean, Standard Deviation, and Range of of Pharmacokinetic Parameters for DHA after Singe 2.0, 4.0 and 8.0 mg/kg IV Administrations of Intravenous Artesunate Daily for 3 Days

Parameter	Units	2.0 mg/kg	4.0 mg/kg	8.0 mg/kg
$AUC_{24}^{1}{}_{(DHA)}$	ng.hr/mL	n = 6 1982 ± 377 [1521 - 2502]	n = 6 4122 ± 809 [2907 - 5397]	n = 6 8992 ± 3355 [5083 - 14054]
$AUC_{24}^{2}{}_{(DHA)}$	ng.hr/mL	n = 6 1865 ± 512 [1380 - 2681]	n = 6 3509 ± 970 [2068 - 4681]	n = 6 8122 ± 2750 [4682 - 12231]
$AUC_{24}^{3}{}_{(DHA)}$	ng.hr/mL	n = 6 2204 ± 524 [1726 - 2956]	n = 6 3748 ± 646 [2718 - 4400]	n = 6 8571 ± 2817 [5522 - 13890]
$AUC_{last}^{1}{}_{(DHA)}$	ng.hr/mL	n = 6 1954 ± 367 [1517 - 2464]	n = 6 4065 ± 771 [2907 - 5301]	n = 6 8842 ± 3265 [5082 - 13739]
$AUC_{last}^{2}{}_{(DHA)}$	ng.hr/mL	n = 6 1847 ± 516 [1366 - 2680]	n = 6 3394 ± 974 [1871 - 4513]	n = 6 8011 ± 2780 [4619 - 12226]
$AUC_{last}^{3}{}_{(DHA)}$	ng.hr/mL	n = 6 2171 ± 519 [1682 - 2893]	n = 6 3701 ± 647 [2650 - 4315]	n = 6 8405 ± 2715 [5462 - 13503]
$AUC_{\infty}^{TOTAL}{}_{(DHA)}$	ng.hr/mL	n = 6 6051 ± 1074 [4776 - 7400]	n = 6 11379 ± 2128 [8253 - 14031]	n = 6 25685 ± 8715 [15286 - 40174]
$C_{max}^{1}{}_{(DHA)}$	ng.hr/mL	n = 6 1735 ± 547 [1280 - 2470]	n = 6 3015 ± 882 [2060 - 4000]	n = 6 6057 ± 2456 [3820 - 9190]
$C_{max}^{2}{}_{(DHA)}$	ng.hr/mL	n = 6 1710 ± 715 [1330 - 3160]	n = 6 2923 ± 1050 [1660 - 4510]	n = 6 5943 ± 2110 [3590 - 9060]
$C_{max}^{3}{}_{(DHA)}$	ng.hr/mL	n = 6 2358 ± 634 [1760 - 3480]	n = 6 2933 ± 810 [2110 - 3920]	n = 6 5762 ± 3426 [3630 - 12600]
$T_{max}^{1}{}_{(DHA)}$	hr	n = 6 0.244 ± 0.132 [0.067 - 0.333]	n = 6 0.208 ± 0.137 [0.083 - 0.333]	n = 6 0.208 ± 0.137 [0.083 - 0.333]
$T_{max}^{2}{}_{(DHA)}$	hr	n = 6 0.167 ± 0.129 [0.083 - 0.333]	n = 6 0.208 ± 0.137 [0.083 - 0.333]	n = 6 0.181 ± 0.238 [0.083 - 0.667]
$T_{max}^{3}{}_{(DHA)}$	hr	n = 6 0.122 ± 0.104 [0.067 - 0.333]	n = 6 0.292 ± 0.102 [0.083 - 0.333]	n = 6 0.250 ± 0.129 [0.083 - 0.333]

Study 1168 – US Army 2006

The primary objective was to determine PK over 6 h after a dose of 2.4 mg/kg in 30 Kenyan adults with uncomplicated falciparum malaria mono-infection. Patients were male or non-pregnant female adults aged 18 to 65 years. On Study Days 0, 1 and 2, subjects received 2.4 mg/kg intravenous artesunate once daily. There were 268 plasma samples analysed. Artesunate was quantifiable in 164 samples with a range between 0.86 and 40,027 ng/ml and DHA was quantifiable in all but one post-

dose sample with a range between 2.24 and 5582 ng/ml. The mean concentration-time profiles of artesunic acid and DHA are shown in the figures.

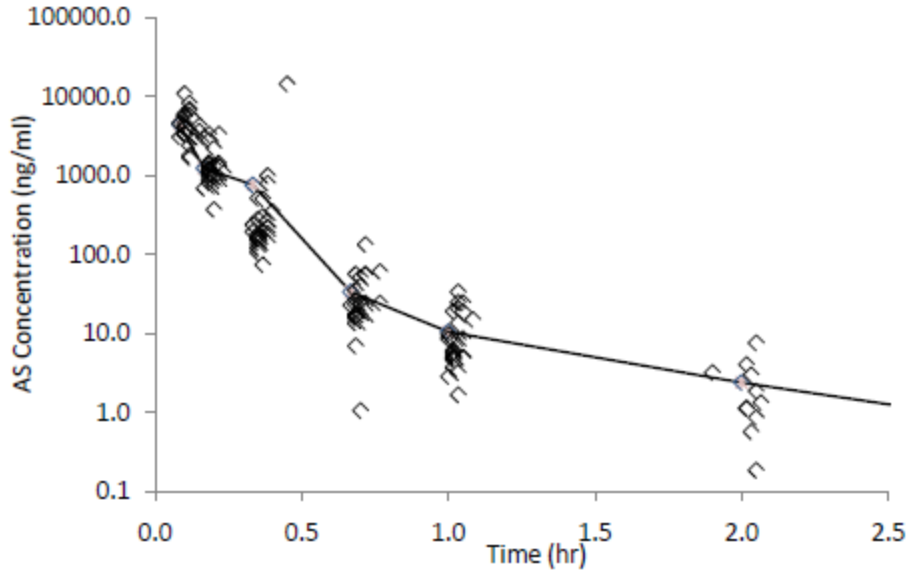


Figure 2: Concentration-time Profile of Artesunic Acid (empty diamond) and mean Concentration curve (solid-line)

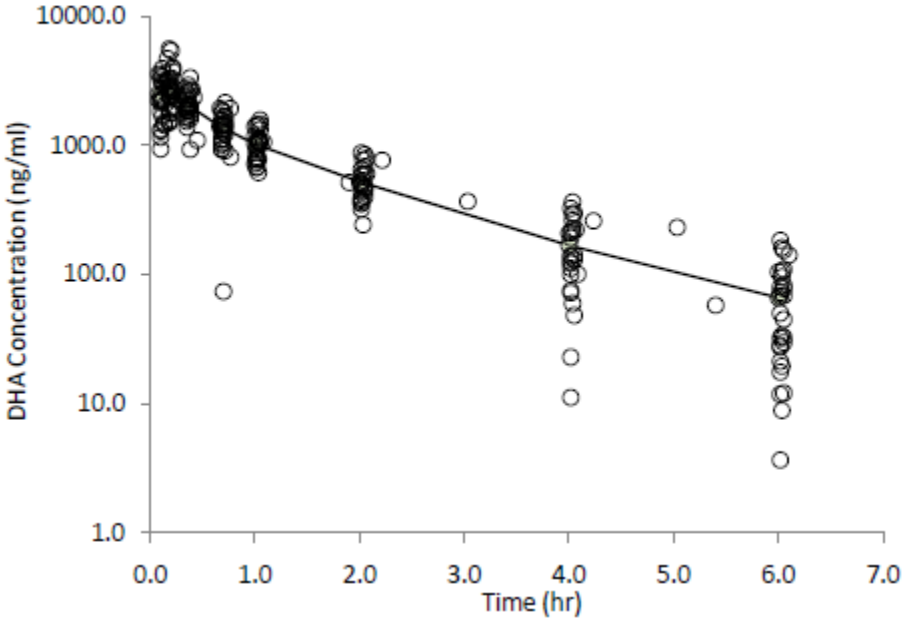


Figure 3: Concentration-time Profile of Dihydroartemisinin (empty cycle) and mean Concentration curve (solid-line)

For artesunic acid, there were very few quantifiable levels reported beyond 1 h. The estimated mean peak concentration of artesunic acid at the end of the infusion (C0.028) was 28,557 ng/ml. Plasma levels declined rapidly with a half-life of less than 20 minutes in most patients.

Table 5: Pharmacokinetic Parameters for Artesunic Acid Calculated using a Comparmental Analysis

Parameters (Units)	Infusion time (hr)	AUC_{inf} (ng·h/ml)	$C_{0.028}$ (ng/ml)	$t_{1/2}$ beta (hr)	CL (ml/min/kg)	Vss (ml/kg)
Mean	0.028	1877.59	28557.84	0.17	1727.80	138.7
SD	0.015	1189.59	28530.73	0.08	982.64	123.80
% CV	54.66	63.36	99.91	47.43	56.87	89.23
Minimum	0.017	477.14	3361.50	0.11	373.00	41.14
Maximum	0.067	3521.41	55873.43	0.43	5029.92	670.44

For DHA the profiles were well defined at all dose levels for up to 6 h. The results for DHA using a non-compartmental analysis and a compartmental analysis are summarised in the two tables below. No significant differences were found between the model-dependent and model-independent calculations. The results also showed no significant differences between $AUC_{0\text{-last}}$ and $AUC_{0\text{-}\infty}$, indicating that elimination was almost complete 6 h after dosing. The mean half-life of DHA was 1.25 h with the peak concentration of 2,796 ng/mL occurring 0.20 hours after the infusion.

Table 6: Pharmacokinetic Parameters for Dihydroartemisinin Calculated using a non-Compartmental Analysis

Parameters (Units)	$AUC_{0\text{-last}}$ (ng·h/ml)	AUC_{inf} (ng·h/ml)	$C_{0.028}$ (ng/ml)	T_{max} (hr)	$t_{1/2}$ beta (hr)	CL (ml/min/kg)	Vss (ml/kg)
Mean	3443.24	3587.37	2789.64	0.20	1.25	723.81	1240.27
SD	933.28	1024.72	931.86	0.08	0.43	205.59	355.49
% CV	27.10	28.56	33.40	38.75	34.02	28.40	28.66
Minimum	2012.51	2207.93	1493.27	0.08	0.61	442.48	500.39
Maximum	5038.87	5424.01	5568.94	0.38	2.19	1185.57	1934.57

Table 7: Pharmacokinetic Parameters for Dihydroartemisinin Calculated using a Compartmental Analysis

Parameters (Units)	$AUC_{0\text{-last}}$ (ng·h/ml)	AUC_{inf} (ng·h/ml)	$C_{0.028}$ (ng/ml)	T_{max} (hr)	$t_{1/2}$ beta (hr)	CL (ml/min/kg)	Vss (ml/kg)
Mean	3496.27	3543.33	2932.06	0.18	1.30	730.79	1047.55
SD	941.23	989.55	849.94	0.06	0.34	204.74	250.24
SD	941.23	989.55	849.94	0.06	0.34	204.74	250.24
% CV	26.92	27.93	28.99	30.06	26.53	28.02	23.89
Minimum	2016.33	2018.70	1602.53	0.12	0.79	464.12	773.26
Maximum	5048.61	5216.48	4748.07	00.35	1.90	1188.88	1349.79

The study confirmed that artesunic acid was rapidly converted to DHA, which was detectable in all patients until 6 h after infusion. The plasma concentration of DHA was higher than that of artesunic acid, with a 1.89 ratio of AUC of DHA/artesunic acid. In contrast, the artesunic acid/DHA Cmax ratio was 10.35.

Distribution

Vd values of ~1 L/kg imply that artesunate and DHA distribute to ~60 L in a 60 kg adult, which is close to adult total body water (42 L).

Li (2006) reported that [^{14}C] artesunate was 73% bound to human plasma proteins. Batty (2004) used 10-[^{3}H]-DHA to assess protein binding in whole blood from 15 healthy volunteers and 22 Vietnamese patients with *P. falciparum* or *P. vivax* uncomplicated malaria. DHA was ~93% protein bound and the α-DHA:β-DHA ratio was 6.5:1 in the patients with malaria. DHA protein binding was 88% in Vietnamese volunteers and 91% in Caucasian volunteers. In the albumin binding studies, the free fraction of DHA was not concentration-dependent.

Using artemisinin radiolabeled with tritiated borohydride, the tritiated DHA showed low concentrations in culture medium with accumulation in erythrocytes infected with *P. falciparum*. Uninfected erythrocytes concentrated the drug less than 2-fold whereas infected erythrocytes achieved more than 300 times the concentration in the culture medium.

Penetration of AS and DHA into CSF was assessed in 6 adults with cerebral or severe malaria who underwent LP on admission and during convalescence at different times (from 15 to 120 minutes) after IV dosing with 120 mg artesunate. Artesunate was not detected in any CSF sample. At 15 min post injection, the DHA concentration in CSF was 4% of that in plasma but the concentration increased with time, suggesting continuing influx but a slower efflux of DHA, thought to reflect a sink effect of DHA uptake transfer by lipid-rich brain structures. DHA levels in CSF reached > 100 nmol/L at 120 min in samples from acute and convalescent phases.

Excretion

In Study 1128 (SAD), clearance of artesunic acid was high at approximately 100 L/hr (>1500 mL/min) after doses of 1.0 mg/kg to 8.0 mg/kg.

The CL parameter for DHA could not be determined unless it was assumed that there was 100% conversion of artesunic acid to DHA within the body and all of the DHA formed was delivered to the systemic circulation prior to elimination. If this assumption holds, the apparent CL of the metabolite was approximately 50 L/hr and the apparent Vd was about 100 L. Thus, in comparison with the parent compound, DHA has a smaller CL and a larger Vd, which together yield a half-life ($T½ = \ln(2) \times Vd/CL$) that is longer than that of artesunic acid.

Metabolism

Based on in-vitro studies with rat and human liver microsomes and recombinant cytochromes:

- Conversion of artesunate to DHA is due to CYP2A6 and blood esterases;
- In human liver microsomal incubations of DHA, DHA-glucuronide was the only metabolite found;
- Formation of DHA-glucuronide was predominantly due to UGT1A9;
- There was no significant metabolism of DHA by CYP isoenzymes or by cytosolic sulfotransferases;
- Urine from patients contained α-DHA-β-glucuronide (α-DHA-G) and a variable amount of the tetrahydrofuran isomer of α-DHA-G while DHA was present in very small amounts.

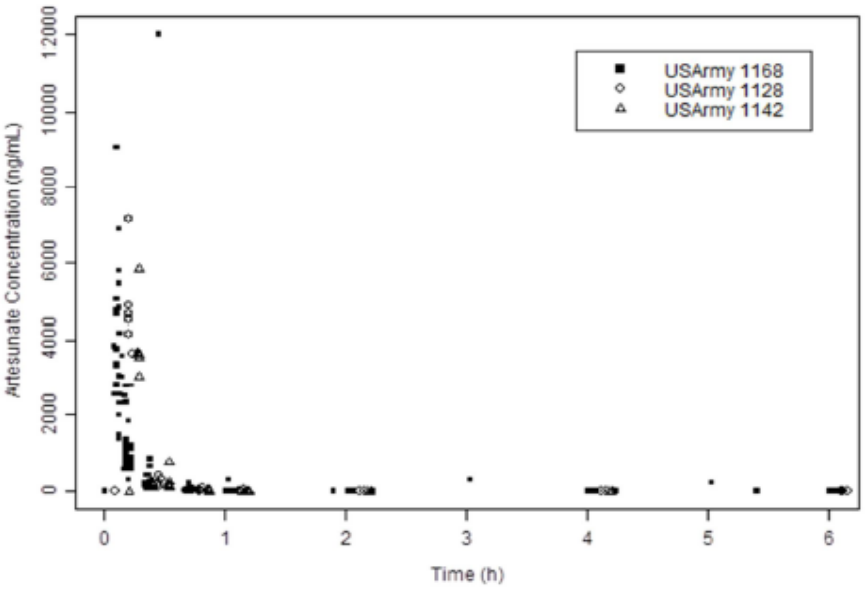
Inter-conversion

According to d'Acquarica *et al*. (2010), the reduction of artemisinin by $NaBH_4$ to form DHA gives rise to a stereochemically labile centre at C-10. This results in two interconverting lactol hemiacetal tautomers (also called epimers or isomers), named DHA2α and DHA2β, with a rate of interconversion that depends on buffer, pH and solvent polarity. Interconversion occurs on a chromatographic time-scale, which has implications for analytical methods. In the solid state, the drug consists exclusively of the β-epimer; however, upon dissolution, the two epimers equilibrate, reaching different solvent-dependent ratios with different rates. Such equilibration also occurs *in vivo*, irrespective of the isomeric purity at

which the drug would have been administered. Batty *et al*. (2004), when investigating protein binding (see above), found that the α-DHA anomer predominated in human samples with ratios of α-DHA:β-DHA that were in the range 5.4 to 6.9. Clinical trials showing superior efficacy to standard of care did not control for interconversion, nor did *in vitro* susceptibility studies. Expert opinion is that any occurrence of interconversion is not important for safety or efficacy in clinical use.

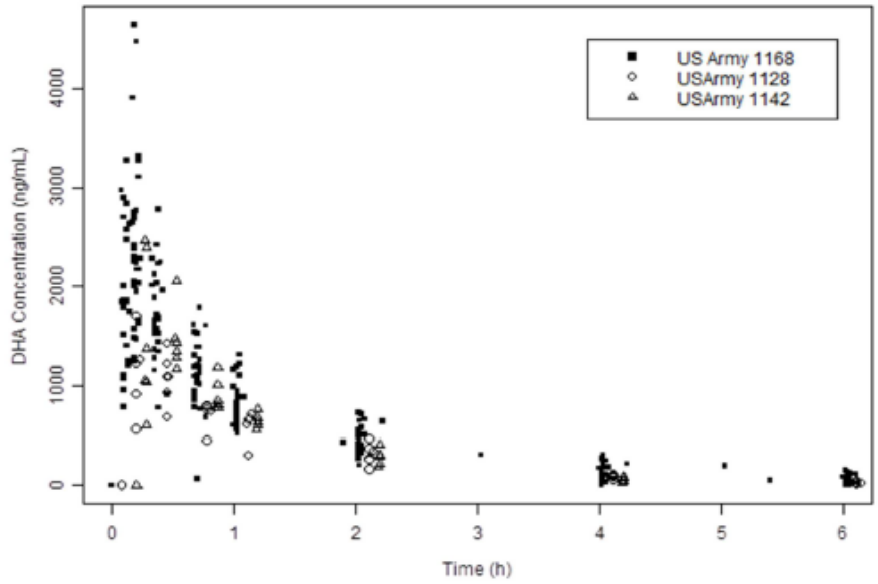
Pharmacokinetics in target population

The applicant provided a comparison between Day 1 PK in healthy volunteers in 1128 and 1142 (IV dose 2 mg/kg) vs. infected patients in 1168 (IV dose 2.4 mg/kg; results normalised to 2 mg/kg).



Note: Time is elapsed time from start of infusion.

Figure 4: Comparison of Artesunate Concentration Versus Time: Phase 1 and Phase 2 Studies



Note: Time is elapsed time from start of infusion.

Figure 5: Comparison of Dihydroartimisinin Concentration Versus Time: Phase 1 and Phase 2 Studies

The applicant also compared artesunate and DHA PK parameters across many studies, including some reported only in the literature and which used the Guilin product. Not only did the dose vary but also the route of administration (some were oral studies), mechanics of administration (e.g. solution and injection/infusion time) and the assay. Since DHA is the most active moiety and since Cmax artesunate may be unreliable, the table shows the DHA data.

Table 8: Summary of DHA PK Results Following IV AS administration

Ref.	Subjects & Regimen	Cmax (ng/mL)	Tmax (min)	Clearance (L/kg/hr)	Volume (L/kg)	Half-life (min)	AUC (ng*hr/mL)
Healthy volunteers							
Binh-2001	17 Vietnamese receiving: 120 mg IV AS over 2 min	1507; 1678 (i)	9; 16			53; 47	
Li-2009	30 receiving 0.5, 1, 2, 4, or 8 mg/kg IV AS over 2 min	428; 802; 1286; 3148; 4744	9.6; 15; 9.6; 7.2; 24	1.3; 0.98; 1.1; 0.86; 0.82	Vss: 1.734; 2.201; 1.860; 1.701; 2.403	57.6; 92.4; 69.0; 82.2; 128.4	385; 1082; 1850; 4886; 10410
Miller-2012	18 receiving 2, 4, or 8 mg/kg IV AS over 2 min for 3 days						
	Day 1	1735, 3015, 6056		0.96, 0.96, 0.9	Vss: 1.6, 2.4, 1.9	72, 102, 84	2121, 4391, 9687
	Day 2	1710, 2923, 5943		1, 1.1, 1	Vss: 2.2, 2.2, 2.5	96, 84, 114	2012, 3740, 8732
	Day 3	2358, 3018, 5762		0.9, 1, 0.96	Vss: 1.7, 1.9, 1.8	78, 72, 78	2385, 3960, 9205
Uncomplicated malaria patients							
Batty-1998a	12 Vietnamese adults with vivax malaria 120 mg IV AS over 2 min	2192	8	1.10	0.92	36.7	1845
Batty-1998b	26 adult uncomplicated falciparum malaria patients in Vietnam, 120 mg IV AS over 2 min	2192	9.0	0.75	0.76	40.2	2377
Krishna-2001	9 Ghanaian children with moderate malaria 2.4 mg/kg IV	215				32	2308
Ilett-2002	23 Vietnamese adults with complicated falciparum malaria; subjects randomised into two groups, both receiving: 120 mg IV AS over 2 min	2758; 2730	7; 9	0.64; 0.48	0.8; 0.55	59; 50	2872; 3298
Li-2014	28 Kenyan adults, 2.4	2932		1.7	Vss: 0.14	78	3543

Ref.	Subjects & Regimen	Cmax (ng/mL)	Tmax (min)	Clearance (L/kg/hr)	Volume (L/kg)	Half-life (min)	AUC (ng*hr/mL)
	mg/kg over 2 min						
Severe malaria patients							
Davis-2001	30 adults with falciparum malaria -120 mg IV AS over 2 min						
	Group 1: 12 with complications	2417	10.4	1.09	0.77	40.0	2078
	Group 2: 8 without complications	2531	9.9	0.73	1.01	64.1	2559
	Group 3: 10 with moderately severe complications (240 mg IV AS infused over 4 h	910	240 (end of infusion)	0.73	0.78	46.2	5573
Nealon-2002	28 paediatric Gabonese patients with severe malaria						
	Group 1: 2.4 mg/kg IV AS	3011	0.5	2.16	Vss: 0.75	20.7	923
	Group 2: 1.2 mg/kg IV AS	1584	1.4	1.08	Vss: 0.77	32.0	737
Newton-2006	17 Thai adults with severe falciparum malaria - 2.4 mg/kg IV AS over 2 min [2.1 (1.4 – 2.8 mg / kg)]	605	Tmax reached by 15 min	5.6	Vss: 1.9	20.4	418
Maude-2009	Adults 2.4 mg/kg IV	2060					2017
Bayakika-2012	14 Ugandan adults, 2.4 mg/kg over 4 min	3140	3.4	1.1		8	3492

Values given as mean unless otherwise specified.

Davis-2001 reported data from 30 Vietnamese adults with slide-positive falciparum malaria treated with IV artesunate. The mean values for DHA elimination $t_{½}$, CL, and Vz were comparable to those after IV artesunate administration to healthy subjects, suggesting that severity of the malaria did not influence PK. There was a non-significant but borderline inverse correlation between time to 50% parasite clearance (PCT_{50}) and AUC for DHA 24 patients (P=0.06).

Nealon-2002 also concluded that the severity of malaria does not have an important influence on the PK of artesunate and DHA. Newton-2006 reported a large inter-individual variability (10-fold) in DHA C_{max} and AUC in patients with severe malaria but no concentration-parasiticidal effect relationship was significant based on comparing C_{max}, AUC, Tlast and t1/2 for artesunate and DHA with PCT, PC_{50} and PC_{90}.

Special populations

Impaired renal and/or hepatic function

Renal and/or hepatic impairment often accompany severe malaria. The severe malaria patients studied by Davis (2001) had mean creatinine levels of 301 µmol/L and mean AST levels of 183 U/L on presentation. For severe malaria patients in Study CDC-060, 20%, 18% and 9% of the evaluable population had mild, moderate and severe renal impairment, respectively, and 7% were on dialysis at baseline. Also, the baseline MELD scores suggested that 11%, 38%, 13% and 7% of patients had mild, moderate, severe and life-threatening liver disease, respectively. Based on DHA AUCs, the applicant states that clinically significant renal and hepatic impairment do not affect DHA exposure.

Elderly

The US Army studies did not provide PK data in elderly persons. No PK data are reported by the applicant from the literature after IV artesunate in persons aged >65 years.

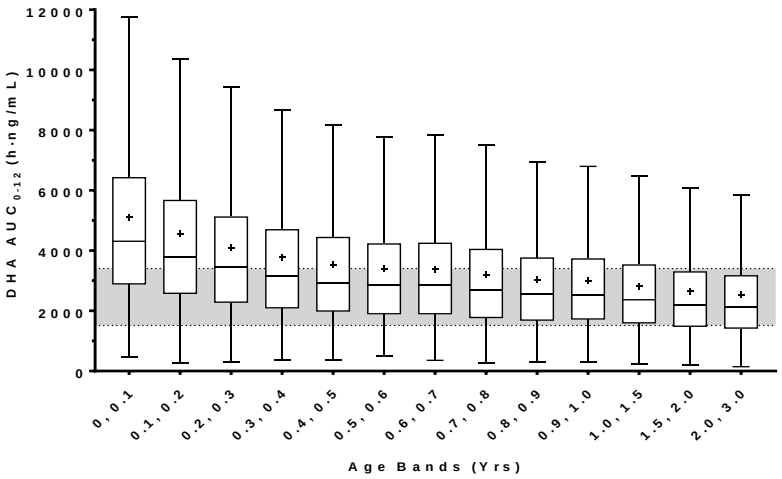
Children

The WHO (2015) recommended that children of <20 kg should receive 3 mg/kg/dose vs. 2.4 mg/kg/dose for heavier persons. The applicant's position is that support for this recommendation is weak, referring to two published POPPK studies in children, one of which was confined to oral dosing.

Zaloumis 2014 conducted an analysis of PK data from 71 adults and 195 children with severe malaria, with a mixture of sparse and rich sampling within the first 12 h after IV artesunate administration. A one-compartment model described the population PK of DHA adequately. Body weight had the greatest impact on DHA PK, resulting in lower DHA exposure for smaller children (6–10 kg) than for adults. With standard dosing (2.4 mg/kg/dose), 6-10 kg children showed a reduction in geometric mean DHA exposure of 13.7% (95% CI: 11.7–15.6%; P <0.001) compared with 21-25 kg children.

Importantly, there was no correlation between exposure and PCT50. In the PD analysis for 142 patients who received 2.4 mg/kg IV artesunate at baseline, the median parasite clearance half-life was 3.1 h with 25% (14/55) of patients clearing 90% of their baseline parasitaemia by 12 h. A non-significant association between *post hoc* DHA Vd and PC_{90} at 12 h was found such that a doubling of Vd was associated with a 0.28-fold decrease in the odds of PC_{90} at 12 h (95% CI: 0.07–1.11; P=0.07). However, post hoc estimates of DHA exposure were not significantly associated with parasitological outcomes.

In 2020, the US FDA performed new PK simulations of IV artesunate dosing in paediatric patients with severe malaria using information obtained from Zaloumis' population-based meta-analysis of DHA PK.



Each box represents the 25th and 75th percentile of the DHA exposure measure. The bar and cross inside the box represent the median and mean respectively, whiskers represent 1.5 times the interquartile range. The gray band represents the interquartile range for patients weighing 20 to 25 kg (8 to 10 years of age) and adults. Clearance was estimated using a combination of allometric weight scaling with a sigmoid function to account for organ maturation.

Figure 6: Predicted Steady-State DHA AUC_{0-12} in Pediatric Patients 0 to 3 Years of Age with Severe Malaria after 2.4 mg/kg Artesunate for Injection

These simulations demonstrated that a dosing regimen of 2.4 mg/kg resulted in comparable or greater predicted steady state DHA AUC_{0-12h} in infants less than 6 months of age compared to that observed in older children or adults. The difference in predicted exposures in infants less than 6 months of age was presumed to be due to immature development of the UGT elimination pathway for DHA.

Pregnancy

A POPPK analysis was conducted using data from Kingasani Maternity Clinic in the DRC obtained from 26 pregnant women in the second or third trimester and from 25 non-pregnant female controls. All subjects received 200 mg oral artesunate. After oral dosing, the effect of pregnancy on DHA CL/F was determined to be significant, with a pregnancy-associated increase in DHA CL/F of 42.3% (19.7 - 72.3%). The plasma concentration profile thus differed slightly between pregnant and non-pregnant women after oral dosing.

However, in a study of malaria in pregnancy, McGready *et al*. (2011) women with uncomplicated falciparum malaria received intravenous artesunate 4 mg/kg on the first day and oral artesunate 4 mg/kg on the second day or *vice versa*. All women then received oral artesunate 4 mg/kg/day for 5 days. Controls were the same women restudied when healthy and at 3 months post-partum.

Artesunate and DHA PK were similar after IV artesunate administration to pregnant women with malaria (n = 20) and to controls (n = 14). The ratio of $t_{½}$ for both analytes was 0.9. The ratios of Vd for artesunate and DHA were 0.64 [P = 0.59] and 0.74 [P =0.12], respectively, and the AUC ratios were 1.2 [P=0.87] and 1.1 [P = 0.25], respectively. However, oral administration resulted in higher total drug exposure in pregnancy. Total DHA exposure was lower at Day 6 in pregnant women with malaria (P < 0.001) compared with Day 0 or 1, but this effect was not observed in the controls (P=0.084).

