27 January 2022
EMA/48303/2023
Committee for Medicinal Products for Human Use (CHMP)

Withdrawal assessment report

Garsun

International non-proprietary name: artesunate

Procedure No. EMEA/H/C/005718/0000

Note

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

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Table of Contents

List of abbreviations

ACT	Artemisinin-based combination therapies
α-DHA-G	α-DHA-β-glucuronide
AER	Auditory evoked response
ANC	Absolute neutrophil count
ARDS	Acute respiratory distress syndrome
ART	Artemisinin derivative
AQUAMAT	African Quinine Artesunate Malaria Trial
BP	Blood pressure
BSAEP	Brain stem auditory evoked potentials
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CNS	Central nervous system
CYP	Cytochrome P450
DHA	Dihydroartemisinin
ECG	Electrocardiogram
FDA	Food and Drug Agency
GCP	Good Clinical Practice
G6PDH	Glucose-6-phosphate dehydrogenase
Hb	Haemoglobin
HIV	Human immunodeficiency virus
HR	Hazard ratio
ICU	Intensive care unit
IM	Intramuscular
IPL	Interpeak latencies
IQR	Interquartile range
ITT	Intent-to-treat
IV	Intravenous
LDH	Lactate dehydrogenase
LOS	Length of stay
MAA	Marketing Authorisation Application
MORU	Mahidol-Oxford Tropical Medicine Research Unit
NaCl	Sodium chloride

OR	Odds ratio
P	Plasmodium
PADH	Post artesunate delayed haemolysis
PD	Pharmacodynamic
PK	Pharmacokinetic
PP	Per protocol
PSUR	Periodic safety update report
RBC	Red blood cell
SAE	Serious adverse event
SEAQUAMAT	South East Asian Quinine Artesunate Malaria Trial
UGT	Uridine 5’-diphospho-glucuronosyltransferase
ULN	Upper limit of normal
t1/2	Elimination half-life
WHO	World Health Organization

1. CHMP Recommendation

Based on the review of the data on quality, safety, efficacy, the application for Garsun, an orphan medicinal product, for the

Treatment of severe malaria caused by *Plasmodium falciparum* in adults and children

is not approvable since "major objections" have been identified which are not yet resolved, which precludes a recommendation for marketing authorisation at the present time. The details of the major objections are provided in the list of outstanding issues (see section 7).

The major objections precluding a recommendation of marketing authorisation, pertain to the following deficiencies:

(1) GMP of manufacturing site.

Valid proof of GMP compliance issued by an EEA national competent authority inspectorate (e.g. a GMP Certificate) for the proposed drug product manufacturer in China must be provided.

(2) Orphan similarity and derogations

On 22 November 2021, while the assessment of Garsun was ongoing, the EC granted a Marketing Authorisation to Artesunate Amivas (artesunate) for the treatment of severe malaria in adults and children. Artesunate Amivas has orphan drug designation.

The applicant should provide a similarity report viz. Artesunate Amivas and if applicable provide ground(s) requesting derogation allowing a Marketing Authorisation in accordance with Point 3 of Article 8 of the Orphan Regulation (EC) No 141/2000.

(3) New Active Substance (NAS)

In view of the Marketing Authorisation of Artesunate Amivas, the applicant should submit a new report on the claim regarding the NAS status for Garsun.

Questions to be posed to additional experts

None

Inspection issues

GMP inspection(s).

A Major Objection is raised in relation to the absence of a valid proof of GMP compliance (e.g. a GMP Certificate) issued by an EEA national competent authority for the finished product manufacturing in China.

The site was inspected by FDA in 2018. The FDA inspection covered only the manufacturing operation for the drug substance bumetanide (non-sterile API by chemical synthesis) and not the drug substance artesunate.

The site was inspected by WHO in 2018, 2020, and in 2021. The inspection performed in 2020 covered the manufacturing process so-called ''batch-to-batch process'' for artesunate. This is the process under

assessment in the application. The site was considered GMP-compliant for this manufacturing process and a GMP compliance statement has been issued by WHO.

It is however noted that the site has so far never been inspected by an EEA National Competent Authority.

France (ANSM), the GMP National Competent Authority for this application, clarified that they cannot perform an on-site inspection due to the pandemic travel restrictions. ANSM considers a distant assessment for the batch-to-batch process at the site, which was covered during the WHO inspection in 2020, also not appropriate for the following reasons:

The site is proposed as the manufacture of the sterile artesunate powder (aseptic process) and the solvents (terminal sterilisation).

The manufacturing steps contain aseptic operations, which are particularly critical to assess as any minor deviation can have an impact on the sterility assurance of the product.

ANSM considered therefore that a distant assessment for the batch-to-batch process at the site is not feasible.

In view of the above, a request for GMP inspection has been adopted by CHMP for the site in China in order to verify the GMP compliance status. The outcome of this inspection is required for the Committee to complete its examination of the application.

GCP inspection

None

New active substance status

Based on the Commission Decision for Artesunate Amivas, it is considered that the active substance artesunate contained in the medicinal product Garsun is not qualified as new active substance. The major objections identified, which preclude the recommendation are detailed in the List of outstanding issues.

Similarity with authorised orphan medicinal products

On 22 November 2021, the EC granted a licence for Artesunate Amivas (artesunate), which is indicated for the initial treatment of severe malaria in adults and children. This product has orphan drug designation in the EU.

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant is requested to submit a critical report, addressing the possible similarity with authorised orphan medicinal product Artesunate Amivas.

2. Executive summary

2.1. Problem statement

Artesunate is a semi-synthetic artemisinin derivative intended for the initial intravenous treatment of severe falciparum malaria in adults and pediatric 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, diarrhoea 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, 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: 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:

- *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 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: Pf* parasitaemia >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.

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 from 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 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 (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. 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 obtained by special means. For example, by allowing import of the WHO-prequalified (2011) Guilin formulation (Garsun).

2.2. About the product

Artemisinin 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. Parasitaemia 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 *Pv* and *Po*.

Garsun is supplied as a sterile powder for constitution containing 60 mg of artesunate per vial. The powder is reconstituted with 1.0 mL of supplied sterile diluent containing 50 mg sodium bicarbonate and further diluted using sodium chloride 0.9% to form a 10 mg/mL solution for intravenous injection and a 20 mg/mL solution for intramuscular injection. The applicant proposed parenteral treatment for at least 3 doses and a switch to an oral combination regimen as soon as possible after that. Regardless of route of administration, the SmPC recommends 2.4 mg/kg at 0, 12 and 24 h and then once daily.

The applicant did not seek advice from the CHMP or from national EU agencies.

The applicant's intravenous artesunate preparation was used in two prospective randomised and active controlled trials in adults and children with severe falciparum malaria in endemic areas (SEAQUAMAT and AQUAMAT). Whilst this application is based on Article 10a (well-established use; see below), these prospective, randomised and active controlled studies provided the pivotal evidence of efficacy that has been behind WHO recommendations and national moves to use of parenteral artesunate as first line treatment for severe malaria until oral treatment is possible.

2.3. General comments on compliance with GMP, GLP, GCP

GMP

A declaration of GMP Compliance for the manufacturer of the drug substance covering the steps in the proposed synthesis (reduction, esterification, recrystallisation) is provided by the Qualified Person responsible for batch release in the EU.

A Major Objection is raised in relation to the absence of a valid proof of GMP compliance (e.g. a GMP Certificate) issued by an EEA national competent authority for the finished product manufacturing in China.

The site was inspected by FDA in 2018. The FDA inspection covered only the manufacturing operation for the drug substance bumetanide (non-sterile API by chemical synthesis) and not the drug substance artesunate.

The site was inspected by WHO in 2018, 2020, and 2021. The inspection performed in 2020 covered the manufacturing process so-called ''batch-to-batch process'' for artesunate. This is the process under assessment in the application. The site was considered GMP-compliant for this manufacturing process and a GMP compliance statement has been issued by WHO.

WHO confirmed that the on-site inspection from August 2021 is for another process (continuous manufacturing). Taking into account the criticality of the site and the risk associated with their products the inspection was on-site. The site was considered GMP-compliant for this manufacturing process and a GMP compliance statement has been issued by WHO.

It is noted that the site in China has so far never been inspected by an EEA National Competent Authority. France (ANSM), the GMP National Competent Authority for this application, clarified that they cannot perform an on-site inspection due to the pandemic travel restrictions. ANSM considers a distant assessment for the batch-to-batch process at the site, which was covered during the WHO inspection in 2020, also not appropriate for the following reasons:

-The site in China is proposed as the manufacture of the sterile artesunate powder (aseptic process) and the solvents (terminal sterilisation).

-The manufacturing steps include aseptic operations, which are particularly critical to assess as any minor deviation can have an impact on the sterility assurance of the product.

ANSM considered therefore a distant assessment for the batch-to-batch process at the site is not feasible.

In view of the above, a request for GMP inspection has been adopted by CHMP for the site in China in order to verify the GMP compliance status. The outcome of this inspection is required for the Committee to complete its examination of the application.

The only other manufacturing sites are the batch control sites in the EU and these are appropriately authorised to complete batch control testing.

GLP

No new non-clinical studies have been conducted. Reference is made to literature, with unknown GLP status. This is considered acceptable, in view of the well-known non-clinical and clinical safety profile.

GCP

The Clinical Overview does not address GCP aspects except to state that compliance with GCP in SEAQUAMAT and AQUAMAT is unknown.

2.4. Type of application and other comments on the submitted dossier

Legal basis

The legal basis for this application refers to:

Article 10a of Directive 2001/83/EC, as amended – an application based on well-established use. The product falls within the mandatory scope for centralised procedures due to its orphan designation.

Well-established use in the EU

Module 1.5.1 contains the applicant's justification for well-established use in the EU, which may be summarised as follows:

Time over which artesunate has been used for the treatment of severe malaria in Europe

A literature search was performed using Medline to evaluate the use of artesunate in the management of severe falciparum malaria. Malaria caused by *Plasmodium falciparum* is a rare disease in the EU. It has been estimated that there are not more than 4000 malaria cases in the EU annually – the vast majority being imported from endemic countries by travellers [Levy et al. 2016]. In addition, 7-15% of *Plasmodium falciparum* cases present as severe malaria [Wångdahl et al. 2012; Sonden et al. 2017; Broderick et al. 2012]. For example, from 2011-2017 there were 1544 patients with severe *P. falciparum* malaria treated in France, of which 70% were treated with artesunate [El Ket et al. 2020]. In a survey from France, Netherlands and Belgium, the number of medical centres using artesunate increased from 1 to 116 in the years from 2007 to 2012 [Kreeftmeijer-Vegter et al. 2013].

In conclusion, treatment with artesunate is well established in the EU and it has been continuously used for more than 10 years. The publications identified cover 13 member states covering more than 95% of the total population. The first documented regular use of artesunate in the treatment of severe malaria in the EU is well before 2010 based on:

- A retrospective case series of children treated in one of the four German tertiary centres with IV artesunate started in 2010 [Bélard et al. 2019]
- The first 8 patients that had been treated with artesunate (at that time still in combination with IV quinine) in Italy during 2008/2009 [Bartolini et al. 2010].
- In Norway nine patients were identified that were treated with artesunate between February 2006 and May 2008 [Mørch et al. 2008].
- In Sweden since 2010 patients with severe disease or vomiting are initially administered intravenous artesunate [Sondén et al. 2017]
- In one study from UK the experience with artesunate (regularly used since 2009) was compared with quinine [Eder et al. 2012]. In addition, UK treatment guidelines mentioned as early as 2007 that the Advisory Committee on Malaria Prevention "*is impressed by accumulating evidence that artesunate offers significant benefit over quinine for patients with very severe malaria or high parasite counts*" [Lalloo et al. 2007].
- Artesunate was available via named patient programs in the Netherlands since 2007 and in Belgium since 2009 [Kreeftmeijer-Vegter et al. 2012; Kreeftmeijer-Vegter et al. 2013].
- Zoller and colleagues provided data from patients treated with artesunate in Denmark, Germany, or Norway during the period 2006 – 2010 [Zoller et al. 2011]
- The TropNet program – with sites from 11 EU member states – was initiated in 2005 [Zoller et al. 2011]. This revealed that in 2006 the proportion of patients treated with intravenous artesunate was 27%, i.e. more than one fourth of the patients [Kurth et al. 2017].

Quantitative aspects of the use of artesunate in Europe

Published literature indicates that almost all sites globally use parenteral artesunate manufactured by Guilin Pharma, who supply B & O Pharm with product for this marketing authorisation. Data on the export of Guilin Pharma artesunate to the EU provide indirect evidence of the use of this antimalarial agent. Such data are available only since due to the fact that the sales data were not appropriately

processed before that time. An overview on the supply of Guilin's artesunate in EU is presented below, showing continuous use of artesunate in the EU during this reporting period.

Exports were made to member states that had not been identified from the literature research which supports widespread use of artesunate in the EU.

Not all EU member states are included in this table because some of the importers of Guilin Pharma artesunate were local distributers not covered individually by the Guilin data. Furthermore, the Dutch company ACE Pharma distributes artesunate (60 mg active substance together with 5% sodium bicarbonate) under the brand name Malacef. As in other EU countries, artesunate is not authorised in the Netherlands but the Dutch Health Care Inspectorate has given permission for supply to hospitals for treatment on a named patient basis. In addition, this product is available in France for treatment of severe malaria under an ATU and it is used in Belgium based on a named patient programme [Kreeftmeijer-Vegter et al. 2013].

Conclusion

The first documented use of artesunate in the EU was immediately subsequent to publication of the SEAQUAMAT study in 2006. Since then it has been frequently administered to patients in the EU despite lack of marketing authorisation. Based on evidences above it is safe to conclude that artesunate has been used extensively and continuously in the EU for a period of more than 10 years.

Increasing scientific interest in artesunate is largely based on the fact that prior to the availability of parenteral artesunate quinine was the recommended treatment for the management of severe *P. falciparum* malaria but it was associated with some significant safety concerns. For example, IM quinine is painful and can cause sterile abscesses, sciatic nerve damage and predispose to lethal tetanus [Yen et al. 1994]. Intravenous administration has to be done as a slow rate-controlled infusion since rapid administration can result in lethal hypertension. Quinine-induced hyperinsulinaemic hypoglycaemia is a serious problem – in particular in pregnant patients [WHO 2015; White et al. 1983; Lalloo et al. 2016; Askling et al. 2012]. In addition, quinine must be administered with care to older patients or those with heart disease due to its potential to cause arrhythmias [Lalloo et al. 2016].

It has been demonstrated that artesunate is clinically superior to quinine in terms of reduction of mortality. This has been observed in various studies and retrospective analyses in Africa, Europe and Asia [Hien et al. 1992; Cao et al 1997; Newton et al. 2003; Dondorp et al. 2005; Dondorp et al. 2010; Eltahir et al. 2010; El Ket et al. 2020]. In a Cochrane report it was concluded that treatment with artesunate significantly reduced the risk of death both in adults (RR 0.61, 95% CI 0.50 to 0.75; 1664 participants, five trials) and children (RR 0.76, 95% CI 0.65 to 0.90; 5765 participants, four trials) [Sinclair et al. 2012]. Nowadays, the use of artesunate for the treatment of severe malaria is standard of care [Sinclair et al. 2012]. This is reflected in various national and global guidelines [Askling et al. 2012; Leblanc et al. 2020; Lalloo et al. 2016; WHO 2015].

In summary, the active substance artesunate has been in well-established medicinal use within the Community in the EU for at least ten years, with recognised efficacy and an acceptable level of safety. Accordingly, a Marketing Authorisation Application based on Article 10a was filed and non-clinical and clinical test and trial results were replaced by appropriate scientific literature.

New active substance status

The applicant requested the active substance artesunate contained in Garsun should be considered a new active substance. At the time of filing this application, there were no licensed products in the EU containing artesunate. However, Artesunate Amivas (artesunate), another product with orphan drug

designation for treatment of malaria, was granted a licence in the EU on 22 November 2021, at which time the assessment of Garsun was ongoing.

Orphan designation

Garsun was designated as an orphan medicinal product EU/3/15/1521 on 28 July 2015 in the following condition: treatment of malaria. The ODD was subsequently transferred from the initial applicant (Ulrich Granzer) to the current applicant (B and O Pharm).

Similarity with orphan medicinal products

The application did not contain a critical report pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, addressing the possible similarity with authorised orphan medicinal products. This was because there were no medicinal products authorised in the EU for the initial treatment of severe malaria or for the treatment of complicated falciparum malaria when the application was filed. However, Artesunate Amivas (artesunate) was granted a licence for treatment of severe malaria on 22 November 2021. Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant is requested to submit a critical report, addressing the possible similarity with authorised orphan medicinal product Garsun.

Information on paediatric requirements

Due to the legal basis for the application, no PIP was required.

3. Scientific overview and discussion

3.1. Quality aspects

3.1.1. Introduction

Artesunate is a semi-synthetic hemisuccinate derivative of artemisinin, a class of antimalarial compounds extracted from the *Artemisia annua* or sweet wormwood plant. It is proposed to be used for the treatment of severe malaria caused by *Plasmodium falciparum* in adults and children. The drug product contains one vial of Artesunate for Injection (60 mg/vial), one ampoule of Sodium Bicarbonate Injection (50 mg/ml, 1 mL) as solvent, and one ampoule of Sodium Chloride Injection (9 mg/ml, 5 mL) as solvent. These are co-packed in one package.

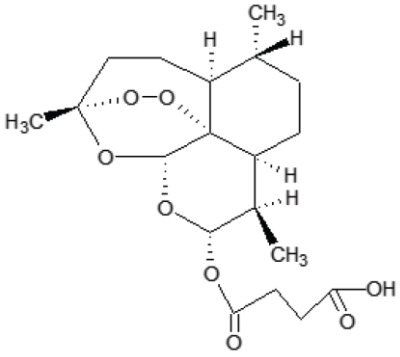
The drug substance is manufactured using a semi-synthetic process.

3.1.2. Active Substance

General Information

International Non-Proprietary Name (INN):	Artesunate
Chemical names:	(3R, 5αS, 6R, 8αS, 9R, 10S, 12R, 12αR)-Decahydro-3,6,9–trimethyl-3,12-epoxy- 12H-pyrano[4,3-j]-1,2- benzodioxepin-10-ol, hydrogen succinate
Molecular formula:	$C_{19}H_{28}O_8$
Relative molecular mass:	384.43

Structure:



Artesunate is a white crystalline powder, very slightly soluble in water, very soluble in dichloromethane, freely soluble in ethanol and acetone. The physicochemical characteristics such as hygroscopicity, pH of solution, specific optical rotation and melting point have been described. The active substance contains eight chiral centers. It is a single enantiomer, α-epimer of artesunate. No polymorphism on active substance is claimed.

Manufacture, process controls and characterisation

A declaration of GMP Compliance for the manufacturer of the drug substance covering the steps in the proposed synthesis (reduction, esterification, recrystallisation) is provided by the Qualified Person responsible for batch release in the EU.

The proposed commercial manufacturing process consists of three steps. Information has been provided on the origin and control of the proposed starting material and on the origin, fate and control of potential impurities from the starting material.

The commercial manufacturing process has been used for about 40 years. The commercial manufacturing process, critical process parameters material control and in-process control have been well established. Information about the justification of these controls has been provided. The control of stereochemistry during the synthesis has been discussed and possible formation of the β isomer further discussed and supported by analytical data.

Validation was performed to confirm that the manufacturing site can produce artesunate drug substance of consistent quality.

The potential impurities in the drug substance are discussed and there are no known potential genotoxic impurities expected from the synthesis. Two impurity structures of uncertain toxicity were initially identified. One substance is adequately controlled, and, as the other could appear as an impurity in the drug substance, Ames testing was used to confirm the absence of mutagenic potential.

Specification, analytical procedures, reference standards, batch analysis, and container closure

The specification includes the required tests for the drug substance. The analytical methods are described. Method validation has been provided to support the relevant test methods.

Stability

Artesunate is found to be stable.

3.1.3. Finished Medicinal Product

Description of the product and Pharmaceutical Development

After reconstitution and dilution one ml of final solution for injection contains 10 mg (intravenous use) or 20 mg (intramuscular use) of artesunate. The proposed parenteral artesunate formulation should be administered until oral treatment can be substituted. It should be administered for a minimum of 24 hours (3 doses).

The Target Product Profile and summary information on the development of the product is provided and provided an understanding of the development of the manufacturing processes and allows an understanding of the process controls employed for routine manufacture.

The dosage form is for intravenous administration only and the dosage characteristics have been justified. The dosage will be administered by a healthcare professional.

Manufacture of the product and process controls

A Major Objection was raised and is maintained in relation to the absence of a valid proof of GMP compliance issued by the EEA national competent authority for the manufacturing site.

Process validation has been completed. The excipients used in the drug product manufacture are ethanol and WFI and these fully comply with the respective Ph. Eur. monographs.

Product specification, analytical procedures, batch analysis

The analytical methods are described and method validations to support the methods are included in the dossier. Batch data supports the quality of the product manufactured.

The potential presence of nitrosamine impurities in the product has been evaluated and it is concluded that there is no/negligible potential for these impurities to occur.

The vial, stopper and cap are sterilised before use. Confirmation is provided of compliance with the Ph. Eur. monograph for rubber closures.

Stability of the product

Acceptable stability data at accelerated and long-term is available

Drug Product (Sodium Bicarbonate)

The product is manufactured at the same sites as the artesunate. The absence of a valid proof of GMP compliance issued by the EEA competent authorities (e.g. a GMP Certificate) to support manufacturing is raised as a Major Objection.

The specification is acceptable.

The analytical procedures have been described and method validation supports the validity of the methods. No reference standards are used in the analytical testing.

The container closure is a Type I glass ampoule.

Drug Product (Sodium Chloride)

The provided pharmaceutical development information is acceptable.

The product is manufactured at the same sites as the artesunate. The absence of a valid proof of GMP compliance issued by the competent authorities (e.g. a GMP Certificate) to support manufacturing is raised as a Major Objection.

The specification is acceptable.

The analytical procedures have been described and method validation supports the validity of the methods. No reference standards are used in the analytical testing.

3.1.4. Discussion and conclusions on chemical, pharmaceutical and biological aspects

The drug product contains one vial of Artesunate for Injection (60 mg/vial), one ampoule of Sodium Bicarbonate Injection (50 mg/ml, 1 mL) as solvent, and one ampoule of Sodium Chloride Injection (9 mg/ml, 5 mL) as diluent. These are co-packed in one package.

3.2. Non clinical aspects

3.2.1. Pharmacology

Artesunate is a semi-synthetic hemisuccinate derivative of artemisinin, a class of antimalarial compounds extracted from the Artemisia annua or sweet wormwood ("Qinghao") plant. Artesunate is used as a first-line drug for the treatment of severe *falciparum* malaria. The artemisinins are distinguished from other antimalarials by their ability to kill all erythrocytic stages of the malaria parasite, including the relatively inactive ring stage and late schizonts, as well as the gametocytes responsible for malaria transmission.

Primary pharmacodynamics

The mechanism of action of artesunate and DHA is likely multifactorial. The endoperoxide bridge is essential for the antiplasmodial activity. Upon activation by haem (liberated as a result of plasmodial protease-mediated degradation of host haemoglobin), artesunate and DHA, like other artemisinins, generate free radicals (e.g., superoxide anions, H2O2 and other intermediates), increase oxidative stress within the infected RBCs, cause alkylation of proteins and lipids as well as ultrastructural changes leading to a decrease in parasite function, growth and survival. The mitochondria, endoplasmic reticulum, as well as digestive vacuole appear to be the sites of damage.

Artesunate and DHA, the active metabolite of artesunate, have shown activity in vitro against all blood stages (asexual forms and gametocytes) of laboratory strains and clinical isolates of *P. falciparum*, with IC50 values between 0.08 and 6.54 ng/mL. Artesunate and DHA are not active against liver stages. Similar in vitro activities have been reported for *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*. Most of the referred publications indicate that asexual forms are more sensitive than gametocytic forms, and the mature gametocytes appear to be the least sensitive.

In vivo studies in rodent and nonhuman primate malaria models support the anti-malaria activity of artesunate and DHA on erythrocytic stage parasites, including severe and cerebral malaria animal models infected with *P. berghei* or *P. yoelii*, immunocompromised chimeric mice infected with P*. falciparum*, and non-human primates infected with *P. coatneyi, P. cynomolgi, P. falciparum* or *P. knowlesi*. However, recrudescence generally occurs after discontinuation of treatment. DHA was effective in curing immunocompromised mice infected with a chloroquine and quinine resistant strain of *P. falciparum*.

In vitro and *in vivo* studies indicate a potential for resistance development to artesunate or DHA, manifested as increased IC50 values, recrudescence, and/or delay in parasite clearance time. The mechanism of resistance is likely to be multifactorial and complex with more than one genotype/phenotype associated with artemisinin resistance. Some studies have shown alterations in some genetic regions, such as *Kelch13* and *Pfmdr1* of *P. falciparum*, while other studies have not been able to relate increased resistance altered *Kelch13*.

Secondary pharmacology

No specific secondary pharmacology studies to evaluate potential off-target and unintentional effects of artesunate are published in the literature. Non-antimalarial effects are, however, being evaluated in several studies for the indication of oncology, anti-inflammatory and immunoregulatory effects.

Safety pharmacology

Numerous publications addressing potential effects on the cardiovascular system and the central nervous system has been provided. At high IV doses (>250 mg/kg in mice, >80 mg /kg in dogs and monkeys), artesunate caused dose related decreased activity and sedation in all species. At very high doses, death due to CNS depression, convulsions and respiratory suppression was observed in guinea pigs, dogs and monkeys.

Although artemisinins are considered to be rather well tolerated, artheether and arthemether have generated a selective pattern of brainstem neuropathology. Similar findings have, however, not been reported for artesunate.

Artesunate had no effect on the hERG-current (IC50 >100 µM). However, an inhibitory effect is seen with DHA (IC50 7.7 µM). While decreases in ECG, heart rate and blood pressure were noted in a single, anaesthetised dog administered artesunate at 320 mg/kg IV, QT prolongation was not observed in dogs following single oral dosing up to 150 mg/kg. This is in contrast to other anti-malaria agents, including quinidine, artemether and artemotil, demonstrating QT prolongation.

Pharmacodynamic drug interaction

In vitro and in vivo studies addressing artesunate in combination with other anti-malaria drugs as well as with drugs for other indications indicate synergistic effects, although antagonistic effects have also been reported. A reduced activity of artesunate is reported in vitro when combined with drugs exerting antagonistic activity, like antioxidant drugs. From the use of artesunate over the past decades as well as from controlled clinical drug-drug interaction (DDI) studies, however, no clinically significant pharmacodynamic drug interactions are reported with co-administration of artesunate.

3.2.2. Pharmacokinetics

Absorption

The pharmacokinetic properties of artesunate were studied in healthy rats, dogs and monkeys using IV, IM or PO route of administration following single or repeated dosing. Furthermore, studies were performed in malaria infected animals, using *P. berghei* infected rats and mice, and *P. coatneyi* infected monkeys.

Following IV and IM dosing, artesunate is rapidly hydrolysed to the main active metabolite DHA, with similar PK profiles for the two routes of administration. Following IV dosing, short terminal half-life is seen for artesunate (within minutes) and DHA (less than an hour) across all species.

In rats, IM relative bioavailability (artesunate + DHA) is high, between 85 and 98%. PO bioavailability is low in all species (rat, dog, monkey), and it is postulated that most oral artesunate is absorbed as DHA due to rapid hydrolysis in the stomach.

Pharmacokinetic parameters in malaria-infected rats and mice were comparable to uninfected animals.

Distribution

Tissue distribution studies with both [^{14}C]-artesunate and [^{14}C]-DHA show rapid and wide distribution following IV dosing. One hour after dosing, substantial amounts were amassed in the small intestine and its content. At later time points drug distribution was significantly increased in other tissues, indicating redistribution and potential enterohepatic circulation. This is further supported by mass balance data showing that excretion is mainly via urine. Long tissue half-lives (50-100 hours) indicate retention. Radioactivity in whole blood was always higher (2.1–4.2 fold) than in plasma throughout the period measured. For both substances, brain levels were about 2-fold higher than that in plasma.

Equivocal results regarding placental transfer has been reported. Following repeated PO artesunate at up to 8 mg/kg/day (GD6-15) to rats, artesunate and DHA were both below the limit of detection (<1 ng/mL) in the amniotic fluid at GD15, indicating limited placental transfer (Chung, 2013). Placental transfer of radioactivity was, however, confirmed in pregnant rats given oral doses of [^{3}H]-artesunate on GD 18 (Clark, 2010). Excluding tissues involved in absorption and excretion, the highest concentrations of radioactivity were observed in tissues involved in haemoglobin synthesis and/or destruction in both the mother and the foetus. Highest foetal levels were observed in blood and liver, for which the foetal blood levels were >3.7-fold higher than maternal blood levels at all time points.

For both substances, high plasma protein binding of artesunate is observed in rat and human plasma in vitro (81 and 73%, respectively).

Metabolism

Following parenteral administration, artesunate is transformed to the active metabolite DHA, mainly via esterases. In vitro and in vivo data indicate minimal contribution of the P450 system.

Conjugation studies showed that drug conjugation of DHA (up to 90% of the dose in plasma and urine) is a major metabolic pathway of [14C]-artesunate, and that conjugation is time-dependent in rats. The main conjugation pathway for DHA is glucuronidation in the liver. The UGT enzymes involved in DHA glucoronidation in humans is UGT1A9 and UGT2B7.

Excretion

In rats, less than 1% of IV artesunate was excreted as unchanged drug. DHA and conjugates of DHA are excreted via urine (48-66%) and faeces (37-42%).

Pharmacokinetic drug interactions

Both CYP inhibition and induction have been reported for artemisinin derivatives, based on in vitro data and clinical data, respectively. Further, DHP had slight but significant inductive effects on BCRP, BSEP and MRP3, and inhibitory effect on BCRP.

3.2.3. Toxicology

Artesunate is intended for IV or IM use. While there are limited non-clinical data available based on intended routes of administration, several studies with oral administration were included in the dossier. Since artesunate is rapidly metabolised to the active metabolite DHA, studies with DHA have also been included. Although toxicokinetic data are scarce, systemic toxicity findings following repeated dosing with artesunate do indicate sufficient exposure.

GLP status is not addressed in the literature references. Most studies have been conducted by academia, and some studies were conducted before introduction of GLP by the OECD in 1992. This is considered acceptable for a bibliographical dossier.

Single and repeat-dose toxicity

IV and IM artesunate have shown low acute toxicity in all species tested. LD50 was ≥475 mg/kg in mice, and minimum lethal IV dose was 160, 480, 480 and 640 mg/kg in monkey, dog, Guinea pig and rabbit, respectively.

Major target organs for toxicity following repeated dosing are the haematopoietic system, the cardiovascular system, the nervous system, the gastrointestinal system and the liver. Most findings are considered reversible and manageable. Mortalities were observed in mice, rats and monkeys at high parenteral doses (≥150 mg/kg/day in mice (28 days), >240 mg/kg in rats (3 days), and 128 mg/kg in monkeys (7 days)). These doses are considered sufficient above human dosing indicating low clinical relevance. In general, studies with oral dosing are better tolerated than intravenous or intramuscular dosing, likely due to the limited oral bioavailability of artesunate/DHA. Long-term oral studies have, however, shown haematological toxicity, hepatotoxicity and nephrotoxicity, similar to findings in parenteral studies.

While there are no unequivocal evidence for neurotoxic effects in malaria patients (WHO 2016), artemisinins induce a characteristic pattern of neuronal lesions in the brain stem in animals. This neurotoxicity appears dependent on dose, route of administration, and physical-chemical properties of the derivate (WHO, 2016). In a comparative study in rats, IM artesunate at 31 mg/kg/day for 7 days was devoid of CNS effects (supported by behavioural and histopathology data), while artheether at equimolar doses caused substantial behavioural and histopathological effects. A similar difference in neurotoxic potential has also been reported in mice, with IM artemether (50−100 mg/kg/day for 28 days) that caused dose-dependent behavioural effects (balance and gait), and neuropathologic damage to the brain stem. In contrast, although behavioural effects of IM artesunate were observed at doses up to 300 mg/kg/day, no histopathological findings were reported.

Reversible haematological toxicity and bone marrow toxicity was observed in rodents, dogs and monkeys, characterised by intravascular haemolysis, haemoglobinuria, reduced RBC parameters, reticulocytopenia, leukopenia, neutropenia, thrombocytopenia, and reduced bone marrow cellularity. At dose levels causing substantial haematological toxicity, reversible renal toxicity was also apparent, characterised by haemoglobinuria, proteinuria and tubular necrosis and regeneration. Rat appeared more sensitive compared to dog and monkey. According to the applicant, haematopoietic toxicity on the progenitors of the erythroid lineage is likely responsible for reticulocytopenia and leucopenia of central origin. However, increased levels of malondialdehyde (MDA) and reduced levels of antioxidants do suggest increased lipid peroxidation of erythrocyte membranes due to reactive oxygen species (Aabolaji, 2014), contributing to observed vascular haemolysis.

Hepatic toxicity (histopathological findings and increased hepatic enzyme levels and altered clinical chemistry parameters) was only apparent in rats following oral dosing. Although recovery has not been addressed, the hepatic effects are considered mild to moderate, and therefore expected to be reversible.

Genotoxicity

The provided references on genotoxicity studies are generally not considered GLP compliant, and not in accordance with ICH S2(R1). No data with regard to mutagenic effects have been provided for artesunate. However, a negative Ames test on the structurally similar artemisinin indicate lack of mutagenic potential for artesunate.

The provided references on in vitro and in vivo genotoxicity studies have shown that artesunate is cytotoxic, and induces micronuclei formations and single strand-breaks. Suggested mechanisms are oxidative damage due to oxygen radical formation.

Carcinogenicity

No data have been provided.

Reproductive toxicity

No data have been provided addressing potential effects of female fertility. Data from repeat-dose toxicity studies as well as genotoxic evaluations in vitro indicate reversible effect on sperm in rodents and monkeys. In a fertility study in male rats, moderate and reversible effects on testosterone levels, sperm parameters (motility, viability, count), and corresponding histopathology was without effect on fertility.

In conventional studies of embryo-foetal development, oral artesunate caused dose-dependent foetal toxicity in rats, rabbits and monkeys, resulting in foetal resorption and abortion, as well as a low incidence of cardiac and skeletal defects at dose levels without maternal toxicity. In rats and rabbits, embryonal loss occurred at all dose levels, and NOAEL could not be determined. In monkeys, NOAEL was 4 mg/kg/day.

Findings in a number of in vitro and in vivo publications includes deletion of primitive erythroblasts, reduction of maternal reticulocyte count and anaemia, embryonic death and foetal resorption during organogenesis, retardation of foetal growth among surviving foetuses and cardiovascular malfunction, skeletal defects, and delays in limb and tail development. Based on short-term dosing at different time windows during gestation, it is suggested that there is a critical time period during which the embryonic erythroblast cells are particularly sensitive to artesunate-induced cytotoxicity, leading to unusually steep dose-response relationship. In the WHO guidance document (WHO 2016), it is suggested that the primary target are the embryonic erythroid precursor cells forming in the blood islands of the yolk sac. It is furthermore suggested, that the mechanistic basis of this damage to erythroid precursor cells is the same as, or at least analogous to, the mechanism for the antimalarial

activity of artemisinins. As maturation of embryonic RBC occurs, the embryo becomes less sensitive to artesunate-induced developmental toxicity.

The clinical relevance of these findings for first trimester pregnancies are not known. As indicated by WHO (2016) and the provided RMP, limited prospective clinical data from use during first trimester of pregnancy have not shown a similar embryotoxic potential in humans. This could potentially be because development of the circulatory system in the growing human embryo takes place between days 21 and 35 post conception, meaning that the most sensitive period for the generation of any cardiovascular defects in humans could possibly be at a time when pregnancy is not yet recognized. Alternatively, the corresponding sensitive period during which primitive erythroblasts are formed and remain circulating in the embryo is longer than that in rodents, which could theoretically allow time for artesunate-damaged cells to be replaced thereby curtailing the teratogenic effects.

Impurities

Beside Artemisitene (impurity of the starting material), Impurity A (Dihydroartemisinin, Artenimol and (10R)-Artenimol), Impurity B (Artemisinin), Impurity C (Anhydrodihydroartemisinin), Impurity D (Dihydroartemisinin impurity A) and Impurity F (Artesunate impurity 1) were identified as possible impurities contained in Artesunate for Injection. Impurity A (Dihydroartemisinin) is the active metabolite of Artesunate. Based upon lack of suspected warning structures, impurities A, B, and C do not pose any genotoxicity hazard.

(Q)SAR assessment has been performed on artemisitene, impurity D and impurity F. Based on two (Q)SAR methodologies, impurity D is not considered to pose a genotoxic risk. Artemisitene was classified as inconclusive, as it was outside domain in one of two (Q)SAR methods. The level of artemisitene is adequately controlled in the specification of the starting material artemisinin. Impurity F was also classified as inconclusive, since it was outside domain in one of two (Q)SAR methods. Impurity D is the degradant of impurity F and does also contain alkyl aldehyde. Considering that impurity D is negative in (Q)SAR, impurity F is considered not to pose any genotoxicity hazard. Both impurities D and F are limited to below identification threshold (ICH Q3B (R2)).