Table 9: Non-compartmental Analysis of AS and DHA PK in Pregnant Women with Malaria and in the Same Women Post-Partum in a Healthy State

	Median (Range)					
	ARS			DHA		
	Pregnant (n = 20)	Post-partum (n = 14)	P value [a]	Pregnant (n = 20)	Post-partum (n = 14)	P value[a]
Body weight (kg)	48.0 (40.0 – 64.0)	46.5 (37.0 – 52.0)	0.011	48.0 (40.0 – 64.0)	46.5 (37.0 – 52.0)	0.011
Dose (mg kg^{-1})	4.00 (3.33 – 4.05)	4.00 (3.96 – 4.05)	0.232	2.96 (2.47 – 3.00)	2.96 (2.93 – 3.00)	0.232
Number of points lambda	3.00 (3.00 – 5.00)	3.00 (3.00 – 5.00)	0.359	4.00 (4.00 – 6.00)	5.00 (4.00 – 5.00)	0.014
C_0 (ng ml^{-1})	15,700 (3,860 – 28,700)	12,200 (5,490 – 23,900)	0.975	3,210 (1,570 – 4,360)	2,930 (856 – 3,980)	0.272
C_0 / dose (ng ml^{-1} /mg kg^{-1})	3,910 (976 – 7,110)	3,070 (1,390 – 5,900)	0.925	1110 (535 – 1,450)	984 (292 – 1,340)	0.246
CL (l h^{-1})	196.0 (101.0 – 410.0)	213.0 (81.5 – 467.0)	0.730	60.6 (32.8 – 107.0)	61.9 (35.3 – 99.7)	0.925
CL (l h^{-1} kg^{-1})	4.19 (2.29 – 10.20)	5.05 (2.20 – 9.93)	0.551	1.20 (0.683 – 2.14)	1.35 (0.905 – 1.99)	0.470
V (l)	35.6 (17.0 – 208.0)	53.3 (22.3 – 94.6)	0.594	87.5 (40.5 – 196.0)	108 (44.9 – 225.0)	0.300
V (l kg^{-1})	0.76 (0.39 – 4.15)	1.18 (0.46 – 1.89)	0.594	1.76 (0.84 – 3.92)	2.37 (1.15 – 4.51)	0.124
$t_{½}$ (h)	0.12 (0.11 – 0.61)	0.13 (0.11 – 0.42)	0.730	1.03 (0.65 – 1.46)	1.15 (0.88 – 1.57)	0.074
$AUC(0,_{\infty})$ (ng ml^{-1} h)	955 (395 – 1,735)	792 (398 – 1,840)	0.975	2,450 (1,370 – 4,340)	2,220 (1,470 – 3,270)	0.363
$AUC(0,_{\infty})$ / dose (ng ml^{-1} h/mg kg^{-1})	239 (98 – 437)	198 (101 – 454)	0.875	831 (467 – 1,460)	741 (502 – 1100)	0.246
Back ext. AUC (%)	68.5 (32.3 – 76.6)	71.2 (51.2 – 76.2)	0.826	10.1 (5.3 – 15.7)	9.7 (4.8 – 13.1)	0.925
Ext AUC (%)	0.05 (0.02 – 0.28)	0.06 (0.03 – 0.20)	0.551	0.92 (0.15 – 2.80)	1.80 (0.69 – 5.79)	0.022

[a] All estimates are given as median (range). P values are given using the Wilcoxon matched-pairs signed-ranks test.

Combined PK values for mildly and moderately ill pregnant patients are shown in the figure.

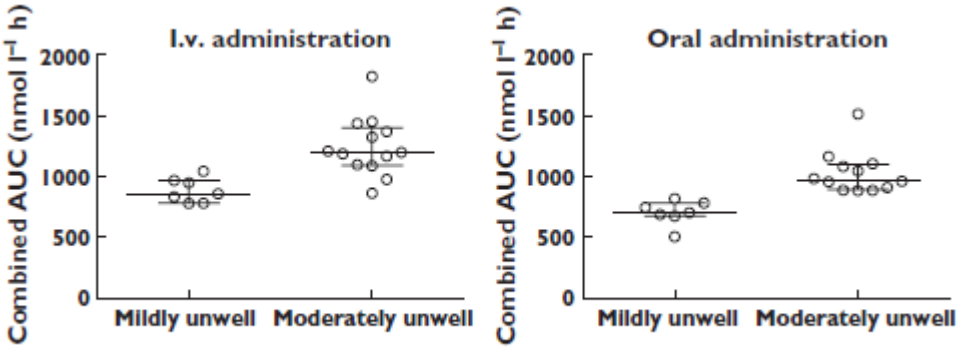


Figure 7: Combined Drug Exposure of AS and DHA

It was concluded that artesunate and DHA PK following IV artesunate dosing did not differ substantially between pregnant and post-partum women. There was a significantly higher combined drug exposure in women who were more unwell indicating reduced drug clearance.

Interactions

With the exception of a few sponsored studies, the information is reported from the literature.

Transporter studies

Artesunate and DHA were not substrates for any of the transporters investigated.

DHA does not inhibit P-gp. Artesunate and DHA at 50 µM and 10 µM did not inhibit OATP1B3, OAT1, OCT2, MATE1 and MATE2K. Artesunate 50 µM and DHA 10 µM displayed moderate inhibition of OAT3 and OCT1 that ranged from 33% to 51% and 21% to 43%, respectively. Artesunate was a weak

inhibitor of OATP1B1 (IC_{50} = 19 µg/mL) and OAT3. Artesunate and DHA had no or moderate interactions with MDR1 and BCRP and they inhibited BSEP in a non-specific manner.

The *in vitro* concentrations (10 µM DHA = 3µg/mL; 50 µM artesunate = 19 µg/mL) are somewhat higher than Cmax for these drugs [1-3 µg/mL], but neither the *in vitro* concentration nor the Cmax accounts for protein binding. Since the incubation medium for the transporter studies used a protein concentration of 1 mg/mL while human plasma/serum has a protein concentration of 70 mg/mL, unbound drug would be much higher in the *in vitro* model than found in human plasma/serum.

CYP studies

In one study, artesunic acid did not inhibit CYP1A2, CYP2C9, CYP2C19, CYP2D6 or CYP3A4 (IC_{50} values >40 µM). DHA moderately inhibited CYP1A2 (IC_{50} value of 10 µM) and CYP2C19 (IC_{50} value of 9 µM). These IC_{50} values are ~5-fold above the DHA unbound maximum concentration (C_{max}) values observed in healthy subjects (1.67 µM) after a dose of 8 mg/kg.

Using human liver microsomes, DHA 10 µM was a poor inhibitor of CYP2B6, CYP2C8, CYP2C9, CYP2D6, CYP2E1 and CYP3A with <15% inhibition. DHA was a moderate inhibitor of CYP2C19 (42% inhibition at 10 µM). DHA was also a relatively strong inhibitor of CYP1A with >50% inhibition at 10 µM. Using recombinant CYP enzymes, DHA was a poor inhibitor of CYP2C9, CYP2D6 and CYP3A4 with <15% inhibition at 10 µM, a moderate inhibitor of CYP2C19 with 22% inhibition at 10 µM and a relatively strong inhibitor of CYP1A2 with >66% inhibition at 10 µM.

The risk of DDI, calculated by the I/Ki method, is shown below. Data are based on estimated K, values and the maximum unbound systemic concentrations of inhibitor ($[I]_{max,u}$) or the maximum unbound concentrations of inhibitors at the inlet to the liver ($[I]_{max, inlet, u}$) for artemisinin, artemether and DHA on rCYP1A2, 2B6 and 2C19.

Table 10: Predicted Change in Drug Exposure and Risk for DDI *in vivo*

				AUC ratio[(i)]			
CYP isoform - inhibitor	**K_i (µM)**	**$[I]_{max,u}$ (µM)[(ii)]**	**$[I]_{max, inlet, u}$ (µM)**	**$[I]_{max,u}$**	**$[I]_{max, inlet, u}$**	**Risk for DDI**	
CYP1A2 - ART	1.0	0.32	5.3	1.3	6.3	High	High
CYP1A2 - DHA	3.4	0.091	0.72	1.0	1.2	Low	Medium
CYP2B6 - ART	6.2	0.32	5.3	1.1	1.9	Medium	Medium
CYP2B6 - ARM	2.7	0.016	0.056	1.0	1.0	Low	Low
CYP2C19 - ART	1.4	0.32	5.3	1.2	4.8	High	High
CYP2C19 - ARM	9.1	0.016	0.056	1.0	1.0	Low	Low
CYP2C19 -DHA	48.9	0.091	0.72	1.0	1.0	Low	Low

(i) Calculated based on $AUC_I/AUC=1 + [I]/K_i$.
(ii) Calculated based on the literature.
ART, artemisinin; ARM, artemether; ARS, artesunate; DHA, dihydroartemisinin.

It was concluded that DHA inhibited 2C19 and 1A2 and artesunate inhibited 1A2 in human liver microsomes with IC_{50} values of 7-13 µM. The data suggest a predicted medium to high risk for DDIs with DHA on CYP1A2.

Artemisinin compounds have been shown to auto-induce their own metabolism, resulting in decreased concentrations and exposure over time. In study 8740133, DHA was evaluated for CYP enzyme induction in human hepatocytes. DHA was not an inducer of CYP1A, CYP2B6, or CYP3A with less than 40% of positive control activity when assayed with hepatocytes from three human donors.

In vivo

Drug-drug interactions have been evaluated in 7 clinical trials published between 2007 and 2014. Very briefly, and noting the route of administration of artesunate in each study:

Asimus 2007 conducted a cocktail study in which adults were randomised to receive oral doses of artemisinin, DHA, arteether, artemether or artesunate for 5 days. A 6-drug cocktail was given orally on Days -6, 1, 5 and 10 to assess the activities of CYP1A2, CYP2A6, CYP2C19, CYP2D6, CYP2E1 and CYP3A. The results suggested that:

- Artemisinin, DHA and artemether are inducers of CYP3A
- Artemisinin, DHA and arteether inhibit CYP1A2

The authors concluded that magnitudes of the mean changes were low but could be of importance for risk of DDIs with drugs that have narrow therapeutic windows.

Davis 2007 reported no effect of mefloquine on oral artesunate based on comparable DHA AUC_{∞} values.

Minzi 2007 found no effect of oral artesunate on sulfadoxine and pyrimethamine and Matar 2014 found no effect of sulfadoxine and pyrimethamine on oral artesunate.

Orrell 2008 conducted a crossover study to evaluate co-administration of single oral doses of amodiaquine (10 mg/kg) and artesunate (4 mg/kg). When given in combination, the mean AUC was lower for DHA [ratio 67% (95% CI 51– 88%); P=0.008] and desethylamodiaquine [ratio 65% (95% CI 46–90%); P=0.015] when compared with monotherapy.

Fehintola 2012 co-administered oral nevirapine and artesunate-amodiaquine 200/600 mg. Nevirapine resulted in reduced clearance of artesunate by 50% (1950 vs. 2995 L/h; P = 0.03), resulting in a 45% increase in the AUC_{0-96} (105 vs. 69 µg·hr/L; P=0.02). The half-life of DHA was shorter after co-administration (1.6 vs. 3.2 h; P=0.004) and a lower conversion of artesunate to DHA was observed (DHA:AS AUC_{0-96} = 5.6 vs. 8.5; P=0.008). Overall, co-administration of oral artesunate with nevirapine resulted in a decrease in C_{max} and AUC of DHA by 59% and 68%, respectively.

Morris 2012 co-administered oral ritonavir with pyronaridine/artesunate. Ritonavir co-administration resulted in a 27% increase in artesunate AUC, a 38% decrease in DHA AUC and a 27% decrease in DHA C_{max}. Ritonavir exposure was increased 3.2-fold in the presence of pyronaridine/artesunate. It was proposed that RTV induced UGTs, which increased DHA glucuronide conjugation.

2.6.2.2. Pharmacodynamics

Mechanism of action

Artemisinin is a sesquiterpene lactone produced by the Chinese medicinal herb *Artemisia annua*. Dihydroartemisinin (DHA) is the active in-vivo metabolite of all clinically used semisynthetic lactol derivatives. Activation of the endoperoxide bridge is essential for artemisinin antimalarial activity.

Artemisinin activation is thought to involve iron-catalyzed reductive scission of the endoperoxide bond, generating carbon-centered radicals that react with susceptible groups in parasite proteins and other biomolecules. The major source of iron in parasites when located in parasitophorous vacuoles inside infected erythrocytes is thought to be in the form of haem species that are liberated following plasmodial protease-mediated degradation of imported host haemoglobin. Most of this proteolysis occurs during the trophozoite stage when parasites endocytose large quantities of haemoglobin from the erythrocyte into an acidic digestive vacuole. Although most of this pool of highly reactive haem is sequestered via incorporation of Fe3+-haem dimers (known as β-haematin) into chemically inert haemozoin crystals, some haem is available in the reduced Fe2+ form to activate artemisinins.

Primary and Secondary pharmacology

In-vitro activity

Artesunate and DHA were tested in in-vitro susceptibility screens at the WRAIR using incorporation of radiolabelled hypoxanthine by the parasites as a biomarker of parasite growth. The artesunate and DHA IC_{50} data against *P falciparum* from W2 and D6 clones using the flow cytometry method and the hypoxanthine incorporation assay were compared. W2 is resistant to chloroquine and pyrimethamine but susceptible to mefloquine. D6 is susceptible to chloroquine and pyrimethamine and has reduced susceptibility to mefloquine and halofantrine.

Table 11: *In Vitro* Mean IC_{50} values for artesunate and DHA against *P falciparum* strains from Indochine (W2) and Sierra leone (D6) by two different methods

Drug	W2 clone of *P falciparum*		D6 clone of *P falciparum*	
	FCM (ng/mL)	^{3}H-HIA (ng/mL)	FCM (ng/mL)	^{3}H-HIA (ng/mL)
Artesunate	0.2	0.25	0.3	0.35
DHA	0.31	0.34	0.26	0.29
Chloroquine	183	203	4.94	5.75
Mefloquine	2.98	3.08	9.91	7.24

Abbreviations: DHA, dihydroartemisinin; FCM flow cytometry method; HIA, ^{3}H- hypoxanthine incorporation assay

Additional multidrug-resistant clones used were TM91-C2 [Thailand] and RCS [Brazil] and IC_{50} values were calculated with comparison to in-house historical test data.

Table 12: WRAIR *In Vitro* Anrimalarial Drug Susceptibility Efficacy Screen Historical Data for Artesunate/Dihydroartemisinin

Source (Test Compound)	*P falciparum* Strain	IC_{50} (ng/mL)
Dihydroxyartemisinin (Shmuklarsky-1993)	W-2 Indochina clone	0.25
	D-6 Sierra Leone clone	0.25
	Vietnam Smith/RE clone	0.29
Artesunate - CIS/WRAIR in-house studies (bottle BM17174)	W-2 Indochina clone	0.749 ± 0.3
	D-6 Sierra Leone clone	0.673 ± 0.3
	TM90-C2A	1.44 ± 0.4
	TM90-C2B	0.563 ± 0.2
	WR75-TM91-C235	1.074 ± 0.09
Artesunate (Brossi-1988)	W-2 Indochina clone	0.47
	D-6 Sierra Leone clone	0.62

Abbreviations: CIS, Chemical Information System; IC_{50}, 50% inhibitory concentration; WRAIR, Walter Reed Army Institute of Research.

Using the WRAIR in-vitro screen, an older lot of artesunate (Starks BM17174/D (1993); non-GMP Guilin No. II) performed very similarly to 2 newer GMP lots (Knoll, new and old) of US Army artesunate.

Table 13: Parasite 50% Inhiitory Concentrations (ng/mL) of Various Artesunate Lots against Parasite Strains at Different Starting Parasitemias (0.8% vs 1.0%)

Parasite Strain:	W2	W2	D6	D6	RCS	TM91
Starting Parasitemia (%):	0.8	1	0.8	1	0.8	0.8
Drug/Bottle	IC_{50} (ng/mL)					
Guilin - Starks (1993)/BM17174/D	0.43	0.49	0.64	0.88	0.55	0.85
Knoll - Old (April 2003)/BQ36281/D	0.33	0.42	0.93	1.11	0.45	1.05
Knoll - New (June 2003)/BQ37377/D	0.33	0.42	0.83	0.99	0.60	0.93

Abbreviation: IC_{50}, 50% inhibitory concentration.

Whilst the clinical efficacy data described in the next section relate to *P. falciparum* infections, published data show that the ED_{50} for artesunate and for DHA was in the low-to-mid nM range for *P. falciparum* and for the other human malarias (*P. vivax, P. ovale, P. malariae, P. knowlesi*), which suggests comparable efficacy of the "other" malarias to *P. falciparum.*

Resistance to the artemisinins

Decreased sensitivity to artemisinins, manifesting clinically as slower rates of parasite clearance, has been documented in multiple Southeast Asian countries. In-vitro studies have shown that parasites isolated from patients with either fast- or slow-clearing infections exhibit similar sensitivity to DHA in standard growth inhibition assays. In contrast, the response of parasites in a ring-stage survival assay (RSA0–3 h) generally correlates well with parasite clearance times.

The primary genetic determinant of resistance was described in laboratory-based selection studies in which the artemisinin-sensitive Tanzanian F32 isolate was pressured in a dose-escalating, 125-cycle regimen of exposure to artesunate over five years. Whole-genome sequence analysis revealed a mutation (M476I) in the propeller domain of the K13 (Kelch13, PF3D7_1343700) gene. Subsequent analyses revealed the presence of several mutations in the K13 propeller domain in isolates from western Cambodia associated with delayed parasite clearance in patients. The discovery of K13 mutations and their implication in conferring artemisinin resistance led to the identification of a substantial number of polymorphisms in parasites from malaria patients in different regions.

In a comprehensive analysis conducted by the Tracking Resistance to Artemisinin Collaboration, multiple mutations were identified throughout the entire K13 gene. Many in the β-propeller domain (beginning at amino acid 442) were associated with parasite clearance half-lives of greater than 5 h. Surveillance studies conducted in 59 countries by the K13 Artemisinin Resistance Multicenter Assessment consortium recently identified 108 non-synonymous K13 mutations, with an overall prevalence of 37% in South East Asia. These mutations showed strong regional differences. For example, in Cambodia-Vietnam-Laos the dominant mutation was C580Y (at ~50% prevalence), whereas in the samples from Thailand-Myanmar-China the dominant mutation was F446I (at 20% prevalence), with minimal C580Y. These data are consistent with multiple *de novo* mutational events accompanied by gene flow between parasite populations. It should be noted that diminished DHA susceptibility has to be evaluated against ring-stages, not against mature stages.

In annual studies conducted in 3 malaria-endemic provinces in the south of Vietnam between 2011 and 2015, 489 patients with uncomplicated *P. falciparum* malaria were enrolled and subjected to detailed assessments over 42 days following treatment with DHA-piperaquine for three days. Between 2011 and 2015, the proportion of patients with detectable parasitaemia on Day 3 rose steadily from 38% to 57% ($P < 0.001$). In Binh Phuoc province, the parasite clearance half-life increased from 3.75 h in 2011 to 6.60 h in 2015 ($P < 0.001$), while treatment failures rose from 0% in 2012 and 2013 to 7% in

2014 and 26% in 2015 (P < 0.001). Recrudescence was associated with in-vitro evidence of artemisinin (and piperaquine) resistance.

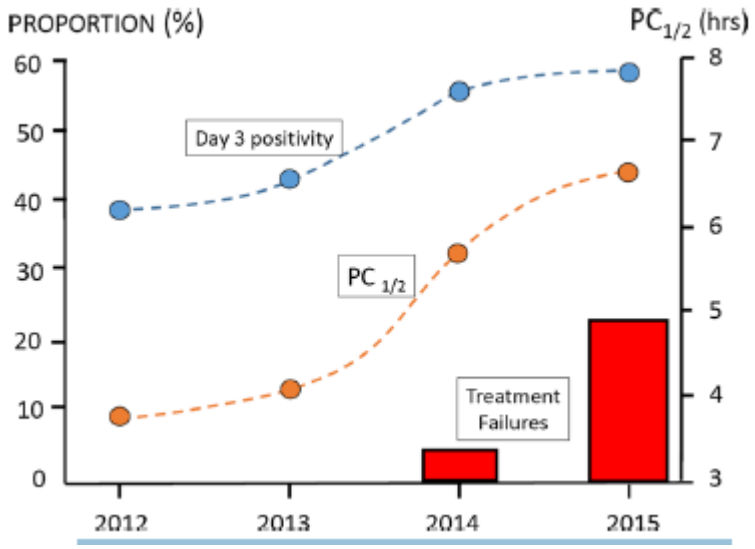
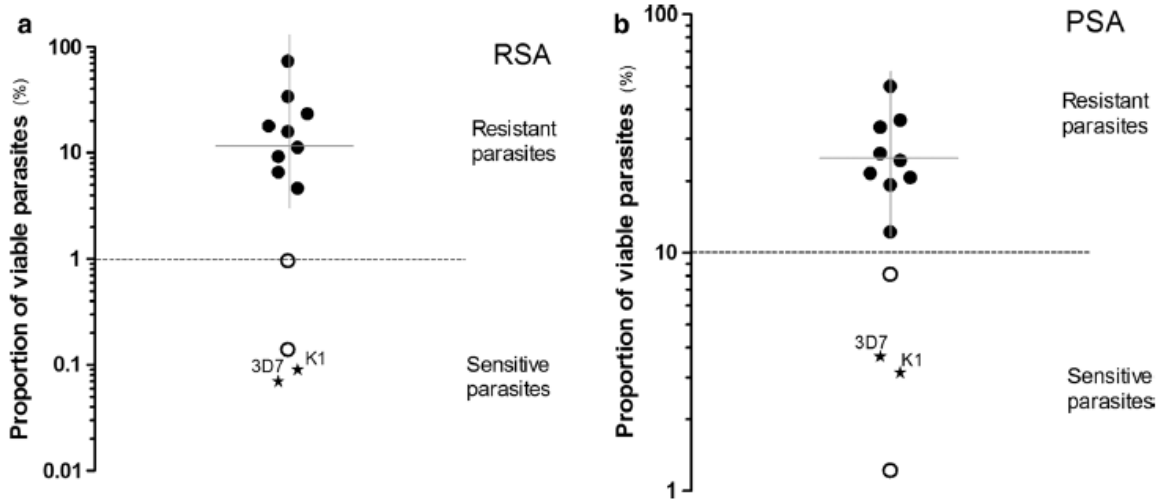


Figure 8: Proportion of Patients with Positive Smear on Day 3, Parasite Clearance Half-life (PC1/2) and the Treatment Failure Rate Following Dihydroartemisinin–Piperaquine Treatment in Binh Phuoc from 2012–2015 in Vietnam



Legend: Ex vivo response to DHA and in vitro response to piperaquine of parasites collected from patients following dihydroartemisinin–piperaquine treatment using ring stage assay (RSA) and piperaquine survival assay (PSA), respectively. Black dots represented for parasites from recrudescent cases, white dots represented for parasite from ACPR cases. The *Plasmodium falciparum* lines of 3D7 and K1 are controls. From Thahn-2017.

Figure 9: Ex Vivo Response to DHA and In Vitro Response to Piperaquine

In the Vietnam treatment failures of 2015, all 14 parasite isolates carried the C580Y Pfkelch 13 gene. Also, 93% (13/14) carried exoE415G mutations, which are markers of piperaquine resistance. In the paper by Thuy-Nhien (2017) the prevalence of K13 mutations was 29% (222/767), 6% (11/188) and 43% (45/105) in the Binh Phuoc, Ninh Thuan and Gia Lai Provinces of Vietnam, respectively. Cys580Tyr had become the dominant genotype with 79.1% (34/43) of isolates in Binh Phuoc Province

and 63% (17/27) of isolates in Gia Lai Province carrying this mutation. This analysis of haplotypes flanking K13 suggested the presence of multiple strains with the Cys580Tyr mutation rather than a single strain expanding across the three sites.

In Africa (Senegal) in 2014, parasites remained 100% susceptible to AS/DHA.

Mean *ex vivo* IC_{50} values were 1.3 and 0.7 nM, respectively, and no isolates had values at or above the resistance cut-off of 12 nM. Furthermore, *P. vivax* has maintained its susceptibility to AS/DHA. Among mutations that have been observed in African isolates, A578S was reported to be the most common but gene-editing experiments showed that it does not mediate a resistance phenotype *in vitro*.

Importantly, several studies in Africa have reported similar parasite clearance rates in patients harbouring wild type or mutant K13 alleles. These data suggest that in Africa K13 mutations are not under significant selection pressure and do not adversely affect the efficacy of artemisinin combination therapy. One possibility is that the combined effects of high levels of immunity in African populations, relatively reduced drug pressure and the frequent occurrence of polyclonal infections (that select against resistance phenotypes that have reduced growth rates), are sufficient to prevent the relatively mild levels of K13-mediated resistance from altering clinical efficacy.

The information generated by the CDC Malaria Surveillance Summaries (2012-2016) for artemisinin resistance in *P. falciparum* submitted to the CDC for resistance testing is summarised in the table below. Artemisinin resistance was detected in 2/137 (1.5%) isolates in 2014 and in 1/149 (0.7%) in 2015. The 3 resistant specimens were from Africa and had the K13-propeller marker. No artemisinin-resistant specimens were detected in 2102, 2013 or 2016.

Table 14: Artemisinin Resistance among *P falciparum* Submitted to CDC (2012-2016)

Year	Resistance Markers Tested	No. Specimens Tested	Regions Represented	No. Specimens Resistant (marker)
2012	Polymorphisms located on: chromosome 10 (MAL10-688956) chromosome 12 (MAL13-1718319)	56	52 – Africa 2 – C. America 2 - Unknown	0
2013	Polymorphisms located on: chromosome 10 (MAL10-688956) chromosome 12 (MAL13-1718319)	76	60 – Africa 1 – C. America 1 – S. America 14 - Unknown	0
2014	Polymorphisms located on: chromosome 10 (MAL10-688956) chromosome 12 (MAL13-1718319) and K13-propeller domain	137	128 – Africa 2 –Asia 7 - Unknown	2 (K13 propeller) Both specimens from Africa
2015	Polymorphisms located on: chromosome 10 (MAL10-688956) chromosome 12 (MAL13-1718319) and K13-propeller domain	149	78 – Africa 5 – C. America 66 - Unknown	1 (K13 propeller) Specimen from Africa

Year	Resistance Markers Tested	No. Specimens Tested	Regions Represented	No. Specimens Resistant (marker)
2016	K13-propeller domain	153	146 – Africa 2 – C. America 5 - Unknown	0 But 1 specimen was positive for the 578S K13 mutation that is not associated with resistance

Secondary pharmacology

No TQT study was conducted. Detailed ECGs were obtained in studies 1128 and 1142.

Study 1128
Baseline ECG data were collected at the same times up to 6 h on the day prior to dosing and after dosing. ECG records were transmitted to the central laboratory (Cardiocore, Bethesda, MD) where cardiac intervals were measured manually by cardiovascular technologists and then over read by Board Certified cardiologists blinded to the study treatment. Measurements were performed on the averaged representative beat of lead II. At each ECG time point, blood was drawn for AS and DHA levels.

For all IV artesunate dose groups, mean placebo-subtracted time-matched differences from baseline in QTcF (msec) showed scattered peak effects seen at various observation times, without a clear dose response. In the highest dose group, 8 mg/kg, the maximal change of 6.55 msec occurred at 40 min.

At 4 h, the maximal increase over placebo in any group was 10.42 msec, seen in the 2 mg/kg group.

There was a statistically significant increase of QTcF (10.39 msec) for the 0.5 mg/kg group at 20 min ($p<0.5$). The only other matched pair with a significant difference was for a QTcF decrease.

No subject in any group had a QTcF >450 msec at any time. One subject in the placebo group had a single increase over baseline in QTcF >30 msec, while no subject in any of the IV artesunate groups had such an increase. The maximum mean change from baseline in heart rate observed in the study was 4.33 bpm in the 8 mg/kg group at 20 minutes.

Scatter plots were constructed and linear regressions were performed to assess whether there was a relationship between changes in QTc and serum concentration of artesunate or DHA. The estimated regression slopes indicated a trend toward smaller changes in QTc with increasing concentrations.

Study 1142
On D-1 and on each dosing day (Days 1, 2 and 3) ECGs were done at 5, 20 and 40 min and at 1, 2, 4, 8 and 24 h post-dose. ECGs were read as in 1128.

For all IV artesunate dose groups, mean placebo-subtracted time-matched differences from baseline in QTcF (msec) were mostly negative. The greatest positive difference was 7.02 msec at 1 h on Day 2 in the 2 mg/kg group. At 8 mg/kg, the greatest positive difference was 4.27 msec at 8 h on Day 1 and all other differences were negative. No subject had a QTcF > 450 msec. One subject in the 4 mg/kg group had a single increase in QTcF > 30 msec on Day 1.

Mean changes from baseline for HR were within -2 bpm and + 10 bpm except for 12 bpm on Day 3 at 5 minutes post-dose for the 8 mg/kg group, 13 bpm on Day 2 at 8 h for the 2 mg/kg group and 16 bpm on Day 3 at 8 h for the 2 mg/kg group. While most of the HR changes were toward positive values, there were no obvious trends by time point and none evident by dose.

Overall, new morphological findings in the IV artesunate cohorts were minimal and similar to placebo. No relationship was seen between increases in QTc and serum concentrations of artesunate or DHA.

Relationship between plasma concentration and effect

Since the activity of antimalarial agents, including the artemisinins, is not exerted in plasma and since the degree of erythrocyte sequestration of these agents changes as the parasite load changes, so investigations of antimalarial effect related to plasma levels is not expected to be fruitful. Furthermore, while the applicant has not provided a specific analysis or discussion of the PK-PD relationship, many of the published studies included in the dossier have failed to detect relationships between blood levels of artesunate and DHA and measures of efficacy, such as PCT.

2.6.3. Discussion on clinical pharmacology

Pharmacokinetics

PK data generated with US Army artesunate

The SAD and MAD Phase 1 studies confirmed that there is rapid conversion of artesunate to DHA after IV administration. Thus, clearance of artesunate was high at doses of 1.0 mg/kg to 8.0 mg/kg.

DHA is the only active metabolite formed after IV artesunate administration and its PK was described in the applicant's studies. Assuming that there is complete conversion of artesunate to DHA within the body and that all DHA reaches the systemic circulation prior to elimination, the apparent CL of DHA was estimated to be ~50 L/hr and the apparent Vd was about 100 L. Thus, in comparison with the parent compound, DHA has a smaller CL and a larger Vd, which together yield a longer half-life.

Nevertheless, the plasma half-life of DHA is relatively short (1-2 h) and it would not *per se* support 12 hourly or daily dosing with IV artesunate. Rather, the rationale for the dose interval reflects the very high concentrations of the artemisinins within parasitised erythrocytes compared to plasma levels.

Since there was no metabolite profiling study conducted, the relative amount of DHA glucuronides to artesunate and DHA in plasma is unknown and the PK of the glucuronides has not been described. However, it is widely reported that glucuronidated DHA is inactive. In the specific case of IV artesunate, and in contrast to the situation for oral artesunate, the omission of a metabolite profiling study and lack of data on PK of the glucuronides could be acceptable.

There was a linear relationship between the IV artesunate dose and plasma artesunate and DHA Cmax and AUC parameters after single doses from 1.0 to 8 mg/kg. Moreover, dose-normalised AUCs for artesunate and DHA were comparable after multiple doses of 2, 4 and 8 mg/kg. Linearity is important for the discussion of paediatric dosing (see below).

Protein binding

Variable estimates of protein binding of artesunate and DHA have been published using different methods applied to blood samples from different populations. Batty (2004) reported that in non-severe *P. falciparum* malaria, albumin levels (39 g/L) were lower than for normal volunteers (49 g/L), but surprisingly the fraction of DHA that was unbound (0.07) was less than in normal volunteers (0.12).

Alterations in protein binding, if they occur, could affect unbound drug levels in plasma and thus affect levels within infected erythrocytes. However, it is relevant to note that the recommended dose of 2.4 mg/kg was shown to be safe and effective in patients in SEAQUAMAT, in which a range of protein

binding could be expected, including among patients with impaired renal function. Therefore, it seems unlikely that variable protein binding would affect the safety or efficacy of the recommended dose.

Healthy subjects vs. patients with malaria

The applicant's data come from adult patients with uncomplicated malaria following the first dose of 2.4 mg/kg IV artesunate. The plasma profiles for artesunate and DHA in healthy subjects and patients with uncomplicated malaria showed that the AUC DHA is about twice that of artesunate. In both populations, there was rapid conversion of artesunate to DHA and the plasma half-life of DHA was between 1-2 h but the DHA Cmax and AUC in patients after 2.4 mg/kg were disproportionately higher than values observed in healthy subjects after a 2 mg/kg dose.

The available data point to considerable inter-individual variability (IIV) in artesunate and DHA PK after IV dosing. However, snce intra-erythrocytic concentrations are critical for efficacy, the IIV for blood levels may not translate into variable efficacy when using the recommended dose.

Special populations

Renal and hepatic impairment
Some degree of renal and/or hepatic impairment is very common at presentation in severe malaria patients. Based on considerations of PK and of protein binding, as well as the clinical experience in treating severe malaria, it can be agreed that no dose adjustment is likely needed.

Paediatric dosing
It is agreed with the applicant that a single dose recommendation of 2.4 mg/kg may be applied regardless of age. There are no clinical safety and efficacy data that point to the need for a higher dose in children < 20 kg. However, there is limited experience with IV artesunate for treatment of malaria in the first 18 months of life and very few data on use in the first 6 months of life. The SmPC reflects the uncertainties but there is no known reason to preclude use below a certain age.

Use in the elderly
It is agreed that no dose adjustment is needed based solely on age. The applicant provided a review of experience with IV artesunate in older adults, including the 17 treated with IV artesunate in SEAQUAMAT. In comparison with 21 who received quinine in this study, there was no reason found to preclude use in older adults.

Use in pregnancy and breastfeeding
The same IV artesunate dose may be used in non-pregnant and pregnant women. The applicant provided a comprehensive review of available information on pregnancies during which IV artesunate was administered in any trimester, along with all known pregnancy outcomes. Among 56 prospective artesunate-exposed first trimester patients, death occurred in 3 (5.4%) of foetuses, and there were 2 infants with congenital abnormalities. These outcomes are not inconsistent with outcomes seen in healthy first trimester patients living in the same geographic areas. Information regarding outcomes for 340 prospective second or third trimester cases treated with IV artesunate was available from 4 publications. Overall, these data point to a benefit in terms of maternal and fetal survival when IV artesunate is used to treat maternal severe falciparum malaria.

Drug-drug interactions
The in-vitro data were not generated in line with CHMP recommendations. Nevertheless, the conclusions reached based on the information available can be broadly supported. The clinical studies were not conducted in an adequate fashion to draw conclusions. The SmPC reflects the uncertainties.

Pharmacodynamics

Antimalarial activity of the artemisinins

The artemisinin compounds have a common and reasonably well understood mechanism of action that results in rapid parasiticidal activity against asexual ring stage parasites. When used orally, the artemisinins have been paired with a range of antimalarial agents that have a different mechanism of action. Wen treatment is commenced with IV artesunate as monotherapy, complete cure depends also on appropriate follow-on treatment once oral treatment is possible.

Most published data on in-vitro activity indicate that all the artemisinins have some antimalarial activity. All are converted to DHA after oral or parenteral administration and most studies report that DHA is more active *in vitro* compared to the parent molecules. In contrast, data reported from US Army studies for two strains (W2 and D6 clones of *P. falciparum*) suggest comparable activity between parent and metabolite using assay two methods. It is unclear if this finding could be related to some degree of conversion of artesunate to DHA during the long incubation period of the assays. Nevertheless, since artesunate is converted to DHA very rapidly after oral or parenteral administration, clinical efficacy is thought to be driven primarily by the metabolite.

Resistance

The applicant provided a summary of what is known about the mechanisms of resistance to the artemisinins and presented an overview of the geographical distribution of clinical resistance, as assessed by delayed parasite clearance. As summarised in the dossier, the risk of acquiring artemisinin-resistant malaria is highest in pockets of SE Asia and lowest in Africa.

PK-PD relationship

Several of the published efficacy studies described in the applicant's summaries in which artesunate (variable source; oral or IV) has been used to treat severe malaria have reported on PK-PD relationships. With few exceptions, the conclusion has been that there is no consistent relationship between plasma artesunate, DHA or the two combined and efficacy parameters (such as PCT). Given that the artemisinins concentrate in infected erythrocytes, the lack of a clear PK-PD relationship based on plasma drug concentrations is not a surprise.

Cardiac conduction

Severe malaria *per se* has been reported to disrupt cardiac conduction, including prolongation of the QTc interval that resolves in line with patient recovery from the infection. Furthermore, several antimalarial agents in widespread use are known to prolong the QTc interval. Data obtained with Eurartesim (as detailed in the EPAR) showed that DHA did not have a significant effect on the QTc interval. The applicant did not conduct a TQT study with Artesunate Amivas but did obtain multiple ECGs in the SAD and MAD studies in healthy subjects and did not detect a potentially clinically important effect on cardiac conduction. There is no need for any cautionary statements in the SmPC.

2.6.4. Conclusions on clinical pharmacology

There are no outstanding issues on the clinical pharmacology to be addressed. Salient points of the clinical pharmacology are reflected in SmPC Section 5.1.

2.6.5. Clinical efficacy

The demonstration of efficacy of IV artesunate is based on two prospective, randomised, controlled trials (RCTs) conducted between 2003 and 2010 by academic consortia of investigators, coordinated from the Mahidol University Bangkok and funded by the Wellcome Trust:

- The South East Asian Quinine Artesunate Malaria Trial - SEAQUAMAT - conducted between 2003 and 2005 in adults (mostly) and children with severe falciparum malaria admitted to hospitals in Asia and SE Asia (*Lancet* 2005; 366: 717-25).
- The African Quinine Artesunate Malaria Trial - AQUAMAT - conducted between 2005 and 2010 in children with severe falciparum malaria admitted to hospitals in Africa (*Lancet* 2010; 376: 1647-57).

SEAQUAMAT and AQUAMAT used intravenous artesunate manufactured in Guilin, China.

Dose-finding or other supplementary information comes from six other studies. Four of these studies used the US Army artesunate but two of the four enrolled patients with uncomplicated malaria.

Study	**Route of administration**	**Patient Population**
CDC-060*	Intravenous	Severe and complicated malaria in all age ranges
Study 1168*	Intravenous	Uncomplicated malaria
Study 1263ab*	Intravenous 4 dose regimens compared	Uncomplicated malaria
EDCTP-MMV07-01*	Intravenous 3 doses vs. 5 doses	Severe malaria in paediatrics
Cao-1997	Intravenous	Severe malaria in paediatrics
Sinclair-2012 (Cochrane meta-analysis)	Parenteral and intra-rectal	Severe and complicated malaria in adults and paediatrics

*These studies used the US Army intravenous artesunate preparation

Two other published studies have reported on use of IV artesunate to treat returning travellers.

- One study from Netherlands and Belgium used the Guilin preparation.

The TropNet severe malaria study used intravenous artesunate from another manufacturer

2.6.5.1. Dose response studies

Study 1263 – US Army 2007

This open-label study in Thailand and Kenya randomised 100 adult and paediatric patients with uncomplicated *P. falciparum* malaria to one of four IV artesunate dose regimens:

- 1.2 mg/kg once daily for 3 days;
- 2.4 mg/kg once daily for 3 days;
- 2.4 mg/kg at 0, 12, 24 and 48 h;
- 4.8 mg/kg once daily for 3 days.

All patients received follow-on therapy with mefloquine in Thailand and with Malarone in Kenya.

Of 100 subjects, 67% were black (Kenyan) and 37% were Asian (Thai). The majority was male (67%) and under the age of 18 (59%), with an overall age range from 5-53 years. All patients achieved the primary endpoint of 90% parasite clearance by 32 h and all but one had 100% clearance by 72 h.

Table 15: Summary of parasite Clearance Times and Parasite Reduction Ratios for the Evaluable Population

	1.2 mg/kg Once Daily for Three Days (N=25)	2.4 mg/kg Once Daily for Three Days (N=25)	2.4 mg/kg Twice on Day 0 and Once Daily for Two Days (N=25)	4.8 mg/kg Once Daily for Three Days (N=25)	Overall (N=100)
PCT_{90} (h)					
n (%)	25 (100.0)	25 (100.0)	25 (100.0)	25 (100.0)	100 (100.0)
mean (SD)	13 (8.2)	11 (4.2)	15 (5.8)	10 (3.7)	12 (6.0)
median	12	12	12	10	12
range	(2, 32)	(2, 20)	(4, 28)	(2, 16)	(2, 32)
coefficient of variation	63	37	40	39	49
PCT_{100} (h)					
n (%)	25 (100.0)	25 (100.0)	25 (100.0)	25 (100.0)	100 (100.0)
mean (SD)	45 (19.6)	40 (13.3)	47 (38.8)	34 (12.3)	41 (23.7)
median	40	40	32	32	36
range	(14, 72)	(12, 72)	(20, 164)	(10, 71)	(10, 164)
coefficient of variation	44	33	83	36	57
PRR_{12h}					
n (%)	25 (100.0)	25 (100.0)	25 (100.0)	25 (100.0)	100 (100.0)
mean (SD)	84 (22.0)	88 (16.4)	79 (22.3)	95 (10.0)	87 (19.0)
median	95	94	92	98	96
range	(31, 100)	(26, 100)	(36, 100)	(52, 100)	(26, 100)
coefficient of variation	26	19	28	11	22
PRR_{24h}					
n (%)	25 (100.0)	25 (100.0)	25 (100.0)	25 (100.0)	100 (100.0)
mean (SD)	96 (8.0)	99 (1.0)	98 (3.4)	100 (0.4)	99 (4.5)
median	100	100	100	100	100
range	(72, 100)	(97, 100)	(85, 100)	(98, 100)	(72, 100)
coefficient of variation	8	1	3	0	5

There was a significant difference in PCT_{90} between the 4.8 mg/kg once daily for 3 days dose cohort and the 2.4 mg/kg twice on Day 0 and once daily for 2 days cohort. The parasite clearance times for 99% clearance and the difference in parasite reduction ratio at 12 h was also significantly different between these cohorts. The PRR at 24 h was significantly different between the 4.8 mg/kg once daily for 3 days dose cohort and the 1.2 mg/kg once daily for 3 days cohort.

There was an apparent dose response in Kenyan subjects with the 4.8 mg/kg once daily for 3 days cohort clearing 99% of the parasites faster than the 1.2 or 2.4 mg/kg once daily for 3 days cohorts and clearing 100% of parasites faster than the 1.2 mg/kg group. Conversely, there was no dose response relationship in Thai subjects.

There were no deaths in this study.

The CSR concluded that the finding that PCT was significantly reduced in the 4.8 mg/kg once daily for 3 days cohort compared to the 2.4 mg/kg twice on Day 0 and daily for 2 days could be due to the existence of artemisinin resistant parasites in Thailand. In contrast, Kenya was (at the time) relatively artemisinin-naïve and no artemisinin resistant malaria parasites had been described.

EDCTP/MMV07-01 – MMV-sponsored study in African children with severe malaria (Kremsner, 2012)
This was a randomised study in African children aged 6 months to 10 years (weight ≥ 5 kg) with ≥ 5,000 parasites/µL on initial blood smear. The study compared two dose regimens as follows:

Group A (n=94) received IV artesunate 2.4 mg/kg at 0, 12, 24, 48, and 72 h (5-dose cohort);

Group B (n=100) received IV artesunate 4.0 mg/kg at 0, 24 and 48 h (3-dose cohort) with matching placebo (saline) given at 12 and 72 h. At Lambarene and Libreville in Gabon the follow-on oral anti-malarial drug was Fansidar. At Blantyre in Malawi the oral antimalarial drug was Riamet (artemether/lumefantrine).

The primary efficacy endpoint was the proportion of patients with parasite clearance (≥99% reduction from the baseline asexual parasite count; PC99) at 24 h after initiation of study drug. The primary efficacy analysis was performed on the ITT population and repeated on the PP population.

All patients were African with an average age of 3.6 years in group A and 3.9 years in group B. The baseline asexual parasite counts were not significantly different between treatment groups. About 40% were considered to have hyperparasitaemia.

In the primary analysis in the ITT and PP populations, there was no significant difference between treatment groups but there was a numerical advantage for the 5-dose regimen. Post-hoc testing for non-inferiority (B vs. A) showed that the lower bounds of the 95% CI for PC99 were within -20%.

Confidence interval of the treatment difference (ITT): -8.49% [-17.87% ;0.89%]
Confidence interval of the treatment difference (PP): -7.23% [-17.01% ; 2.55%]

Table 16: Description and test for proportion of patients with PC_{99} from the baseline asexual parasite count at 24h per treatment groups (ITT population)

	Treatment Group		All	Test
	A N=93	B N=89	N=182	
PC99 at T24				Chi-2
NO	13 / 93 (14.0%)	20 / 89 (22.5%)	33 / 182 (18.1%)	P = 0.137
YES	80 / 93 (86.0%)	69 / 89 (77.5%)	149 / 182 (81.9%)	
PC99 reached	80 / 93 (86.0%)	69 / 89 (77.5%)	149 / 182 (81.9%)	Chi-2
CI 95%	[79.0% , 93.1%]	[68.9% , 86.2%]	[76.3% , 87.5%]	P = 0.137

Table 17: Description and test for proportion of patients with PC_{99} from the baseline asexual parasite count at 24h per treatment groups - PP

	Treatment Group		Total	Test
	A N=86	B N=85	N=171	
PC99				Chi-2
NO	13 / 86 (15.1%)	19 / 85 (22.4%)	32 / 171 (18.7%)	P = 0.225
YES	73 / 86 (84.9%)	66 / 85 (77.6%)	139 / 171 (81.3%)	
PC99 reached	73 / 86 (84.9%)	66 / 85 (77.6%)	139 / 171 (81.3%)	Chi-2
CI 95%	[77.3% , 92.5%]	[68.8% , 86.5%]	[75.4% , 87.1%]	P = 0.225

The median time to PC100 was 36 h with no statistically significant difference between the survival curves (p=0.902). When looked at by age sub-class, 51% aged 0.5-2 years, 51.6% 2-3 years, 60.3% 3-5 years and 43% ≥5 years reached PC100 36 h after the first dose of the study drug. The median times to PC99, PC90 and PC50 were not statistically significant different between groups. The percent reduction in asexual parasite count at 24 h was slightly higher in group A (99.2%) vs. group B (97.6%) (p=0.544). At 48 h the rates were 100% vs. 98.8% (p=0.610).

The median fever clearance time was 12h for both groups (p=0.627). More than 57% aged 0.5-2, 2-3 and 3-5 years reached fever clearance within 12 h after the first dose and more than 40% aged 3-5 years and ≥5 years reached fever clearance within 6 h.

2.6.5.2. Main studies

Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial
*South East Asian Quinine Artesunate Malaria Trial (**SEAQUAMAT**) group*

Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): an open-label, randomised trial

Methods

In SEAQUAMAT, eligible patients were aged at least 2 years with a positive blood antigen test for *Plasmodium falciparum* (HRP2; Paracheck, Orchid Biosystems, Goa, India) and a diagnosis of severe malaria according to the admitting physician. Slides were read retrospectively in the reference laboratories in Thailand.

In AQUAMAT, eligible patients were aged < 15 years with a positive rapid diagnostic test for *Plasmodium falciparum* LDH (Optimal, Diamed, Cressier, Switzerland) and severe malaria in the admitting physician's clinical opinion.

Treatment
Artesunate 2.4 mg/kg bodyweight (Guilin) was given on admission, at 12 h and 24 h and then once daily until oral medication could be taken. Administration was IV (both studies) or by IM injection into the anterior thigh (an alternative in AQUAMAT only).

In SEAQUAMAT, IV artesunate was followed by oral artesunate 2 mg salt per kg per day to complete a total course (including parenteral treatment) of 7 days. Both regimens were combined with oral doxycycline 100 mg twice daily for 7 days once the patient could take oral medication with the exception of i) children aged < 8 years; ii) pregnant women; iii) Chittagong and Orissa. In AQUAMAT, after a minimum of 24 h of parenteral treatment, oral Coartem (artemether-lumefantrine) was given at 1.5/9 mg/kg twice daily for 3 days with milk or fat.

In both studies, quinine dihydrochloride was given parenterally in a 20 mg/kg loading dose followed by 10 mg/kg three times a day until starting oral quinine 10 mg/kg every 8 h to provide a total of 7 days.

Outcomes/endpoints
In both studies, the primary endpoint was death from severe malaria, defined as all-cause in-hospital mortality measured to time of hospital discharge. Secondary endpoints were incidence of neurological sequelae, combined death or neurological sequelae, other significant sequelae (unspecified), recovery times (times to eat, speak, sit and discharge), time to death and development of severe complications not present on admission.

Sample size
In SEAQUAMAT, the initial plan was to include 2000 patients with severe malaria to be able to show a 33% reduction in mortality from 12% to 8% with a power of 80% and a confidence of 95%, allowing a drop out (ineligibility) rate of 5%. This estimate was based on about half of the enrolled patients subsequently fulfilling modified WHO criteria for severe malaria and an anticipated 24% mortality rate from severe malaria based on local figures.

In AQUAMAT, 5306 children with severe malaria were needed to show, with 80% power and 95% confidence, a 25% reduction in mortality from 8% to 6%. This was based on 16% mortality in severe malaria with about half the enrolled patients anticipated to fulfil the criteria for severe malaria.

Randomisation

This was done in both studies using sealed envelopes containing unique study numbers that referred to sealed boxes containing the study drug, case record form and all disposables needed for drug administration and blood sampling.

Blinding (masking)
Both studies were open label with respect to the investigators and persons who administered treatment. Persons conducting microscopy of blood slides and data analysts remained unaware of treatment allocation until the end of the study.

Statistical methods

SEAQUAMAT
There was a pre-specified analytical plan. A revised final SAP was generated to support the US Army analyses. The primary analysis was conducted in the ITT population, defined as all randomised patients. An analysis of the primary endpoint was also conducted in the PP population, comprising patients confirmed to have *P. falciparum* from the peripheral blood smear. Subgroup analyses were conducted in subsets defined by various demographic characteristics and according to severe malaria and less severe malaria groups, according to fulfilment of the criteria for severe malaria (a modification of definitions used by Hien and colleagues was applied).

The primary endpoint was analysed as a binary variable with a two-tailed test with alpha=0.05. The Cochran-Mantel-Haenszel (CMH) procedure was used to compare mortality rates between the treatment groups, stratifying by study site. Odds ratio and relative risk point estimates and associated 95% confidence intervals were reported for the primary endpoint.

According to the protocol, an interim analysis was to be evaluated by an external data safety monitoring committee (DSMC) after inclusion of 500 patients and at other time points chosen by the committee. There was no formal pre-specified plan to control the type 1 error. The DSMC's remit included advising to stop the trial if there was evidence beyond reasonable doubt that the results would change clinical practice. The trial was stopped on May 11, 2005 following a second interim analysis.

AQUAMAT
The approach was similar to that for SEAQUAMAT but the criteria for defining severe falciparum malaria were not identical between studies.

Results – SEAQUAMAT

The first interim analysis was conducted in June 2004 after inclusion of 659 patients (129 deaths). A second interim analysis was conducted using an analysis database that was frozen in February 2005. There were 244 reported deaths for the 1294 patients included in the database. The number of accrued deaths exceeded the number of deaths projected for a completed trial. The DSMC recommended stopping the trial based upon the strength and robustness of the efficacy findings and supporting safety results. After the trial was halted, unblinded analysis results prepared by the SEAQUAMAT group were published based on data for 1461 patients (271 deaths), of which 79 (5%) did not have falciparum malaria confirmed by the presence of parasites on the blood smear.

Baseline data
Severe falciparum malaria was confirmed for 70% artesunate and 74% quinine group patients.

Table 18: Categorical baseline characteristics

	Artesunate (n=730)	Quinine (n=731)
Sex		
Male	546 (75%)	529 (72%)
Female	184 (25%)	202 (28%)
Child (age <15 years)	97 (13%)	105 (14%)
Pregnant	23 of 133 (17%)	26 of 143 (18%)
Pretreatment with antimalarial drug	167 (23%)	142 (19%)
Pretreatment with quinine	103 (14%)	84 (11%)
Pretreatment with artemisinin derivative	25 (3%)	42 (6%)
Pretreatment with chloroquine	43 (6%)	21 (3%)
Pretreatment with sulphadoxine-pyrimethamine	9 (1%)	10 (1%)
Pretreatment with mefloquine	0	5 (1%)
Pretreatment with an effective antimalarial*	125 (17%)	118 (16%)
Severe malaria†	509 (70%)	541 (74%)
Malaria parasites on blood film	708 (97%)	716 (98%)
Hyperparasitaemia (>10%)	121 (17%)	108 (15%)
Complications on admission		
Coma (Glasgow coma scale <11 or Blantyre coma scale <3)	284 (39%)	304 (42%)
Convulsions	89 (12%)	87 (12%)
Jaundice (clinical)	355 (49%)	349 (48%)
Severe anaemia (haemoglobin <50 g/L)	40/683 (6%)	54/675 (8%)
Shock (clinical)	78 (11%)	92 (13%)
Acidosis (base excess less than −3·3 mmol/L)	308/662 (47%)	334/648 (52%)
Hypoglycaemia (blood glucose <2·2 mmol/L)	8/701 (1%)	17/693 (3%)
Respiratory distress	79 (11%)	96 (13%)
Blackwater fever	20 (3%)	16 (2%)
History of anuria	99 (14%)	135 (18%)

Data are number (%). *Pretreatment with quinine, an artemisinin derivative, or mefloquine. Excludes chloroquine and sulphadoxine-pyrimethamine, which are ineffective throughout this region. †See panel for definition.

Table 19: Continuous baseline characteristics

	Artesunate (n=730)	Quinine (n=731)
Age (years); mean (95% CI)	27·9 (26·8–29·0)	27·9 (26·8–29·0)
Days of fever	5 (3–7, 0–120)	5 (3–7, 0–120)
Days of coma	0 (0–0·75, 0–4)	0·1 (0–1, 1–7)
Physical examination		
Weight (kg)	50 (43–55, 9–85)	50 (43–60, 9–100)
Temperature (°C)	38 (37·2–38·9, 35–41·5)	37·9 (37–38·9, 33·8–41·5)
Adult respiratory rate per min	24 (20–32, 12–103)	26 (20–32, 12–68)
Systolic blood pressure (mm Hg)	100 (90–115, 30–200)	100 (90–110, 30–180)
Diastolic blood pressure (mm Hg)	60 (60–70, 0–120)	60 (54–70, 0–100)
Glasgow coma scale (n=1425)	12 (9–15, 3–15)	12 (8–15, 3–15)
Blantyre coma scale (n=36)	4 (3–5, 1–5)	4 (3–5, 0–5)
Investigation		
Parasite count (per µL); geometric mean (95% CI)	39 850 (33 300–47 700)	31 050 (25 800–37 450)
Sodium (mmol/L)	134 (130–137, 108–159)	134 (130–138, 100–165)
Potassium (mmol/L)	3·9 (3·4–4·3, 2–8·8)	3·8 (3·4–4·3, 2–7·4)
Chloride (mmol/L)	101 (98–104, 77–130)	101 (98–105, 71–128)
Blood urea nitrogen (mmol/L of urea)	9·2 (5·4–17·8, 1·1–104)	10·4 (5·7–21·4, 1·1–86·8)
Haematocrit (%)	30 (22–36, 9–60)	29 (21–36, 5–62)
Haemoglobin (g/L)	100 (71–120, 26–200)	100 (70–120, 80–200)
pH	7·407 (7·352–7·448, 6·5–7·696)	7·4 (7·347–7·45, 6·542–7·582)
$paCO_2$ (mm Hg)	33 (28–38, 6–68)	32 (27–37, 7–83)
Total CO_2 (mmol/L)	22 (18–25, 2–45)	22 (17–25, 3–42)
Base excess (mmol/L)	−3 (−8 to 0, −30 to 22)	−4 (−9 to 0, −30 to 12)
Anion gap (mmol/L)	12·5 (0 to 16, −30 to 38)	13 (−1 to 16, −28 to 58)

Data are median (IQR, range) unless otherwise stated.

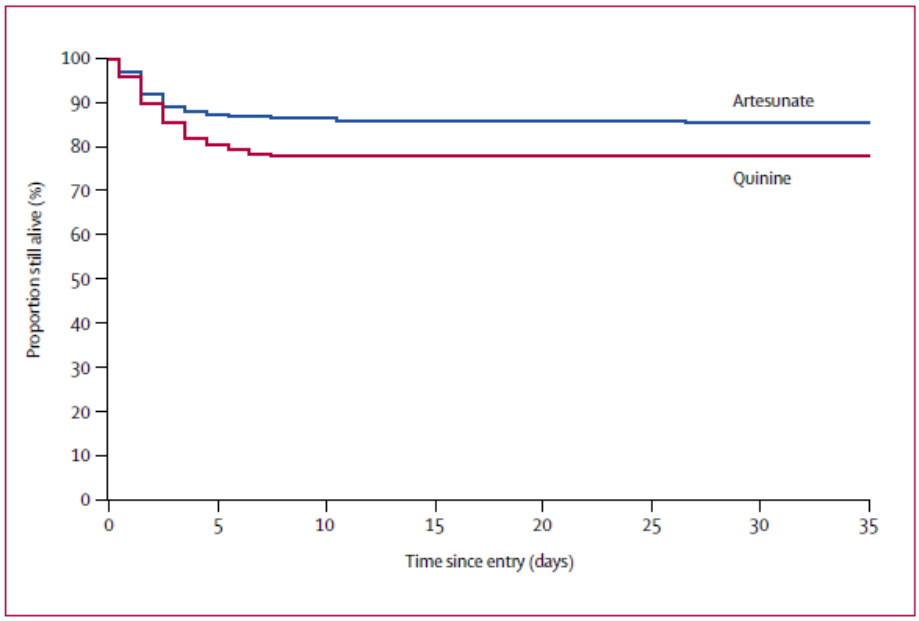
Mortality

Only in-hospital mortality was considered as the primary endpoint. One patient was discharged to die at home and this patient was excluded from the primary analysis. In the ITT population, mortality was 14.7% in the artesunate group compared with 22.4% in the quinine group (relative risk 0.67, 95% CI 0.54–0.83; odds ratio 0.60, 95% CI 0.45-0.79). The analysis of mortality in smear positive cases gave similar findings (relative risk 0.69, 0.55–0.85; odds ratio 0.62; 95% CI 0.47-0.82). The Breslow-Day (BD) test of homogeneity across the strata was not significant (p=0.340).

Table 20: Results by treatment group

	Artesunate (n=730)	Quinine (n=731)	Mantel-Haenszel stratified OR/hazard ratio [hr] (95% CI)	p (stratified)	p for homogeneity
In-hospital death	107 (15%)	164 (22%)	0·60 (0·45–0·79)	0·0002	0·39
Death within 48 h of entry	61 (8%)	75 (10%)	0·81 (0·57–1·16)	0·25	0·67
Death after 48 h of entry	46 (6%)	89 (12%)	0·48 (0·33–0·70)*	0·0001	0·73
In-hospital death (blood-smear positive)	105 of 689 (15%)	157 of 693 (23%)	0·62 (0·47–0·82)	0·0007	0·29
Neurological sequelae	7 (1%)	3 (<1%)	2·3 (0·59–8·8)	0·22	0·34
Combined outcome: in hospital death or neurological sequelae	114 (16%)	167 (23%)	0·63 (0·48–0·82)	0·0007	0·36
Fetal death	5 of 23 (22%)	5 of 26 (19%)	1·33 (0·28–6·18)	0·72	0·34
Time to discharge (days); median (IQR, range)	5 (4–8, 0–54)	5 (4–8, 0–45)	hr 0·93 (0·83–1·04)	0·20	0·77
Time to speak (days); median (IQR, range)	1 (0–2, 0–35)	1 (0–2, 0–21)	hr 0·97 (0·84–1·13)	0·73	0·82
Time to eat (days); median (IQR, range)	2 (0–3, 0–21)	2 (0–4, 0–47)	hr 0·91 (0·79–1·04)	0·17	0·69
Time to sit (days); median (IQR, range)	2 (0–3, 0–30)	2 (0–3, 0–45)	hr 0·91 (0·80–1·05)	0·19	0·82
Convulsions after entry	31 (4%)	43 (6%)	0·70 (0·44–1·12)	0·14	0·09
Shock developing after entry	26 (4%)	36 (5%)	0·72 (0·43–1·21)	0·22	0·59
Hypoglycaemia after entry	6 (<1%)	19 (3%)	0·31 (0·12–0·78)	0·009	0·94
Blackwater fever developing after entry	49 (7%)	33 (5%)	1·58 (0·94–2·65)	0·08	0·54
Dialysis after entry	60 (8%)	48 (7%)	1·25 (0·85–1·85)	0·25	0·011
Vasopressor treatment after entry	23 (3%)	24 (3%)	0·92 (0·52–1·64)	0·78	0·28
Mechanical ventilation after entry	26 (4%)	39 (5%)	0·65 (0·39–1·1)	0·11	0·40

Data are number (%) unless otherwise stated. Analysis by intention to treat unless otherwise indicated. Results stratified by study site. *Excludes patients who died within 48 h.