Other toxicity studies

Two studies indicate potential effects on the immune system, leading to both stimulatory effects in vitro and suppressive effects in vivo. The doses and exposure times were higher in vitro and, in the animals, compared to the clinical situation. Clinical experience does not indicate excessive immune-stimulatory effects.

3.2.4. Ecotoxicity/environmental risk assessment

A log Kow is determined to 2.36, based on the shake-flask method and procedures as described in OECD guideline 107. A PECsw of 1.4 µg/L was calculated for artesunate, which is above the action limit of 0.01 µg/L. A refined PEC_{SW} of 0.0011599 µg/L is further presented, based on data on the prevalence of severe malaria in France, the country in Europe with highest prevalence in Europe in 2017 and 2018. Considering absence of PBT risk and a refined PECsw <0.01 µg/L, a phase II risk assessment is deemed not necessary.

3.2.5. Discussion on non-clinical aspects

Pharmacology

Data on safety pharmacology have shown sedative effects on CNS and respiration, with reduced heart rate and blood pressure at doses significantly above intended human dosing, indicating low clinical relevance.

Pharmacokinetics

Considering that this is a WEU procedure based on bibliographic documentation, validation status of the applied analytical methods has not been addressed.

Three daily IM doses of artesunate to uninfected rats and three daily IV doses of artesunate to *P. berghei*-infected rats led to reduced exposure levels for both artesunate and DHA (Li 2005, 2008b). Reduced DHA exposure levels were also reported in rats following oral dosing for 5 days (Xing 2011). Although limited data, a similar effect was seen in monkeys following repeated PO dosing. Reduced exposure levels have also been reported in humans.

According to Clark (2010), there is a substantial discrepancy between plasma concentrations of artesunate and DHA compared to radioactivity measurements in blood following oral dosing of artesunate and [^{3}H]-artesunate, respectively. It is postulated that the most plausible explanation between TK data and radioactivity data is that the large majority of the radiolabelled material detected in blood in the QWBA study, which is no longer in the form of artesunate or DHA, has become covalently bound to macromolecules, probably as a result of free radical formation following reduction of the endoperoxide bridge. This may also be the reason for equivocal results regarding placental transfer in rats, where both artesunate and DHA were below the limit of detection in the amniotic fluid in one study, while substantial levels of radioactivity were detected in foetal tissues in another study.

The main conjugation pathway for DHA is glucuronidation in the liver, and in humans the UGT enzymes involved in DHA glucoronidation is UGT1A9 and UGT2B7. Several non-clinical studies have reported reduced exposure levels of artesunate and DHA following repeated dosing, indicating auto-induction. Further, in healthy humans (Zhang 2014), repeated treatment with DHA led to lower exposure and higher metabolic capability to form its metabolite DHA-glucuronide after repeated oral doses. Taken together, although there are no confirming data available, a potential autoinduction of UGT enzymes involved cannot be excluded.

Toxicology

Major target organs for toxicity following repeated dosing of artesunate are the haematopoietic system, the cardiovascular system, the nervous system, the gastrointestinal system and the liver.

Based on submitted documentation, NOAELs and margins of safety cannot be established. Considering intended short-term treatment of parenteral artesunate, expected reversibility of haematological and hepatic findings, and substantial clinical experience indicating low clinical risk, the lack of toxicokinetic data and exposure margins are considered acceptable for a WEU application.

The water-soluble artesunate appears less potent with regard to neurotoxicity than the water-insoluble artemether and arteether. Based on existing non-clinical data, and no unequivocal evidence for neurotoxic effects in malaria patients (WHO 2016), neurotoxicity is not considered to be of major concern for artesunate.

Artesunate has shown genotoxic potential in vitro, and inconclusive results in vivo. At present, results from in vivo studies with adequate systemic exposure, i.e. with IV administration are not publicly available. Thus, the potential relevance of the in vitro findings for the intended clinical use of Garsun is not known, but a threshold-based mechanism of action may be assumed. SmPC section 5.3 has been amended accordingly.

In view of short-term treatment and an expected threshold-based mechanism for genotoxicity, the lack of carcinogenicity studies with artesunate is considered acceptable.

Animal studies have shown reversible effects on the male reproductive system, not affecting male fertility in rats. These findings are adequately addressed in SmPC section 4.6.

Substantial reproduction toxicity is seen in conventional studies on embryo-foetal development, across all species (rat, rabbit and monkey). The primary target for toxicity is likely the embryonic erythroid precursor cells forming in the blood islands of the yolk sac. As maturation of embryonic RBC occurs, the embryo becomes less sensitive to artesunate-induced developmental toxicity. Based on findings in animal studies and low to non-existing exposure margins, a potential risk during organogenesis cannot be excluded. The number of known prospectively exposed first trimester pregnancies is too limited to allow any clinical risk evaluation. For second and third trimester, however, a substantial number of exposures is known. Together with existing non-clinical data indicating early stage embryolethality due to deletion of primitive erythroblasts, this indicates lower risk during later pregnancy periods. SmPC section 4.6 regarding pregnancy is amended accordingly.

There are no data on juvenile animals. The intended patient population comprises children at all ages (including newborns). Based on the well-known safety profile of artemisinins, intended indication (severe malaria caused by *Plasmodium falciparum*), and existing clinical data, the lack of toxicity data from juvenile animal is considered acceptable.

The applicant has addressed the mutagenic potential of impurities A, B, C, D, artemisitene impurity F. Based upon lack of suspected warning structures, impurities A, B, and C are not considered to pose a genotoxic risk. Based on two (Q)SAR methodologies, impurity D is not considered to pose a genotoxic risk. For artemisitene and impurity F however, both were considered as inconclusive. Artemisitene is adequately controlled in the starting material and is therefore not considered to be of any concern. As part of D180 LoOI response, the applicant has provided data from a miniaturized Ames assay, confirming that impurity F was without mutagenic potential in Salmonella typhimurium test strains TA98 and TA100. Consequently, due to negative QSAR for the related impurity D, negative outcome in one of two QSAR methods for impurity F, and negative outcome in relevant bacterial strains in a miniaturized Ames assay at sufficient concentration, impurity F is not considered to pose a genotoxic risk.

3.2.6. Conclusion on non-clinical aspects

An extensive amount of literature data have been provided by the applicant. As artesunate is well-known active substance, the applicant has not provided additional studies and further studies are not required. Overview based on literature review is, thus, appropriate.

Garsun may be granted a marketing authorisation from a non-clinical point of view.

3.3. Clinical aspects

Tabular overview of clinical studies

The applicant did not conduct any clinical studies. The applicant provides information from published studies that involved Guilin artesunate (Garsun).

3.3.1. Pharmacokinetics

The assays used to measure artesunate and DHA in published studies have been variable and generally few details are available on assay performance.

IV dosing

Davis (2001)

The authors used IV artesunate (Guilin) to treat severe falciparum malaria in 30 Vietnamese adults.

- 12 with complications (severe; group 1) and 8 without complications (moderately severe; group 2) received 120 mg of artesunate by injection. Group 2 received a second dose of 120 mg at 4 h.
- 10 with moderately severe complications (group 3) received 240 mg artesunate in a 4-h infusion.

Artesunate and DHA were assayed by HPLC and the authors applied non-compartmental PK analyses.

Table 1: Pharmacokinetic parameters for ARTS and DHA following i.v. injection of 120 mg (312.5 µmol) ARTS in groups 1 and 2 and a 4-h infusion of 240 mg (625 µmol) in group*

Drug and parameter	Group 1	Group 2	Group 3
ARTS			
$t_{1/2}$ (min)	2.3 ± 0.9	4.3 ± 2.6	3.2 ± 1.6
CL (liters/h/kg)	1.63 ± 0.96[c]	2.49 ± 1.68	3.07 ± 0.90
V_z (liter/kg)	0.08 ± 0.04[b,c]	0.24 ± 0.17	0.23 ± 0.10
DHA			
$t_{1/2}$ (min)	40.0 ± 24.8	64.1 ± 28.8	46.2 ± 18.7
CL (liters/h/kg)	1.09 ± 0.53	0.73 ± 0.28	0.73 ± 0.28
V_z (liters/kg)	0.77 ± 0.28	1.01 ± 0.34	0.78 ± 0.31
AUC (µmol · h/liter)	7.3 ± 4.1	9.0 ± 3.9	19.6 ± 8.6
C_{max} (µmol/liter)	8.5 ± 2.9	8.9 ± 3.8	3.2 ± 1.5[d]
T_{max} (min)	10.4 ± 7.5	9.9 ± 3.4	240[d]
Pharmacodynamics[e]			
No. of patients	8	6	10
Initial parasitemia (no. of parasites/µl)	9,260 (120–139,330)	89,730 (520–176,810)	74,710 (950–219,670)
PCT_{50} (h [range])	9 (2–20)[c,f]	6 (1–12)	6 (1–12)
FCT (h [range])	34 (20–216)	36 (20–95)	32 (16–72)

[a] Data are given as means ± SDs. Pharmacodynamic parameters are also shown as group medians and (ranges).
[b] $P < 0.01$ versus group 2.
[c] $P < 0.05$ versus group 3.
[d] End-of-infusion values.
[e] Excludes those patients given unscheduled doses of ARTS during the first 24 h.

Mean artesunate $t_{1/2}$ values were short (range 2.3 to 4.3 min). The DHA $t_{1/2}$ (range 40 to 64 min), CL (range, 0.73 to 1.01 L/h/kg) and Vd (range 0.77 to 1.01 L/kg) were similar across the three patient groups. It was concluded that artesunate and DHA PK are not influenced by the severity of malaria.

Newton (2006)

The authors reported on the PK of artesunate and DHA in adults with severe falciparum malaria in

Western Thailand from 1997-2000. Plasma artesunate and DHA levels were determined for 17 patients who received an initial dose of 2.4 mg/kg (Guilin) as an IV bolus in 5 mL.

Artesunate was rapidly converted to DHA. An open, one-compartmental model with first order kinetics gave the best fit to the plasma artesunate concentration-time data for 11 patients with sufficient data.

An open, one compartmental model with first order kinetics gave a better fit to the plasma DHA concentration-time data than a two-compartmental model for 11 patients whilst a two-compartmental model gave a better fit for the other six patients. The DHA Cmax was reached by 15 minutes in all patients. The data were consistent with very rapid hydrolysis of artesunate to DHA and underestimation of the maximum artesunate concentration. The median (range) observed DHA Cmax was 2,128 (513-5,789) nmol/L, the $t_{1/2}$ was 0.34 (0.14-0.87) h, the mean Vss was 1.9 L/kg, CL was 5.6 L/kg/h and $AUC_{0-\infty}$ was 1,469 (505-4,797) h*nmol/L.

Table 2: Median and range values for artesunate dose and artesunate and DHA pharmacokinetic variables after intravenous artesunate administration in severe malaria

Variable	Artesunate	DHA
Actual drug dose mg/kg	2.1 (1.4–2.8)[a]	–
Actual drug dose nmol/kg	5,525 (3,706–7,458)[a,b]	7,453 (4,999–10,061)[a,b,c]
$C_{0.25}$ (nmol/L)[d]	110 (13–520)	2,128 (513–5,789)
C_0 (nmol/L)	338 (124–2,557)[f]	–
Tlast (hr)	0.5 (0.25–2.0)	2.0 (1.0–6.0)
K_{10} (hr-1)	3.10 (1.13 –8.43)[f]	2.02 (0.79–4.98)
K_{12} (hr^{-1})[e]	–	1.39 (0.55–2.06)
K_{21} (hr^{-1})[e]	–	1.79 (0.89–3.98)
$t_{1/2}$(hr)	0.22 (0.08 – 0.61)[f]	0.34 (0.14–0.87)
α (hr^{-1})[e]	–	5.27 (3.28–7.62)
β (hr^{-1})[e]	–	0.73 (0.44–1.87)
$AUC_{0-\infty}$ (nmol.hr/L)	128 (36–450)	1,469 (505–4,797)
Vss (L/kg)	15.2 (2.2–39.0)[f]	1.9 (0.8–11.5)
V1 (L/kg)[e]	–	1.2 (0.6–2.5)
V2 (L/kg)[e]	–	0.8 (0.4–1.7)
Cl (L/kg/h)	64 (17–180)	5.6 (2.0–16.6)
MRT (hr^{-1})	0.32 (0.12–0.89)[f]	0.52 (0.39–2.11)
AIC	51 (10–58)[f]	88 (44–108)

[a] assuming that amount of artesunate in the vials was 88.5% of that stated; [b] To convert artesunate nmol//L to ng/mL divide by 2.601 and to convert DHA nmol/L to ng/mL divide by 3.517; [c] assuming complete conversion of artesunate to DHA; [d] Includes one dataset at 0.33 hr rather than 0.25 hr; [e] Refers only to data from the 6 two-compartmental models, the remaining variables refer to the one and two-compartmental and non-compartmental models combined; [f] Six patients had too few data points to allow estimation of these variables

$C_{0.25}$ (observed plasma concentration at t=0.25 h); C_0 (predicted plasma concentration at t_0); T_{last} (last time point with detectable drug); K_{10} (elimination rate constant); K_{12} (rate constant, central to peripheral compartment); K_{21} (rate constant, peripheral to central compartment); $t_{1/2}$ (elimination half-life, 0.693/β and 0.693/K_{10} for two-and one-compartment models, respectively); α (alpha rate constant); β (beta rate constant); $AUC_{0-\infty}$ (area under the time-drug concentration curve); Vss (volume of distribution at steady state/kg body weight); V1 (volume of distribution of first compartment/kg body weight); V2 (volume of distribution of second compartment/kg body weight); Cl (clearance/kg body weight); MRT (mean residence time); AIC (Akaike Information Criterion)

Morris (2011) pointed out that the AUCs observed in this study were lower and more variable than in other studies, which could reflect decomposition during transport to South Africa and lack of sampling before 15 min post dose.

Maude (2009)

The study primarily investigated whether artesunate prolonged the QT interval when used to treat 21 Bangladeshi adults with severe falciparum malaria.

The study included evaluation of artesunate and DHA in plasma following IV artesunate dosing using LC-MS/MS. Patients received 2.4 mg/kg (Guilin) at 0, 12 and 24 h and then once daily until oral switch to artemether-lumefantrine was possible. Pharmacokinetic analysis of serial plasma artesunate and DHA concentrations was performed by non-compartmental analysis. ECGs were obtained at these same time points after first and last doses. There was no effect of treatment on the QTc interval regardless of the correction method applied. Artesunate was assumed to be converted completely into DHA. The DHA median Tmax was 11.9 min.

Table 3: Pharmacokinetics of dihydroartemisinin (DHA) and artesunate shown as mean (95% CI) maximal observed plasma concentration (C_{max}) and predicted area under the drug concentration time curve extrapolated to infinity (AUC)

	DHA		Artesunate	
Dose	C_{max} (µg/mL)	AUC (µg/mL/min)	C_{max} (µg/mL)	AUC (µg/mL/min)
First	2.06 (1.70–2.43)	121 (90.7–152)	1.02 (0.584–1.46)	45.1 (18.5–71.7)
Last	2.29 (1.93–2.64)	136 (94.4–178)	1.37 (0.390–2.34)	30.7 (13.4–48.1)

Byakika-Kibwika (2012)

The authors determined the PK of artesunate and DHA in adult Ugandan patients with severe falciparum malaria treated with IV artesunate (2.4 mg/kg). The PK analysis used a non-compartmental infusion model. There was large inter-individual variability in DHA exposure.

Table 4: Pharmacokinetic parameters of artesunate and dihydroartemisinin

Parameter	Artesunate	Dihydroartemisinin
	Median (range)	Median (range)
Dose (mg)	140 (111–190)	103 (82–140)
C_{max} (ng/mL)	3260 (1020–164000)	3140 (1670–9530)
T_{max} (hr)	0.25 (0–6.07)	0.14 (0–6.07)
CL (L/hr)	180 (1–652)	32.25 (16–55)
V (L)	68.5 (0.18–818)	59.7 (26–117)
$T_{1/2}$ (hr)	0.25 (0.11–1.82)	1.31 (0.89–2.87)
$AUC_{0\text{-last}}$ (ng·h/mL)	727 (290–111256)	3492 (2183–6338)

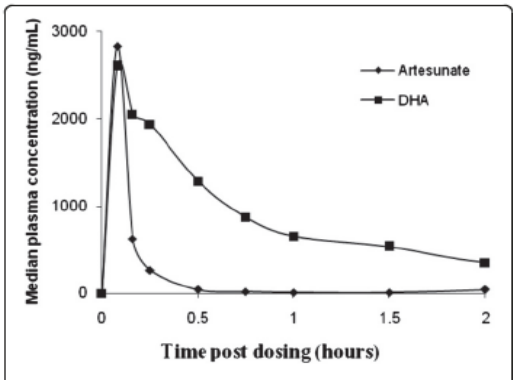


Figure 1: Mean artesunate and dihydroartemisinin plasma concentration versus time. Vertical bars represent standard error.

Zaloumis (2014)

The authors reported on a POPPK study including pooled data from 71 non-pregnant adults and 195 children with severe malaria from 6 studies conducted in Africa and Asia (Davis 2001; Kremsner 2012, Krishna 2001; Nealon 2002; Maude 2009; Simpson 2006).

The studies mainly used artesunate IV dosing of 2.4 mg/kg, but some patients received 4 mg/kg or 120 mg (see table below). The analysis focused on DHA concentrations assuming complete conversion *in vivo* of artesunate to DHA because there were limited data available on plasma artesunate concentrations. The administered dose of DHA was calculated from the relative difference in molecular weight vs. artesunate (284/384). A one-compartment model described the concentration-time data of DHA adequately, assuming IV bolus dose administration and additive error on the natural log scale.