Patients either died in hospital or were discharged well, so all deaths included. To construct plot survival time of all discharged patients was set to 35 days.

Figure 10: Survival curve of in-hospital mortality

The hospital death rate on day 0 was 3.2% and 4.0% for artesunate and quinine groups, respectively.

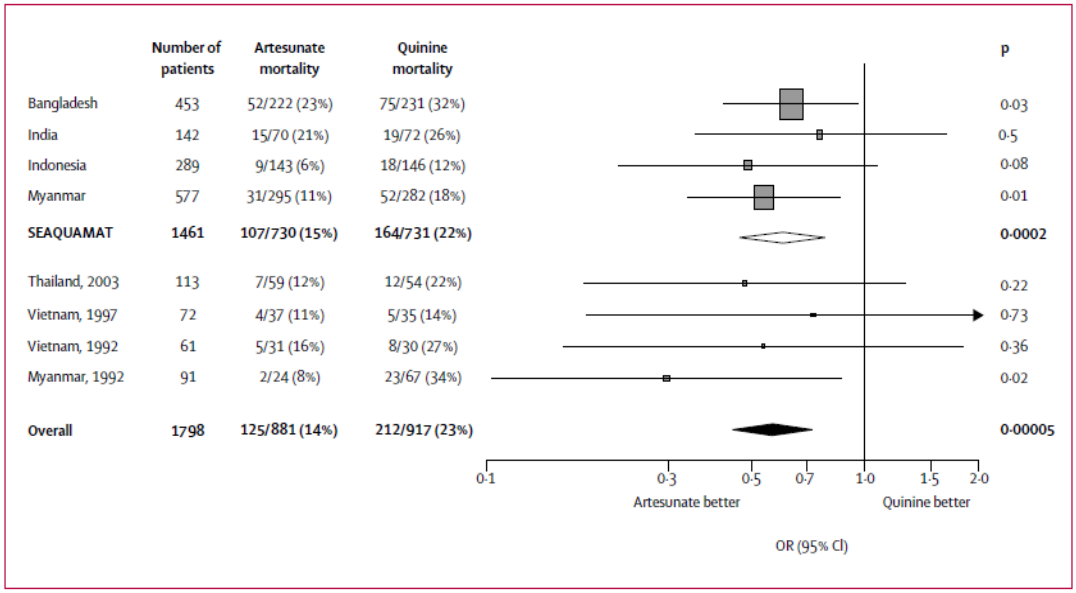
Rates on day 1 were 5.2% and 6.3%, respectively, after which rates diverged (2.7% vs. 4.7% on day 2, 1.2% vs. 3.4% on day 3 and 1.0% vs. 3.4% on days 4-6. The proportions discharged alive were 85.3% for the artesunate group and 77.6% for the quinine group.

In the severe malaria (SM) subgroup, the mortality rate was 19.8% in artesunate patients compared to 28.1% in quinine patients. The relative risk ratio from the stratified CMH analysis was 0.73 with 95% confidence interval (0.59, 0.90). The odds ratio estimate (95% confidence interval) from the analysis was 0.64 (0.48, 0.87). The stratified CMH analysis for the SM population was significant (p=0.0031) and the Breslow-Day result (p=0.122) did not demonstrate heterogeneity in the odds of mortality between the sites. Nine (3%) of the 332 smear-positive patients who did not fulfil the criteria for severe malaria on admission died compared to 24% (253/1050) of patients who fulfilled the criteria for severe malaria.

There were 17 patients in the artesunate group and 21 in the quinine group aged 65+ years. The numbers in the individual age groups 65 years and older were small, but 12/17 (70.6%) patients in the artesunate group and 12/21 (57.1%) patients in the quinine group survived.

There were 202 subjects aged <15 years in the study of whom 89 were <6 years. The overall mortality in children was 8% (16/202) with no significant difference between treatments.

Overall mortality varied significantly between countries (from 9.3% in Indonesia to 28% in Bangladesh), but there was no significant heterogeneity in treatment effect.



Site of boxes proportional to number of events in individual trial and thus contribution to overall effect. Diamond-summary stratified OR and 95% CI.

Figure 11: Forest plot of mortalities comparing parenteral quinine and artesunate in treatment of severe malaria in SEAQUAMAT and previously published studies

Other endpoints

Ten patients were discharged from hospital with residual neurological sequelae, seven in the artesunate group and three in the quinine group (p=0.23). Five had psychiatric sequelae, four had persisting problems with balance (one with psychiatric sequelae and a tremor) and two had a hemiparesis. Combining deaths and neurological sequelae, there were 114 (15.6%) adverse outcomes in the artesunate group and 167 (22.8%) in the quinine group (relative risk 0.68, 0.55–0.85).

Table 21: Summary of Significant Sequelae and Mortality – ITT Population

	Artesunate		Quinine			
	(N=730)		(N=731)		Relative Risk	
Outcome	n	%	n	%	RR	95CI
Neurological sequelae	7	1.0	3	0.4	2.34	(0.61, 9.00)
Neurological sequelae - Blindness	0	0.0	0	0.0	--	(-- , --)
Neurological sequelae - Cranial Nerve Involvement	0	0.0	0	0.0	--	(-- , --)
Neurological sequelae - Ataxia	2	0.3	0	0.0	--	(-- , --)
Neurological sequelae - Poor Balance	4	0.5	3	0.4	1.34	(0.30, 5.94)
Neurological sequelae - Paresis	2	0.3	0	0.0	--	(-- , --)
Neurological sequelae - Neuropsychiatric	4	0.5	4	0.5	1.00	(0.25, 3.99)
Neurological sequelae - Other	4	0.5	0	0.0	--	(-- , --)
Sequelae - Other	36	4.9	28	3.8	1.29	(0.79, 2.09)
Any Sequelae	43	5.9	30	4.1	1.44	(0.91, 2.26)
Died	107	14.7	164	22.4	0.65	(0.52, 0.81)
Combined Outcome - Death or Sequelae	114	15.6	167	22.8	0.68	(0.55, 0.85)

The incidence of any significant sequelae was 5.9% vs. 4.1% for the artesunate and quinine groups respectively (p=0.1171), yielding a relative risk ratio (artesunate/quinine) of 1.44 with 95% confidence interval (0.91, 2.26).

The incidence of any sequelae was higher in the artesunate group than in the quinine group among SM patients (7.1% vs. 3.7%) but lower in the non-SM subgroup (3.2% vs. 5.3%).

Table 22: Summary of Significant Sequelae and Mortality by Severe Malaria on Admission Status – ITT Population

	Severe Malaria on Admission							
	No (N=411)				Yes (N=1050)			
	Study Drug				Study Drug			
	Artesunate		Quinine		Artesunate		Quinine	
	n	%	n	%	n	%	n	%
Outcome								
Neurological sequelae	1	0.5%	1	0.5%	6	1.2%	2	0.4%
Neurological sequelae - Blindness	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Neurological sequelae - Cranial Nerve Involvement	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Neurological sequelae - Ataxia	0	0.0%	0	0.0%	2	0.4%	0	0.0%
Neurological sequelae - Poor Balance	0	0.0%	1	0.5%	4	0.8%	2	0.4%
Neurological sequelae - Paresis	0	0.0%	0	0.0%	2	0.4%	0	0.0%
Neurological sequelae - Neuropsychiatric	2	0.9%	0	0.0%	2	0.4%	4	0.7%
Neurological sequelae - Other	1	0.5%	0	0.0%	3	0.6%	0	0.0%
Sequelae - Other	6	2.7%	10	5.3%	30	5.9%	18	3.3%
Any Sequelae	7	3.2%	10	5.3%	36	7.1%	20	3.7%
Died	6	2.7%	12	6.3%	101	19.8%	152	28.1%
Combined Outcome - Death or Sequelae	7	3.2%	13	6.8%	107	21.0%	154	28.5%

Supporting Listing Number 16.2.5.2
Notes: Interaction of treament and severe malaria status is significant for sequelae-other (p=0.0343) and any sequelae (p=0.0359) in logistic regression models with treatment, severe malaria status, and interaction in the model.
There was an insufficient number of events to reliably test other sequelae categories in the model.
The interaction of treatment and severe malaria status is not significant for the combined outcome (p=0.42) in a logistic regression model with treatment, severe malaria status, and interaction in the model.

Among those who survived, there were no significant differences between the treatment groups for times to eat or sit or to hospital discharge.

The pregnancy outcome for 23 artesunate and 26 quinine patients with data included 6 (26.1%) artesunate and 8 (30.8%) quinine group cases of foetal death while 5 in each group had an abortion.

The treatment effect was as good in patients who had received effective antimalarial treatment before admission as in those who had not (ratio of ORs 0.6, 95% CI 0.3–1.21). Pre-treatment included quinine in 187 patients, an artemisinin derivative in 67, chloroquine in 64 and sulphadoxine-pyrimethamine in 19 (some patients received more than one drug). Post-hoc (not pre-specified) analyses of pre-treatment drug subgroups revealed no significant patterns with respect to the treatment effect.

Patients with hyperparasitaemia (admission parasitaemia ≥10%) had a significantly greater treatment effect with artesunate than non-hyperparasitaemic patients (ratio of ORs 0.34, 0.17–0.69, p=0.001).

There was a significant excess of hypoglycaemia after study entry in the quinine group compared with the artesunate group (RR 3.2, 95% CI 1.3–7.9). Such a trend was seen in SM and non-SM subgroups. In contrast, there were no differences between the study groups in the incidence of haemodynamic shock, convulsions and blackwater fever or in the use of supportive therapies (mechanical ventilation, vasopressor support and dialysis).

Results - AQUAMAT

There were 5425 patients enrolled and included in the ITT population. Most of the patients not eligible for the PP population had negative or missing blood smears.

Baseline data

There were no major differences in baseline data between randomised treatment groups in terms of demographics or clinical status at study baseline. With a mean age of ~3 years, the youngest patients were >18 months old.

Table 23: Baseline Characteristics in the two treatment groups

	Quinine (N=2713)	Artesunate (N=2712)
Female sex	1295 (48%)	1315 (48%)
Age (years)	2·9 (1·7–4·3)	2·8 (1·6–4·2)
Fever before enrolment (days)	3 (2–4)	3 (2–4)
Coma before enrolment (h)	5·0 (2·5–10)	5·0 (2·0–9·5)
Pretreatment with antimalarials		
None	1270 (47%)	1281 (47%)
Ineffective*	371 (14%)	387 (15%)
Effective*	959 (37%)	938 (36%)
Complications on admission		
Coma†	945 (35%)	880 (32%)
Convulsions	879 (32%)	811 (30%)
Jaundice	59 (2%)	55 (2%)
Severe anaemia (haemoglobin <50 g/L)	692 (29%)	737 (30%)
Shock	339 (12%)	323 (12%)
Decompensated shock	88 (35%)	90 (39%)
Severe acidosis (BE <–8 mmol/L)	975 (43%)	1009 (44%)
Hypoglycaemia (<3 mmol/L)	278 (10%)	277 (10%)
Respiratory distress‡	428 (16%)	439 (16%)
Severe prostration§	1668 (61%)	1683 (62%)
Blackwater fever	116 (4%)	121 (4%)
Hyperparasitaemia (>10%)	573 (24%)	584 (25%)
Clinical examination		
Weight (kg)	12·6 (4·6)	12·4 (4·8)
Temperature (°C)	38·0 (1·1)	38·0 (1·1)
Blood pressure (mm Hg)		
Systolic	95 (16)	95 (16)
Diastolic	56 (14)	56 (14)
Coma depth (total N, median [IQR])		
Blantyre coma score	1704, 4 (2–5)	1713, 4 (2–5)
Glasgow coma score	1005, 11 (8–15)	999, 11 (8–15)
Comorbidity		
Immune compromised (from history)	49 (2%)	45 (2%)
Severe malnutrition	43 (2%)	54 (2%)

(Continues in next column)

	Quinine (N=2713)	Artesunate (N=2712)
(Continued from previous column)		
Suspected pneumonia	226 (8%)	227 (8%)
Confirmed by radiograph	29 (13%)	29 (13%)
Clinical sepsis	355 (13%)	302 (11%)
Confirmed by culture	33 (9%)	32 (11%)
Suspected meningitis	166 (6%)	169 (6%)
Confirmed meningitis	3 (2%)	6 (4%)
Other significant comorbidities	71 (3%)	80 (3%)
Laboratory assessments		
Parasitaemia (parasites per µL; geometric mean, range)	49110 (0–1858880)	47922 (0–1494640)
Sodium (mmol/L)	132 (6·5)	131 (6·5)
Potassium (mmol/L)	4·1 (0·9)	4·1 (0·9)
Chloride (mmol/L)	105 (10)	105 (10)
Blood urea nitrogen (mmol/L)	6·1 (4·9)	6·1 (4·6)
Haemoglobin (g/L)	70 (31)	68 (29)
pH	7·36 (0·14)	7·36 (0·14)
$PaCO_2$ (mm Hg)	28·2 (10·1)	27·9 (9·1)
HCO_3 (mmol/L)	16·6 (5·7)	16·6 (5·6)
Plasma BE (mmol/L)	−8·6 (7·3)	−8·5 (7·3)
Anion gap (mmol/L)	17·2 (5·0)	17·0 (4·9)

Data are number (%), median (IQR), or mean (SD), unless otherwise indicated. BE=base excess. $PaCO_2$=partial pressure of carbon dioxide. HCO_3=bicarbonate. *See webappendix p 12 for classification of categories. †Depth of coma was assessed either by Blantyre coma score (for preverbal children, n=3417) or Glasgow coma scale (n=2004). ‡Respiratory distress was defined as costal indrawing, use of accessory muscles, nasal alar flaring, deep breathing, or severe tachypnoea. §Severe prostration was defined as inability to breastfeed for children younger than 6 months or inability to sit for older children.

Mortality

Of the total 5425 patients recruited, 527 (9.7%) died. Death rates were 230/2712 (8.5%) in the artesunate group vs. 297/2713 (10.9%) in the quinine group (relative risk 0.78, 95% CI 0.66–0.91; odds ratio 0.75, 95% CI 0.63–0.90, in favour of artesunate; p=0.0022). This represents a relative reduction in mortality of 22.5% (95% CI 8.1–36.9%). There was no heterogeneity between study sites (p=0.99). The per-protocol analysis (excluding those who died before receiving study treatment, those with incomplete initial antimalarial treatment with the study or with negative or missing blood slides for *P falciparum*) gave a very similar result to the ITT population.

The survival analysis for overall mortality during admission by antimalarial treatment gave the same result as the Mantel-Haenszel analysis (HR stratified by study site 0.76, 95% CI 0.64–0.91; p=0.0022). Mantel-Haenszel analysis of the predefined subgroups showed no evidence of any differences in odds ratios between subgroups and these results were confirmed by Cox regression.

The endpoint review committee identified 16 children (7 artesunate, 9 quinine) in whom death was unlikely to be related to severe malaria. Omission of these cases from the analysis had no effect on the magnitude of the survival benefit with artesunate.

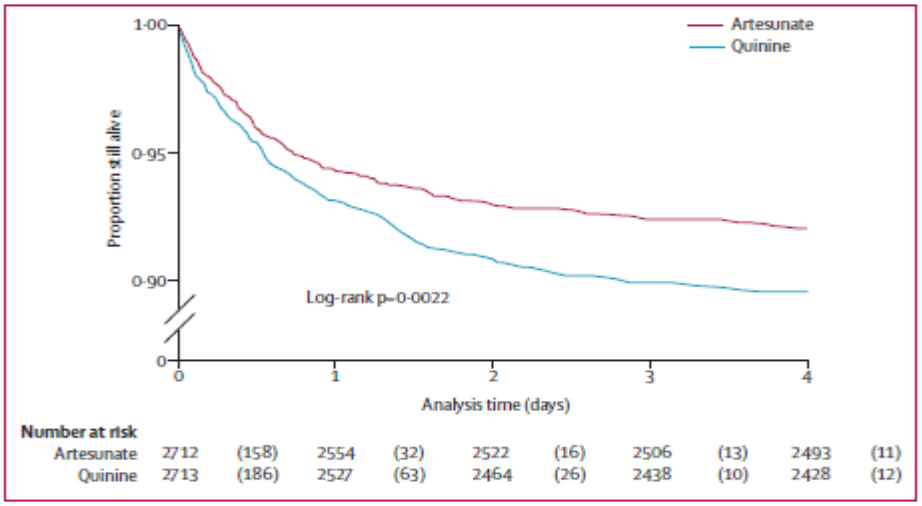
There was a benefit for artesunate on mortality among children meeting criteria for severe malaria.

HIV serology was assessed routinely in Beira, Muheza and Kilifi. Of 2095 patients tested, 125 (6%) were positive (64 artesunate, 61 quinine) and mortality in these patients was high regardless of treatment group.

Table 24: Mortality and complications according to treatment group

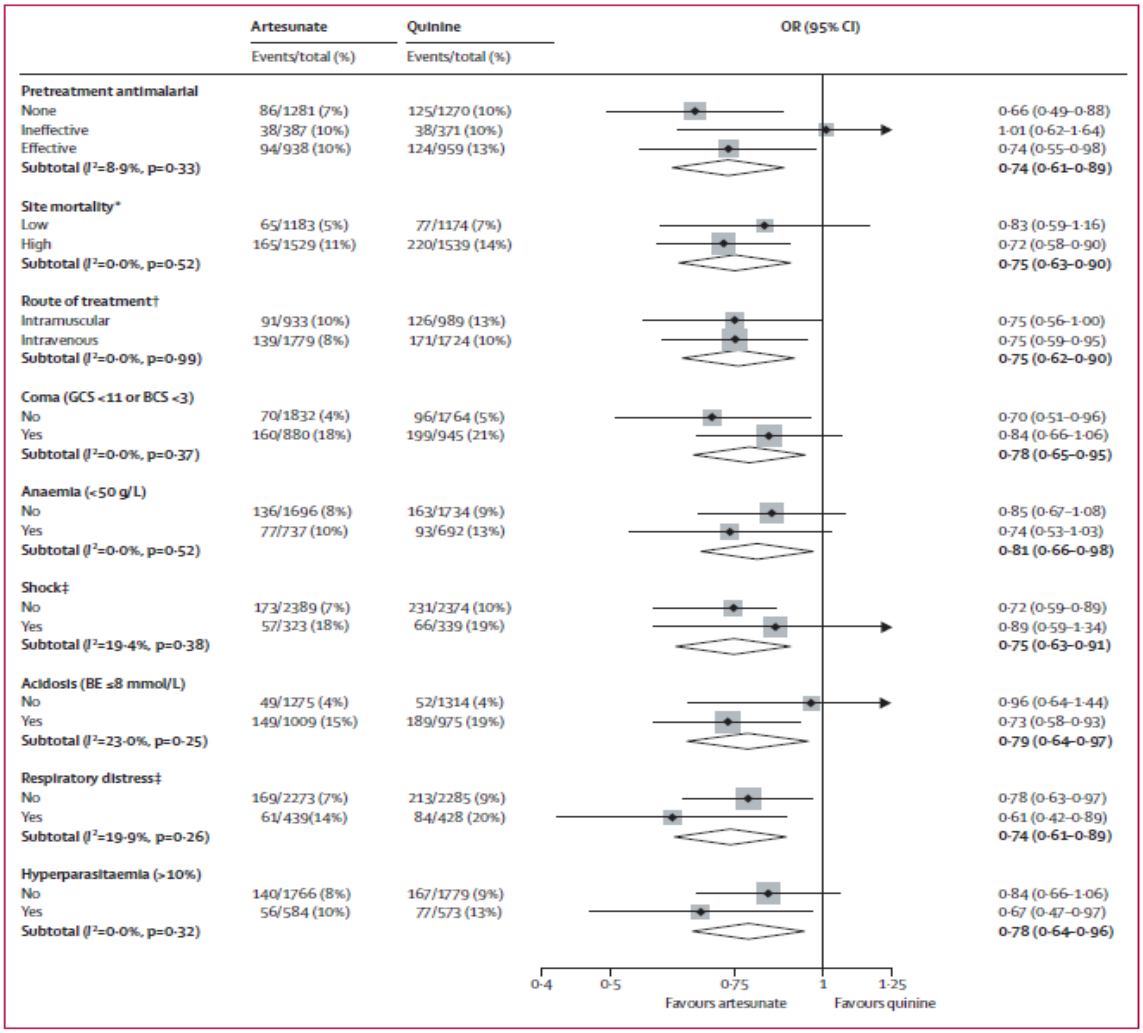
	Quinine (n/N, %)	Artesunate (n/N, %)	OR (95% CI)	p value
Mortality, ITT analysis	297/2713 (10·9%)	230/2712 (8·5%)	0·75 (0·63–0·90)	0·0022
Mortality, per-protocol analysis	260/2552 (10·2%)	208/2563 (8·1%)	0·78 (0·64–0·94)	0·0099
Death or sequelae at 28 days	316/2695 (11·7%)	253/2689 (9·4%)	0·78 (0·65–0·93)	0·0056
Malaria-attributable mortality*	288/2704 (10·7%)	223/2705 (8·2%)	0·75 (0·63–0·91)	0·0025
Mortality in strictly defined severe malaria†	291/2338 (12·4%)	226/2280 (9·9%)	0·77 (0·64–0·93)	0·0055
Case fatality in HIV-positive children‡	19/61 (31%)	16/64 (25%)	0·74 (0·33–1·62)	0·45
Development of coma§	91/1768 (5·1%)	65/1832 (3·5%)	0·69 (0·49–0·95)	0·0231
Deterioration of coma score	208/2713 (7·7%)	166/2712 (6·1%)	0·78 (0·64–0·97)	0·0245
Convulsions developing or persisting >6 h after admission	273/2713 (10·1%)	224/2712 (8·3%)	0·80 (0·66–0·97)	0·0199
Hypoglycaemia	75/2713 (2·8%)	48/2712 (1·8%)	0·63 (0·43–0·91)	0·0134
Severe anaemia (<50 g/L) after admission§	98/1734 (5·7%)	78/1696 (4·6%)	0·81 (0·59–1·11)	0·18
Blackwater fever§	18/2597 (0·7%)	30/2591 (1·2%)	1·69 (0·94–3·05)	0·076

ITT=intention to treat. *The likelihood that malaria contributed to or directly caused the death was assessed by an independent endpoint review committee blinded to the treatment allocation. †As defined in panel 1. ‡HIV status was assessed only in Beira, Muheza, and Kilifi (n=2095). §Development of coma, anaemia, and blackwater fever was assessed only in patients without these disorders on admission.



The numbers in parentheses are the deaths during the indicated time. In eight patients the exact time of death during the night was missing and was estimated as 2359 h.

Figure 12: Kaplan-Meier curves comparing survival in African children with severe falciparum malaria treated with either parenteral artesunate or quinine



The forest plot shows odds ratios and 95% CIs. The size of the squares is proportional to the size, and therefore weight, of the subgroup. The diamonds show the combined differences. The efficacy of antimalarial pretreatment was classified before study unblinding (webappendix p.12). Hyperparasitemia means greater than 10% of red cells parasitized. OR=odds ratio. GCS=Glasgow coma scale. BCS=Blantyre coma scale. BE=base excess. *Site mortality classified as low if the site mortality rate was lower than the overall study mortality rate, and high if the site mortality rate was higher than the overall study mortality rate. †Classified according to centre policy (ten sites); classified according to individual data (one site). ‡ Decompensated or compensated shock. I^2 denotes the percentage of total variation across subgroups resulting from heterogeneity rather that chance, with the p value of significance.

Figure 13: Treatment effect in protocol-specified subgroups

Other endpoints

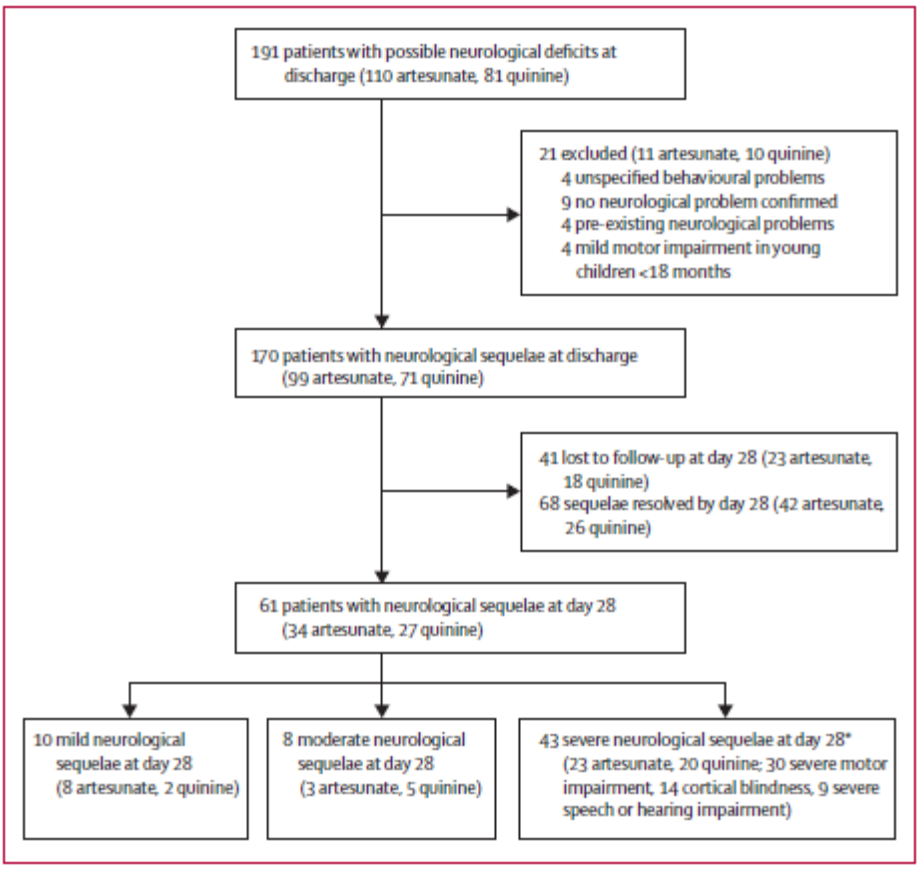
The development of coma, deterioration in coma score and convulsions all occurred more frequently in patients who received quinine than in those who received artesunate.

In cerebral malaria survivors, the time from randomisation until the child was able to localise a painful stimulus or was able to speak was slightly longer overall in patients treated with artesunate than in those given quinine when compared by survival analysis.

In the 4898 survivors, 170 (99 artesunate, 71 quinine) had not yet made full neurological recoveries at the time of hospital discharge. Of these 170 patients, 129 (76 artesunate, 53 quinine) were followed up between 3 and 8 weeks after enrolment at which time 68 (53%) had recovered fully, 18 (14%; 11 artesunate, 7 quinine) were mildly or moderately impaired and 43 (33%; 20 quinine, 23 artesunate) had severe neurological deficits.

The overall rate of persistent neurological sequelae in survivors assessed at 28 days after cerebral malaria was 3.2% (24/706 artesunate, 23/737 quinine) and the rate for severe neurological sequelae was 2.3% (17/706 artesunate, 17/737 quinine).

Of the 14 patients with any neurological sequelae who did not have cerebral malaria initially (10 artesunate, 4 quinine), 7 had multiple convulsions (3 quinine, 4 artesunate) and all had severe prostration on admission.



*Some patients had severe impairment in more than one domain.

Figure 14: Neurological sequelae at discharge and after 28 days (range 3-8 weeks) in children with severe falciparum malaria

The times to eat or to sit unsupported did not differ between treatment groups.

Table 25: Recovery times in surviving patients according to treatment group

	Quinine (median, IQR)	N	Artesunate (median, IQR)	N	HR (95% CI)	p value
Time to discharge (days)	3·0 (2·0–5·0)	2412	3·0 (2·0–5·0)	2478	1·04 (0·99–1·10)	0·059
Time to eat (h)	12 (2–24)	2269	9 (0–24)	2358	0·99 (0·93–1·06)	0·74
Time to sit unsupported (h)	22 (6–44)	2312	18 (6–42)	2373	1·02 (0·95–1·08)	0·60
Time to localise pain (h)*	12 (6–24)	726	12 (6–24)	698	0·87 (0·78–0·98)	0·0093
Time to speak (h)*	18 (11–36)	695	20 (8–42)	664	0·88 (0·79–0·99)	0·016

*Time to localise pain and time to speak was assessed only for surviving patients with coma on admission (Blantyre coma scale <3 or Glasgow coma scale <11).

Summary of main efficacy results

Title: SEAQUAMAT				
Study identifier	**Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial** *South East Asian Quinine Artesunate Malaria Trial (SEAQUAMAT) group*			
Design	Prospective, multicentre, open-label, randomised active controlled study			
	Duration of main phase:		June 2003-May 2005	
Hypothesis	Non-inferiority			
Treatments groups	IV Artesunate vs. IV Quinine Both given with oral doxycycline from time of oral switch except in pregnant women, children aged < 8 years and at 2 centres		IV artesunate 2.4 mg/kg at 0, 12 and 24 h, then daily until oral switch IV quinine 20 mg/kg loading dose, then 10 mg/kg q8h until oral switch	
Endpoints and definitions	Primary endpoint	Mortality from severe malaria	Defined as in-hospital mortality	
	Secondary endpoints		Neurological sequelae, combined death or neurological sequelae, recovery times and rate of severe complications	
Database lock	Not stated			
Results and Analysis				
Analysis description	**Primary Analysis**			
Analysis populations	ITT for in-hospital mortality PP with positive blood smear at baseline Subset of the intent to treat population confirmed to have had severe *P. falciparum* malaria based on WHO criteria applicable at the time			
Descriptive statistics and estimate variability	Treatment group	Artesunate	Quinine	
	Number	730 689 509	731 693 541	Randomised Blood smear positive Severe malaria
	In-hospital death	107/730 (15%)	164/731 (22%)	OR 0.60 (0.45, 0.79); P = 0.0002
	In-hospital death blood smear positive	105/689 (15%)	157/693 (23%)	OR 0.62 (0.47, 0.82); P = 0.0007
	Neurological sequelae	7/730 (1%)	3/731 (<1%)	HR 2.3 (0.59, 8.8) p=0.22

Title: AQUAMAT			
Study identifier	**Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): an open-label, randomised trial**		
Design	Prospective, multicentre, open-label, randomised active controlled study		
	Duration of main phase:		October 2005 to July 2010
Hypothesis	Non-inferiority		
Treatments groups	Artesunate		Quinine
	IV or IM Artesunate vs. IV or IM Quinine Both were followed by artemether-lumefantrine 6 doses over 3 days		Artesunate 2.4 mg/kg at 0, 12 and 24 h, then daily until oral switch Quinine 20 mg/kg loading dose, then 10 mg/kg q8h until oral switch
Endpoints and definitions	Primary endpoint	Mortality from severe malaria	Defined as in-hospital mortality
	Secondary endpoints		Neurological sequelae, combined death or neurological sequelae, recovery times and rate of severe complications
Database lock	Not stated		

Results and Analysis

Analysis description	**Primary Analysis**			
Analysis populations	ITT for in-hospital mortality With positive blood smear at baseline Per protocol with severe malaria			
Descriptive statistics and estimate variability	Treatment group	Artesunate	Quinine	
	Number	2712 2695 2563	2713 2688 2552	Randomised Blood smear positive PP
	In-hospital death	230/ 2712 (8.5%)	297/ 2713 (10.9%)	OR 0.78 (0.66, 0.91); P = 0.0022
	In-hospital death in severe malaria	291/2338 (12.4%)	226/2280 (9.9%)	OR 0.77 (0.64, 0.93); P = 0.0055
	Coma (new)	91/1768 (5.1%)	65/1832 (3.5%)	HR 0.69 (0.49, 0.95) p=0.02
	Convulsions (new)	273/ 2713 (10.1%)	224/ 2712 (8.3%)	HR 0.80 (0.66, 0.97) p=0.02

2.6.5.3. Supportive studies

R-CDC-060 – US Army 2007-2011

This was a retrospective analysis of US patients who received intravenous artesunate Amivas for initial treatment of severe malaria under the US CDC IND. It was expected that the majority would have malaria due to *P. falciparum* but those with undetermined species or species other than *falciparum* were also eligible. Eligible patients had to meet at least one criterion in each of groups A, B and C:
Criteria A malaria confirmed by microscopy

Criteria B need for IV treatment

Criteria C need for IV artesunate

The initial dose regimen was 2.4 mg/kg at 0 h, 12 h, 24 h and 48 h for a total of 4 doses. The choice of an appropriate follow-on oral antimalarial drug was at the discretion of the treating physician. After 3 days of treatment with IV artesunate, if the subject still could not tolerate oral medications, the potential options included: 1) to continue the IV artesunate, not to exceed a total course of 7 days; or 2) to switch to treatment with IV doxycycline (7 days) or IV clindamycin (7 days).

The primary outcome was safety. The secondary clinical benefit outcomes included efficacy endpoints.

Of the 104 patients who received IV artesunate, appropriate consent forms had not been signed in two cases, leaving a safety population of 102 patients of which 93 (91%) completed IVAS treatment.

Of the 102 patients, the majority (61%) was male, 63% were Black/African American and the mean age was 38.1 years (range 1 to 72 years) with 10/102 aged <15 years, including 4 aged 1-3 years. Three patients were pregnant.

There were 87 patients in the evaluable population and 74 in the PP population. Prior exposure to quinidine was reported for 47/102 (46%) in the safety analysis population and 43/87 (49%) in the evaluable analysis population.

Table 26: Summary of Subjects Meeting Inclusion Criteria by Analysis Population

	Safety Analysis Population (N=102)[a]	Evaluable Analysis Population (N=87)	Per Protocol Analysis Population (N=76)
Section A (Malaria diagnosis)			
Malaria confirmed by microscopy	98	87	74
Strong clinical suspicion	2	0	2
No evidence of malaria	2	0	0
Section B (Need for IV treatment)			
Unable to take oral medication	36	33	31
Parasitemia ≥5%	67	65	51
Impaired consciousness	25	24	21
Seizures	5	5	5
Circulatory collapse/shock	26	24	22
Pulmonary edema/ARDS[b]	12	9	9

	Safety Analysis Population (N=102)[c]	Evaluable Analysis Population (N=87)	Per Protocol Analysis Population (N=76)
Acidosis	37	35	27
Acute renal failure	25	25	18
Abnormal bleeding/DIC[d]	4	3	2
Jaundice	48	43	37
Severe anemia (hemoglobin <7 g/dL)	10[e]	8	8
Did not answer or did not know	4	0	0
Section C (IVAS desirable)[f]			
IVAS obtainable more quickly	61	52	57
Quinidine treatment failure	0	0	0
Quinidine intolerance[g]	17	15	17
Contraindications to quinidine[h]	2	2	2
Did not answer or did not know	22	18	0

[a] Some subjects met the criteria for more than one Analysis Population.
[b] ARDS=acute respiratory distress syndrome.
[d] DIC=disseminated intravascular coagulation.
[e] One subject (Subject 21094) was listed as having severe anemia at baseline; however, her baseline hemoglobin was actually 8.2 g/dL.
[f] Multiple answers were not given.
[g] 12 of these 17 subjects had QTc interval prolongation by more than 25% of the baseline value, 2 had persistent hypotension unresponsive to fluid resuscitation, 3 had an unspecified reaction to quinidine during therapy.
[h] Of these 2 subjects, 1 had an allergy or hypersensitivity to quinidine or cinchona alkaloids and 1 had a left bundle-branch heart block or other severe intraventricular conduction defects.

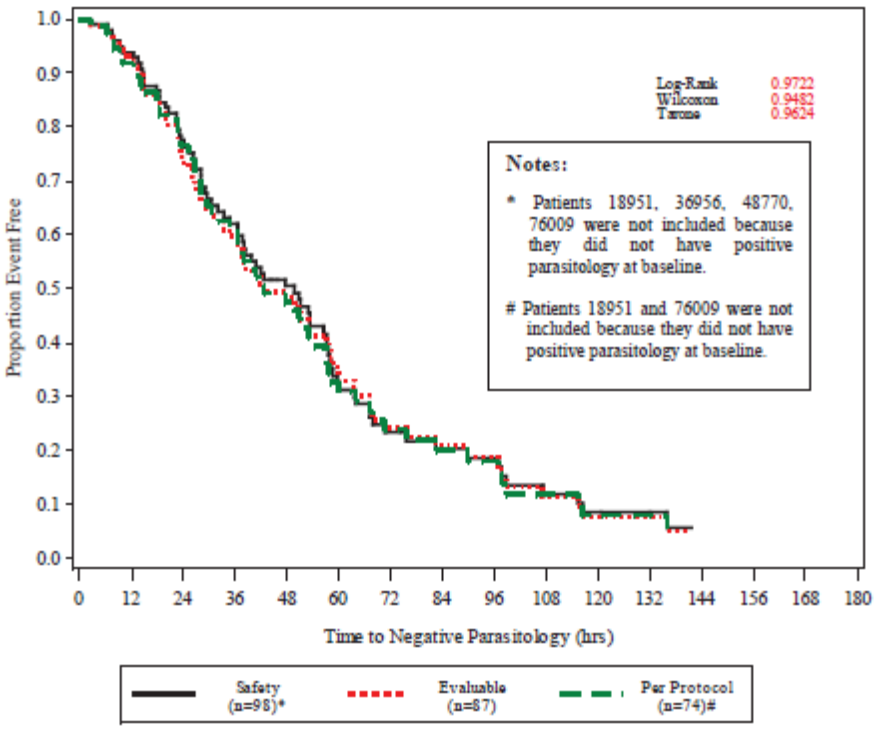
Of 87 patients in the evaluable population, 39 (45%) were able to switch to follow-on oral therapy but 38/87 (including 4 deaths) were excluded from the analysis because they received concomitant IV antimalarial medications during treatment with IV artesunate. Of the 10 who did not switch, 8 could not take oral medication, one died and one had no reason recorded.

Seven of the 102 patients died, including 5 who died before completing 4 IV artesunate doses. One other patient died after the data abstraction period. All deaths were considered due to the severity of malaria. Five of the 7 had *P. falciparum* with baseline hyperparasitaemia from 12% to 90% and one patient had *P. vivax.* All showed significant baseline neurological deterioration (seizures, impaired consciousness, ventilator support) and moderate or severe hepatic injury as assessed by MELD score. Three of the 7 who died had brain oedema or brainstem herniation. The only two patients in the safety population who were aged >70 years both died and one other death was in a patient aged >60 years. One of two patients in the study with HIV died as did 2/11 with diabetes. The three pregnant women treated all survived.

The median time to negative parasitology was 49.6 h in the safety population, 42.7 h in the evaluable population and 42.7 h in the per protocol population. There were no statistically significant differences in the time to negative parasitology between those with and without severely impaired renal or hepatic function at baseline. Those with severe clinical/neurological deterioration at baseline (seizures, impaired consciousness, ventilator support) did not have a statistically significant longer time to negative parasitology vs. those without baseline deterioration.

Table 27: Kaplan-Meier Analysis of Time to Negative Parasitology by Analysis Population

	Safety (N=102)	Evaluable (N=87)	Per Protocol (N=76)
Number of subjects analyzed (*)	98	87	74
Number of Events (%)	78 (80)	71 (82)	60 (81)
Number Censored (%)	20 (20)	16 (18)	14 (19)
Median Time to Event (hours)	49.6	42.7	42.7
95% Confidence Interval	(36.7, 57.1)	(33.5, 57.9)	(34.7, 57.1)



(*) subjects with malaria confirmed by microscopy; Source: Section 14.2.1, Table 8 and Figure 1.

In the evaluable analysis population (n=87), patients who received concurrent antimalarial treatments (n=38) during IV artesunate administration did not show a statistically significant difference in time to negative parasitology compared with the 49 who did not but there was a numerical difference (43 h vs. 51 h, respectively; p=0.942). Administration of a concomitant antimalarial agent did not improve the negative parasitology rates or ICU discharge rates. Patients exposed to quinidine (n=43) before or during IV artesunate did not show a statistically significant difference in time to negative parasitology compared with 44 without quinidine exposure (41 h vs. 50 h, respectively; p=0.608).

In the evaluable analysis population, 39/49 (80%) transitioned to oral antimalarial therapy within the abstraction period with a mean time to initiation of 3.8 days (range 2 to 5 days). Baseline severe renal or hepatic impairment or neurological deterioration did not significantly affect median time to starting follow-on oral antimalarial therapy. There was a borderline statistically significant (p=0.050) difference between those not exposed (3 days) vs. exposed to quinidine (4 days) before or during IV artesunate therapy but in the PP population the median was 4 days in both subgroups.

Cao et al., 1997

In this open, randomised study, Vietnamese children (n=109) aged from 3 months to 14 years with severe *P. falciparum* malaria received one of the following:

- Artesunate suppositories (n=37), (40 mg/kg initially and 20 mg/kg at 4, 24, 48 and 72 h) followed by oral mefloquine (15 mg/kg) at 96 h;
- Artesunate intramuscular (Guilin; 3 mg/kg initially and 2 mg/kg at 12, 24, 48 and 72 h) followed by oral mefloquine (15 mg/kg) at 96 h;
- Quinine dihydrochloride (20 mg salt/kg in 5% glucose IV infusion over 4 h, followed by 10 mg/kg every 8 h for 7 days) followed by a single dose of pyrimethamine/sulfadoxine (Fansidar) on day 7.

On admission, 24 children (22%) had cerebral malaria, 64 (59%) were jaundiced and 14 (13%) had visible haemoglobinuria. Overall, 11 patients (10%) died, most of them from multi-organ failure. One in the quinine group died before the first dose. There was no statistically significant difference in mortality rates among the treatment groups.

Table 28: Outcome of Vietnamese children with severe malaria

	Artemisinin	Artesunate	Quinine
No. of children	37	37	35
No. survived well	34 (92%)	32 (87%)	30 (86%)
No. survived with neurological sequelae	1 (3%)	1 (3%)	0 (–)
No. died	2 (5%)	4 (11%)	5 (14%)
Time to die, from admission (h)[a]	13 (12 to 14; 12–14)	22 (2 to 68; 2–68)	32 (2 to 75; 2–75)
Fever clearance time (h)			
See notes (b,d)	4 (0 to 8; 0–84)	4 (4 to 8; 4–198)	8 (4 to 12; 0–96)
See notes (c,d)			
All patients	48 (30 to 90; 0–406)	84 (61 to 107; 4–198)	81 (54 to 107; 0–246)
Excluding superinfections	48 (30 to 90; 0–302)	66 (50 to 106; 4–144)	60 (36 to 106; 0–168)
Coma resolution (h)[a,e]	26 (6 to 44; 1–48)[f]	42 (5 to 108; 4–228)[g]	31 (4 to 66; 4–66)[h]
Parasite clearance time (h)[d]			
50%	7·0 (5·3 to 8·8; 2·3–28·4)	5·7 (4·1 to 8·0; 2·0–15·3)	13·2 (9·2 to 18·6; 2·4–103·0)
90%	14·9 (12·7 to 16·2; 5·2–65·0)	12·0 (10·5 to 17·8; 3·7–35·0)	27·7 (23·3 to 34·0; 7·5–107·0)
100%	48 (31 to 54; 8–84)	36 (24 to 48; 16–126)	84 (66 to 90; 12–240)
Period in hospital (d)[a,d]	7 (6 to 8; 5–18)	8 (6 to 9; 5–20)	8 (7 to 12; 5–24)

[a]Median (95% confidence interval and range in parentheses).
[b]Time until temperature first dropped to 37·5°C or below.
[c]Time until temperature first dropped to 37·5°C or below and remained ≤37·5°C for at least 24 h.
[d]Values of *P* are given in the Figure legend.
[e]Time for Blantyre coma score to become 5/5.
[f]*n*=6.
[g]*n*=10.
[h]*n*=2.

PCTs and FCTs were shorter in the groups that received an artemisinin. Four patients in the quinine group had RI1 resistance (persistence of parasites). No patient in the artemisinin or artesunate groups failed to clear parasites within 7 days of commencing treatment.

COCHRANE Meta-analysis (Sinclair 2012)

This meta-analysis was conducted over a 10-year period (1992-2012). The authors found 31 clinical trials of intravenous or intramuscular artesunate for treatment for severe or complicated malaria from which they selected 8 that were prospective randomised trials. Six trials were conducted in Asia (Cao *et al*. 1997; Dondorp *et al*. 2005 [SEAQUAMAT]; Hien *et al*. 1992; Newton *et al*. 2003; Sinclair *et al*. 2012) and two were in Africa (Dondorp-2010 [AQUAMAT]; Eltahir-2010).

In the 8 randomised and controlled trials, 1664 adults and 5765 children were enrolled to receive parenteral artesunate or parenteral quinine.

Artesunate compared with quinine for treating severe malaria

Patient or population: Children with severe malaria
Settings: Malaria endemic areas
Intervention: Artesunate
Comparison: Quinine

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)
	Assumed risk	Corresponding risk			
	Quinine	Artesunate			
Death	**109 per 1000**	**83 per 1000** (71 to 98)	**RR 0.76** (0.65 to 0.9)	5765 (4 studies[1])	**high**[2,3,4,5]
Neurological sequelae at day 28	**11 per 1000**	**14 per 1000** (8 to 22)	**RR 1.23** (0.74 to 2.03)	4857 (1 study[6])	**moderate**[7,8,9,10]
Neurological sequelae at discharge	**28 per 1000**	**38 per 1000** (28 to 51)	**RR 1.36** (1.01 to 1.83)	5163 (3 studies[11])	**moderate**[2,3,4,12]
Time to hospital discharge (days)	See comment	See comment	Not estimable	113 (3 studies[11])	**moderate**[2,13,4,14]
Hypoglycaemia episodes	**30 per 1000**	**19 per 1000** (13 to 26)	**RR 0.62** (0.45 to 0.87)	5765 (4 studies[1])	**high**[2,3,4,15]

*The **assumed risk** was calculated by dividing the total number of events in the control group (across studies) by the total number of patients in the control group (across studies). This was numerically very similar to the median control group risk but is easier to link with the corresponding forest plot. The **corresponding risk** (and its 95% CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; RR: Risk Ratio

[1] One large multicentre trial (Dondorp 2010) and two small trials (Cao 1997, Eltahir 2010) have assessed artesunate vs quinine in children aged <15 years. In addition one large multicentre study included a subgroup of children in this age group (Dondorp 2005)
[2] No serious study limitations: All the trials adequately concealed allocation to be considered at low risk of bias. The trials were unblinded but this is unlikely to bias this objective outcome
[3] No serious inconsistency: There was no statistical heterogeneity between the trials ($I^2 = 0\%$).
[4] No serious indirectness: Most of the data is from Dondorp 2010 which had centres in Mozambique, the Gambia, Ghana, Kenya, Tanzania, Nigeria, Uganda, Rwanda and the Democratic Republic of Congo, and used the established standard doses of artesunate and quinine (with loading dose). Of note the median age of children in this trial was 2.9 years in the quinine group and 2.8 in the artesunate group.
[5] No serious imprecision: Both limits of the 95% CI of the pooled effect imply an appreciable clinical benefit with artesunate. The Number Needed To Treat to prevent one childhood death is 38.
[6] Only one large multicentre trial (Dondorp 2010) reports this outcome.
[7] Serious study limitations: 41/170 (24%) patients with neurological sequelae at discharge were not available for assessment at day 28.
[8] No serious inconsistency: Not applicable as only one trial.
[9] No serious indirectness: This trial (Dondorp 2010) had 11 centres throughout Africa and used the standard dosing of artesunate and quinine. The nature of the neurological sequelae is not described.
[10] No serious Imprecision: The 95% CI around the absolute effect is narrow. The worst case scenario is a 1.2% increase in neurological sequelae at day 28
[11] Three trials (Dondorp 2010, Dondorp 2005, and Cao 1997) report this outcome
[12] Serious imprecision: The effect estimate is of a clinically important harm. However the 95% CI includes the possibility of no clinically important difference between the two interventions.
[13] No serious inconsistency: None of the trials found evidence of an important difference between the two treatment groups
[14] Serious imprecision: We were unable to pool the data as they were only reported as medians and range/intra quartile range. There is no evidence of a clinically important benefit with artesunate on this outcome.
[15] No serious imprecision: The result is statistically significant in favour of artesunate. The current sample size is adequately powered to detect a 40% risk reduction with 80% power and 95% confidence.

Overall, there was a reduced mortality in adults from severe malaria of about 40% (RR, 0.61; 95% CI, 0.50-0.75) and a reduced mortality in children of about 25% (RR, 0.76; 95% CI, 0.65-0.90). There was a small increase in neurologic sequelae in children treated with artesunate at the time of hospital discharge, most of which, however, slowly resolved with little or no difference between artesunate and quinine 28 days later.

2.6.6. Discussion on clinical efficacy

Design and conduct of clinical studies

GCP

The applicant considers that three studies in the dossier are pivotal, all of which met the ethical requirements of Directive 2001/20/EC but they were not conducted fully in accordance with GCP.

SEAQUAMAT (comparative using Guilin artesunate)
The sponsor (Wellcome Trust) provided the clinical protocol, blank case report forms and a legacy data file with subject level data, which allowed the applicant to construct a clinical study report (CSR) plus subject level data and listings. It was not possible to employ traditional GCP inspection methods for the sites during the trial and the sponsor was unwilling to give permission for a study audit by the US Army or any subsequent Regulatory Authority.

AQUAMAT (comparative using Guilin artesunate)
The study was sponsored by the Wellcome Trust and co-ordinated by the Mahidol-Oxford Tropical Medicine Research Unit in Bangkok, which provided logistic support and data management. The data from this study are available only in the 2010 publication. It was not possible to employ traditional GCP inspection methods for the duration of the trial. The sponsor denied requests for further patient level data, the data sets, the FSCR and permission to audit the AQUAMAT study.

R-CDC-060 (non-comparative, retrospective; used US Army artesunate)
The data presented in the CSR were compiled by the Department of the Army, in collaboration with the CDC. The principles of GCP were followed to the extent possible in both trial conduct and documentation procedures. The final CSR is not fully compliant with GCP due to the nature of the study and various methods of data collection across the sites involved.

Of the other studies in the dossier, four were conducted by the US Army – two PK studies and two efficacy studies in patients with uncomplicated malaria. Whilst these are not pivotal, the applicant explained that there were audits of 1128 and 1142 by a US-based CRO.

In summary, it is not possible to verify the degree of GCP compliance of SEAQUAMAT and AQUAMAT either retrospectively or by instituting GCP inspections. CDC-060 was conducted under CDC's IND but the degree of GCP compliance at all the sites that treated patients with IV artesunate cannot be verified.

Pivotal randomised controlled studies - *SEAQUAMAT* and *AQUAMAT*

These were both prospective, randomised trials that compared initial treatment with parenteral artesunate with widely recommended and well-established comparative regimens for treatment of severe *P. falciparum* malaria. Taken together, the two trials cover a wide age range. In general, the main features of the design of these studies seem to have been appropriate.

The open label design of these studies was due to the practical difficulties contingent on a double dummy approach, including the fact that in SEAQUAMAT the blind would have to encompass the different oral follow-on regimens (see below). Since the focus of the clinical comparison was on in-hospital mortality, the open label design does not threaten the conclusions on efficacy.

Both studies used the same parenteral artesunate regimen, with 2 doses of 2.4 mg/kg given at 0 and 12 h and then once daily dosing until oral medication was possible. Both studies also used the same initial comparative regimen, with a 20 mg/kg loading dose of quinine followed by 10 mg/kg q8h until oral treatment was possible. However, while AQUAMAT required that 24 h parenteral treatment was

given before an oral switch, there does not seem to have been a minimum duration of parenteral treatment required in SEAQUAMAT although the applicant's CSR reports that the mean number of doses of artesunate was 3.9.

It appears that SEAQUAMAT (based on the protocol from 2003 and the applicant's CSR but not mentioned in the publication) and AQUAMAT (mentioned in the publication) allowed IM as an alternative to IV administration of the initial parenteral treatment. However, it appears that no patients received IM dosing in SEAQUAMAT and only ~10% were dosed IM in AQUAMAT. The SmPC recommends only IV dosing, which is acceptable and appropriate.

There is an important difference between the two studies in terms of oral follow-on treatment. In SEAQUAMAT, parenteral artesunate was followed by oral artesunate and parenteral quinine was followed by oral quinine, both given with oral doxycycline unless this was contraindicated. The contribution of the oral follow-on regimens to the overall in-hospital mortality rates and to the other clinical endpoints cannot be determined in this study design. Furthermore, there was no on-treatment serial parasite counting conducted, which means that the initial impact of the two parenteral treatments on parasite density cannot be compared.

In contrast, all patients in AQUAMAT followed initial parenteral treatment with a full course or oral artemether-lumefantrine (Coartem). Coartem is highly effective for the *de novo* treatment of uncomplicated malaria and use of the same follow-on regimen in both treatment arms could be expected, if anything, to reduce the differences observed between randomised groups in terms of outcomes measured after oral switch. Therefore, any differences detected between the two randomised treatment arms likely reflect the impact on the initial parenteral treatment.

The primary efficacy endpoint in both studies was in-hospital all-cause mortality, which was thought to reflect death rates from the presenting episodes of severe malaria. This primary endpoint is acceptable. Based on this primary endpoint, both studies were planned with sample sizes to provide 80% power to show a reduction in mortality for initial parenteral treatment with artesunate vs. quinine. The estimated mortality rates reflected recent data relevant to the populations enrolled.

Randomisation was based on opening of sealed envelopes at study sites. This is not at all ideal but, given the timing of the studies and the distribution of sites, it likely reflects the only practical means available.

In both studies, the primary analysis was conducted in the ITT (all randomised) population and also in the PP population who had the rapid test for *P. falciparum* confirmed by finding asexual parasites in blood smears. Additional analyses were conducted in those who were determined retrospectively to meet the protocol-defined criteria for severe malaria and in other subgroups.

Studies with Artesunate Amivas

Dose and regimen - 1263 and EDCTP-MMV07-01

Study 1263 was conducted in 100 adults and children (actual age range 5-53 years) with uncomplicated *P. falciparum* malaria.

There was no active control arm but it compared four IV artesunate dose regimens in an open-label design. The results of effects on parasite load up to 48 h after the first dose allow for an assessment of the impact of the IV artesunate regimens.

EDTCP-MMV07-01 was conducted by the *Medicines for Malaria Venture* in children 6 months-10 years with severe malaria regardless of species. There was no active control arm but it compared 5 doses of 2.4 mg/kg within 72 h with 3 doses of 4 mg/kg within 48 h in a double blind design. The primary endpoint was the rate of 99% parasite clearance (PC99) at 24 h after the first dose (i.e. after 2 doses of 2.4 mg/kg or after 1 dose of 4 mg/kg).

1168
This uncontrolled 2006 study in adults with uncomplicated *P. falciparum* malaria evaluated 3 doses of IV artesunate 2.4 mg/kg q24h for initial treatment, followed by oral Malarone. Since the recommended regimen for severe malaria is to give 2 doses in the first 24 h and then daily doses, the data on initial effects on parasite counts are of interest.

CDC060
While this was a retrospective uncontrolled study, it provides some data on the use of the recommended IV artesunate dose regimen for initial treatment of malaria. The intent was to limit IV treatment to 4 doses of 2.4 mg/kg at 0 h, 12 h, 24 h and 48 h but extension of parenteral treatment was allowed if patients were unable to switch to one of the oral regimens as recommended by CDC. Not all patients entering this study necessarily had severe malaria and infections were not confined to P. falciparum. While the primary endpoint was safety, the study captured effects on treatment on parasite counts as well as the clinical outcomes.

Since this study was conducted in returning travellers, the importance for the application is that it describes outcomes in persons not likely to have any or, at least, any substantial pre-existing naturally acquired immunity to malaria.

Efficacy data and additional analyses

Pivotal randomised controlled studies

SEAQUAMAT
The great majority of the 1461 randomised patients (~95%) had *P. falciparum* confirmed by microscopy of blood smears and adults accounted for 1269/1461 patients. Therefore, the analysis of efficacy in the ITT population essentially reflects adults with complicated malaria, i.e. using the term *complicated* to refer to the need for initial parenteral treatment. The analysis of efficacy in the PP population essentially reflects adults with complicated *P. falciparum* malaria. Nevertheless, the indication sought is *treatment of severe malaria* and only about 70% of the ITT population met at least one of the criteria for severe malaria.
The second interim analysis, which assessed efficacy based on data up to February 2005 and which led to stopping the study in May 2005, included 1294 subjects (~70% had severe malaria), which was about half of the initial planned sample size of patients with confirmed severe malaria. In contrast, the number of deaths (244) in this total population was higher than had been projected initially at the time of trial completion. The applicant's CSR mentions that the p-value derived from the second interim analysis was 0.0007 and that the test statistic would have crossed the stopping boundary had either the O'Brien-Fleming or the Haybittle-Peto procedure been applied.

In contrast, the publication of this study describes results for the 1461 patients enrolled when the study was stopped, including 271 deaths. The applicant's CSR is based on re-analyses of data from the total 1461 patients. The applicant states that the treatment effect estimate obtained from this later database was less than that observed in the interim analysis that led to stopping the study but the details of the second interim analysis were not published and are not available to the applicant.

In the ITT and PP populations, the in-hospital death rates were statistically significantly lower in the artesunate group, with an absolute difference between groups of 7-8 percentage points. Both analyses indicated that the relative risk of dying in the artesunate group was about 2/3 of that in the quinine group with upper bounds of the 95% confidence intervals that did not exceed 0.82. With so few patients having neurological sequelae, the comparison of the combined death and sequelae rates was driven by the death rates.

The benefit of parenteral artesunate on risk of mortality was evident within the first 24 h of starting treatment although most of the treatment effect reflected reduction in mortality after the first 24-48 h on study. This finding is important since, in this study, the two treatment groups had separate oral follow-on regimens (i.e. artesunate or quinine as per initial assignment), which would have contributed to the overall survival and clinical response rates.

Severe malaria was confirmed for 509 (70%) in the artesunate group and 541 (74%) in the quinine group. The mortality rates in those with severe disease were 19.8% with artesunate compared to 28.1% with quinine, which again showed a statistically significant benefit.

The mortality rates varied significantly between countries (from 9.3% in Indonesia to 28% in Bangladesh), which the publication mentions may have reflected availability of intensive care. Nevertheless, the absolute risk reduction in each country associated with artesunate vs. quinine treatment was in the range from 5-9 percentage points.

AQUAMAT
The final study population was more homogeneous than that in SEAQUAMAT, being confined to African children aged from 18 months to <5 years. Although about one third had received potentially effective antimalarial treatment for the presenting episode, the condition of the children at study baseline indicates that the treatment was not controlling the disease. There were no important differences between treatment groups in baseline host or disease characteristics. Thus, as in SEAQUAMAT, it appears that the method of randomisation was adequate. As expected, the mean and range of baseline parasitaemia was even higher in these children compared to the population enrolled into SEAQUAMAT.

The ITT population death rates in both treatment groups were lower compared to SEAQUAMAT, being 8.5% vs. 10.9%, representing a statistically significant benefit for starting treatment with artesunate rather than quinine when each was followed by a complete and highly effective oral regimen with Coartem. A very similar result was obtained in the PP population with confirmed *P. falciparum*. Importantly, the mortality rates in the subset that met the pre-defined criteria for severe malaria, which comprised ~85% of the total 5425 randomised, were 9.9% for artesunate and 12.4% for quinine, which was a statistically significant difference (p=0.0055). Moreover, in contrast to SEAQUAMAT, the survival curves diverged from the time of the first dose onwards.

As in SEAQUAMAT, on-treatment parasite counts were not determined. See the discussion of safety regarding neurological sequelae.

<u>Studies with Artesunate Amivas</u>

Dose and regimen - 1263 and EDCTP-MMV07-01
In 1263 in uncomplicated falciparum malaria, all of the IV artesunate regimens resulted in PC90 by 32 h. While the PCT90 was shorter with the 3x4.8 mg/kg dose vs. the 4x2.4 mg/kg, all except one patient had total clearance by 72 h. Although there seemed to be slightly slower clearance in Thailand vs. Kenya, the parasite reduction ratios were not statistically significantly different from 24 h onwards. Overall, 3 or 4 doses of 2.4 mg/kg in the first 48 h appeared to be highly effective and no patients died in this study.

In children with severe malaria, the MMV study indicated a numerical advantage for the 4 mg/kg dose group based on the primary endpoint of PC99 at 24 h. However, the median time to PC100 was 36 h in both dose groups and there were only 6-h differences between regimens for the median times to PC90 and PC99. Also, the median FCT was 12 h in both groups. Two patients in the 4 mg/kg group died. One died on day 2 for unknown reason and the other died on day 1 following a grand mal convulsion. It

was concluded from the overall results and the age subgroup analyses that both regimens were suitable for the initial treatment of severe malaria in the age range studied.