Table 5: Study site, number of patients, study population, study design, and ARS administration (i.v., i.r., and i.m.) and each study contributing data to the pooled analysis

Research team[a]	Study site	Number of patients[b]	Study population	Study design	Dosing scheme
Kremsner *et al.*[19]	Gabon	ITT–182	Children with severe malaria	Randomized controlled trial	I: 2.4 mg/kg i.v. at 0, 12, 24, 48, and 72 h
	Malawi	PP–177			II: 4 mg/kg i.v. at 0, 24, and 48 h
Krishna *et al.*[20]	Ghana	34	Children with moderately severe malaria	Crossover trial	I: 10 mg/kg i.r. at 0 h, 2.4 mg/kg i.v. at 12 h
					II: 20 mg/kg i.r. at 0 h, 2.4 mg/kg i.v. at 12 h
					III: 2.4 mg/kg i.v. at 0 h, 20 mg/kg i.r. at 12 h
Nealon *et al.*[22]	Gabon	28	Children with severe malaria	Crossover trial	I: 2.4 mg/kg i.v. at 0 h, 1.2 mg/kg i.m. at 12 h
					II: 2.4 mg/kg i.m. at 0 h, 1.2 mg/kg i.v. at 12 h
Maude *et al.*[21]	Bangladesh	21	Adults with severe malaria	Clinical study	2.4 mg/kg i.v. at 0, 12, 24, 48 h, and then every 24 h
WHO[23]	Bangkok, Thailand	48	Adults with moderately severe malaria	Crossover trial	I: 2.4 mg/kg i.v. at 0 h, 10 mg/kg i.r. at 12 h
					II: 10 mg/kg i.r. at 0 h, 2.4 mg/kg i.v. at 12 h
					III: 2.4 mg/kg i.v. at 0 h, 20 mg/kg i.r. at 12 h
					IV: 20 mg/kg i.r. at 0 h, 2.4 mg/kg i.v. at 12 h
Davis *et al.*[18]	Vietnam	30	Adults with either severe or moderately severe malaria	Phase I: clinical study	120 mg/kg i.v. at 0 h
				Phase II: randomized controlled trial	I: 120 mg/kg i.v. at 0 and 4 h II: 240 mg/kg i.v. infusion over 4 h at 0 h

ARS, artesunate; i.m., intramuscular; i.r., intrarectal; i.v., intravenous; ITT, intention to treat; PP, per protocol; WHO, World Health Organization.
[a]Research teams are referred to by first author of the paper the data were published in or by organization that conducted the study (e.g., WHO). Note superscripts in this column are not footnotes, but references. [b]Number reported in published paper or internal WHO report.

The covariates in each of the four subgroups were examined for association with PK parameters using stepwise covariate selection. All models included an allometric function of body weight on mean CL and Vd and an additional age maturation function on the population mean CL. The population mean Vd for men was estimated to be 14% lower (95% CI: 24–3% lower) compared with women (population mean Vd for men, 9.9 L vs. 11.6 L for women), implying that men tend to achieve a higher *C*max. However, the population mean CL was the same for men and women, so that AUCs should be similar. Between-patient variability reduced substantially after inclusion of body weight as an allometric function on population mean CL and Vd (from 91–60% for CL and 86–45% for Vd) and an additional age-related enzyme-maturation effect on CL (decreased to 59%) but inclusion of sex did not further reduce the between-patient variability.

The exclusion of patients in period two of the crossover trials resulted in stepwise covariate model selection identifying haemoglobin and body temperature to be associated with the population mean CL.

VPCs of the final model fitted to the dataset including and excluding patients in period two of the crossover trials exhibit no model misspecification.

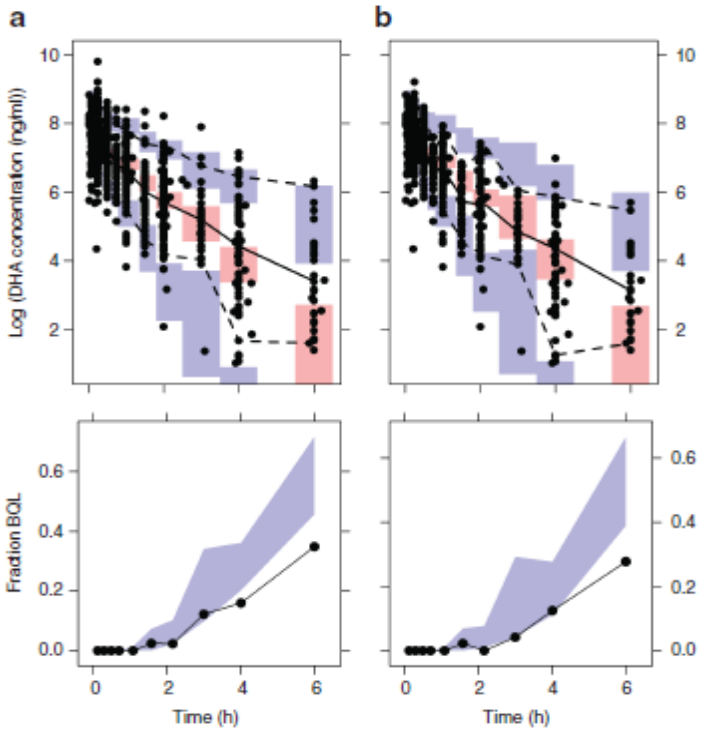


Figure 1 Visual predictive checks (VPCs) of final models fitted to (**a**) the PK dataset including patients in period two of the crossover trials (total $n = 266$) and (**b**) excluding patients in period two of the crossover trials (total $n = 223$). In the upper panel, the solid black circles are the observed $\log_e$ DHA plasma concentration (ng/ml) above the respective limit of quantification for each study (see **Supplementary Table S4**), and the following are plotted for bins across the independent variable time: 50th (solid black line), 2.5th, and 97.5th (dashed black lines) percentiles of the observed concentrations; and simulation-based 95% confidence intervals for the predicted 50th (pink region), 2.5th, and 97.5th (blue regions) percentiles. In the lower panel, the solid black line is the observed fraction of DHA concentrations (at each time point of sample collection) below quantification limit (BQL), and the blue region is the simulation-based 95% confidence interval for the predicted fraction of BQL DHA samples. DHA, dihydroartemisinin; PK, pharmacokinetic.

Figure 2

The median (range) post hoc estimates of DHA Cmax, AUC_{0-12h} h*ng/mL and $t_{1/2}$ for IV administration derived from the pooled PK model (3,053 ng/mL [1,155–6,313]; 2,610 h*ng/ml [851–7,911]; and 0.63 h [0.26–1.35], respectively) were consistent with those from the six individual studies.

Body weight had the greatest impact on DHA PK. Children with body weight between 6 and 10 kg showed a reduction in geometric mean DHA exposure of 13.7% (95% CI: 11.7–15.6%; p< 0.001) compared with children with body weight between 21 and 25 kg, following the standard 2.4 mg/kg dose. Comparable DHA exposure in smaller children and adults after IV artesunate was achieved under a dose modification, employing higher doses in lower weight children (weight-band dosing) according to Hendriksen 2013 (average of 3 mg/kg) (**Figure 1**, **Figure 2**).

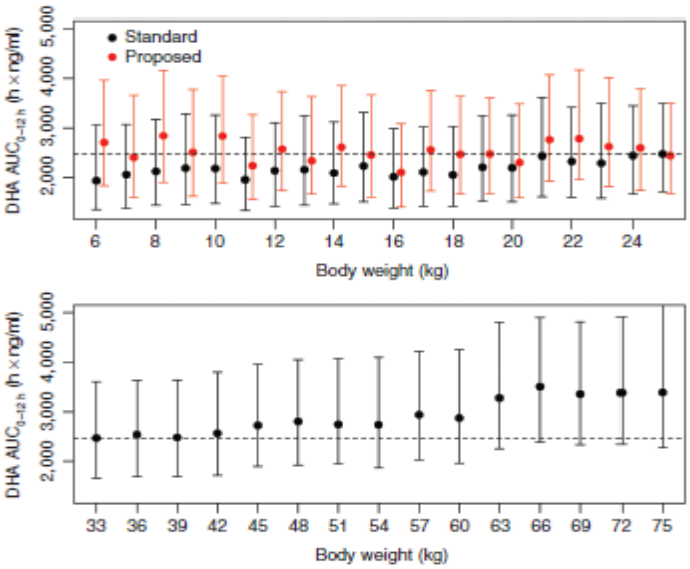


Figure 2 Simulated total first dose exposures (AUC_{0-12h}) of DHA after the standard 2.4 mg/kg dosing in children at different body weights (black) and the adjusted dosing regimen proposed in Hendriksen *et al.*[7] (red). Solid circles represent the median, and the error bars indicate the 25th and 75th percentiles of the 1,000 simulations at each body weight. The dashed line is the median exposure for the 25 kg weight group after the standard 2.4 mg/kg dose. DHA, dihydroartemisinin.

Figure 3

The applicant provides the following summary table.

Table 6: Pharmacokinetic Properties of Artesunate with IV Administration

PK Parameters	Dose and Route					
	IV 2.4 mg/kg	IV 2.4 mg/kg	IV 2.4 mg/kg	IV 2.4-4.0 mg/kg	IV 4.0 mg/kg	IV 4.0 mg/kg
	Disease/ Patients					
	Severe, Adults	Severe, Adults	Severe, Adults	Severe, Adults, children	Uncomplicated, Pregnant	Healthy, Post-partum
	Median (range) for artesunate					
AUC_{0-12h} (h*ng/mL)	$AUC_{0-\infty}$: 49.2 (13.8-173.0)[*&]	$AUC_{0-\infty}$: 751.7 (308.3-1195.0)[&]	AUC_{0-last}: 727 (290–111256)	N/A	1,090 (912-1180)	1,090 (1,040-1,130)
C_{max} (ng/mL)	129.9 (47.7-983.0) [*†]	1,020 (584-1,460)	3,260 (1,020–164,000)	N/A	17,800 (14,800-18,100)	17,800 (17,600-18,000)
$t_{½}$ (hours)	0.22 (0.08-0.61)	N/A	0.25 (0.11–1.82)	N/A	0.18 (0.12-0.78)	0.25 (0.12-0.74)
	Median (range) for DHA					
AUC_{0-12h} (h*ng/mL)	$AUC_{0-\infty}$: 417.7 (143.6-1,364.0)[*&]	$AUC_{0-\infty}$: 2,016.7 (1,511.7-2533,3)[&]	AUC_{0-last}: 3,492 (2,183–6,338)	2609.83 (851.26-7910.63)	2,250 (1,860-2,850)	2,240 (1,930-2,810)
C_{max} (ng/mL)	605.1 (145.9-1,646.1)[*† †]	2,060 (1,700-2,430)	3,140 (1,670–9,530)	3,053.44 (1,155.01-6,312.63)	2,370 (1,980-2,420)	2,360 (2,300-2,450)
T_{max} (hours)	0.25	0.20 (0.08-1.00)	0.14 (0–6.07)	N/A	N/A	0.10 (0.10-0.20)
$t_{½}$ (hours)	0.34 (0.14-0.87)	N/A	1.31 (0.89–2.87)	0.63 (0.26-1.35)	1.27 (1.16-1.44)	1.26 (1.15-1.38)
Source	Newton 2006	Maude 2009	Byakika-Kibwika 2012	Zaloumis 2014	Kloprogge 2015	Kloprogge 2015

AUC = area under the curve, Cmax=maximum plasma concentration, DHA=dihydroartemisinin; IV=intravenous; N/A=not reported; PK=pharmacokinetic; $t_{½}$=terminal elimination half-life; T_{max}=time to maximum plasma concentration. *Plasma exposure (AUCs) in this study were lower than in other studies, potentially due to decomposition during plasma sample transport to South Africa and lack of sampling before 15 min post dose.
†C_0 = concentration at 0 hours; † $C_{0.25}$ = concentration at 0.25 hours; &Units converted to uniform scale. ‡Units converted to uniform scale by Morris 2011

IM dosing

Hendriksen (2013) reported a POPPK analysis using PK data obtained after the first dose of IM artesunate for the treatment of African children with severe malaria. Artesunate and DHA concentrations were obtained by sparse sampling of 70 Tanzanian children aged 6 months to 11 years (84% were <5 years) who participated in the AQUAMAT trial. All had severe falciparum malaria and were treated with IM artesunate (2.4 mg/kg at 0, 12 and 24 h). The timing of sampling is not reported but it continued until 12 h, when the second dose was due.

The model included 274 post-dose samples, randomly distributed over the first 12 h of the study. A one-compartment disposition model for both artesunate and DHA was adequate to describe the observed plasma concentration–time data. All combinations of two-compartment disposition models displayed significant model misspecification despite significantly lower objective function values. Zero-order distribution/ absorption from the injection site(s) to the central compartment provided the best description of the data, but too few samples were collected during the absorption phase and the absorption rate was therefore fixed for an accurate estimation. Inter-individual variability could be estimated reliably for artesunate and DHA CL and for artesunate Vd, showing correlation between

CL and Vd.

When body weight was used as a fixed allometric function on all elimination clearance (power of 0.75) and apparent volume of distribution (power of 1) parameters, a significantly better model fit was observed.

DHA clearance increased 10.2% per unit (g/dl) of decrease of haemoglobin. Inter-individual variability in CL decreased from 63.6% coefficient of variation to 55.3% coefficient of variation when this covariate was included, suggesting that it accounts for a limited but significant part of the observed variability.

The final model described the observed data well, with adequate goodness-of-fit diagnostics.

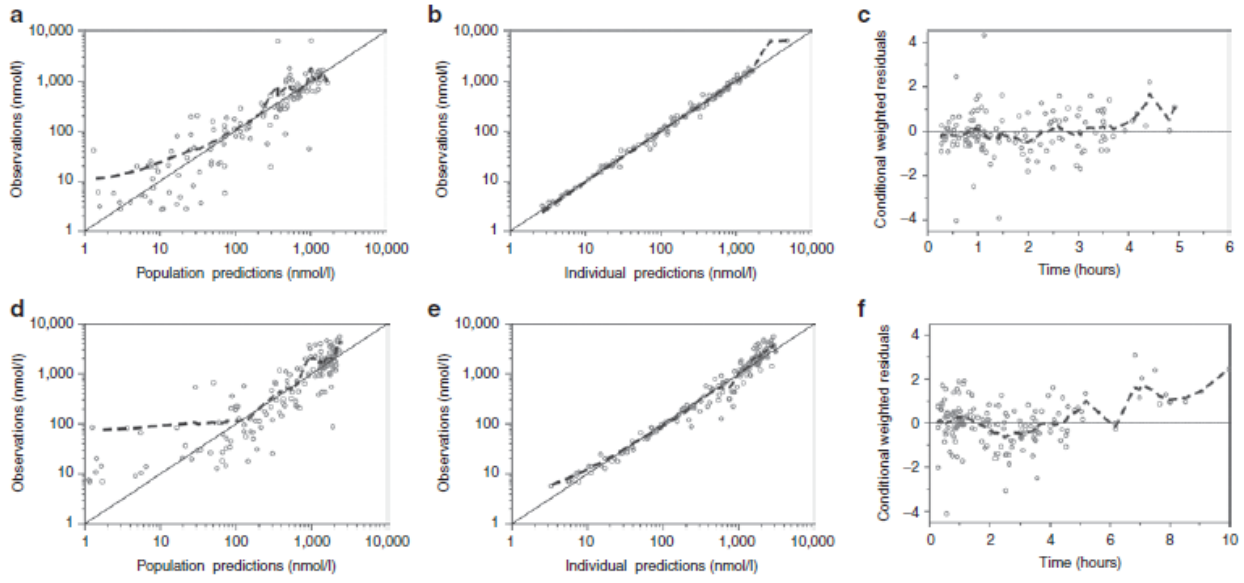


Figure 1 Goodness-of-fit diagnostics of the final population pharmacokinetic model of (**a**,**b**,**c**) artesunate and (**d**,**e**,**f**) dihydroartemisinin in children with severe malaria. The broken line represents a locally weighted least-squares regression; the solid line is the line of identity. The observed concentrations, population predictions, and individual predictions were transformed into their logarithms (base 10).

Figure 4

A prediction-corrected visual predictive check of the final model resulted in no model misspecification and good simulation properties.

Artesunate and DHA exposures after a dose of 2.4 mg/kg were simulated at each body weight level (1,000 simulations each at 1-kg intervals from 6 to 25 kg) using a uniform distribution of haemoglobin concentrations within the observed range (2.72–13.6 g/dl) to account for the observed covariate relationship.

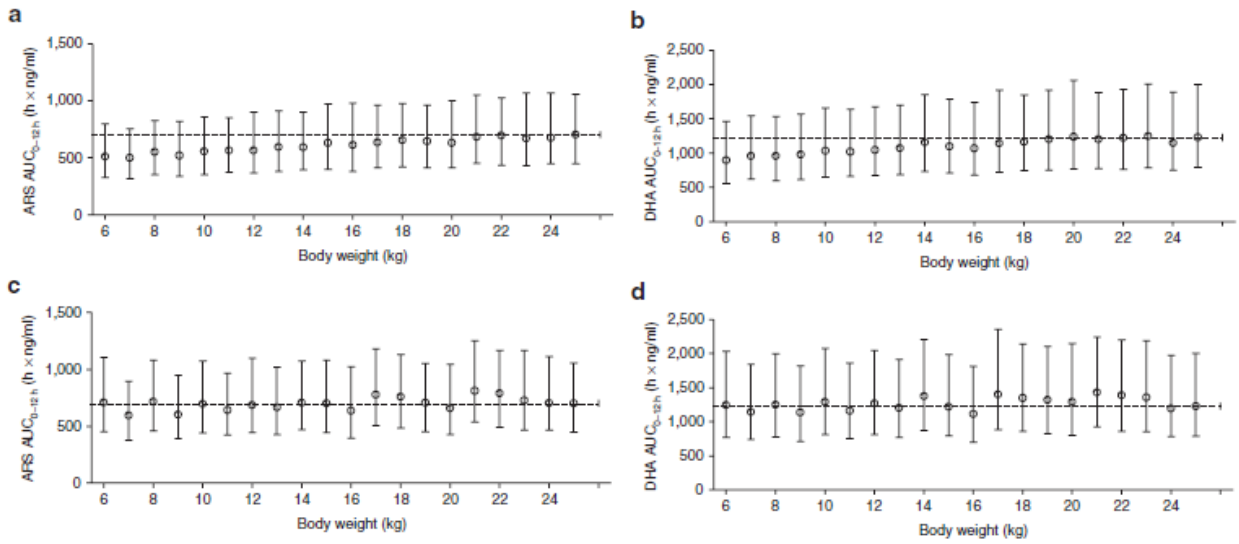


Figure 3 Simulated total first-dose exposure levels (AUC_{0-12h}) of (**a**) ARS and (**b**) DHA after the standard 2.4 mg/kg dosing in children at different body weights. Simulated total first-dose exposure levels (AUC_{0-12h}) of (**c**) ARS and (**d**) DHA after the suggested adjusted dose regimen (Table 3). Open circles represent median values, and bars indicate the 25th to 75th percentiles of simulations (1,000 simulations at each body weight). The broken line represents the median exposure for the largest weight group (i.e., 700 h × ng/ml and 1,230 h × ng/ml for ARS and DHA, respectively). ARS, artesunate; AUC_{0-12h}, area under the concentration–time curve from time point 0 to 12 h; DHA, dihydroartemisinin.