1168
The mean baseline *P. falciparum* parasitaemia in the 30 adults with uncomplicated malaria was 23,484. The median was 5,938 and the range was from 405-270,515. The mean parasite reduction ratio at 48 h for the 30 evaluable subjects was 99.998%, supporting the adequacy of initial treatment with 3 IV artesunate doses of 2.4 mg/kg at 24-h intervals in adults.

CDC-060
This study is not pivotal. The results must be viewed with much caution, not least due to the number and types of protocol deviations, including concomitant antimalarial agents during the IV treatment phase. Nevertheless, the baseline features indicate that a substantial proportion of the patients were severely unwell at the time of starting artesunate. This is underlined by the fact that 5 patients died before completing 4 doses of artesunate.

While the applicant reports median times to negative parasitology for the safety, evaluable and PP populations (as defined in the CSR), with point estimates from 42-50 h, these numbers are based on populations that included those who received other antimalarial agents with IV artesunate. Thus, it should be noted that the 38/87 patients in the evaluable analysis population who received concurrent antimalarial treatments during IV artesunate administration did not show a statistically significant difference in time to negative parasitology compared with the 49/87 who received IV artesunate alone. However, there was a numerical difference (43 h vs. 51 h, respectively; p=0.942). In the 49 with no concomitant antimalarial agent during IV artesunate administration, 39/49 (80%) transitioned to oral antimalarial therapy with a mean time to initiation of 3.8 days (range 2 to 5 days).

The intent of the labelling is that IV artesunate should be given alone until such time that a switch to an appropriate oral follow-on regimen is possible. To support this usage in returning travellers, the applicant compared all the efficacy endpoints for the subsets of the evaluable population (this being the population of most interest) that did (n=38) and did not (n=49) receive concomitant antimalarial agents during IV artesunate treatment. There was no evidence of a benefit for concomitant administration of another antimalarial agent with IV artesunate.

2.6.7. Conclusions on the clinical efficacy

Efficacy in the initial treatment of severe malaria

The two pivotal trials were conducted in populations that differed in age range and geographical distribution. Different approaches were taken to the oral follow-on treatment and only retrospectively defined subsets met the pre-defined criteria for severe malaria, these subsets being most relevant to the indication statement. Nevertheless, both studies gave results that support the proposed IV artesunate dose regimen for the initial treatment of severe *P. falciparum* malaria in children aged from ~18 months, adolescents and adults.

Inevitably, these studies were conducted in endemic areas where some degree of partial immunity to malaria is acquired as subjects get older. It is important that the AQUAMAT study was confined to children aged < 5 years with substantial parasite counts at baseline and in whom very limited naturally acquired immunity would be expected. In this study, in which all children received the same highly effective oral follow-on regimen, initial treatment with parenteral artesunate was more effective than initial treatment with parenteral quinine in terms of in-hospital mortality. In SEAQUAMAT, in which

most subjects were adults, parenteral followed by oral artesunate was more effective than parenteral followed by oral quinine in terms of in-hospital mortality. These studies support the efficacy of initial treatment of severe *P. falciparum* malaria with IV artesunate.

The four efficacy studies that used the US Army artesunate had no active control arms. Two were confined to uncomplicated malaria (1263 and 1168), two compared different IV artesunate regimens (1163 and EDCTP-MMV07-01) and one was a retrospective collation of clinical cases (CDC-060). The most informative of these studies was not sponsored by the US Army but by MMV. This African study provided a direct comparison of 5 doses of 2.4 mg/kg with the first two doses given 12 h apart and 3 doses of 4 mg/kg given 24 h apart. The effect on parasite counts supported use of either dose regimen in children aged 6 months to 10 years with severe *P. falciparum* malaria.

The indication is not specific to *P. falciparum* malaria. Inevitably, almost all of the clinical data pertain to treatment of severe *P. falciparum* since this species is responsible for the vast majority of severe malaria cases. The in-vitro data support lack of speciation in the indication.

The clinical trial efficacy data only support use from ~18 months and there is very limited published information on use in infants, reflecting the fact that severe malaria in the first 6 months of life is unusual. However, severe malaria does occur occasionally in this age group and it is possible that EU travellers could take very young infants to endemic regions to visit families. Therefore, there is no *a priori* objection to an indication from birth. Hence, it is agreed with the applicant that a single dose recommendation of 2.4 mg/kg may be applied regardless of age. There are no clinical safety and efficacy data that point to the need for a higher dose in children < 20 kg. The SmPC reflects the uncertainties, but for the life-threatening condition there is no known reason to preclude use below a certain age.

2.6.8. Clinical safety

2.6.8.1. Patient exposure

There were 282 subjects/patients who received at least one dose of the US Army IV artesunate in Phase 1 (n=50), Phase 2 (n=130) or in CDC-060 up to 2010 (n=102). Overall, 117/282 (41.5%) had total IV artesunate exposure equivalent to the recommended regimen (2.4 mg/kg × 4 doses or total exposure of 9.6 mg/kg), while 128 (45.4%) received less and 37 (13.1%) received more than the recommended regimen. However, 60/128 who received less than the recommended regimen did receive 2.4 mg/kg × 3 doses (total exposure of 7.2 mg/kg). The applicant also reports additional safety information from CDC-060 during 2011 to 2016.

Table 29: Studies Sponsored by the Army or CDC and Providing Safety Data

Study Identifier	Study Design	Population (location)	Sample Size	Dose Regimens
1128	Randomised, double-blind, placebo-controlled, single dose escalation study	Healthy subjects (United States)	IV AS, N=30 n=6 per arm Placebo, N=10	IV AS 0.5, 1.0, 2.0, 4.0, or 8.0 mg/kg or placebo IV single dose
1142	Randomised, double-blind, placebo-controlled,	Healthy subjects (United States)	IV AS, N=20 2.0 mg/kg, N=6; 4.0 mg/kg ,	IV AS 2.0, 4.0, or 8.0 mg/kg or placebo IV once daily for 3 days

Study Identifier	Study Design	Population (location)	Sample Size	Dose Regimens
	multiple dose escalation study		N=7; 8.0 mg/kg, N=7 Placebo, N=6	
1168	Non-randomised, open-label study	Adults with uncomplicated falciparum malaria (Kenya)	N=30	IV AS 2.4 mg/kg once daily for 3 days Additional follow-on oral treatment
1263ab	Randomised, open-label, dose-ranging study	Adults and children with uncomplicated falciparum malaria (Thailand, Kenya)	IV AS, N=100 n=25 per arm	IV AS 1.2, 2.4, or 4.8 mg/kg once daily for 3 days or IV AS 2.4 mg/kg at 0, 12, 24, and 48 hours Additional follow-on oral treatment
R-CDC-060	Retrospective study	Adults and children with severe or complicated malaria (United States)	N=102 who received IV AS	IV AS 2.4 mg/kg at 0, 12, 24, and 48 hours

EDCTP-MMV07-01 in 194 children provides supportive safety data for the US Army product.

Added to these studies, after reviewing 31 trials identified by a 2013 meta-analysis of publications from 1992-2012, 8 prospective randomised trials were considered useful to assess safety. Most of the data across these 8 trials came from SEAQUAMAT and AQUAMAT and only two others (Cao, 1997 and Newton, 2003) described AEs.

2.6.8.2. Adverse events

Cohort of 282 treated with US Army IV artesunate

In Study 1128, the most frequently reported treatment emergent AEs (in at least 10%) across all dose groups were nervous system disorders including dizziness (33%), dysgeusia (33%) and headache (17%). Dizziness and dysgeusia appeared to be dose-related. All AEs except for one were graded as mild in severity. The exception occurred in 8.0 mg/kg dose group and concerned a moderate allergic reaction (urticaria) that started on the day of dosing and resolved the same day after treatment with diphenhydramine.

In Study 1142 AEs reported by more than 10% in any dose group included nervous system disorders (dysgeusia, headache, dizziness, paresthesia and somnolence), anaemia and respiratory tract infection. Of these AEs, only dysgeusia appeared to be dose-dependent. From 5/7 to 6/6 in the artesunate dose groups had at least one AE considered drug-related. All AEs reported at least possibly related to drug were graded as "mild" in severity.

Phase 2 studies

Overall, 128/130 (98%) patients with uncomplicated malaria experienced at least one AE. Anaemia affected 20%-43.3% of patients across the dose groups while leukopenia affected up to 32%. In study 1263ab some degree of change in RBCs, WBCs and platelets occurred in all patients who received

artesunate. Typically, haematologic parameters were low at study entry, were followed by hyper-response and then decreased again, ultimately returning to approximately normal. The RBC changes were considered typical of malaria patients and were attributed to the disease. Non-haematologic AEs reported with all artesunate regimens included abdominal pain, vomiting, diarrhoea, pyrexia, headache, dizziness, cough and rhinorrhoea.

Most AEs (75.7%-86.3%) were of mild intensity. The highest percentage of severe AEs (6.8%) occurred with the highest dose (14.4 mg/kg) in Study 1263ab. Most of the severe AEs (17/23; 73.9%) were blood and lymphatic system disorders. In particular, neutropenia affected 60% of patients who received 4.8 mg/kg daily × 3 days and 20% had severe neutropenia. Thrombocytopenia was reported in 44.0% of patients at the highest IV AS dose.

Table 30: Severe Adverse Reported in Patients with Uncomplicated Malaria in Phase 2 Trials, by Increasing IV AS Exposure

Total IV AS Exposure	**3.6 mg/kg (n=25)**	**7.2 mg/kg (n=55)**		**9.6 mg/kg (n=25)**	**14.4 mg/kg (n=25)**
Artesunate Dosing Regimen	1.2 mg/kg daily × 3 days at 0, 24, and 48 h	2.4 mg/kg daily × 3 days at 0, 24, and 48 h		2.4 mg/kg at 0, 12, 24, and 48 h	4.8 mg/kg daily × 3 days at 0, 24, and 48 h
Study	1263ab	1168 (n=30)	1263ab (n=25)	1263ab	1263ab
Number (%) of subjects with:					
Any SAE	5 (20.0)	6 (20.0)	2 (8.0)	2 (8.0)	7 (28.0)
Blood and lymphatic system disorders	5 (20.0)	5 (16.6)	1 (4.0)	0	6 (24.0)
Anemia	1 (4.0)	1 (3.3)	0	0	0
Neutropenia	4 (16.0)	4 (13.3)	0	0	5 (20.0)
Thrombocytopenia	0	0	1 (4.0)	0	1 (4.0)
General Disorders and Administration Site Conditions	0	0	1 (4.0)	0	1 (4.0)
Pyrexia	0	0	1(4.0)	0	1 (4.0)
Infections and Infestations	0	0	0	1 (4.0)	0
Plasmodium malaria infection	0	0	0	1 (4.0)	0
Nervous System Disorders	0	0	0	1 (4.0)	0
Headache	0	0	0	1 (4.0)	0
Skin and Subcutaneous Tissue Disorders	0	1 (3.3)	0	0	0
Stevens-Johnson Syndrome	0	1 (3.3)	0	0	0

Six AEs were considered possibly related to artesunate, of which 3 concerned infusion/injection site pain and there was one report each of hypotension, vomiting and eosinophilia. Another 4 AEs were considered definitely related to artesunate and concerned eosinophilia and dysgeusia, neither of which showed a dose-dependent trend.

Study CDC-060

Overall, 94/102 patients [92.2%] experienced at least one AE and the most frequently reported AEs were those associated with severe malaria (i.e. anaemia, LFTs increased and thrombocytopenia).

Table 31: Study R-CDC-060, Most Frequently Reported AEs (in ≥ 5%)

System Organ Class/ Preferred Term, n (%)	Occurrence[a]	Safety (N=102)
At Least One AE	94/102 = 92.2% [85.3%, 96.0%]	94 (92)
Blood and Lymphatic System Disorders	75/102 = 73.5% [64.2%, 81.1%]	75 (74)
Anaemia		66 (65)
Thrombocytopenia		18 (18)
Leukocytosis		10 (10)
Lymphopenia		7 (7)
Neutropenia		5 (5)
Investigations	52/102 = 51.0% [41.4%, 60.5%]	52 (51)
Transaminases increased		28 (27)
AST increased		22 (22)
Respiratory, Thoracic and Mediastinal disorders	20/102 = 19.6% [13.1%, 28.3%]	20 (20)
Acute respiratory distress syndrome		8 (8)
Hepatobiliary Disorders	17/102 = 16.7% [10.7%, 25.1%]	17 (17)
Hyperbilirubinaemia		14 (14)
Renal and Urinary Disorders	13/102 = 12.7% [7.6%, 20.5%]	13 (13)
Renal failure acute		10 (10)
Vascular Disorders	8/102 = 7.8% [4.0%, 14.7%]	8 (8)
Gastrointestinal Disorders	8/102 = 7.8% [4.0%, 14.7%]	8 (8)
Cardiac Disorders	6/102 = 5.9% [2.7%, 12.3%]	6 (6)
Infections and Infestations	7/102 = 6.9% [3.4%, 13.5%]	7 (7)
Metabolism and Nutrition Disorders	7/102 = 6.9% [3.4%, 13.5%]	7 (7)

Of the 278 AEs reported, there were 69 (25%) assessed as Grade 1 in severity, 83 (30%) as Grade 2, 70 (25%) as Grade 3 and 56 (20%) as Grade 4. The 7 fatal AEs were designated as Grade 5.

Table 32: CDC-060: Grade 3 and Grade 4 AEs and AES that Resulted in Death

System Organ Class/ Preferred Term	Grade 3	Grade 4	Total Grade 3 and Grade 4 AEs	Grade 5 AEs that Resulted in Death
All Reported AEs of this Severity	70	56	126	7
Blood and Lymphatic System Disorders	30	23	53	0
Anaemia	20	13	33	0
Thrombocytopenia	6	6	12	0
Lymphopenia	3	1	4	0

System Organ Class/ Preferred Term	Grade 3	Grade 4	Total Grade 3 and Grade 4 AEs	Grade 5 AEs that Resulted in Death
Disseminated intravascular coagulation	0	2	2	0
Neutropenia	1	0	1	0
Leukocytosis	0	1	1	0
Investigations	15	3	18	0
Transaminases increased	10	2	12	0
AST increased	4	0	4	0
Blood creatinine increased	1	0	1	0
Pulse absent	0	1	1	0
Hepatobiliary Disorders	9	6	15	0
Hyperbilirubinaemia	9	4	13	0
Cholecystitis	0	1	1	0
Acute hepatic failure	0	1	1	0
Respiratory, Thoracic and Mediastinal Disorders	1	11	12	1
Acute respiratory distress syndrome	1	6	7	1
Respiratory distress	0	2	2	0
Pneumonia aspiration	0	1	1	0
Pulmonary hemorrhage	0	1	1	0
Pneumothorax	0	1	1	0
Renal and Urinary Disorders	6	3	9	0
Renal failure acute	5	3	8	0
Oliguria	1	0	1	0
Infections and Infestations	1	3	4	0
Lung infection	1	0	1	0
Sepsis syndrome	0	1	1	0
Septic shock	0	1	1	0
Fungaemia	0	1	1	0
Metabolism and Nutrition Disorders	2	1	3	0
Metabolic acidosis	1	0	1	0
Generalized edema	1	0	1	0
Hyperkalemia	0	1	1	0
Psychiatric Disorders	2	1	3	0
Delirium	1	0	1	0
Mental status changes	1	1	2	0
Cardiac Disorders	2	1	3	2
Cardiac arrest	0	0	0	2
Cardiac failure congestive	0	1	1	0
Atrial fibrillation	1	0	1	0
Cardiomyopathy	1	0	1	0
Nervous System Disorders	0	3	3	1
Brain oedema	0	2	2	1
Depressed level of consciousness	0	1	1	0
Vascular Disorders	2	1	3	0
Hypotension	0	1	1	0
Peripheral ischaemia	2	0	2	0

System Organ Class/ Preferred Term	Grade 3	Grade 4	Total Grade 3 and Grade 4 AEs	Grade 5 AEs that Resulted in Death
General Disorders and Administration Site Conditions	0	0	0	1
Multi-organ failure	0	0	0	1
Injury, Poisoning, and Procedural Complications	0	0	0	2
Brain herniation	0	0	0	2

Of the 66 reports of anaemia, 20 (30%) were Grade 3 and 13 (20%) were Grade 4. Also, blood and lymphatic system disorders accounted for the highest percentage of Grade 3 and 4 AEs (53/126, or 42.1%) of any SOC, followed by Investigations (18 or 14.3%). Among the 57 reports of anaemia considered related, the relationship was unlikely for 38 and possible for 19, with none considered of probable or definite relationship. The two AEs assessed as probably related were Grade 3 increases in hepatic transaminases in one patient and a Grade 1 increase in AST in the other. No AEs were considered definitely related to artesunate.

Table 33: Study R-CDC-060: CBEC Attributions of Causality for the Three Most Frequently Reported Adverse Events

Adverse Event (No. reported)	Causality According to Data Analysis Definition		Relationship to IV AS as Determined by CBEC				
	Unrelated, n (%)	Related, n (%)	Not Related n (%)	Unlikely n (%)	Possibly n (%)	Probably n (%)	Definitely n (%)
Anemia (n=66)	9 (14)	57 (86)	9 (14)	38 (58)	19 (29)	0	0
Increased transaminases (n=28)	3 (11)	25 (89)	3 (11)	8 (29)	16 (57)	1 (4)	0
Increased AST only (n=22)	3 (14)	19 (86)	3 (14)	5 (23)	13 (59)	1 (5)	0

EDCTP –MMV07-01

At least one AE was reported for 133/194 children (68.6%), including 68 in Group A (5 doses; 68.0%) and 65 in group B (4 doses; 69.1%) (p=0.878). In contrast to CDC-060, the two most common AEs were infections and infestations (29% Group A; 38% Group B) and gastrointestinal disorders (22%; 24%). Anaemia was reported in 19% in Group A and 11% in Group B). Thrombocytopenia, leukocytosis, lymphopenia, neutropenia and transaminases increased were not observed.

Table 34: AEs (in ≥ 5%) by SOC and PT in CDC-060 versus EDCTP-MMV07-01

	R-CDC-060	EDCTP-MMV07-01	
System Organ Class/ Preferred Term, n (%)	**(N=102)**	**Group A (N=100)**	**Group B (N=94)**
IV AS Regimen	2.4 mg/kg at 0, 12, 24, and 48 h	2.4 mg/kg at 0, 12, 24, 48, and 72 h	4.0 mg/kg at 0, 24 and 48 h
At Least One AE	94 (92)	68 (68)	65 (69)
Blood and Lymphatic System Disorders	75 (74)	23 (23)	14 (15)
Anaemia	66 (65)	19 (19)	10 (11)
Thrombocytopenia	18 (18)	0	0
Leukocytosis	10 (10)	0	0
Lymphopenia	7 (7)	0	0
Neutropenia	5 (5)	0	0

	R-CDC-060	EDCTP-MMV07-01	
System Organ Class/ Preferred Term, n (%)	**(N=102)**	**Group A (N=100)**	**Group B (N=94)**
Splenomegaly	0	4 (4)	5 (5)
Investigations	52 (51)	2 (2)	1 (1)
Transaminases increased	28 (27)	0	0
Aspartate aminotransferase (AST) increased	22 (22)	0	0
Respiratory, Thoracic and Mediastinal Disorders	20 (20)	21 (21)	18 (19)
Acute respiratory distress syndrome	8 (8)	2 (2)	0
Cough	0	14 (14)	14 (15)
Hepatobiliary Disorders	17 (17)	1 (1)	2 (2)
Hyperbilirubinaemia	14 (14)	0	0
Renal and Urinary Disorders	13 (13)	4 (4)	5 (5)
Renal failure acute	10 (10)	0	0
Vascular Disorders	8 (8)	6 (6)	10 (11)
Phlebitis	0	5 (5)	8 (9)
Gastrointestinal Disorders	8 (8)	22 (22)	23 (24)
Abdominal pain	1 (1)	8 (8)	12 (13)
Abdominal distension	1 (1)	7 (7)	3 (3)
Diarrhea	3 (3)	5 (5)	2 (2)
Vomiting	1 (1)	5 (5)	0
Infections and infestations	7 (7)	29 (29)	36 (38)
Bronchitis	0	6 (6)	5 (5)
Upper respiratory tract infection	0	1 (1)	6 (6)
Malaria	0	5 (5)	6 (6)
General Disorders and Administration Site Conditions	1 (1)	14 (14)	10 (11)
Pyrexia	0	12 (12)	6 (6)
Nervous System Disorders	3 (3)	5 (5)	6 (6)
Convulsion	0	4 (4)	2 (4)
Cardiac Disorders	6 (6)	2 (2)	0
Metabolism and Nutrition Disorders	7 (7)	3 (3)	1 (1)

SEAQUAMAT and AQUAMAT

The applicant coded the raw data from SEAQUAMAT provided by the Wellcome Trust in accordance with MedDRA preferred terms and summary statistics were prepared. The frequency of AEs was generally similar between the IV artesunate and IV quinine groups.

Table 35: SEAQUAMAT Summary of Adverse Events that Occurred In ≥5% of Patients

	After Hospital Admission and Initiation of Treatment		At Hospital Discharge	
System Organ Class/Preferred Term	**IV Artesunate n=730**	**IV Quinine n=731**	**IV Artesunate n=730**	**IV Quinine n=731**
Number of Subjects with at Least one AE	296 (40.5%)	339 (46.4%)	39 (5.3%)	27 (3.7%)
Renal and urinary disorders	178 (24.4%)	178 (24.4%)	3 (0.4%)	1 (0.1%)
Renal failure	140 (19.2%)	157 (21.5%)	3 (0.4%)	0
Haemoglobinuria	49 (6.7%)	32 (4.4%)	0	0
Infections and infestations	107 (14.7%)	129 (17.6%)	4 (0.5%)	4 (0.5%)
Sepsis	61 (8.4%)	76 (10.4%)	0	0

	After Hospital Admission and Initiation of Treatment		At Hospital Discharge	
System Organ Class/Preferred Term	**IV Artesunate n=730**	**IV Quinine n=731**	**IV Artesunate n=730**	**IV Quinine n=731**
Nervous system disorders	77 (10.5%)	94 (12.9%)	9 (1.2%)	3 (0.4%)
Coma	54 (7.4%)	68 (9.3%)	0	0
Seizure	31 (4.2%)	45 (6.2%)	0	0
Respiratory, thoracic and mediastinal disorders	37 (5.1%)	55 (7.5%)	1 (0.1%)	0
Respiratory failure	26 (3.6%)	39 (5.3%)	0	0
Vascular disorders	39 (5.3%)	47 (6.4%)	0	0
Blood and lymphatic system disorders	20 (2.7%)	21 (2.9%)	7 (1.0%)	5 (0.7%)
Anaemia	20 (2.7%)	21 (2.9%)	6 (0.8%)	5 (0.7%)

AEs reported in the SEAQUAMAT and AQUAMAT trials showed little comparability to those in CDC-060.

Table 36: AEs in CDC-060 vs. AQUAMAT

AE MedDRA System Organ Class and Preferred Term	Percent of Patients in Study R-CDC-060	Percent of Patients in AQUAMAT	
	IV AS (n=102)	**Artesunate N=2712**	**Quinine N=2713**
Blood and Lymphatic Tissue Disorders			
Acute anaemia	65%	5.7%	4.6%
Nervous System Disorders			
Coma	0	5.1%	3.5%
Convulsion	0	10.1%	8.3%
Infections and Infestations			
Blackwater fever	0	0.7%	1.2%
Investigations			
Hypoglycemia	0	2.8%	1.8%
Ear and Labyrinth Disorders			
Tinnitus	0	0	0.3%

Specific types of AEs

Haematological

In Phase 1 studies, the incidence of anaemia was 50% in those who received a multi-dose IV artesunate vs. 10% after a single dose. Anaemia occurred in 20.0%-43.3% in Phase 2 studies and in 64.7% of patients in Study CDC-060.

When patients with severe malaria receive effective treatment, Hb initially falls and typically reaches a nadir on Day 3 to Day 7 before rising to stabilise at about 6 weeks. This pattern was observed in CDC-060, where the low mean Hb value of 10.3 g/dL at baseline fell to a nadir of 8.6 g/dL (mean decrease of 16.5%) on Day 5 and Day 6, and then edged upward to 8.7 g/dL on Day 7. The observed drop in Hb took place during the period of parasite clearance (range 36.7-57.1 h to achieve total elimination of parasites on blood smears, which is consistent with the pathogenic mechanisms of "typical" malarial anaemia associated with *P. falciparum* infection. The drop in Hb prior to Day 7 was also consistent with

the initial pattern of decline (i.e. drop of 16%-21% before Day 8) that was seen in a European study of anaemia occurring in travellers with severe *Pf* treated with IV artesunate in France.

Thrombocytopenia was not reported in Phase 1 but occurred as an AE in up to 44% in Phase 2 and in 17.7% in CDC-060, suggesting that it was an effect of the malaria disease process. Thrombocytopenia is a very common finding in malaria and the study findings were consistent with the literature. Furthermore, as patients with severe malaria in CDC-060 improved over time, their platelet counts quickly improved from a median value of 42,500/µL at baseline to 101,000/µL by Day 3 and 219,500/µL at Day 7. All cases of thrombocytopenia in CDC-060 were considered unrelated or unlikely related to IV artesunate.

Neutropenia was reported in 5% in Phase 1 and 4.9% in CDC-060 vs. up to 60% of patients in the Phase 2 studies, primarily with IV artesunate doses >2.4 mg/kg daily. Although decreases in WBCs (lymphopenia or neutropenia) were reported in 12/102 (11.8%) patients in CDC-060, only 1 case of lymphopenia was considered life threatening. All 5 cases of neutropenia and 2 of the 7 cases of lymphopenia were considered possibly related to IV artesunate.

Reticulocyte count is the haematological parameter that is most consistently found to be altered by IV artesunate dosing across studies.

As a drug class, the artemisinins have shown direct inhibitory effects on human erythroid precursors *in vitro* and can inhibit bone marrow responses (predominantly affecting red blood cell precursors) in animal models. Animal pre-clinical data have suggested that reversible reticulocytopenia might be a common side effect of IV artesunate, and this effect was confirmed in the initial Phase 1 studies, where reticulocytopenia occurred in healthy volunteers after IV artesunate administration.

For example, in 1142 with IV artesunate dosed at 2, 4, or 8 mg/kg for 3 days, mild to moderate dose-dependent reticulocytopenia was observed after the third dose in healthy subjects. A reticulocyte count nadir was reached in all dose cohorts by Day 7, but this effect reversed by Day 10. The reticulocyte count was not assessed in 1168. In study 1263ab in uncomplicated malaria in Kenyan and Thai adults and children there was a slight decrease in reticulocyte count on Day 3 in patients receiving the two higher IV artesunate doses (2.4 and 4.8 mg/kg).

In summary, consistent with *in vitro* observations, a short-lived decrease in reticulocyte count following artesunate exposure has been documented in nonclinical studies and in clinical studies of IV artesunate. Transient dose-dependent decreases in reticulocytes were seen in healthy subjects in the Phase 1 studies and patients with uncomplicated malaria also experienced this effect.

Post-artemisinin delayed haemolysis (PADH)

PADH has been described primarily in patients from non-endemic areas who acquire malaria while travelling, with rates from < 5% to 27% in different studies. The mean observed drop in Hb is ~30% and this occurs at a mean of 16.3 ± 5.5 days after start of treatment. Affected patients present with high mean parasitaemia (20.5% ± 10.9%) that clears in a mean of 4.1 ± 2.3 days following treatment with artesunate. Almost all patients have elevated LDH and low or undetectable haptoglobin. In many reported cases of PADH there are potential confounding causes of haemolysis and/or anaemia including G6PD deficiency, HIV co-infection, transfusions, immune-mediated processes and haemolysis-related concomitant medications. Although patients survive PADH, from 5-50% need transfusions. In CDC-060, 2/102 (2%) patients were assessed as showing the AE of "haemolysis" but their presentation was not consistent with PADH.

Hepatic

No AEs of hepatobiliary disorders or LFT abnormalities were reported in Phase 1. In Phase 2 studies there were sporadic reports of jaundice, hepatomegaly or ALT increased in <4% per dose group. In

CDC-060, hepatobiliary disorders AEs were reported in 17/102 (17%) patients, of which most (14/17) were hyperbilirubinaemia (one of the recognised features of severe malaria). Four of the 14 cases of hyperbilirubinemia were assessed as Grade 4 (life-threatening/SAE) while 9 were assessed as Grade 3 (severe) but only 4 were considered probably related to IV artesunate and none was considered definitely related. One patient (a 31-year-old male) developed Grade 4 acute hepatic failure. He was jaundiced at baseline, with hyperparasitaemia, impaired consciousness, circulatory collapse, acidosis, acute renal failure and abnormal bleeding/DIC. Onset of the patient's acute hepatic failure was on Day 2 and he expired on Day 3. Overall, the observed elevations in bilirubin and hepatic transaminases were considered related to malaria and its sequelae.

Table 37: Study R-CDC-060: Summary of Hepatic Adverse Events by Severity and Causality

Adverse Event	No. Patients Reporting this AE	AE Severity, n (%)				AE Relationship to IV AS, n (%)		
		Grade 1 (Mild)	Grade 2 (Moderate)	Grade 3 (Severe)	Grade 4 (Life-Threatening)	Not Related or Unlikely Related	Possibly Related	Probably Related
Hepatobiliary Disorders	17							
Hyperbilirubinaemia	14	0	1 (7.1)	9 (64.3)	4 (28.6)	7 (50.0)	3 (21.4)	4 (28.6)
Cholecystitis	2	1 (50.0)	0	0	1 (50.0)	2 (100.0)	0	0
Acute hepatic failure	1	0	0	0	1 (100.0)	1 (100.0)	0	0
Investigations	50							
Transaminases increased	28	3 (10.7)	8 (28.6)	10 (35.7)	2 (7.1)	11 (39.3)	16 (57.1)	1 (3.6)
AST increased	22	9 (40.9)	9 (40.9)	4 (18.2)	0	8 (36.4)	13 (59.1)	1 (4.5)

Nervous system effects

Nervous system disorders were reported in 3 (2.9%) patients in Study CDC-060, including 2 reports of brain oedema and 1 report of impaired consciousness that were Grade 4 but considered unrelated to IV artesunate. Across AQUAMAT, SEAQUAMAT, EDCTP-MMV07-01 and CDC-060, the most frequently reported nervous system disorders were coma and seizure or convulsion, with incidences slightly higher in the quinine group than the artesunate group. In SEAQUAMAT, 10.5% of artesunate-treated patients developed neurological defects after admission but only 1.2% had defects that persisted at discharge compared to 12.9% and 0.4%, respectively, in the quinine group.