Figure 5

Children with body weights between 6 and 10 kg showed a mean reduction of 20.4% ($P < 0.0001$) in DHA exposure compared with body weights 21-25 kg (median (25th to 75th percentile) exposure: 3,380 (2,130–5,470) ng × h/ml in the 6–10 kg patients, 3,780 (2,430–6,060) ng× h/ml in the 11–15 kg patients, 4,100 (2,570–6,590) ng × h/ml in the 16–20 kg patients, and 4,240 (2,700–6,840) ng × h/ml in the 21–25 kg patients).

Using the final model, various dosing regimens were evaluated based on body weight bands. The proposed regimen resulted in similar exposures in all weight bands after the first dose of IM artesunate (panels c and d in Figure 5 above). The same simulations were performed assuming a uniform distribution of body weights at different levels of haemoglobin. These resulted in lower DHA exposures with decreasing haemoglobin levels (see figure below).

During the study 9 of the 70 children died. There was no statistical difference between survivors and non-survivors with respect to total exposure (artesunate $P = 0.8060$, DHA $P = 0.4828$) or maximum concentration (artesunate $P = 0.7655$, DHA $P = 0.6865$) after the first dose. Similarly, an exposure–response relationship could not be established using NONMEM in a time-to-event approach. The authors commented that this might be a consequence of the relatively low number of deaths and the high proportion of pre-treatments with different antimalarial drugs, doses and administration routes. Moreover, an exposure-response relationship with parasite response could not be established.

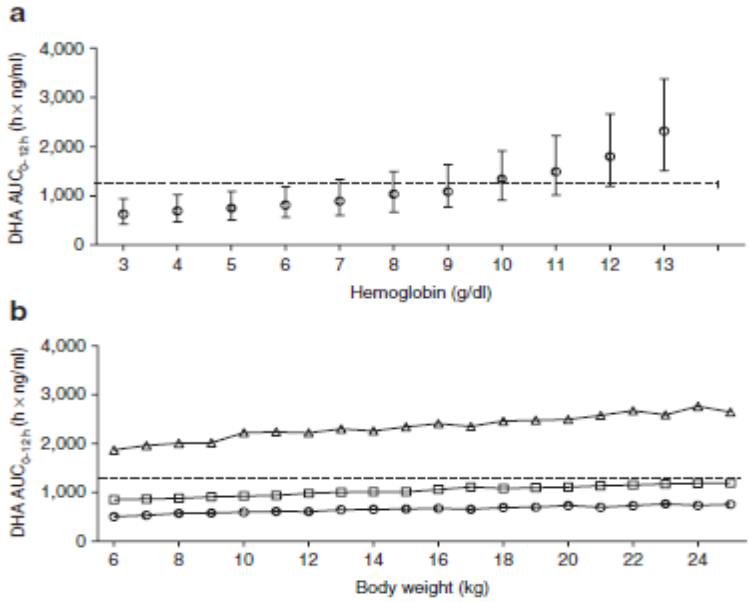


Figure 4 (**a**) Simulated total first-dose exposure levels (AUC_{0-12h}) of DHA after the standard 2.4 mg/kg dosing in children at different hemoglobin levels. Open circles represent median values, and bars indicate the 25th to 75th percentiles of simulations (1,000 simulations at each hemoglobin level). (**b**) Simulated total first-dose exposure (AUC_{0-12h}) of DHA in children at different body weights at very low (open circles: 3 g/dl), low (open squares: 8 g/dl), and normal (open triangles: 13 g/dl) hemoglobin levels. The broken line represents the median exposure for the largest weight group (i.e., 1,230 h × ng/ml). AUC_{0-12h}, area under the concentration–time curve from time point 0 to 12 h; DHA, dihydroartemisinin.

Figure 6

The applicant provided the following summary table related to IM dosing.

Table 7: Pharmacokinetic Properties of Artesunate with IM Administration

PK Parameters	Dose and Route			
	IM 2.4 mg/kg	IM 2.4 mg/kg	IM 120 mg	IM 2.4 mg/kg
	Median (range) for artesunate			
	Severe, Children	Severe, Adults	Uncomplicated, Adults	Severe, Children
AUC_{0-12h} (h*ng/mL)	570 (281-2170)	856[f]	999[f]	544[f]
C_{max} (ng/mL)	943 (329-5090)	2195[f]	884[f]	661[f]
$t_{½}$ (hours)	0.43 (0.24-0.73)	0.50[f]	0.68[f]	0.80[f]
	Median (range) for DHA			
AUC_{0-12h} (h*ng/mL)	890 (297-2510)	1496[f]	2474[f]	1123[f]
C_{max} (ng/mL)	547 (284-890)	870[f]	1166[f]	626[f]
T_{max} (hours)	0.61 (0.32-1.04)	0.58[f]	0.75[f]	0.68[f]
$t_{½}$ (hours)	0.43 (0.15-1.18)	0.88[f]	1.07[f]	0.67[f]
Source	Hendriksen 2013	Hien 2004	Ilett 2002	Nealon 2002

AUC = area under the curve, C_{max}=maximum plasma concentration; DHA=dihydroartemisinin; IM=intramuscular; PK=pharmacokinetic; $t_{½}$=drug terminal elimination half- life
T_{max}=time to maximum plasma concentration.
[f]Units converted to uniform scale by Morris et al, 2011.

Comparison of IM and IV dosing

The applicant refers to two studies in which IM artesunate was administered and plasma PK was compared with IV dosing.

Nealon (2002) studied the bioavailability of artesunate (Guilin) in Gabonese children aged 19-49 months with severe malaria.

Group 1 – 14 children were randomised to receive IV artesunate 2.4 mg/kg followed 12 h later by an IM dose of 1.2 mg/kg

Group II – 14 children were randomised to receive IM artesunate 2.4 mg/kg followed by an IV dose of 1.2 mg/kg

Of these 28 children, PK data were analysed from 10 in Group I and 11 in Group II.

For IV artesunate - An open two-compartment model with no lag time and first-order elimination kinetics best fit the data. The median Cmax was ~2-fold higher in those given 2.4 mg/kg IV (Group I) vs. those given 1.2 mg/kg (Group II) but there was no significant difference in AUC between dose levels. The Vc and Vss were ~2-fold higher in patients who had already received a dose.

For IM artesunate - An open two-compartment model with no lag time, first-order absorption and first-order elimination kinetics best fit the data. There were no significant differences in Cmax, AUC and elimination half-life estimates between Groups I and II or between IV and IM routes. The bioavailability estimations for IM dosing (from AUCIM/AUCIV) were similar for Groups I and II, with a combined median value of 66% (range 24 to 742%).

For DHA after IV dosing - An open two-compartment model with no lag time, first-order appearance and first order elimination kinetics best fit the data. Cmax values were ~2-fold higher for Group I vs. Group II but there were no significant differences for AUC. The distribution half-life was significantly shorter for Group I patients. The authors considered that PK parameters for DHA after IV artesunate were comparable to those obtained in Ghanaian children with moderate malaria, suggesting that the severity of disease does not have an important influence on DHA PK.

For DHA after IM dosing - An open two-compartment model with no lag time, first-order appearance and first-order elimination kinetics best fit the data. Cmax and AUC estimates were significantly higher (by ~2-fold) for Group II. The overall estimate of the relative bioavailability of DHA was a median of 86% (range 11 to 462%). The median Vss was 1.3 L/kg (range 0.5 to 7.9 L/kg) and the median clearance was 0.028 L/kg/min (range 0.001 to 1.58 L/kg/min). The authors concluded that the variability in estimated absolute bioavailability of DHA after IM dosing is probably due to drug disposition because similar variations in AUCs were observed for DHA after IV artesunate dosing.

The longer estimate of DHA t1/2 after IM artesunate suggested that in some patients, absorption from the injection site may be rate limiting for elimination (flip-flop kinetics).

Table 8: Pharmacokinetics of ARS and DHA in children with severe malaria*

Drug, route, and group	Actual dose (μmol/kg [mean ± SD])	$V_{c/f}$ (liters/kg)	V_c (liter/kg)	$V_{ss/f}$ (liter/kg)	V_{ss} (liter/kg)	CL/f (liter/kg/min)	CL (liter/kg/min)
ARS							
i.v.							
Group I (n = 10)	5.82 ± 1.72	NA	0.10 (0.03–0.6)	NA	0.17 (0.09–4.1)	NA	0.052 (0.002–0.17)
Group II (n = 11)	3.31 ± 0.32	NA	0.21 (0.4–0.4)	NA	0.44 (0.06–7.7)	NA	0.071 (0.001–0.14)
Combined	NA	NA	0.14 (0.03–0.6)	NA	0.40 (0.06–7.7)	NA	0.046 (0.001–0.17)
i.m.							
Group I	3.38 ± 0.33	1.3 (0.5–3.2)	2.31 (0.2–9.3)	2.07 (0.9–5.1)	2.66 (0.2–11.8)	0.040 (0.005–0.34)	0.042 (0.001–0.93)
Group II	6.95 ± 0.74	2.1 (0.3–6.4)	1.37 (0.7–20.2)	3.98 (0.3–9.1)	2.16 (0.7–28.6)	0.058 (0.003–0.17)	0.045 (0.006–0.53)
Combined	NA	1.9 (0.3–6.4)	1.80 (0.2–20.2)	2.67 (0.3–9.1)	2.55 (0.2–28.6)	0.046 (0.003–0.34)	0.043 (0.001–0.93)
DHA							
i.v.							
Group I	5.82 ± 1.72	NA	0.47 (0.05–1.2)	NA	0.75 (0.3–1.3)	NA	0.036 (0.012–0.15)
Group II	3.31 ± 0.32	NA	0.47 (0.3–1.5)	NA	0.77 (0.5–1.9)	NA	0.018 (0.011–0.04)
Combined	NA	NA	0.47 (0.05–1.5)		0.77 (0.3–1.9)	NA	0.025 (0.011–0.15)
i.m.							
Group I	3.38 ± 0.33	1.2 (0.4–6.3)	1.44 (0.1–55.1)	1.32 (0.5–7.9)	1.32 (0.5–7.9)	0.036 (0.001–0.18)	0.037 (0.02–1.58)
Group II	6.95 ± 0.74	1.2 (0.03–3.2)	1.10 (0.1–2.4)	1.28 (0.5–4.2)	1.28 (0.5–4.2)	0.025 (0.006–0.13)	0.0201 (0.001–0.096)
Combined	NA	1.2 (0.03–6.3)	1.36 (0.1–55.1)	1.32 (0.5–7.9)	1.30 (0.5–7.9)	0.025 (0.006–0.18)	0.0280 (0.001–1.58)

[a] Abbreviations: $V_{c/f}$, fractional volume of the central compartment; V_c, volume of the central compartment; V_{ss}, volume of distribution at steady state; CL/f, fractional clearance; C L, total body clearance of the drug from plasma; $t_{1/2}\alpha$, distribution half-life; $t_{1/2}\beta$, elimination half life; t_{abs}, half-life of appearance; AUC, area under the plasma concentration-time curve; T_{max}, time to reach maximum concentration following drug administration; C_{max}, peak drug concentration in plasma after administration of a single dose.

$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (min)	t_{abs} (min)	AUC (μmol · min/liter)	Relative bioavailability (%)	T_{max} (min)	C_{max} (μmol/liter)
2.1 (0.9–5.4)	1.5 (0.06–1668)	NA	162.7 (49.9–5071.4)	NA	NA	77.2 (13.5–266.3)
1.4 (0.5–2.1)	11.5 (0.68–2023)	NA	86.7 (47.2–2660.4)	NA	NA	38.98 (16.4–49.8)
1.6 (0.5–5.4)	2.9 (0.06–2023)	NA	NA	NA	NA	NA
7.7 (2.3–17.6)	25.2 (4.23–501.00)	2.7 (0.87–5.99)	83.5 (9.2–747.3)	54.1 (24.4–741.8)	7.2 (4.1–11.4)	1.6 (0.6–2.97)
5.2 (1.4–52.8)	48.2 (13.37–319.65)	2.5 (1.28–41.52)	84.9 (39.2–2264.7)	94.4 (31.3–250.2)	8.0 (3.9–78.9)	1.72 (0.7–10.1)
5.96 (1.4–52.8)	42.3 (4.23–501.00)	2.7 (0.87–41.52)	NA	65.7 (24.4–741.8)	7.2 (3.9–78.9)	NA
0.3 (0.1–28.0)	20.7 (0.28–35.81)	0.2 (0.05–4.96)	194.8 (52.4–498.8)	NA	0.5 (0.1–14.3)	10.59 (5.0–75.4)
7.2 (0.5–54.4)	32.0 (14.11–53.55)	0.4 (0.05–2.17)	155.6 (84.2–334.2)	NA	1.4 (0.3–7.3)	5.57 (1.8–8.5)
0.8 (0.1–54.4)	22.8 (0.28–52.55)	0.2 (0.05–4.96)	NA	NA	1.3 (0.1–14.3)	NA
17.6 (2.4–36.6)	31.9 (18.2–110.4)	15.4 (3.8–47.9)	83.6 (17.4–298.2)	84.7 (11.4–462.1)	25.9 (10.8–71.9)	1.2 (0.1–2.9)
8.7 (3.2–21.6)	40.2 (1.4–148.8)	25.1 (5.1–47.3)	236.9 (46.7–582.7)	109.4 (36.1–408.3)	40.5 (11.5–68.2)	2.2 (0.8–3.6)
10.8 (2.4–36.6)	35.8 (1.4–148.8)	18.4 (3.8–47.9)	NA	86.37 (11.4–462.1)	35.1 (10.8–71.9)	NA

Ilett (2002)

The authors investigated IM vs. IV artesunate (Guilin) in 24 Vietnamese adult patients with uncomplicated falciparum malaria. Patients were randomised to receive either IV artesunate (120 mg over 2 min; dissolved in 1 mL sodium bicarbonate solution and diluted to 10 mL in 5% dextrose) or IM artesunate (120 mg in 1 mL sodium bicarbonate injected into the gluteal muscle) initially and then received a second dose by the alternate route after 8 h.

Conversion of artesunate to DHA was assumed to be complete. Mean bioavailability of DHA following IM artesunate injection was 88%.

Table 9: Pharmacokinetic parameters for ARTS and DHA following i.v. and i.m. ARTS (120 mg (312.5µmol); Group 1 and i.v. ARTS (120 mg (312.5 µmol) followed by rectal DHA (563 µmol (160 mg); Group 2. Data are given as mean ± s.d. unless otherwise indicated.

	Intravenous ARTS (n=11)		*Intramuscular ARTS (n=11)*	
Group 1	*ARTS*	*DHA*	*ARTS*	*DHA*
Dose (µmol kg^{-1})	5.7±0.8	–	5.7±0.8	–
$t_{1/2}$ (min)	3.2±2.1	59±23	41±18	64±21
MRT (min)	4.1±1.2	83±28	59±29	114±22
CL (l h^{-1} kg^{-1})	2.8±1.5	0.64±0.22	2.9±1.2[c]	0.73±0.21[c]
V (l kg^{-1})	0.22±0.16	0.8±0.2	2.6±1.2[c]	1.1±0.4[c]
AUC (µmol l^{-1} h)	2.7±1.4	10.1±4.0	2.6±1.9	8.7±3.4
C_{max} (µM)	42±24[a]	9.7 (7.7–12.0)[b]	2.3 (2.0–4.8)[b]	4.1 (3.2–4.6)[b]
t_{max} (min)	–	7.0 (5.5–11.2)[b]	12 (10–15)[b]	45 (34–60)[b]
Bioavailability	–	–	–	0.88±0.19

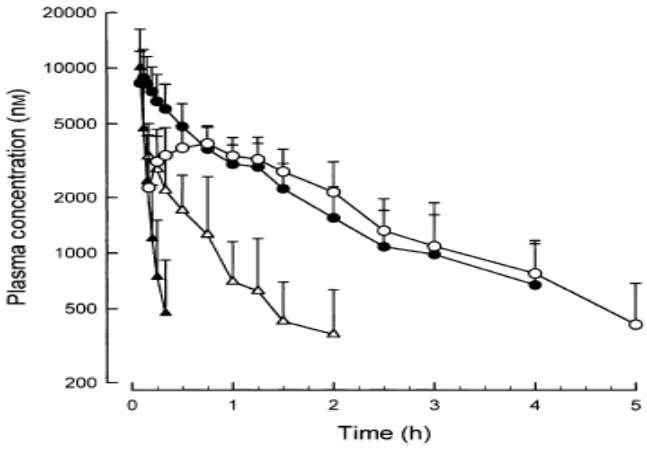


Figure 1 Plasma concentration-time profile for artesunate (▲) and dihydroartemisinin (●) following 312.5 µmol (120 mg) i.v. ARTS, and for artesunate (△) and dihydroartemisinin (○) following 312.5 µmol i.m. ARTS, administered to 11 Vietnamese patients with uncomplicated falciparum malaria. Data are shown as mean+s.d..

Figure 7

Distribution

The applicant provides no information in the application dossier on the distribution of artesunate or DHA beyond reporting Vd values in published studies.

Excretion

The applicant does not discuss the elimination of artesunate and DHA other than to describe metabolism (see below) and to refer to published studies stating that DHA is excreted in urine after glucuronidation.

Metabolism

Artesunate is hydrolysed very rapidly and completely back to DHA in the circulation *in vivo* by plasma esterases (Navaratnam 2000; Morris 2011; Li 2009; Zaloumis 2014; Byakika-Kibwika 2012; Hendriksen 2013). In a study by Ilett, Ethell 2002, urine was collected from 17 Vietnamese adult patients with falciparum malaria who had received 120 mg of IV artesunate. Analysis of metabolites by

HPLC-MS identified α-DHA-β-glucuronide (α-DHA-G) and a product characterized as the tetrahydrofuran isomer of α-DHA-G. Unchanged DHA was present only in very small concentrations. The tetrahydrofuran isomer appeared to be at least partly a product of non-enzymic reactions occurring in urine. Metabolism of DHA was also assessed *in vitro* by incubating human liver microsomes with $^{[12\text{-}3H]}$DHA and cofactors for either glucuronidation or cytochrome P450 (CYP)-catalysed oxidation.