Content of section 4.8 of the SmPC

To support the final list of ADRs, the applicant provided a synthesis of ADRs across US army and other studies from which data could be obtained. This is shown in the table below.

Table 38: Summary of AEs Considered Drug Related by Study Definition of Studies that Used Army Product

	Adults					Children	
	Healthy Volunteers		Uncomplicated *P. falciparum* Malaria		Severe Malaria	Severe Malaria	
MedDRA System Organ Class/ Preferred Term	1129 2 mg/kg to 8 mg/kg – (N=18)	1142 2 to 8 mg/kg Overall (N=20	1168 Artesunate 2.4 mg/kg (N=30)	1263ab 1.2 mg/kg to 4.8 mg/kg (N=100)	CDC-060 2.4 mg/kg (N=102)	EDCTP-MMV07-01 (N=194)	All Studies (N=464)
Blood And Lymphatic System Disorders	2 (11%)	6 (30%)	18 (60.0%)	44 (44%)	50 (49.0%)	21 (15.8%)	141 (30.4%)
Anaemia	2 (11%)	2 (10%)	8 (26.7%)	19 (19%)	42 (41.2%)	18 (13.5%)	91 (19.6%)
Disseminated intravascular coagulation					2 (2.0%)		2 (0.4%)
Eosinophilia			1 (3.3%)	3 (3%)			4 (0.9%)
Leukopenia		1 (5%)	2 (6.7%)	12 (12%)			15 (3.2%)
Leukocytosis				1 (1%)	2 (2.0%)		3 (0.6%)
Lymphocytosis				1 (1%)			1 (0.2%)
Lymphopenia			1 (3.3%)		4 (3.9%)		5 (1.1%)
Neutropenia		1 (5%)	16 (53.3%)	17 (17%)	5 (4.9%)		39 (8.4%)
Splenomegaly						3 (2.3%)	3 (0.6%)
Thrombocytopenia				4 (4%)	3 (2.9%)		7 (1.5%)
Thrombocythaemia				2 (2%)			2 (0.4%)
Cardiac Disorders			6 (20.0%)		3 (2.9%)		9 (1.9%)
Atrial fibrillation					1 (1.0%)		1 (0.2%)
Bradycardia			5 (16.7%)		1 (1.0%)		6 (1.3%)
Cardiac failure congestive					1 (1.0%)		1 (0.2%)
Cardiomyopathy					1 (1.0%)		1 (0.2%)
Tachycardia			1 (3.3%)				1 (0.2%)
Ear And Labyrinth Disorders						1 (0.8%)	1 (0.2%)
Haematotympanum						1 (0.8%)	1 (0.2%)
Eye Disorders	1 (6%)		2 (10%)				3 (0.6%)
Asthenopia		1 (5%)					1 (0.2%)
Eye Pain		1 (5%)					1 (0.2%)
Lacrimation Increased	1 (6%)						1 (0.2%)
Photophobia		1 (5%)	1 (5%)				2 (0.4%)
Investigations		2 (10%)		2 (2%)	42 (41.2%)	3 (2.3%)	49 (10.6%)
ALT Increased		1 (5%)		2 (2%)	20 (19.6%)		23 (5.0%)
AST Increased		1 (5%)					1 (0.2%)
Blood creatinine increased					1 (1.0%)		1 (0.2%)
Heart Sounds Abnormal						1 (0.8%)	1 (0.2%)
Body Temperature Increased		1 (5%)					1 (0.2%)
Transaminases increased					23 (22.5%)		23 (5.0%)
White blood cell count decreased					1 (1.0%)		1 (0.2%)
Gastrointestinal Disorders	4 (22%)		4 (13.3%)	6 (6%)	5 (4.9%)	34 (25.6%)	53 (11.4%)
Abdominal Discomfort	1 (6%)						1 (0.2%)
Abdominal Distension					1 (1.0%)	10 (7.5%)	11 (2.4%)
Abdominal Pain						14 (10.5%)	14 (3.0%)
Abdominal Pain Upper				1 (1%)		2 (1.5%)	3 (0.6%)
Abdominal Tenderness						1 (0.8%)	1 (0.2%)
Cheilitis			1 (3.3%)				1 (0.2%)
Constipation						3 (2.3%)	3 (0.6%)
Diarrhoea				3 (3%)	3 (2.9%)	3 (2.3%)	9 (1.9%)
Diarrhoea Haemorrhagic						1 (0.8%)	1 (0.2%)

	Adults					Children	
	Healthy Volunteers		Uncomplicated *P. falciparum* Malaria		Severe Malaria	Severe Malaria	
MedDRA System Organ Class/ Preferred Term	1129 2 mg/kg to 8 mg/kg – (N=18)	1142 2 to 8 mg/kg Overall (N=20	1168 Artesunate 2.4 mg/kg (N=30)	1263ab 1.2 mg/kg to 4.8 mg/kg (N=100)	CDC-060 2.4 mg/kg (N=102)	EDCTP-MMV07-01 (N=194)	All Studies (N=464)
Enteritis			1 (3.3%)				1 (0.2%)
Flatulence						2 (1.5%)	2 (0.4%)
Gingival Bleeding				1 (1%)			1 (0.2%)
Haematemesis						1 (0.8%)	1 (0.2%)
Nausea	2 (11%)			1 (1%)		1 (0.8%)	4 (0.9%)
Tongue Coated	1 (6%)						1 (0.2%)
Vomiting	2 (11%)		2 (6.7%)	2 (2%)	1 (1.0%)	5 (3.8%)	12 (2.6%)
General Disorders And Administration Site Conditions	4 (22%)					12/133	16 (3.4%)
Asthenia	1 (6%)						1 (0.2%)
Chills			1 (3.3%)				1 (0.2%)
Fatigue	3 (17%)					1 (0.8%)	4 (0.9%)
Feeling Abnormal	1 (6%)						1 (0.2%)
Infusion Site Pain			2 (6.7%)	1 (1%)			3 (0.6%)
Infusion Site Irritation				1 (1%)			1 (0.2%)
Infusion Site Phlebitis				1 (1%)		1 (0.8%)	2 (0.4%)
Oedema Peripheral						1 (0.8%)	1 (0.2%)
Pyrexia	1 (6%)		4 (13.3%)			10	15 (3.2%)
Hepatobiliary Disorders					5 (4.9%)	3 (2.3%)	8 (1.7%)
Hepatomegaly						3 (2.3%)	3 (0.6%)
Hyperbilirubinaemia					5 (4.9%)		5 (1.1%)
Infections And Infestations			1 (3.3%)			20 (15.0%)	21 (4.5%)
Herpes Simplex			1 (3.3%)				1 (0.2%)
Abscess						1 (0.8%)	1 (0.2%)
Abscess Limb						1 (0.8%)	1 (0.2%)
Bronchitis						3 (2.3%)	3 (0.6%)
Cutaneous Larva Migrans						1 (0.8%)	1 (0.2%)
Folliculitis						1 (0.8%)	1 (0.2%)
Helminthic Infection						1 (0.8%)	1 (0.2%)
Impetigo						1 (0.8%)	1 (0.2%)
Otitis Media						1 (0.8%)	1 (0.2%)
Pneumonia						2 (1.5%)	2 (0.4%)
Relapsing Fever						1 (0.8%)	1 (0.2%)
Respiratory Tract Infection						1 (0.8%)	1 (0.2%)
Rhinitis						6 (4.5%)	6 (1.3%)
Skin Infection						1 (0.8%)	1 (0.2%)
Subcutaneous Abscess						1 (0.8%)	1 (0.2%)
Tinea Capitis						2 (1.5%)	2 (0.4%)
Upper Respiratory Tract Infection						4 (3.0%)	4 (0.9%)
Urinary Tract Infection						1 (0.8%)	1 (0.2%)
Injury, Poisoning And Procedural Complications						3 (2.3%)	3 (0.6%)
Arthropod Bite						1 (0.8%)	1 (0.2%)
Open Wound						1 (0.8%)	1 (0.2%)

	Adults					Children	
	Healthy Volunteers		Uncomplicated *P. falciparum* Malaria		Severe Malaria	Severe Malaria	
MedDRA System Organ Class/ Preferred Term	1129 2 mg/kg to 8 mg/kg – (N=18)	1142 2 to 8 mg/kg Overall (N=20	1168 Artesunate 2.4 mg/kg (N=30)	1263ab 1.2 mg/kg to 4.8 mg/kg (N=100)	CDC-060 2.4 mg/kg (N=102)	EDCTP-MMV07-01 (N=194)	All Studies (N=464)
Vascular Injury						1 (0.8%)	1 (0.2%)
Metabolism And Nutrition Disorders	1 (6%)				2 (2.0%)	2 (1.5%)	5 (1.1%)
Anorexia	1 (6%)	1 (5%)	1 (3.3%)				3 (0.6%)
Acidosis					1 (1.0%)		1 (0.2%)
Decreased Appetite		1 (5%)					1 (0.2%)
Hyperlactacidaemia						1 (0.8%)	1 (0.2%)
Hypoglycaemia						1 (0.8%)	1 (0.2%)
Metabolic acidosis					1 (1.0%)		1 (0.2%)
Musculoskeletal And Connective Tissue Disorders	2 (11%)			1 (1%)		2 (1.5%)	5 (1.1%)
Arthralgia	1 (6%)						1 (0.2%)
Myalgia	2 (11%)						2 (0.4%)
Neck Pain				1 (1%)		1 (0.8%)	2 (0.4%)
Back Pain						1 (0.8%)	1 (0.2%)
Nervous System Disorders	13 (72%)		1 (3.3%)	7 (7%)		8 (6.0%)	29 (6.3%)
Coma						1 (0.8%)	1 (0.2%)
Convulsion						3 (2.3%)	3 (0.6%)
Dizziness	6 (33%)	3 (14%)		3 (3%)			12 (2.6%)
Dysgeusia	10 (56%)	16 (80%)		4 (4%)			30 (6.5%)
Febrile Convulsion						1 (0.8%)	1 (0.2%)
Grand Mal Convulsion						2 (1.5%)	2 (0.4%)
Headache	4 (22%)	5 (25%)	1 (3.3%)				10 (2.2%)
Memory Impairment						1 (0.8%)	1 (0.2%)
Muscle Contractions Involuntary						1 (0.8%)	1 (0.2%)
Paraesthesia	1 (6%)	2 (10%)					3 (0.6%)
Parosmia		1 (5%)					1 (0.2%)
Somnolence		2 (10%)					2 (0.4%)
Psychiatric Disorders	1 (6%)			1 (1%)	2 (2.0%)		4 (0.9%)
Depression	1 (6%)						1 (0.2%)
Insomnia	1 (6%)			1 (1%)			2 (0.4%)
Renal And Urinary Disorders					5 (4.9%)	8 (6.0%)	13 (2.8%)
Dysuria						1 (0.8%)	1 (0.2%)
Haemoglobinuria						7 (5.3%)	7 (1.5%)
Oliguria					1 (1.0%)		1 (0.2%)
Renal failure					4 (3.9%)		4 (0.9%)
Respiratory, Thoracic And Mediastinal Disorders	2 (11%)				4 (3.9%)	11 (8.3%)	17 (3.7%)
Acute respiratory distress syndrome					1 (1.0%)		1 (0.2%)
Cough	1 (6%)					7 (5.3%)	8 (1.7%)
Epistaxis						3 (2.3%)	3 (0.6%)
Nasal Congestion	1 (6%)						1 (0.2%)
Pulmonary haemorrhage					1 (1.0%)		1 (0.2%)
Respiratory Distress						1 (0.8%)	1 (0.2%)
Sinus Congestion	1 (6%)						1 (0.2%)

	Adults					Children	
	Healthy Volunteers		Uncomplicated *P. falciparum* Malaria		Severe Malaria	Severe Malaria	
MedDRA System Organ Class/ Preferred Term	1129 2 mg/kg to 8 mg/kg – (N=18)	1142 2 to 8 mg/kg Overall (N=20	1168 Artesunate 2.4 mg/kg (N=30)	1263ab 1.2 mg/kg to 4.8 mg/kg (N=100)	CDC-060 2.4 mg/kg (N=102)	EDCTP-MMV07-01 (N=194)	All Studies (N=464)
Sneezing						1 (0.8%)	1 (0.2%)
Skin And Subcutaneous Tissue Disorders	6 (33%)	1 (5%)	1 (3.3%)	2 (2%)	1 (1.0%)	4 (3.0%)	15 (3.2%)
Ecchymosis	1 (6%)						1 (0.2%)
Erythema	1 (6%)						1 (0.2%)
Hyperhidrosis	2 (11%)						2 (0.4%)
Lip Blister	1 (6%)						1 (0.2%)
Night Sweats	1 (6%)						1 (0.2%)
Pruritus	1 (6%)					2 (1.5%)	3 (0.6%)
Rash Pruritic		1 (5%)				1 (0.8%)	2 (0.4%)
Rash				2 (2%)			2 (0.4%)
Rash maculo-papular					1 (1.0%)		1 (0.2%)
Skin Swelling						1 (0.8%)	1 (0.2%)
Stevens-Johnson Syndrome			1 (3.3%)				1 (0.2%)
Urticaria	1 (6%)						1 (0.2%)
Vascular Disorders	1 (6%)			1 (1%)	1 (1.0%)	14	17 (3.7%)
Epistaxis					1 (1.0%)		1 (0.2%)
Flushing	1 (6%)	1 (5%)					2 (0.4%)
Hot Flush		1 (5%)					1 (0.2%)
Hypertension					1 (1.0%)		1 (0.2%)
Hypotension			8 (26.7%)	1 (1%)	2 (2.0%)		11 (2.4%)
Phlebitis						12	12 (2.6%)
Thrombophlebitis						1 (0.8%)	1 (0.2%)
Vein Disorder						1 (0.8%)	1 (0.2%)

Study 1129: Healthy Adult Volunteers, Single Dose 2 mg/kg, 4 mg/kg, and 8 mg/kg
Study 1142: Healthy Adult Volunteers, 2 mg/kg, 4 mg/kg, and 8 mg/kg daily for 3 days
Study 1168 Adults with Uncomplicated *Pf* malaria, 2.4 mg/kg once a day for 3 days
Study 1263ab: Adults, with uncomplicated *Pf* malaria 1.2 mg/kg once daily 3 days; 2.4 mg/kg once daily 3 days; 2.4 mg/kg 2 x Day 1, then once daily 2 more days; 4.8 mg/kg once daily 3 days
EDCTP-MMV07-01: Children ages 6 months to 9 years with Severe *Pf* Malaria: 2.4 mg/kg initially and at 12, 24, 48, and 72; 4.0 mg/kg initially and at 24 and 48 h

The final tabulation of ADRs in the SmPC was further edited to reflect ADRs occurring in ≥ 1% and to remove rare or very rare single occurrences not felt to be drug related. This tabulation was then modified to add ADRs that can be expected to occur based on other published data, with special attention to rare or very rare events, severe and non-severe hypersensitivity reactions and post artesunate delayed haemolysis (PADH) as directed.

2.6.8.3. Serious adverse event/deaths/other significant events

Deaths

No deaths occurred in the Phase 1 or Phase 2 trials.

There were 7 deaths in CDC-060, none of which was judged to be related to the administration of IV AS and all were considered due to severe malaria. The most frequently recorded AEs leading to death were brain oedema or brainstem herniation, which occurred in 3 of the 7 who died. All of the patients who died showed significant baseline neurological impairment (seizures, impaired consciousness, ventilator support) and all had either moderate or severe hepatic injury as assessed by MELD score. Five had *P. falciparum* with baseline hyperparasitaemia ranging from 12% to 90% and one had confirmed *P. vivax.* Two of the patients who expired were aged >70 years and these were the only

patients in the study who were more than 70 years old. One other patient who died was aged > 60 years. In contrast, death occurred in only 2/11 diabetics in the study while none who had pre-existing Hepatitis A or B infection, asthma, G6PD deficiency or asplenia or who were pregnant died.

Table 39: Patient Deaths in Study R-CDC-060

Patient ID/ Gender/Age	Adverse Event	Relationship	Severity (eGFR) [a]	Severity (MELD) [b]	Concurrent antimalarial medication during IV AS Administration	Quinidine exposure	Seizures or impaired consciousness or ventilator support
35905/F/32	ARDS[c]	Not related	Normal	Moderate	Yes	Yes	Yes
50390/F/61	Multi-organ failure	Not related	Moderate	Moderate	No	Yes	Yes
54565/M/71	Cardiac arrest	Not related	Moderate	Moderate	Yes	No	Yes
62013/M/31	Brain herniation	Not related	Normal	Moderate	Yes	Yes	Yes
65400/M/31	Brain herniation	Not related	Severe	Not available	Yes	Yes	Yes
71784/M/54	Brain edema	Not related	Mild	Moderate	Yes	Yes	Yes
75590/M/72	Cardiac arrest (death)	Not related	Moderate	Severe	No	Yes	Yes

During the subsequent 6 years (2011-2016), the death rate among IV artesunate-treated patients with severe malaria was 6.1%. None of the 23 deaths that occurred from 2007 to 2016 was in children.

In EDCTP-MMV07-01, two patients died in the 3-dose cohort. One was a female with sickle cell disease age 3 or 4 years who presented with severe anaemia (Hb 4.2 g/dL), hyperparasitaemia, fever, nausea, vomiting and fatigue. She received all 3 doses of IV artesunate but fell into an unarousable coma and suffered cardiac arrest on Day 2. Her death was classified as possibly related to the study drug. The other was a 34-month-old male who died on Day 1 following a grand mal seizure. His death was attributed to cerebral malaria and respiratory distress, and it was considered unrelated to the study drug. There were no deaths in the 5-dose cohort.

In SEAQUAMAT the death rate was 15% for artesunate compared to 22% for quinine. In AQUAMAT the death rates were 8.5% vs. 10.9%, respectively.

SAEs

There was one SAE in a female patient treated for uncomplicated malaria in 1168 who was hospitalised after developing bullous erythema multiforme (preferred term "Stevens-Johnson Syndrome") after her IV artesunate had concluded and she had begun receiving Malarone. This SAE was considered probably related to Malarone and possibly related to IV artesunate. It resolved following a brief hospitalisation.

2.6.8.4. Laboratory findings

In 1128, the only notable finding was a dose-dependent transient decease in reticulocyte count after IV artesunate that peaked on day 4 and mostly returned to baseline by day 8.

In 1142, the only notable trend was for drug-dependent transient reticulocytopenia after IV artesunate that returned to at least baseline by day 10 in most cases. On Day 10, reticulocyte counts in the artesunate groups were higher than at baseline and higher vs. the placebo group.

In 1168 and in 1263 there were no pronounced drug effects on haemoglobin or haematocrit. Platelets and neutrophils were low at baseline and then showed a hyper-response following treatment. There were also cases of eosinophilia observed, which is typical of patients with malaria. An increase in total bilirubin was observed on Day 3 and resolved by Day 7, which was attributed to malaria. There was a slight depression in reticulocytes on Day 3 in subjects receiving highest doses.

In CDC-060, mean values of most blood chemistry parameters increased marginally from baseline to Day 7, an effect that paradoxically reflected treatment success, i.e. as subjects recovered and were discharged, the submitted samples represented readings from those with a more difficult and longer clinical course. During this period, the maximum BUN value for the population increased, driven by readings from a relatively small number of patients who had persistent renal effects from malaria. Acidosis (a feature of severe malaria) was reported in 3.9% of patients.

2.6.8.5. Discontinuation due to adverse events

In the applicant's cohort of 282 treated with US Army IV artesunate, there was only one withdrawal because of an AE. This occurred in study 1142 and was due to upper respiratory tract infection that was considered unrelated to IV artesunate.

2.6.9. Discussion on clinical safety

Relevant safety database

There were 282 healthy subjects and patients with uncomplicated or severe malaria exposed to the US Army product in 4 sponsored trials and in CDC-060. Another 194 children with severe malaria were exposed in the MMV-sponsored study that used US Army product. None of these individuals received the final product to be placed on the EU market.

There is some information on safety (albeit mainly from publications and therefore limited in nature) for several thousand patients with malaria, many of whom had severe malaria. The minimum age of treated patients seems to have been 6 months (in the MMV study) but the majority of the data come from children aged from ~18 months, adolescents and adults. The adult data come mainly from subjects aged < 50 years.

General features of the safety profile

In the US Army sponsored studies, non-serious AEs mapping to the nervous system were common, including headache, dizziness and dysgeusia. In the single and multiple dose studies in healthy subjects, reversible reticulocytopenia during treatment was observed. This phenomenon is recognised to occur in association with artemisinin administration along with anaemia, reduced neutrophil counts and eosinophilia that are usually mild and short-lived.

In the two US Army studies in patients with uncomplicated malaria, the effect of artesunate on the haematological findings was confounded by the effect of malaria and recovery from malaria. Anaemia affected 20%-43.3% of patients across the dose groups while leukopenia affected up to 32%. In particular, neutropenia affected 60% of patients who received 4.8 mg/kg daily × 3 days and 20% had severe neutropenia. Thrombocytopenia was reported in 44.0% of patients at the highest IV AS dose.

Nevertheless, there was no clear or consistent relationship between the haematological effects and total administered artesunate dose. It seems that counts recovered over time.

In CDC-060, the most frequently reported AEs were anaemia, LFTs increased and thrombocytopenia. While 29% of reported cases of anaemia were considered possibly related to artesunate, ~60% of increases in LFTs were considered at least possibly drug-related by the CBEC. Neutropenia and lymphopenia were also reported. Of the 66 total reports of anaemia, 20 (30%) were Grade 3 and 13 (20%) were Grade 4. Blood and lymphatic system disorders accounted for the highest percentage of Grade 3 and 4 AEs (53/126; 42.1%) of any SOC, followed by Investigations (18/126; 14.3%). Reticulocyte counts were not routinely assessed in CDC-060 so the rates cannot be compared with the other studies.

In contrast to the US Army-sponsored trials, the two most common AEs reported in EDCTP–MMV07-01 were infections and infestations (29% Group A; 38% Group B) and gastrointestinal disorders (22%; 24%). Laboratory testing was conducted at baseline and on days 2, 7 and 28 in this study. Anaemia was reported as an AE in 19% in Group A and 11% in Group B, about half of which were deemed to be possibly related to treatment, while thrombocytopenia, leukocytosis, lymphopenia, neutropenia and transaminases increased were not reported.

Similarly, the AEs reported in the SEAQUAMAT and AQUAMAT trials showed little comparability to those reported in CDC-060. The applicant coded the raw data from SEAQUAMAT provided by the Wellcome Trust in accordance with MedDRA preferred terms and summary statistics were prepared. The frequency of AEs in this trial was generally similar between the IV artesunate and IV quinine groups. After initiation of treatment, renal failure was the most commonly reported AE in the artesunate (19.2%) and quinine groups (21.5%). However, some patients had BUN > 60 mg/dL at admission, suggesting that they already had renal failure at baseline.

Overall, it seems that the investigators involved in the trials of severe malaria conducted in endemic regions had a rather different focus in terms of reporting AEs and ADRs. The general picture suggests that in these trials many observations made on treatment were regarded as normal changes in response to severe malaria and its treatment such that reporting of AEs and ADRs was confined to deteriorations and development of complications.

Post-artemisinin delayed haemolysis (PADH)

Typically, anaemia is present in at the time of presentation with severe malaria, worsens with effective antimalarial treatment and then recovers over a period up to at ~6 weeks.

However, if delayed haemolysis associated with severe malaria that has been successfully treated with IV artesunate or another artemisinin occurs, there is a late drop in haemoglobin at 2-3 weeks after the first dose. While most cases have been described in returning travellers, there were no confirmed cases of PADH in CDC-060 although some would have been expected based on estimated rates for PADH in the literature ranging from 0.9%-27.8%.

Neurological sequelae

Severe malaria can lead to neurological sequelae regardless of the antimalarial agents used for treatment. These sequelae are especially likely to occur in patients who already have major neurological problems when they first present for treatment or who do not respond to their initial antimalarial regimen. However, in the 1980s and 1990s, as global interest in the value of the artemisinins for treatment of severe and uncomplicated malaria increased, nonclinical data emerged that raised some questions over whether the artemisinins themselves might be associated with an enhanced risk of residual neurological deficits. The findings were examined closely by WHO's expert committees, which concluded that the clinical evidence did not support a need for concern. Since then, many millions have been treated for uncomplicated malaria with various artemisinin combination regimens and the WHO has recommended parenteral artesunate for first line treatment of severe malaria.

Nevertheless, the applicant has paid close attention to the AEs and ADRs pertaining to the nervous system observed in clinical trials in patients with malaria. Across AQUAMAT, SEAQUAMAT, EDCTP-MMV07-01 and R-CDC-060, the most frequently reported nervous system disorders were coma and seizure or convulsion. In the comparative studies, rates were slightly higher rates in the quinine group than the artesunate group.

In SEAQUAMAT, 10.5% of artesunate-treated patients and 12.9% treated with quinine developed neurological defects after admission but only 1.2% and 0.4% in respective groups had defects that persisted at discharge. Of 10 patients (7 artesunate and 3 quinine) discharged from hospital with residual neurological sequelae, 5 had psychiatric sequelae, four had persisting problems with balance (one of whom had both psychiatric sequelae and a tremor) and two had a hemiparesis. Moreover, the incidence of any significant sequelae was 5.9% vs. 4.1% for the artesunate and quinine groups respectively (p=0.1171), yielding a relative risk ratio (artesunate/quinine) of 1.44 with 95% confidence interval (0.91, 2.26). However, the applicant points out that the finding likely reflects a lower mortality rate among cerebral malaria patients in the artesunate group, resulting in more individuals who survived but had significant sequelae at hospital discharge. This supposition is supported by the observation that the incidence of sequelae was higher in the artesunate group than in the quinine group among the severe malaria patients (7.1% vs. 3.7%) but lower in the non-severe patients (3.2% vs. 5.3%).

In AQUAMAT, the numbers with convulsions that developed after admission or were present on admission and persisted for more than 6 h were 224 in the artesunate group vs. 273 in the quinine group, while 65 artesunate vs. 91 quinine patients developed coma or had a deterioration of their coma score after starting antimalarial treatment. In the 4898 survivors, 99 artesunate vs. 71 quinine patients had not yet made full neurological recoveries at the time of hospital discharge. However, at longer-term follow-up there was no appreciable difference between groups in numbers with residual neurological deficits. The overall rate of persistent neurological sequelae in survivors assessed at 28 days after cerebral malaria was 3.2% (24/706 artesunate, 23/737 quinine) and the rate for severe neurological sequelae was 2.3% (17/706 artesunate, 17/737 quinine).

Of the 14 patients with any neurological sequelae who did not have cerebral malaria initially (10 artesunate, 4 quinine), 7 had multiple convulsions (3 quinine, 4 artesunate) and all had severe prostration on admission.

In CDC-060 seizures, impaired consciousness or ventilator support at baseline were reported for 35% of the safety analysis population. Three patients were reported to have AEs mapping to the nervous system, including two with brain oedema and one with depressed consciousness, all of which were ascribed to malaria.

In conclusion, noting the impact of successful treatment of severe malaria and the need to view data on neurological deficits not present at baseline in light of those who survived, the data do not support an association between treatment of severe malaria with IV artesunate and enhanced risk of neurological sequelae.

Deaths and SAEs

Deaths were reported only in the trials that enrolled patients with severe malaria. Death was most likely to occur in those with severe neurological deficits and/or hyperparasitaemia when starting treatment with IV artesunate. There is no indication that the deaths were due to IV artesunate.

The risk of SAEs cannot be determined from available information.

Section 4.8 of the SmPC

This was completely revised during the assessment, with a detailed tabulation of ADRs reported in various studies and a justification for the final table in section 4.8 of the SmPC.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

2.6.10. Conclusions on the clinical safety

The appraisal of the safety of the applicant's IV artesunate is not straightforward. The most reliable data come from the four studies sponsored by the US Army, but none enrolled the target population with severe malaria. The non-comparative safety data obtained under the CDC protocol indicate that some AEs and ADRs reflected the underlying infection, which in some patients showed worsening from baseline even if they recovered eventually and in other patients preceded death. Moreover, the timing of onset of some AEs and ADRs was after completion of initial IV artesunate, which makes the assessment of causality even more difficult. Nevertheless, it could be considered that the safety data from CDC-060 are more complete than the data reported from SEAQUAMAT and AQUAMAT, in which it may be concluded that investigators tended to report only AEs that could not be ascribed to the course of the disease.

The overall opinion is that IV artesunate itself is probably not associated with major safety concerns but it clearly (as with all other pharmacological agents) carries a risk for hypersensitivity reactions, which may sometimes be severe. There seems to be a risk for headache, dizziness and dysgeusia. There is also possibly a risk for LFT increases over and above any reaction to severe malaria. Also, there seem to be early on-treatment drops in blood cell counts that recover quickly but with the additional risk of PADH with onset after successful recovery.

With regard to safe use in paediatric population, there are very few case reports of use of artesunate in children aged < 6 months in the EU, although there is more experience with oral artemisinin usage in infants (i.e. not with IV artesunate but with oral artesunate and other oral artemisinin preparations). The available safety and efficacy data do not raise concerns. No dose adjustment is recommended based on age or weight. Appropriate warnings have been added to relevant SmPC sections.

2.7. Risk Management Plan

2.7.1. Safety concerns

Summary of safety concerns	
Important identified risks	None
Important potential risks	Reproductive toxicity (especially in the first trimester)
Missing information	None

2.7.2. Pharmacovigilance plan

Study /Status	Summary of objectives	Safety concerns addressed	Milestones	Due date(s)
Category 3 - Required additional pharmacovigilance activities				
Intravenous Artesunate Pregnancy Registry Planned	To assess risk of pregnancy and maternal complications and adverse effects on the foetus, neonate, and infant.	Reproductive toxicity (especially in the first trimester)	U.S. protocol submission	November 2021
			Final international protocol submission	November 2022
			Start of data collection (U.S.)	April 2022
			Interim study reports	April 2023 April 2024 April 2025 April 2026 April 2027 April 2028
			End of data collection	May 2029
			Final study report	November 2029
Intravenous Injection Fertility and Early Embryonic Development Study of Artesunate in Sprague Dawley Rats Planned	To test for the potential toxic effects/disturbances resulting from Artesunate treatment of Sprague Dawley CD (Crl:CD[SD]) female rats before cohabitation, through mating and to implantation	Reproductive toxicity	U.S. final protocol submission	10 September 2021
			Experimental Starting Date	24 November 2021
			Experimental Completion Date	05 July 2022
			Animal Arrival	24 November 2021
			Oestrous Cycle Evaluation	29 November 2021
			Initiation of Dosing	13 December 2021
			Initiation of Cohabitation	27 December 2021
			First Possible Gestation Day 13 Euthanasia	10 January 2022
			Completion of In-Life Phase	23 January 2022
			Data Summary	31 January 2022
			Dose Formulation Draft Report	08 March 2022
			Dose Formulation Final Report	05 April 2022
			Draft Report	05 April 2022
			Final Report	05 July 2022

2.7.3. Risk minimisation measures

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Reproductive Toxicity (especially in the first trimester)	Routine risk communication: None. Routine risk minimisation activities recommending specific clinical measures to address the risk: None. Other routine risk minimisation measures beyond the Product Information: Pack size: Each box contains 2 vials of Artesunate Amivas powder and 2 vials of phosphate buffer. Three boxes (6 vials) are enough for 3 doses of 2.4 mg/kg for a person up to 91.3 kg. Legal status: Restricted medical prescription.	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None. Additional pharmacovigilance activities: Intravenous Artesunate Pregnancy registry Intravenous Injection Fertility and Early Embryonic Development Study of Artesunate in Sprague Dawley Rats

2.7.4. Conclusion

The CHMP considers that the risk management plan version 0.5 is acceptable.

2.8. Pharmacovigilance

2.8.1. Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.8.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did request alignment of the PSUR cycle with the international birth date (IBD). The IBD is 23.06.2020. The new EURD list entry will therefore use the IBD to determine the forthcoming Data Lock Points.

2.8.3. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.8.4. Labelling exemptions

A request of translation exemption of the labelling as per Art.63.1 of Directive 2001/83/EC has been submitted by the applicant and has been found acceptable by the QRD Group for the following reasons:

The company requested all packaging components, including the package leaflet (PL), in English only on the basis of article 63.1. The group accepted the request on the basis that it is an ultra-orphan medicinal product due to the low incidence of severe malaria in the EU (estimated at ~1250 cases per year). It is, therefore, likely to have only minimal use annually across Europe in a limited number of countries. Consequently, producing a product with regional translations applicable to all EU Member States would be complicated for the product supply chain and distribution management. The group also took into account that this product will only be administered by health care professionals and will not be delivered directly to the patient.

Only two Member States, Belgium and Greece, agreed on the translation exemption (EN only) for the immediate and outer labelling, however, the PL should always be provided in their national language(s). It can also be considered acceptable to provide the PL alongside the pack, if this is technically a better option for the company.

The labelling subject to translation exemption as per the QRD Group decision above will however be translated in all languages in the Annexes published with the EPAR on EMA website, but the printed materials will only be translated in the language(s) as agreed by the QRD Group.

2.8.5. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Artesunate Amivas (artesunate) is included in the additional monitoring list as it contains a new active substance which, on 1 January 2011, was not contained in any medicinal product authorised in the EU.

Therefore, the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

Artesunate Amivas is a semi-synthetic artemisinin derivative given intravenously. The applicant proposes that it is used *for the initial treatment of severe malaria in adults and children*.

3.1.1. Disease or condition

Malaria is a potentially fatal illness caused by protozoal infection of red blood cells (RBC) with parasites belonging to the genus *Plasmodium*, transmitted to humans by the bite of a *Plasmodium*-infected female anopheline mosquito usually between dusk and dawn. Five species of *Plasmodium* infect humans: *Plasmodium falciparum* (*Pf*), *P. vivax* (*Pv*), *P. ovale* (*Po*), *P. malariae* (*Pm*), and *P. knowlesi* (*Pk*). Most severe malaria is due to *P. falciparum*, although severe malaria due to other species is recognised. In Europe in 2018, among 4,516 confirmed cases for which the *Plasmodium* species was reported, 3,793 (84.0%) had *P. falciparum* and 339 (7.5%) had *P. vivax.*

According to the World Malaria Report 2018, there were 219 million cases of malaria globally in 2017 (uncertainty range 203–262 million) and 435,000 malaria deaths, representing a decrease in malaria cases and deaths rates of 18% and 28% since 2010, respectively. The burden is heaviest in Africa. In the EU and in the US, malaria occurs in returning travelers or very recent immigrants from malaria endemic areas. Severe malaria occurred in 293, 259 and 306 US residents in 2014, 2015 and 2016, respectively. In Europe in 2018, the total number of all malaria cases (uncomplicated and severe combined) was 8,349. However, as in the US, the proportion of severe cases in Europe was very low, typically 10% of the total.

Complicated malaria, defined as malaria for which initial oral treatment is not possible, includes malaria that does and does not meet the WHO (2015) criteria for severe malaria. The WHO defines severe falciparum malaria according to one or more clinical features occurring in the presence of asexual parasitaemia:
Impaired consciousness: Glasgow coma score <11 in adults or Blantyre coma score <3 in children.
Multiple convulsions: >2 episodes in 24 h.
Prostration: Generalised weakness - unable to sit, stand or walk without assistance.
Significant bleeding: Including recurrent or prolonged bleeding from the nose, gums or venipuncture sites, haematemesis or melaena.
Shock: Compensated shock (defined as capillary refill ≥3 seconds or temperature gradient on leg but no hypotension) or decompensated shock (defined as systolic blood pressure <80 mmHg in adults or < 70 mmHg in children, with evidence of impaired perfusion).
Pulmonary oedema: Radiologically confirmed or oxygen saturation <92% on room air with respiratory rate >30/min, often with chest in drawing and crepitations on auscultation.
These clinical features are accompanied by one or more laboratory findings that may include:

- *Hypoglycaemia*: Blood or plasma glucose <2.2 mmol/L (40 mg/dL).
- *Acidosis:* A base deficit of >8 mEq/L or plasma bicarbonate <15 mmol/L or venous plasma lactate ≥5 mmol/L. Severe acidosis manifests as respiratory distress (rapid, deep, labored breathing).
- *Severe malarial anaemia:* Haemoglobin ≤5 g/dL or haematocrit ≤15% in children <12 years (≤7 g/dL and <20%, respectively, in adults) with parasitaemia > 10,000/µL.
- *Hyperparasitaemia: P. falciparum* parasitaemia >5%.
- *Renal impairment:* Blood creatinine >265 µmol/L (3 mg/dL) or BUN >20 mmol/L.
- *Jaundice:* Bilirubin >50 µmol/L (3 mg/dL) with >100,000 parasites/µL.

3.1.2. Available therapies and unmet medical need

Quinine was the mainstay of treatment of severe malaria until the rediscovery of artemisinin in China in 1972 and the subsequent synthesis of artemether and artesunate. Artemisinin derivatives are now widely recognised to be the most rapidly acting of all the antimalarial drugs. The SEAQUAMAT Consortium compared IV AS to IV quinine for severe malaria in 1461 (mostly adult) Asian patients and found that parenteral artesunate was statistically superior to quinine in preventing death. This was followed by a study of similar design in 5,425 African children in 2010 (AQUAMAT), which also showed that parenteral artesunate was superior to quinine in preventing mortality. Based on these and other trials, the WHO issued a strong recommendation for the use of IV or IM AS in patients with severe malaria for at least 24 h and until oral medication is possible. The recommended oral follow-on treatment consists of 3 days of an artemisinin-based combination therapy.

In the European Union, there are no medicinal products specifically authorised for the initial treatment of severe malaria. There is one antimalarial available for IV administration but it is licensed only in France as SURQUINA (IV quinine). While many EU centres have moved to use of IV artesunate for

initial treatment of severe malaria, the available products are either the WHO-prequalified (2011) Guilin formulation or artesunate formulations manufactured or approved in other jurisdictions.

3.1.3. Main clinical studies

The demonstration of efficacy of IV artesunate is based on two prospective, randomised, controlled trials:

- The South East Asian Quinine Artesunate Malaria Trial - SEAQUAMAT - was conducted between 2003 and 2005 in adults and children with severe falciparum malaria admitted to hospitals in Asia and SE Asia. The study was published in 2005 (*Lancet* 2005; 366: 717-25). The applicant gained access to the data from this study from the sponsor and constructed a supplementary report.
- The African Quinine Artesunate Malaria Trial - AQUAMAT - was conducted between 2005 and 2010 in children with severe falciparum malaria admitted to hospitals in Africa. The study was published in 2010 (*Lancet* 2010; 376: 1647-57). The applicant was unable to gain access to the data from this study so the only information available comes from the publication.

Of many other studies reported in the application dossier, two studies were conducted with US Army artesunate in severe malaria:

- CDC-060 was a retrospective uncontrolled study in returning US travelers of any age who received IV artesunate under the CDC protocol. The applicant was given access to data by the CDC.
- EDCTP-MMV07-01 was conducted in African children aged < 5 years by MMV and it compared two different regimens of IV artesunate with no active control arm. A CSR constructed by MMV is available.

The applicant is unable to verify GCP compliance in any of these important studies. Furthermore, for reasons explained by the applicant, inspections of study sites or of other bodies involved in the conduct of these studies are not feasible. Nevertheless, CDC-060 was conducted under a US IND and it appears that the deficit is with regards to the variable level of data collection at various sites.

Moreover, four studies in the dossier (two PK studies in healthy subjects and two studies with IV artesunate for uncomplicated malaria) were sponsored by the US Army and conducted under an IND. These were subjected to independent audits by a CRO. However, a definitive conclusion on GCP compliance for these sponsored studies is not possible.

3.2. Favourable effects

Pivotal randomised controlled studies - *SEAQUAMAT* and *AQUAMAT*

These were both prospective, randomised trials that compared initial treatment with parenteral artesunate with widely recommended and well-established comparative regimens for treatment of severe *P. falciparum* malaria. Taken together, the two trials cover a wide age range. In general, the main features of the design of these studies seem to have been appropriate.

Both studies used the same parenteral artesunate regimen, with 2.4 mg/kg given at 0, 12 h and 24 h, and then once daily dosing until oral medication was possible. This is the posology recommended in the applicant's SmPC. It appears that SEAQUAMAT (based on the protocol from 2003 and the applicant's CSR but not mentioned in the publication) and AQUAMAT (mentioned in the publication) allowed IM as an alternative to IV administration of the initial parenteral treatment but no patient received IM in SEAQUAMAT and only ~10% were dosed IM in AQUAMAT.

Both studies also used the same initial comparative regimen, with a 20 mg/kg loading dose of quinine followed by 10 mg/kg q8h until oral treatment was possible. However, while AQUAMAT required that

24 h parenteral treatment was given before an oral switch, there does not seem to have been a minimum duration of parenteral treatment required in SEAQUAMAT.

There is an important difference between the two studies in terms of oral follow-on treatment. In SEAQUAMAT, parenteral artesunate was followed by oral artesunate and parenteral quinine was followed by oral quinine, both given with oral doxycycline unless this was contraindicated. The contribution of the oral follow-on regimens to the overall in-hospital mortality rates and to the other clinical endpoints cannot be determined in this study design. Furthermore, there was no on-treatment serial parasite counting conducted, which means that the initial impact of the two parenteral treatments on parasite density cannot be compared.

In contrast, all patients in AQUAMAT followed initial parenteral treatment with a full course or oral artemether-lumefantrine (Coartem). Coartem is highly effective for the *de novo* treatment of uncomplicated malaria and use of the same follow-on regimen in both treatment arms could be expected, if anything, to reduce the differences observed between randomised groups in terms of outcomes measured after oral switch. Therefore, any differences detected between the two randomised treatment arms likely reflect the impact on the initial parenteral treatment.

The primary efficacy endpoint in both studies was in-hospital all-cause mortality, which was thought to reflect death rates from the presenting episodes of severe malaria. This primary endpoint is acceptable.

Based on this primary endpoint, both studies were planned with sample sizes to provide 80% power to show a reduction in mortality for initial parenteral treatment with artesunate vs. quinine. The estimated mortality rates reflected recent data relevant to the populations enrolled.

In *SEAQUAMAT*, the second interim analysis was based on data for 1294 patients up to February 2005 and led to stopping the study in May 2005. The publication of this study describes results for the 1461 patients enrolled when the study was stopped, including 271 deaths.

The applicant's CSR is based on re-analyses of data from the total 1461 patients. The great majority of the 1461 randomised patients (~95%) had *P. falciparum* confirmed by microscopy of blood smears and adults accounted for 1269/1461 patients. About 70% of the ITT population met at least one of the criteria for severe malaria.

In the ITT and PP populations, the in-hospital death rates were statistically significantly lower in the artesunate group, with an absolute difference between groups of 7-8 percentage points. Both analyses indicated that the relative risk of dying in the artesunate group was about 2/3 of that in the quinine group with upper bounds of the 95% confidence intervals that did not exceed 0.82. Most of the treatment effect reflected reduction in mortality after the first 24-48 h on study. This finding is important since, in this study, the two treatment groups had separate oral follow-on regimens (i.e. artesunate or quinine as per initial assignment), which would have contributed to the overall survival and clinical response rates.

Severe malaria was confirmed for 509 (70%) in the artesunate group and 541 (74%) in the quinine group. The mortality rates in those with severe disease were 19.8% with artesunate compared to 28.1% with quinine, which was a statistically significant benefit. The mortality rates varied significantly between countries (from 9.3% in Indonesia to 28% in Bangladesh), which the publication mentions may have reflected availability of intensive care. Nevertheless, the absolute risk reduction in each country associated with artesunate vs. quinine treatment was in the range from 5-9 percentage points.

In *AQUAMAT*, the final study population was more homogeneous than that in SEAQUAMAT, being confined to African children aged from 18 months to <5 years. Although about one third had received potentially effective antimalarial treatment for the presenting episode, the condition of the children at

study baseline indicates that the treatment was not controlling the disease. As expected, the mean and range of baseline parasitaemia was even higher in these children compared to the mainly adult population enrolled into SEAQUAMAT.

The ITT population death rates in both treatment groups were lower compared to those in SEAQUAMAT, being 8.5% vs. 10.9%, representing a statistically significant benefit for starting treatment with artesunate rather than quinine when each was followed by oral Coartem. A very similar result was obtained in the PP population with confirmed *P. falciparum*. Importantly, the mortality rates in the subset that met the pre-defined criteria for severe malaria, which comprised ~85% of the total 5425 randomised, were 9.9% for artesunate and 12.4% for quinine, which was a statistically significant difference (p=0.0055). Moreover, in contrast to SEAQUAMAT, the survival curves diverged from the time of the first dose onwards.

Severe malaria treated with Artesunate Amivas

EDTCP-MMV07-01 was conducted in children 6 months-10 years with severe malaria regardless of species. The study compared 5 doses of 2.4 mg/kg within 72 h with 3 doses of 4 mg/kg within 48 h in a double blind design. The primary endpoint was the rate of 99% parasite clearance (PC99) at 24 h after the first dose (i.e. after 2 doses of 2.4 mg/kg or after 1 dose of 4 mg/kg).

There was a numerical advantage for the 4 mg/kg dose group based on PC99 at 24 h. However, the median time to PC100 was 36 h in both dose groups and there were only 6-h differences between regimens for the median times to PC90 and PC99. Also, the median FCT was 12 h in both groups. It was concluded that both regimens were suitable for the initial treatment of severe malaria in the age range studied.

CDC-060 was a retrospective uncontrolled study of initial IV artesunate with at least 4 doses of 2.4 mg/kg at 0 h, 12 h, 24 h and 48 h. Not all patients entering this study necessarily had severe malaria and infections were not confined to *P. falciparum*. While the primary endpoint was safety, the study captured effects on treatment on parasite counts as well as the clinical outcomes.

Since this study was conducted in returning travellers, the importance for the application is that it describes outcomes in persons not likely to have any or, at least, any substantial pre-existing naturally acquired immunity to malaria.

The results must be viewed with much caution, not least due to the number and types of protocol deviations, including concomitant antimalarial agents during the IV treatment phase. Nevertheless, the baseline features indicate that a substantial proportion of the patients were severely unwell at the time of starting artesunate. This is underlined by the fact that 5 patients died before completing 4 doses of artesunate. While the applicant reports median times to negative parasitology for the safety, evaluable and PP populations (as defined in the CSR), with point estimates from 42-50 h, these numbers are based on populations that included those who received other antimalarial agents with IV artesunate.

Thus, it should be noted that the 38/87 patients in the evaluable analysis population who received concurrent antimalarial treatments during IV artesunate administration did not show a statistically significant difference in time to negative parasitology compared with the 49/87 who received IV artesunate alone. However, there was a numerical difference (43 h vs. 51 h, respectively; p=0.942). In the 49 with no concomitant antimalarial agent during IV artesunate administration, 39/49 (80%) transitioned to oral antimalarial therapy with a mean time to initiation of 3.8 days (range 2 to 5 days).

To support initial monotherapy with IV artesunate, the applicant compared all the efficacy endpoints for the subsets of the evaluable population (this being the population of most interest) that did (n=38) and did not (n=49) receive concomitant antimalarial agents during IV artesunate treatment. There was no benefit for adding an antimalarial agent to IV artesunate, supporting initial monotherapy usage.

3.3. Uncertainties and limitations about favourable effects

The GCP compliance of SEAQUAMAT and AQUAMAT cannot be verified. Nevertheless, these trials were conducted by academic consortia under the sponsorship of the Wellcome Trust. There is no reason to consider that the data would not be reliable.

With a primary endpoint of in-hospital death, the focus of these studies was on mortality ascribable to the acute malaria episode. Both studies report the total death and sequelae rates at 28 days and both showed an advantage for initial treatment with parenteral artesunate vs. quinine. While the primary analyses included patients who did not meet the criteria for severe malaria, both studies showed a benefit for artesunate in the defined severe malaria subsets. The criteria for severe malaria applied were not the same between studies and were not identical to those published by WHO in 2015 but they seem to have been sufficient to identify the subsets presenting with the most severe disease.

Whilst the most relevant data come from CDC-060, this was a retrospective study in a population of returning travellers with malaria and with a very wide age range. Based on the summary of how patients met inclusion criteria, the majority seem likely to have had at least one feature indicating severe malaria. With 102 patients in the safety population, the death rate during the data abstraction period was 7/102 (6.9%) while the death rate in the evaluable population was 6/87 (6.9%). The median time to negative parasitology was < ~2 days in the evaluable and PP populations, with or without use of concurrent antimalarial agents, supporting the impact of parenteral artesunate.

The MMV study had no active control group. However, in African children aged from 6 months to < 10 years with severe *P. falciparum* malaria based on the protocol-defined criteria, the effects on parasite counts indicated that both regimens were effective. The study lends support to the use of the proposed weight-based posology in the SmPC regardless of age. Moreover, the applicant has summarised several studies reported in the literature that support the 2.4 mg/kg IV artesunate regimen in children.

3.4. Unfavourable effects

In the US Army sponsored studies, non-serious AEs mapping to the nervous system were common, including headache, dizziness and dysgeusia. In the single and multiple dose studies in healthy subjects, reversible reticulocytopenia during treatment was observed. This phenomenon is recognised to occur in association with artemisinin administration along with anaemia, reduced neutrophil counts and eosinophilia that are usually mild and short-lived. In contrast to the early on-treatment drop in reticulocyte count that was observed in uninfected subjects and sometimes in patients with malaria (e.g. this was seen in study 1263), the reticulocyte count tends to peak after 1 to 2 weeks during recovery from severe malaria.

In the two US Army studies in patients with uncomplicated malaria, the effect of artesunate on the haematological findings was confounded by the effect of malaria and recovery from malaria. Anaemia affected 20%-43.3% of patients across the dose groups while leukopenia affected up to 32%. In particular, neutropenia affected 60% of patients who received 4.8 mg/kg daily × 3 days and 20% had severe neutropenia. Thrombocytopenia was reported in 44.0% of patients at the highest IV AS dose.

Nevertheless, there was no clear or consistent relationship between the haematological effects and total administered artesunate dose. It seems that counts recovered over time.

In CDC-060, the most frequently reported AEs were anaemia, LFTs increased and thrombocytopenia. While 29% of reported cases of anaemia were considered possibly related to artesunate, ~60% of increases in LFTs were considered at least possibly drug-related by the CBEC. Neutropenia and lymphopenia were also reported. Of the 66 total reports of anaemia, 20 (30%) were Grade 3 and 13 (20%) were Grade 4. Blood and lymphatic system disorders accounted for the highest percentage of

Grade 3 and 4 AEs (53/126; 42.1%) of any SOC, followed by Investigations (18/126; 14.3%). Reticulocyte counts were not routinely assessed in CDC-060 so the rates cannot be compared with the other studies.

In contrast to the US Army-sponsored trials, the two most common AEs reported in EDCTP–MMV07-01 were infections and infestations (29% Group A; 38% Group B) and gastrointestinal disorders (22%; 24%). Laboratory testing was conducted at baseline and on days 2, 7 and 28 in this study. Anaemia was reported as an AE in 19% in Group A and 11% in Group B, about half of which were deemed to be possibly related to treatment, while thrombocytopenia, leukocytosis, lymphopenia, neutropenia and transaminases increased were not reported.

Similarly, the AEs reported in the SEAQUAMAT and AQUAMAT trials showed little comparability to those reported in CDC-060. The applicant coded the raw data from SEAQUAMAT provided by the Wellcome Trust in accordance with MedDRA preferred terms and summary statistics were prepared. The frequency of AEs in this trial was generally similar between the IV artesunate and IV quinine groups.

After initiation of treatment, renal failure was the most commonly reported AE in the artesunate (19.2%) and quinine groups (21.5%). However, some patients had BUN > 60 mg/dL at admission, suggesting that they already had renal failure at baseline.

PADH is a recognised ADR that may occur after effective treatment with IV artesunate or another artemisinin. It is characterised by a late drop in haemoglobin at 2-3 weeks after the first dose. Most cases have been described in returning travellers, with estimated rates ranging from 0.9-27.8%.

The applicant has paid close attention to neurological sequelae. Across AQUAMAT, SEAQUAMAT, EDCTP-MMV07-01 and R-CDC-060, the most frequently reported nervous system disorders were coma and seizure or convulsion. In the comparative studies, rates were slightly higher rates in the quinine group than the artesunate group. Overall, viewing data on rates for neurological deficits not present at baseline in light of those who survived, the data do not support an association between treatment of severe malaria with IV artesunate and enhanced risk of neurological sequelae.

3.5. Uncertainties and limitations about unfavourable effects

There were 282 healthy subjects and patients with uncomplicated or severe malaria exposed to the US Army product in 4 sponsored trials and in CDC-060. Another 194 children with severe malaria were exposed in the MMV-sponsored study that used US Army product. Thus, the safety database for which capture of AEs and ADRs is most complete is small. None of these individuals received the final product to be placed on the EU market.

However, there is some information on safety (albeit mainly from publications and therefore limited in nature) for several thousand patients with malaria, many of whom had severe malaria. The minimum age of treated patients seems to have been 6 months (in the MMV study) but the majority of the data come from children aged from ~18 months, adolescents and adults. The adult data come mainly from subjects aged < 50 years.

The applicant has pointed to stark differences in the types of AEs and ADRs reported from studies conducted in endemic areas vs. those sponsored by the US Army and CDC. Overall, it seems that the investigators involved in the trials of severe malaria conducted in endemic regions regarded many observations made on treatment as normal changes in response to severe malaria and its treatment such that reporting of AEs and ADRs was confined to deteriorations and development of complications.

These differences in reporting make it difficult to determine which ADRs merit mention in section 4.8 of the SmPC, including differentiation of true ADRs from AEs. Nevertheless, the applicant conducted a

review of ADRs captured across the studies and the final table of ADRs in section 4.8 of the draft SmPC is now agreed subject to a few additions.

3.6. Effects Table

Table 40: Effects Table for Artesunate Amivas

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
Mortality	In-hospital mortality rate	n/N (%)	Artesunate	Quinine	Several uncertainties over study and analysis conduct Published data only for AQUAMAT	
ITT PP Severe malaria			107/730 (15%) 105/689 (15%) 101/509 (19.8%)	164/731 (22%) 157/693 (23%) 152/541 (28.1%)	The oral follow-on treatment differed by treatment arm in SEAQUAMAT	SEAQUAMAT
ITT PP Severe malaria			230/2712 (8.5%) 208/2563 (8.1%) 226/2280 (9.9%)	297/2713 (10.9%) 260/2552 (10.2%) 291/2338 (12.4%)	The same oral follow-on was used in both arms in AQUAMAT	AQUAMAT
Unfavourable Effects						
AE	Rates are for AEs reported	n/N (%)	Artesunate	CBEC judgement	Uncontrolled study but relevant patient population of returning travelers	CDC-060
Anaemia			66/102 (65%)	57/66 related		
Thrombocytopenia			18/102 (18%)			
LFTs increased			28/102 (27%)	25/28 related		
AST increased			22/102 (22%)	19/22 related		
Bilirubin increased			14/102 (14%)			
Renal failure			10/102 (10%)			
ARDS			8/102 (8%)			

3.7. Benefit-risk assessment and discussion

Efficacy in the initial treatment of severe malaria

This application rests on two prospective randomised and active controlled trials conducted in endemic regions – SEAQUAMAT and AQAUAMAT – for which the degree of GCP compliance cannot be verified.

The two trials were conducted in populations that differed in age range and geographical distribution. Different approaches were taken to the oral follow-on treatment and only retrospectively defined subsets met the pre-defined criteria for severe malaria, these subsets being most relevant to the indication statement.

Nevertheless, both studies gave results that support the proposed IV artesunate dose regimen for the initial treatment of severe *P. falciparum* malaria in children aged from ~18 months, adolescents and adults.

Inevitably, these studies were conducted in endemic areas where some degree of partial immunity to malaria is acquired as subjects get older.

It is important that the AQUAMAT study was confined to children aged < 5 years with substantial parasite counts at baseline and in whom very limited naturally acquired immunity would be expected. In this study, in which all children received the same highly effective oral follow-on regimen, initial treatment with parenteral artesunate was more effective than initial treatment with parenteral quinine in terms of in-hospital mortality. In SEAQUAMAT, in which most subjects were adults, parenteral followed by oral artesunate was more effective than parenteral followed by oral quinine in terms of in-hospital mortality.

These studies support the efficacy of initial treatment of severe *P. falciparum* malaria with 2.4 mg/kg IV artesunate.

The two efficacy studies that used the US Army artesunate for treatment of severe malaria had no active control arms. The most informative of these studies was not sponsored by the US Army but by MMV. This African study provided a direct comparison of 5 doses of 2.4 mg/kg with the first two doses given 12 h apart and 3 doses of 4 mg/kg given 24 h apart. The effect on parasite counts supported use of either dose regimen in children aged 6 months to 10 years with severe *P. falciparum* malaria.

Overall, the demonstration of efficacy can be accepted. It is agreed that no lower or upper age or weight limits for use and the same weight-based dose regimen applies to all patients. It is also acceptable that the indication statement is not confined to *P. falciparum*, which causes most cases of severe malaria globally and which was the species involved in the vast majority of cases treated with IV artesunate in the clinical trials included in this application. There are other data, including in-vitro studies, supporting an expectation that IV artesunate will exert a similar antimalarial effect regardless of the exact species of Plasmodium. It is also acceptable that the indication refers to treatment of "severe malaria" to define patients who may benefit from initial treatment with IV artesunate since there is a broadly-accepted definition of severe malaria provided in WHO guidance documents and since the product will be used in patients who cannot be treated orally and *after consultation with a physician with appropriate experience in the management of malaria*.

Safety of IV artesunate

The appraisal of the safety of the applicant's IV artesunate is not straightforward. The most reliable data come from the four studies sponsored by the US Army but none enrolled the target population with severe malaria. The most relevant data come from CDC-060. However, some AEs and ADRs seem to reflect the underlying infection, which in some patients showed worsening from baseline even if they recovered eventually and in other patients preceded death. Moreover, the timing of onset of some AEs and ADRs was after completion of initial IV artesunate, which makes the assessment of causality even more difficult. Nevertheless, it could be considered that the safety data from CDC-060 are more complete than the data reported from SEAQUAMAT and AQUAMAT using IV or IM Guilin artesunate, in

which it may be concluded that investigators tended to report only AEs that could not be ascribed to the course of the disease.

The overall opinion is that IV artesunate itself is probably not associated with major safety concerns but it clearly (as with all other pharmacological agents) carries a risk for hypersensitivity reactions, which may sometimes be severe. There seems to be a risk for headache, dizziness and dysgeusia. There is also possibly a risk for LFT increases over and above any reaction to severe malaria. Also, there seem to be early on-treatment drops in blood cell counts that recover quickly but with the additional risk of PADH with onset after successful recovery.

Section 4.8 of the SmPC has been completely revised based on a detailed re-appraisal of SDRs reported across sponsored and published studies with IV artesunate preparations.

3.7.1. Importance of favourable and unfavourable effects

Artesunate Amivas is intended only for use in patients who require parenteral treatment for severe malaria. It is for use only so long as oral treatment is not possible, after which a full course of an appropriate regimen must be completed to achieve complete cure.

With insertion of advice in section 4.2 of the SmPC to use the product only under appropriate supervision, it is anticipated that only those patients who need IV artesunate will receive it and they will receive the follow-on oral regimen appropriate to the infecting species.

3.7.2. Balance of benefits and risks

Severe malaria carries a considerable mortality rate, even in returning travellers who are diagnosed and treated promptly and with access to intensive care as necessary. For the majority who survive, hospitalisation may be prolonged by development of complications. The available data have several shortcomings but the overall evidence supports a conclusion that IV artesunate is a highly effective treatment for severe and that the recommended posology has an acceptable safety profile.

From the available data, the benefit-risk balance is favourable.

3.8. Conclusions

The overall benefit/risk balance of Artesunate Amivas is positive, subject to the conditions stated in section 4. 'Recommendations'.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Artesunate Amivas is favourable in the following indication:

"Artesunate Amivas is indicated for the initial treatment of severe malaria in adults and children (see sections 4.2 and 5.1).

Consideration should be given to official guidance on the appropriate use of antimalarial agents."

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription.

It is recommended that Artesunate Amivas should be used to treat patients with severe malaria only after consultation with a physician with appropriate experience in the management of malaria.

Other conditions and requirements of the marketing authorisation

- **Periodic Safety Update Reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States

Not applicable.

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that artesunate is to be qualified as a new active substance in itself as it is not a constituent of a medicinal product previously authorised within the European Union.