Glucuronidation was also studied by incubation with recombinant human uridine 5'-diphospho (UDP)-glucuronosyltransferases (UGTs). In addition, sulphation was assessed by incubation with human liver cytosolic sulfotransferases. Metabolites were detected by HPLC-MS and/or HPLC with radiochemical detection. In human liver microsomal incubations, DHA-G (as diastereomer) was the only metabolite identified. α-DHA-G was formed after incubation of DHA with UGT1A9 or UGT2B7, but not with UGT1A1 or UGT1A6. The glucuronides formed did not have antimalarial activity, and this has also been found in studies comparing bioassays, where the antimalarial activity in plasma samples was explained entirely by the parent compound and DHA (Batty 1998; Grace 1999, Maggs 1997; Meshnick 1996). It was concluded that glucuronidation is the main pathway of DHA metabolism and that α-DHA-G is a major metabolite of DHA in humans and its formation is catalysed by UGT1A9 and UGT2B7. After glucuronidation, DHA is excreted in the urine. There was no significant metabolism of DHA by CYP oxidation or by cytosolic sulfotransferases in this study.

Li 2003a used in-vitro systems, rat and human liver microsomes (RLM, HLM) and recombinant cytochrome P450 (rCYP) isoenzymes to determine intrinsic clearance (CLint) and to identify the relative contribution of isoenzymes in the metabolism of 15 anti-parasitic agents, including artemisinin and artesunate. The *in vitro* $t_{1/2}$ value for rat and human liver microsomes of artesunate were 12.2 min and 20.9 min, respectively, with possible contribution from CYP2A6, 1B1, 2B6, 2E1 and 4A11. CLint calculated from the $t_{1/2}$ of human liver microsomes was 33.2 µL/min/mg protein. The predicted human clearance (CLH) is 12.3 mL/min/kg, which the authors noted to be significantly lower than the observed *in vivo* CL in patients of 41.5 (range 13-70) mL/min/kg.

The applicant's summary of this paper states that artesunate was an intermediate clearance drug mainly metabolized by esterases in human blood to DHA and that the contribution of CYP2A6 is minimal. It is also stated that artesunate is a substrate of CYP2A6 and possibly CYP1B1, 2B6, 2E1 and 4A11 but it is not a substrate of CYP1A2, CYP2C8, CYP2C9, CYP2C19 and CYP2D6.

Impaired renal or hepatic function

Severe *P. falciparum* malaria is caused mainly by extensive parasitised erythrocyte sequestration and consequent dysfunction of vital organs, including dysfunction of the kidney and liver. Therefore, the PK of artesunate in severe malaria patients reflect a population that commonly has some degree of temporary impairment of hepatic and/or renal function. Published studies of artesunate in malaria patients do generally not implement artesunate dose modifications according to liver or kidney function. This is also in line with published recommendations that no dose modification is necessary with artemisinins in patients with renal or hepatic impairment (e.g., Lewis 2020; WHO 2015).

Elderly

In numerous studies evaluating efficacy of artesunate in the treatment of severe malaria elderly patients were enrolled (El Ket 2020; Kreeftmeijer-Vegter 2020; Makowiecki 2018; Wagdahl 2019; Sonden 2017; Kurth 2017). Even though no specific analysis of the age category ≥65 years was identified there were no data indicating a specific safety or efficacy concern. Hence, in line with the treatment recommendation in current national and global guidelines, there is no need for dose adaption in elderly patients (WHO 2015; Leblanc 2020; Askling 2012).

Children

In addition to the reports mentioned above from Zaloumis and Hendricksen, the paper by Kremsner *et al*. (2016), which is included in the literature references in Module 5, describes PK data and a POPPK analysis using results with IV or IM Guilin artesunate administered to children aged from 6 months to 15 years with severe *P. falciparum* malaria. The POPPK analysis was performed in a subset of 288 patients with samples collected at 3 study centres and at one of 5 time points, with randomisation such that each child was sampled at one time only (at one of 30, 60, 120, 240 or 360 min after each of the three doses). There were 851 samples analysed for artesunate, DHA and DHA glucuronide. A subset of these children had their G6PD status determined. Samples came from 92 in the 5-dose group and from 99 and 97 in the 3-dose IM and IV groups, respectively. The three regimens appeared to provide comparable exposures in the first days of treatment except that the Vd was higher in the IM dose groups. Of the covariates tested, parasitaemia influenced Vd only after IV dosing.

The authors concluded that the POPPK analysis was consistent with prior publications specific to parenteral artesunate dosing. There was no relationship detected between PK parameters and efficacy.

Table 10: Population pharmacokinetic analysis of parenteral artesunate in severe malaria

Table 3. Population pharmacokinetic analysis of parenteral artesunate in severe malaria.

Arm	Drug Metabolite	Volume of Distribution (Liters) (95% CI)	Clearance (Liters/Hour) (95% CI)
Three-dose i.v.	ARS	32.0 (22.3–41.6)	42.0 (28.9–55.1)
	DHA	12.0 (10.2–13.8)	9.9 (8.4–11.3)
	DHAG	36.6 (32.0–41.3)	10.8 (9.0–12.7)
Three-dose or five-dose i.m	ARS	21.1 (18.2–24.0)	33.3 (29.5–37.1)
	DHA	25.3 (22.4–28.2)	8.5 (7.4–9.5)
	DHAG	66.5 (58.2–74.8)	10.1 (8.3–11.9)

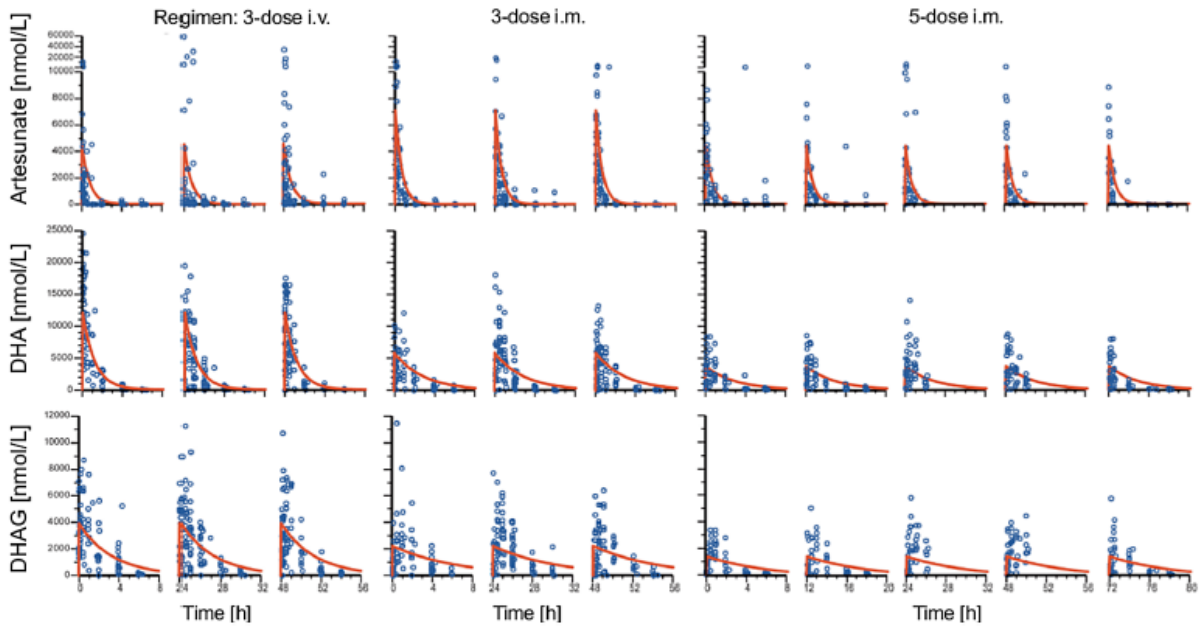


Fig 7. Population pharmacokinetic profiles of artesunate, dihydroartemisinin, and dihydroartemisinin glucuronide. Plots of observed concentration–time profiles for ARS and its major metabolites, DHA and DHAG, are presented according to treatment regimen. Estimated population mean PK profiles are shown by red lines. The three columns of results for each regimen represent the findings after each dose.

Figure 8

- **Pregnancy**

Kloprogge 2015 reported PK data generated after administration of Guilin artesunate to 20 pregnant women on the Thailand-Myanmar border with non-severe malaria of which 15 were restudied 3 months post-partum.

Pregnant 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. This study used simultaneous population PK drug-metabolite modelling. Overall, pregnancy had no effect on the PK properties of artesunate or DHA after IV artesunate administration.

McGready 2011 analysed the same dataset as above and also reported that 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 t½ 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. It was concluded that artesunate and DHA PK following IV artesunate dosing were not different between pregnant and non-pregnant women.

Interactions

- **In vitro**

Artesunate and DHA as victims

Artesunate is metabolized mainly *via* esterases, with minimal contribution of CYP2A6 in humans. Following intravenous injection, artesunate is metabolized to DHA very quickly, with a half-life of about 10 minutes. The process of transformation to DHA by plasma esterases has been demonstrated (Jansen 2007), but is known as well by unspecific esterases and has been shown to occur chemically during storage of the blood following collection. DHA is further metabolized by glucuronidation, mainly by UGT1A9 and UGT2B7.

Li and colleagues (Li 2003a) identified artesunate as a substrate of CYP2A6 and minor activity at CYP1B1, 2B6, 2E1 and 4A11 but not as a substrate of CYP1A2, CYP2C8, CYP2C9, CYP2C19, and CYP2D6.

Transformation by CYP2A6 is known to contribute only to a minor extent. For this reason, the risk of drug-drug interactions by co-mediation of drugs inhibiting CYP2A6 is assumed to be of minor clinical relevance as the artesunate fraction not transformed by CYP2A6 is assumed to be then metabolized by esterases. A relevant risk is only given for ultra-metabolizers of CYP2A6, where adverse reactions upon oral artesunate administrations were reported (Yusof 2012), assumedly due to rapid accumulation of DHA. Accordingly, for co-administration with known CYP2A6 inducers, the impact of increased CYP2A6 enzyme activity is assumed to be of minor importance for efficacy of the artesunate treatment, due to the short period of treatment (single days), the low contribution of CYP2A6 on the artesunate metabolism and the short half-life of artesunate in the organism.

For the potential of DDIs with the glucuronidation of DHA, inhibitor studies of the glucuronidation of DHA in human liver microsomes showed low IC50 values (<500 µM) for substrates such as ethinyloestradiol, diclofenac, ketoprofen, oxazepam and diazepam. Repeated treatment with DHA led to lower exposure and higher metabolic capability to form its metabolite DHA-glucuronide after repeated oral doses. An auto-inductive effect for DHA metabolism is assumed to be relevant for intravenous artesunate as well. Due to increased adverse effects observed in ultra-metabolizers of CYP2A6 (see Yusof 2012 above), inhibition of UGT1A9 or UGT2B7 by co-medication with artesunate is assumed to be the most clinically relevant risk due to accumulation of DHA.

Induction by co-medication with strong inducers (additionally to potential auto-induction by DHA) might decrease DHA exposure below levels needed for antimalarial efficacy. Co-administration of oral artesunate with ritonavir (Morris 2012) resulted in a 27% and 38% decrease in Cmax and AUC, respectively, of DHA. It is expected that co-administration of ritonavir with intravenous artesunate may also decrease the Cmax and AUC of DHA. Thus, co-administration of intravenous artesunate with ritonavir should be avoided.

Artesunate and DHA as perpetrators

Ericsson and colleagues (Ericsson 2014) evaluated the CYP inhibitory potential of several artemisinins on human liver microsomes. The inhibitory potential of artesunate and DHA was determined on CYP1A2, 2A6, 2B6, 2C9, 2C19, 2D6 and 3A4 simultaneously using a substrate cocktail approach. Artesunate showed an inhibitory effect on CYP1A2 with a remaining activity after incubation with 80 µM of artesunate of 41.7 ± 1.2% and an IC50 of 12.8 ± 1.2 µM (4.9 µg/mL), thus was classified to be a weak inhibitor. DHA showed an inhibitory effect on CYP1A2 and CYP2C19 with a remaining activity after incubation with 80 µM DHA of 15.7 ± 0.5% and 42.7 ± 9.0%, respectively, in human liver microsomes. The IC50 values determined were 7.2 ± 1.1 µM (2.1 µg/mL; but 42.1 µmol/L (12.0 µg/mL) by Navaratnam 2000) for CYP1A2 and 13.0 ± 1.3 µM (3.7 µg/mL) for CYP2C19. No inhibitory effect on CYP2A6, 2B6, 2C9, and 2C19 (2C19 only applicable for artesunate), 2D6 and 3A4 was observed in either human liver microsomes or recombinant CYP enzymes.

Navaratnam and colleagues (Navaratnam 2000) further reported absent inhibiting activity by DHA on CYP2D6 and CYP2E1 at concentrations up to 250 µmol/L (71.1 µg/mL).

Following intravenous treatment with 2.4 mg/kg, maximum plasma levels of about 3 µg/mL were determined for artesunate and for DHA. With IC50 values of 4.9 µg/mL (artesunate, CYP1A2), 2.1 µg/mL and 12.0 µg/mL (DHA, CYP1A2) and 3.7 µg/mL (DHA, CYP2C19), an inhibitory effect by artesunate and its main metabolite DHA at clinically reached maximum plasma concentrations cannot be excluded. Nevertheless, the IC50 values reported are in the upper or above the range of plasma levels reported in humans for both artesunate and DHA. Furthermore, metabolism of artesunate to DHA and glucuronidation of DHA is known to be rapid, so an inhibitory influence of artesunate and DHA on CYP1A2 or DHA on CYP2C19 can reasonably assumed to be low and only short-termed. The short duration of the treatment (about four treatments, over three days) further decreases the risk for significant potential of interaction. Taken together, the data suggest a low *in vivo* potential for interaction of artesunate and DHA with cytochrome P450 enzymes.

As mentioned above, DHA may act as auto-inducer to UGT1A9 and UGT2B7 during repeated treatment, especially when artesunate is given twice daily. For co-mediation with drugs mainly metabolized by UGT1A9 and/or UGT2B7, potential interaction by competing for the same enzymes might occur, whereas auto-induction by DHA might compensate this. The clinical relevance for the auto-induction is therefore assumed to be low.

Senarathna and colleagues (Senarathna 2016) evaluated the effect of artesunate on P-glycoprotein (P-gp) using Caco-2 cell monolayers, together with known P-pg substrates and inhibitors. The authors showed that permeability of artesunate was moderate, around 10 x 10-6 cm/sec. An upregulation of P-gp was observed when DHA was co-incubated with mefloquine and amodiaquine (both combinations used for the treatment of *Plasmodium falciparum*). P-gp upregulation was not observed for artesunate combinations, but to a small degree by artesunate alone. Artesunate did not demonstrate P-gp substrate and inhibitory properties at the therapeutic concentration and is absorbed following passive diffusion.

Wang (Wang 2019) investigated the interaction of artemisinin derivatives and P-gp and reported that results of artesunate from bidirectional permeability assays are inconsistent with those from cytotoxicity assays. The reason for this inconsistency was assumed to be that in addition to P-gp there are several other transporters in Caco-2 and MDCK-MDR1 cells, which might be also involved in the transport of artemisinin and artemether. The authors reviewed available literature on the P-gp interactions of artemisinin derivatives and showed that none of the artemisinin derivatives investigated exhibited typical substrate properties of P-gp. Thus, artesunate and DHA are assumed to be not transported by P-gp. An upregulation of P-gp by artesunate or DHA cannot be excluded.

Rijpma 2014 evaluated the inhibitory potential of DHA 100 µM on the activities of ATP binding cassette transporters, including P-gp (ABCB1), BSEP (ABCB11), MRP1-4 (ABCC1-4) and BCRP (ABCG2). The results for DHA are summarised as follows:

- DHA did not inhibit P-gp-mediated N-methyl quinidine transport but slightly induced transport activity to 112% ($p = 0.033$).
- DHA inhibited 30% of solvent-exposed BCRP-mediated transport capacity ($p < 0.001$).
- DHA did not inhibit BSEP-mediated of taurocholic acid transport activities but slightly induced transport activity to 114% ($p < 0.001$).
- DHA did not inhibit MRP1-4 transporter activities.
- DHA induced MRP3-mediated translocation of oestradiol 17-β-D glucuronide 121% ($p = 0.020$).

- **In vivo**

Asimus 2007

This was a cocktail study in which 75 healthy adults were randomised to receive therapeutic oral doses of artemisinin, DHA, arteether, artemether or artesunate for 5 days (Days 1–5). A 6-drug cocktail consisting of caffeine, coumarin, mephenytoin, metoprolol, chlorzoxazone and midazolam was administered orally on Days -6, 1, 5 and 10 to assess the activities of CYP1A2, CYP2A6, CYP2C19, CYP2D6, CYP2E1 and CYP3A, respectively. The plasma concentrations of parent drugs and corresponding metabolites and 7-hydroxycoumarin urine concentrations at 4 h only were quantified by LC-MS/MS.

For DHA, the 1-OH-midazolam/midazolam 4-h plasma concentration ratio (CYP3A) was increased on Day 5 [1.25 (1.06–1.47)] vs. Day -6; the paraxanthine/caffeine ratio (CYP1A2) was decreased on Day 1 [0.73 (0.59–0.90)] vs. Day -6; the α-OH-metoprolol/metoprolol ratio (CYP2D6) was lower on Day 1 compared with Day -6. The authors state that the results suggested that:

- Artemisinin, DHA and artemether are inducers of CYP3A
- Artemisinin, DHA and arteether inhibit CYP1A2
- Artemisinin and arteether, but not artesunate or DHA, induce CYP2C19.

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.

Elsherbiny 2008 conducted a model-based assessment of CYP2B6 and CYP2C19 induction by artemisinins using mephenytoin as a probe for CYP2B6 and CYP2C19 enzymatic activity.

The data from two clinical studies were pooled:

- One dataset (rich data study, 8–16 samples/occasion/subject) contained data from 14 Vietnamese healthy male volunteers who received mephenytoin (200 mg) before and during ten days of oral

artemisinin (250 mg for 9 days and 500 mg for one day) administration.

- The second dataset (sparse data study, 3 samples/occasion/subject) were collected from 74 Vietnamese healthy volunteers who received mephenytoin before, during and after five days administration of artemisinin (500 mg), dihydroartemisinin (60 mg), arteether (100 mg), artemether (100 mg) or artesunate (100 mg).

The model used in the study showed that all studied artemisinin derivatives, including DHA and artesunate, induce CYP2B6. Results also confirmed CYP2C19 induction by arteether and artemether as well as CYP2B6 and CYP2C19 induction by artemisinin. The production rate of CYP2B6 was increased 19.9% by dihydroartemisinin and 16.9% by artesunate but they had no effect on CYP2C19 activity.

3.3.2. Pharmacodynamics

Mechanism of action

Artemisinin and its derivatives are sesquiterpene lactones characterised by an endoperoxide bridge in the trioxane structure. Their pharmacodynamic action requires cleavage of the endoperoxide bridge by an electron donor for activation. In malaria parasites, activation is via haem-Fe2+. Proteins involved in the parasite mitochondrial electron transport chain have also been suggested as potential sites of electron donation. Ring-form stages are less sensitive to artemisinins than more mature stages (e.g. trophozoites), likely because they consume less haemoglobin and therefore produce less activation.

Activated artesunate and DHA generate free radicals (e.g. superoxide anions, H_2O_2 and other intermediates), increase oxidative stress within infected erythrocytes, cause alkylation of proteins and lipids and ultrastructural changes leading to decrease in parasite function, growth and survival. The mitochondria, endoplasmic reticulum and digestive vacuoles appear to be the sites of damage.

Primary pharmacology

Artemisinin derivatives are active against the asexual blood stages of all malaria parasites that infect humans. They are also gametocidal but they do not affect the liver stages of malaria parasite development. The uptake of artemisinins into infected erythrocytes varies with different developmental forms of the *P. falciparum* parasite.

Effect of DHA on ultrastructure of blood stage parasites

Ellis et al. (1985) reported ultrastructural changes in *P. falciparum* after exposure to ^{3H}DHA *in vitro*. The ^{3H}-HA was localised in the parasite limiting membranes and the digestive vacuole membranes of infected cells but not uninfected cells. In mice infected with asexual stage parasites of *P. berghei* and treated with a single dose of artemisinin (10 mg/kg) on Day 4 post-infection, there was an increase in free and bound ribosomes as well as swollen endoplasmic reticulum and mitochondria within 30 minutes of treatment. By 8 to 12 h, the parasite organelles were disorganised with perinuclear vacuolation and aggregation of nuclear material. Almost all parasites had disappeared by 24h. Maeno *et al*. (1993) reported alteration in morphology of the blood stage parasites of a Brazilian clone (ItG2) of *P. falciparum* incubated with ^{3H}DHA. There was localisation of DHA in approximately 70% of the food vacuoles and 40% of mitochondria. The study suggested that the ultrastructural changes occur 2-4 h before the reduction of parasite counts.

Artesunate activity *in vitro*

Various studies using a range of methods have reported the *in vitro* activity of artesunate and DHA against the blood stage parasites of several laboratory strains of *P. falciparum* and against clinical isolates of *P. falciparum.* Generally, the activities of artesunate and DHA appear to be similar.

The artesunate IC_{50} values for laboratory strains and clinical isolates have ranged between ≥ 0.08 ng/mL and ≤ 5.6 ng/mL, respectively. The DHA IC_{50} values for laboratory strains and clinical isolates have ranged from ≥ 0.16 ng/mL and ≤ 6.54 ng/mL, respectively.

Janse *et al*. (1994) reported that the activity of DHA and artesunate decreased in older cultures containing trophozoites compared to younger cultures containing the ring-stage of *P. berghei*. The activity of DHA against trophozoites was 3.7-fold higher than that of artesunate.

Skinner *et al*. (1996) determined the percent inhibition of parasite growth by measuring incorporation of ^{3H}hypoxanthine added to the cultures at the same time as the test artemisinin. There was rapid inhibition of growth of rings, schizonts and trophozoites (>80%) within 2 – 4 h of exposure to DHA.

Mehra and Bhasin (1993) reported the activity of DHA against the gametocytes of the FCD-3 isolate of *P. falciparum*. Complete killing of gametocytes was reported at DHA and artemisinin concentrations of 100 ng/mL at 96 h; gametocyte inhibition at 48 h was lower than at 96 h. The DHA IC_{50} and IC_{90} values, based on a 96-h reading, were 6.6 and 19 ng/mL, respectively, while those for artemisinin were 13 and 22 ng/mL, respectively. Lelièvre *et al*. (2012) reported that artesunate and DHA were >200-fold less active (IC_{50}s: 4163 ng/mL and 1012 ng/mL, respectively) against mature gametocytes compared to asexual forms of the 3D7A strain of *P. falciparum.*

Mechanisms of parasite clearance following treatment with artesunate

The damaged and dead parasites in circulating erythrocytes are cleared predominantly by the spleen, although the liver, bone marrow and other lymphoid tissue play an important secondary role. In falciparum malaria the sequestered mature trophozoites are killed *in situ* and then disintegrate slowly. They leave behind erythrocyte membranes adherent to the vascular endothelium and sometimes trapped malaria pigment in the sequestered vessels. Clearance of this material is performed by circulating phagocytes (monocytes and polymorphonuclear leukocytes).

Resistance to artemisinins

Published studies suggest that resistance to artesunate and DHA in *P. falciparum* is manifested as recrudescence and/or delay in parasite clearance time (PCT). A delay in PCT and/or recrudescence has been shown to be associated with alterations in some genetic regions, such as *Kelch13* and *Pfmdr1* of *P. falciparum* isolates.

Lim *et al*. (2010) reported higher artesunate IC_{50} values for *P. falciparum* isolates from Western Cambodia compared to isolates from Eastern Cambodia. The IC_{50} values for isolates from patients who failed artesunate + mefloquine therapy (37 with recrudescence) were 2-fold higher than those from 558 patients who were cured. Of 851 isolates genotyped for single nucleotide polymorphisms (SNPs) in four genes (*pfdhfr*, *pfcrt*, *pfmdr1* and *pfatpase6*), the distributions of the SNPs differed between Eastern and Western Cambodia. There was no statistically significant correlation between increased pfmdr1 copy numbers and increased artesunate IC_{50} but the *pfmdr1* codon *Y184F* appeared to be associated with higher IC_{50} (n = 113) in Eastern Cambodia.

Birnbaum *et al*. (2020) reported that inactivation of 8 proteins in the *kelch13* defined compartment rendered the ring-stage parasites resistant to artemisinin and adding wild type *kelch13* rendered the resistant parasites fully sensitive to DHA. The *kelch13* proteins were associated with endocytosis of haemoglobin from the host cell.

Relationship between plasma concentration and effect

Laboratory studies have demonstrated that the maximum parasite killing effect of artemisinins is reached at a certain minimum concentration (Klonis 2013, Dogovski 2015, Cao 2017).

A modelling study by Dogovski *et al*. (2015) linked differences in parasite clearance half-life between artemisinin sensitive and resistant infections to the splenic removal of unviable wild-type (artesunate sensitive) and unviable mutant (artesunate resistant) parasites. The model suggested that part of the slower log-linear parasite clearance in artemisinin resistant parasites was explained by slower transformation of drug-damaged parasites into pyknotic bodies, which are cleared by the spleen through pitting. Based on this and on similar PK/PD modelling studies, it has been suggested that twice daily dosing of artemisinins should increase parasite killing substantially.

There are few clinical dose-response assessments for artesunate. Angus (2002) showed that an oral dose of 2 mg/kg was the lowest dose producing a maximal effect on PCTs in adult Thai patients with uncomplicated falciparum malaria. In this study, 47 adult patients with acute uncomplicated falciparum malaria and parasitaemia >1% were randomised to receive a single oral dose of artesunate from 0 and 250 mg together with a curative dose of oral mefloquine. An inhibitory sigmoidal maximum effect PD model (a typical dose-response curve) was fitted to the relationship between dose and shortening of the PCT. No incremental reduction in PCT was found with single oral doses of artesunate > 2 mg/kg. To account for between-patient PK/PD, parasite stage and artemisinin sensitivity, a 4 mg/kg oral dose was proposed. As oral artesunate has 60% bioavailability, this equates with a 2.4 mg/kg parenteral dose of artesunate.

3.3.3. Discussion on clinical pharmacology

Pharmacokinetics

Since this application is based on well-established use, the assessment of pharmacokinetics of artesunate and DHA is focussed on whether the data substantiate the recommendations and statements made in the SmPC.

Route of administration

Regardless of information in the literature on studies that have compared PK after IV and IM administration of artesunate and on efficacy in studies that used or included IM use, only IV administration is well-established in the EU. Therefore, the SmPC refers only to IV use.

Distribution

Morris (20011) estimated artesunate binding to human plasma proteins to be 75% at concentrations <125 ng/mL using equilibrium dialysis and $^{[14C]}$ artesunate but the estimate was 62% at higher concentrations. DHA plasma protein binding, when measured by similar means, was 82% at concentrations <25 ng/mL and 66% at higher concentrations. However, when assessed by ultrafiltration in patients with malaria infection (falciparum or vivax), Vietnamese healthy volunteers and Caucasian volunteers the DHA percent bound was determined to be 93%, 88% and 91%, respectively.

Navaratnam and colleagues (2000) reported that accumulation of DHA in parasitized erythrocytes was >300-fold compared to the medium concentration but was <2-fold for uninfected erythrocytes following in-vitro incubation with 11.2 nmol/L DHA. The uptake process was shown to be reversible and saturable. These data show that DHA substantially accumulates in infected erythrocytes.

The volume of distribution for artesunate has been calculated as 29 L (mean of 68.5 L, 9.6 L, 8.8 L), range 9-69 L, and for DHA as 50 L (mean of 45.1 L, 59.7 L, 44.3 L), range 44-60 L, respectively.

Elimination

For artesunate, the plasma half-life following intravenous administration ranges between 11 and 15 minutes (range 5 to 109 minutes). The plasma half-life of DHA ranges between 20 and 79 minutes

(range 8 to 172 minutes). For artesunate a mean clearance of 159 L/h (mean of 180 L/h, 170 L/h, 126 L/h) and for DHA a mean clearance of 46 L/h (mean of 44.8 L/h, 32.25 L/h, 60.9 L/h) is calculated.

Metabolism

Artesunate is predominantly transformed to DHA by esterases, including unspecific plasma esterases, following absorption. There is possibly a minor contribution of CYP2A6.

DHA is glucuronidated. In human liver microsomal incubations, DHA-G (as diastereomer) was the only metabolite identified and α-DHA-G was formed after incubation of DHA with UGT1A9 or UGT2B7 but not with UGT1A1 or UGT1A6.

Special populations

Renal and hepatic impairment

Based on data from studies in patients with severe malaria, as well as the known metabolism of artesunate, dosage adjustment is not considered necessary in patients with renal/hepatic impairment.

Paediatric dosing

Although some PK studies suggested that 3 mg/kg might be needed in subjects <20 kg to achieve plasma exposures comparable to those seen with 2.4 mg/kg in subjects of > 20 kg, further modelling conducted and published by FDA during their review of another intravenous artesunate preparation supported no dose adjustment by body weight. Furthermore, there are no data supporting any difference in treatment response with 3 mg/kg rather than 2.4 mg/kg, there are no published studies that support the potential value of the higher dose and the pivotal efficacy study in children (AQUAMAT) used only 2.4 mg/kg regardless of age and weight.

Use in the elderly

It is agreed that no dose adjustment is needed based solely on age.

Drug-drug interactions

Based on the literature on in-vitro studies and a few clinical studies, almost all conducted with oral artemisinins, there is a limited risk for clinically important drug-drug interactions to occur. However, co-administration with inducers of UGT should be avoided and co-administration with strong inhibitors of UGT should be avoided whenever possible. Caution is advised when co-administering intravenous artesunate with substrates of CYP3A4 or CYP1A2 that have narrow therapeutic windows.

In conclusion

The applicant addressed many issues and further revised the SmPC. There remain multiple issues to be addressed via further SmPC amendments.

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 treatment is commenced with parenteral artesunate as monotherapy, complete cure depends on appropriate follow-on treatment once oral treatment is possible. When used orally, the artemisinins have been paired with a range of antimalarial agents that have a different mechanism of action and longer half-life.

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, published data for

artesunate and DHA suggest rather comparable in-vitro activity between parent and metabolite. However, it is unclear if this 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 parenteral administration, with very low and rather transient plasma exposures, clinical efficacy is thought to be driven primarily by DHA.

Resistance

Resistance manifesting as delayed PCT and/or recrudescence has been associated with various genetic findings, but the exact mechanism(s) of resistance are not known. Most reported issues for suspected artemisinin resistance have come from SE Asia. Returning EU travellers are more likely to have acquired falciparum malaria in Africa than in SE Asia, with attendant implications for the risk of acquiring artemisinin-resistant strains.

PK-PD relationship

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.

3.3.4. Conclusions on clinical pharmacology

There are no remaining issues.

3.3.5. Clinical efficacy

The applicant's *Summary of clinical efficacy in severe malaria* consists of a tabulated summary of some published trials, papers that describe a series of cases and two meta-analyses.

The demonstration of efficacy of parenteral 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 - study 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 African Quinine Artesunate Malaria Trial - AQUAMAT - study 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).
- SEAQUAMAT and AQUAMAT used artesunate manufactured in Guilin, China.

To supplement these large RCTs, the following studies or meta-analyses were included in Module 5:

Study	**Route of administration**	**Patient Population**
El Ket-2020	Intravenous	Series of cases in French returning travellers treated with IV artesunate or IV quinine between 2011-2017
Kurth-2015	Intravenous	Series of cases in European returning travellers treated with IV artesunate between 2006-2014
Eder-2012	Intravenous (Guilin)	Series of cases in UK returning travellers treated with IV artesunate or IV quinine between

		1991-2009 (quinine) and 2009-2011 (artesunate)
Zoller-2011	Intravenous (Guilin)	Series of cases in EU returning travellers treated with IV artesunate 2006-2010
Twomey-2015	Intravenous (US Army formulation)	Series of cases in US returning travellers treated under CDC IND (CDC-060) with IV artesunate 2007-2010
Sinclair-2012 (Cochrane meta-analysis)	Parenteral and intra-rectal	
Roussel-2017 (meta-analysis)	Intravenous and intra-rectal	

Main studies

Methods

Study participants

SEAQUAMAT

Eligible patients were aged at least 2 years with a positive blood antigen stick test for *Plasmodium falciparum* histidine rich protein 2 (HRP2; Paracheck, Orchid Biosystems, Goa, India) and a diagnosis of severe malaria, according to the admitting physician.

AQUAMAT

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

Treatments

Artesunate 2.4 mg/kg was given on admission, then at 12 h, 24 h and thereafter once daily until oral medication could be taken reliably. It was given as a bolus into an indwelling intravenous cannula or by intramuscular injection into the anterior thigh (an alternative allowed and used only in AQUAMAT.

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, providing a total cumulative dose of 17–18 mg/kg. In AQUAMAT, after a minimum of 24 h of parenteral treatment, oral Coartem was given in a full standard dose (1.5/9 mg/kg twice daily for 3 days with milk or fat).

In SEAQUAMAT, quinine dihydrochloride was given in a 20 mg/kg loading dose followed by 10 mg/kg three times a day until starting oral therapy at the same dose to provide a total of 7 days. In AQUAMAT, the same quinine IV regimen was used but was followed by Coartem (see above).

In SEAQUAMAT, both regimens were combined with oral doxycycline 100 mg twice daily for 7 days.

Outcomes/endpoints

The primary endpoint in both studies was death from severe malaria, defined as all-cause in-hospital mortality measured to time of hospital discharge.

Sample size

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%.

AQUAMAT

Inclusion of more than 5306 children with severe malaria was needed to show, with 80% power and 95% confidence, a 25% reduction in mortality from 8% to 6%.

Randomisation

Patients were randomised to intravenous artesunate or quinine using a computer-generated randomisation list and sealed envelopes. The corresponding numbered sealed box was opened. This box contained the study drug, case record form (labelled with the unique study number) and disposables needed for drug administration and blood sampling.

Statistical methods

SEAQUAMAT

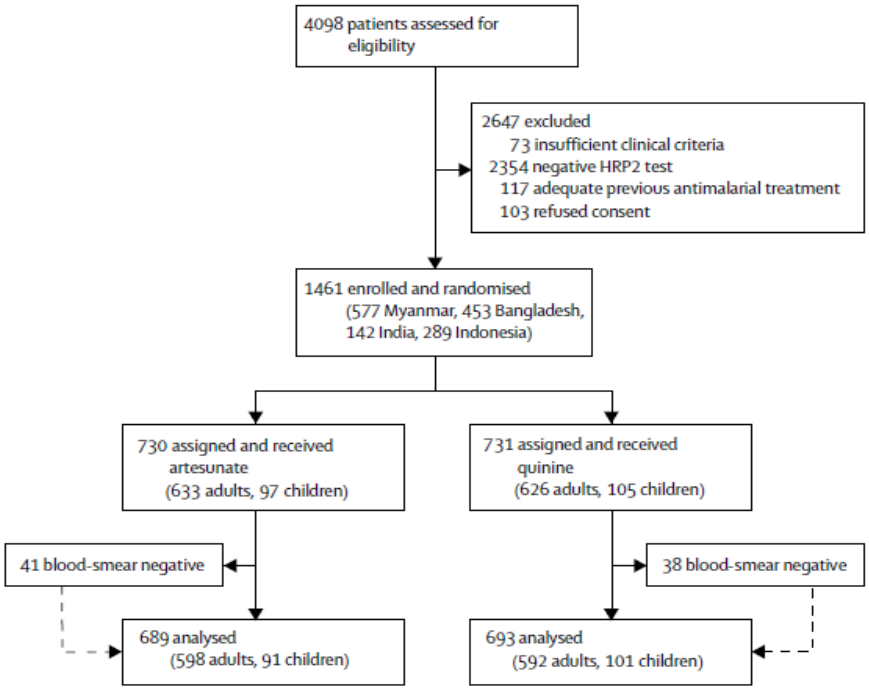
The primary analysis was conducted in the ITT population, defined as all randomised patients. An analysis of the primary endpoint was also conducted in the per-protocol population, which required patients to have a diagnosis of falciparum malaria based on finding asexual *P. falciparum* parasites in the peripheral blood smear. The primary endpoint was analysed 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 site. The odds ratio (artesunate/quinine) from the stratified CMH analysis was presented with a 95% confidence interval. Subgroup analyses of the primary endpoint stratified by site and using the ITT population were performed for a number of factors, including severe/less severe malaria.

AQUAMAT

Similar methods were used to analyse the primary endpoint as in the SEQUAMAT study. The criteria for defining severe falciparum malaria were not identical to those used in SEAQUAMAT due to the different age range.

Results – SEAQUAMAT

There were 1461 patients randomised, of which falciparum malaria was not confirmed by the presence of parasites on the blood smear in 79 (5%) subjects in the ITT population.



The tables below show the baseline characteristics for the ITT population. Severe malaria was confirmed for 509 (70%) in the artesunate group and 541 (74%) in the quinine group. The PP population comprised ~97% of ITT patients per treatment group.

Table 11: 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 12: 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

In the ITT population, mortality was 15% in the artesunate group compared with 22% in the quinine group (relative risk 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).

Table 13: 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.

The primary efficacy analysis in the ITT population was repeated in the severe malaria (SM) subgroup, in which the mortality rate was 20% in artesunate patients compared to 28% in quinine patients. The odds ratio estimate (95% confidence interval) from the analysis was 0.65 (0.48, 0.87). 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 23 and 29 patients in the artesunate and quinine groups, respectively, who died on the day of admission (OR 0.78, 0.45–1.36; p=0.38). Most of the treatment effect resulted from reduction in mortality after the first 24–48 h after entry to the study.

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.

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).

Using data from the two centres that were discontinued during the trial (with 155 patients and 13 deaths), subgroup analyses gave ORs for the treatment effect that did not differ significantly between the subgroups with the exception of hyperparasitaemic patients.

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

The absolute risk reduction associated with artesunate treatment was fairly consistent, ranging from 5% to 9%. The numbers needed to treat to save one life ranged from 11.1 to 20.2 between the countries.

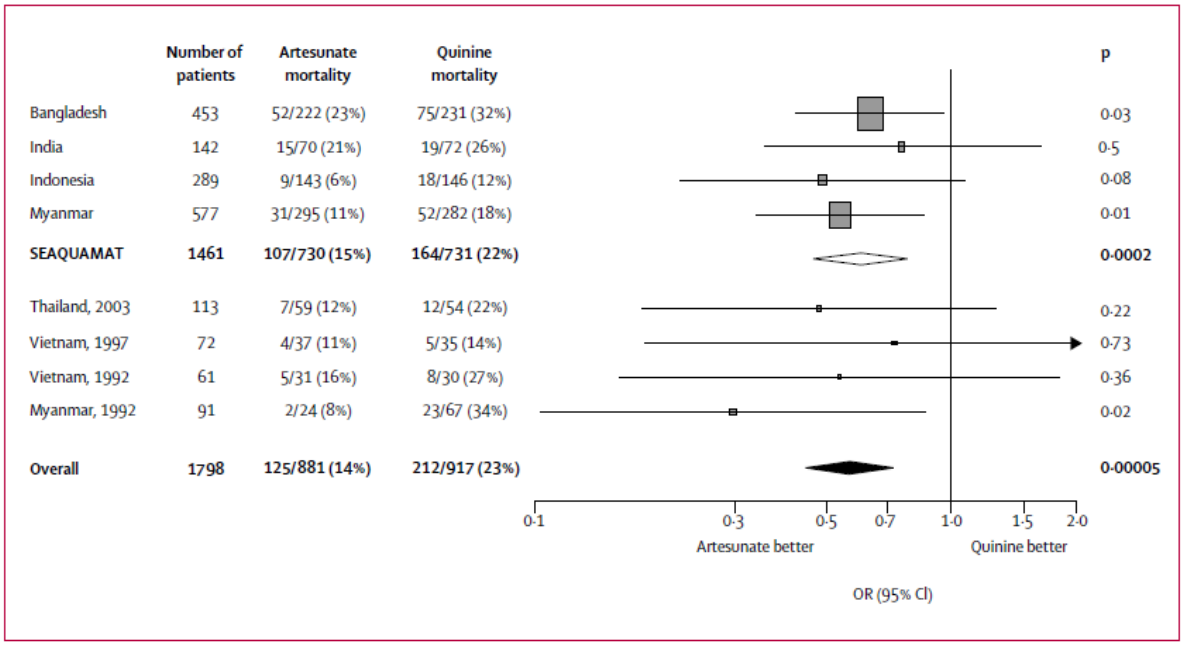


Figure 3: **Forest plot of mortalities comparing parenteral quinine and artesunate in treatment of severe malaria in SEAQUAMAT and previously published studies**[23,26–28]

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

Figure 9

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 individuals had psychiatric sequelae, four had persisting problems with balance (one of whom had both psychiatric sequelae and a tremor) and two had a hemiparesis.

Combining deaths and neurological sequelae, there were 114 (16%) adverse outcomes in the artesunate group and 167 (23%) in the quinine group (p=0.23).

Among those who survived, there were no significant differences between the treatment groups for times to eat, sit and be discharged.

There was a significant excess of hypoglycaemia after study entry in the quinine group compared with the artesunate group (RR 3.2, 95% CI 1.3–7.8). 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).

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 fetal death while 5 in each group had an abortion.

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 (i.e. the diagnosis was not confirmed at the central laboratories).

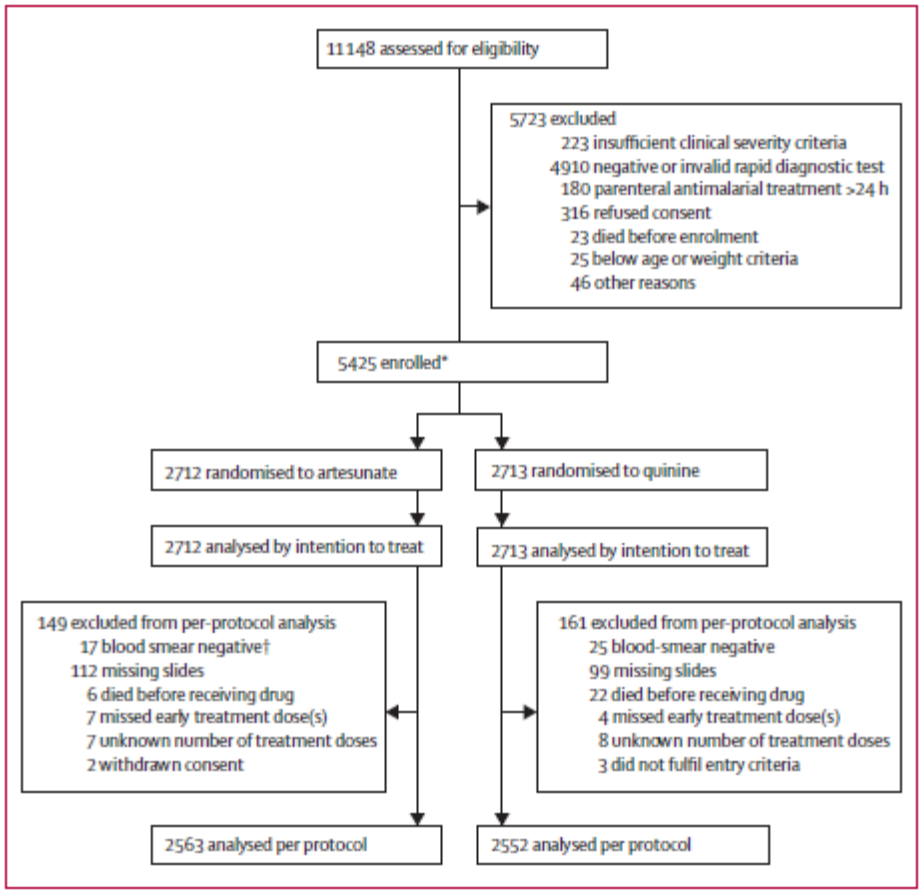


Figure 1: **Trial profile**
*663 in Beira, 442 in Kilifi, 436 in Kumasi, 921 in Muheza, 540 in Korogwe, 502 in Banjul, 450 in Ilorin, 386 in Rwanda, 663 in Mbarara, and 422 in Kinshasa. †Two patients also had other criteria.

Figure 10

The 11 participating centres were located in nine African countries (Mozambique, The Gambia, Ghana, Kenya, Tanzania, Nigeria, Uganda, Rwanda, and Democratic Republic of the Congo).

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 at around 3 years, all children were <4.5 years of age with an almost equal gender split. Although just over a third had already received potentially effective treatment, one quarter had hyper parasitaemia at study baseline, while about one third had coma and/or convulsion with about two-thirds being in prostration.

Table 14: 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.

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; OR 0.75, 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%) and corresponds to an overall number needed to treat to prevent one death of 41 (95% CI 25–112). There was no heterogeneity between study sites (p=0.99). The per-protocol analysis gave a very similar result to the ITT population.

According to Figure 12 (see below), 1779 and 1724 children in the artesunate and quinine groups, respectively, were treated IV and 933 vs. 989 were treated IM based on study site policy (rather than on actual documented route). The mortality rates with IV treatment were 8% for artesunate and 10% for quinine while those for IM treatment were 10% and 13%, respectively. Eight patients (5 artesunate, 3 quinine) were taken from hospital against advice and could not be followed up further so they were censored in the survival analysis at the time of discharge. The survival analysis (see first figure below) 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 (data not shown).

Overall, 497 children (224 artesunate, 273 quinine) had convulsions that either developed after admission, irrespective of duration, or were present on admission and persisted for more than 6 h. Also, 156 patients (65 artesunate, 91 quinine) developed coma or had a deterioration of their coma score after starting antimalarial treatment. 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. There was no significant difference between treatments for rate of development of blackwater fever after hospital admission. Hypoglycaemia after starting antimalarial treatment was significantly less frequent in patients who received artesunate than in those who received quinine.

Table 15: 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.

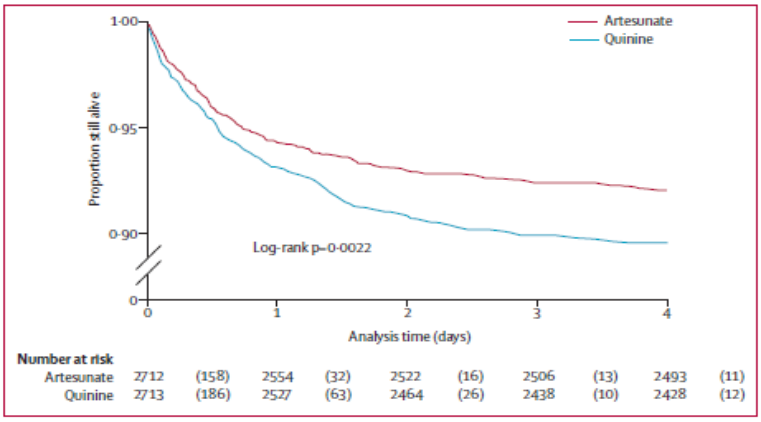


Figure 2: **Kaplan-Meier curves comparing survival in African children with severe falciparum malaria treated with either parenteral artesunate or quinine**
The numbers in parentheses are the deaths during the indicated time. In eight patients the exact time of death during the night was missing and was estimated as 2359 h.

Figure 11

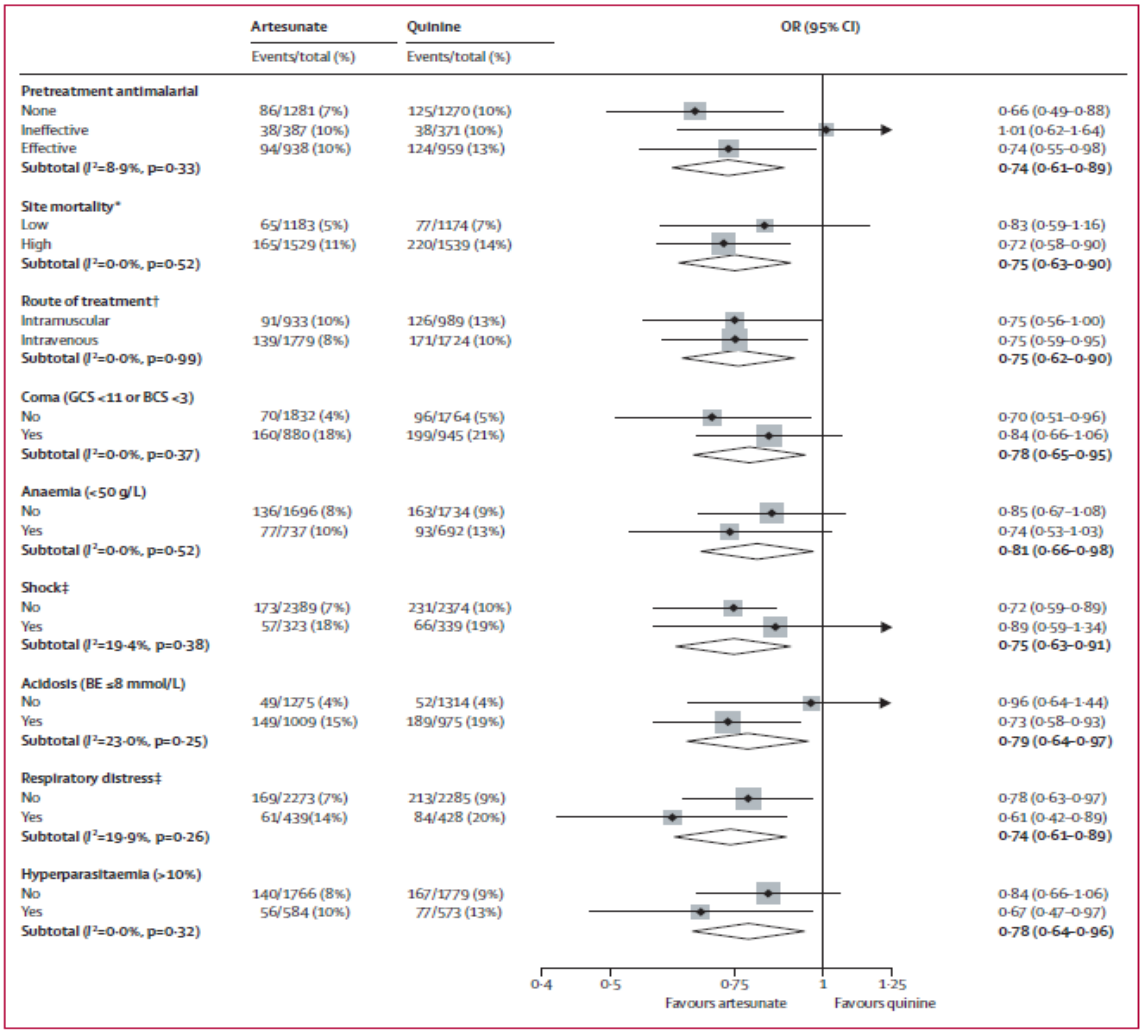


***Figure 3*: Treatment effect in protocol-specified subgroups**
The forest plot shows odds ratios and 95% CIs. The size of the squares is proportional to the size, and therefore weight, of the subgroup. The diamonds show the combined differences. The efficacy of antimalarial pretreatment was classified before study unblinding (webappendix p 12). Hyperparasitaemia means greater than 10% of red cells parasitised. 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 than chance, with the p value of significance.

Figure 12

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.

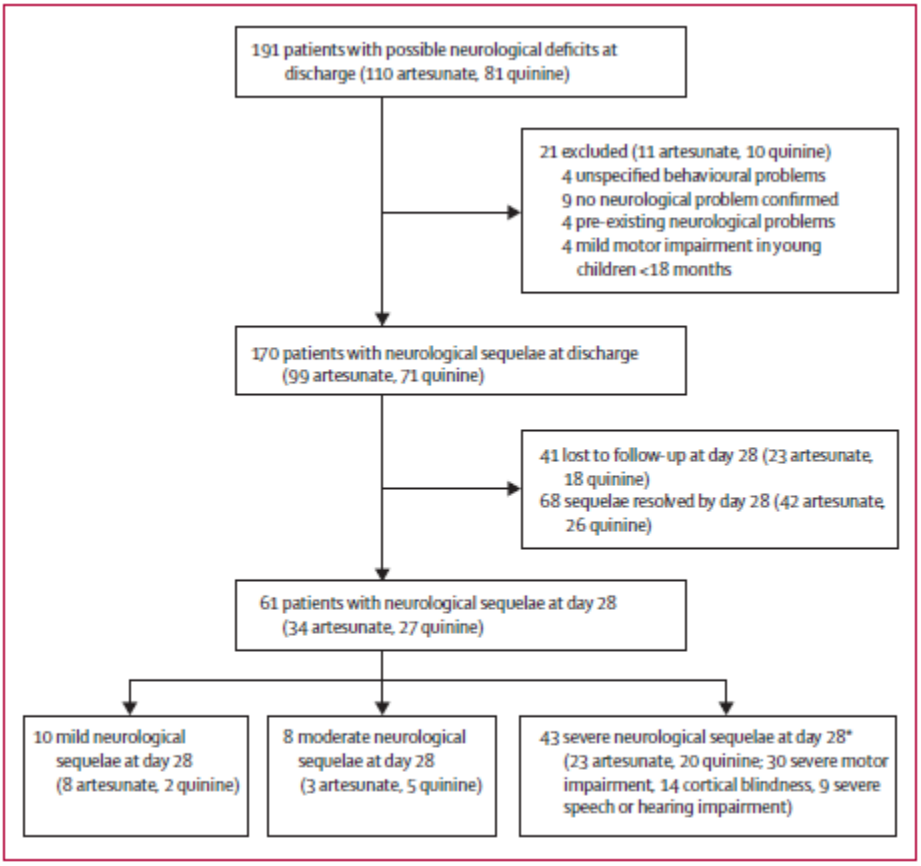


Figure 4: **Neurological sequelae at discharge and after 28 days (range 3–8 weeks) in children with severe falciparum malaria**

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

Figure 13

Blood transfusions were given to 1487/2712 (55%) patients assigned to artesunate and 1495/2713 (55%) assigned to quinine. A fluid bolus at the start of treatment was given to 589/2712 (22%) patients assigned to artesunate and 596/2713 (22%) assigned to quinine. There was also no difference between treatments for proportions given antibacterial treatments (1606 artesunate, 1653 quinine; p=0·20).

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

Table 16: 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%)	HR 0.60 (0.45, 0.79); P = 0.0002
	In-hospital death blood smear positive	105/689 (15%)	157/693 (23%)	HR 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	226/2280 (9.9%)	291/2338 (12.4%)	OR 0.77 (0.64, 0.93); P = 0.0055
	Coma (new)	65/1832 (3.5%)	91/1768 (5.1%)	HR 0.69 (0.49, 0.95); p=0.02
	Convulsions (new)	224/2712 (8.3%)	273/2713 (10.1%)	HR 0.80 (0.66, 0.97); p=0.02

Supportive studies

Study	Route of administration	Patient Population
El Ket-2020	Intravenous	Series of cases in French returning travellers treated with IV artesunate or IV quinine between 2011-2017
Kurth-2015	Intravenous	Series of cases in European returning travellers treated with IV artesunate between 2006-2014
Eder-2012	Intravenous (Guilin)	Series of cases in UK returning travellers treated with IV artesunate or IV quinine between 1991-2009 (quinine) and 2009-2011 (artesunate)
Zoller-2011	Intravenous (Guilin)	Series of cases in EU returning travellers treated with IV artesunate 2006-2010
Twomey-2015	Intravenous (US Army formulation)	Series of cases in US returning travellers treated under CDC IND (CDC-060) with IV artesunate 2007-2010
Sinclair-2012 (Cochrane meta-analysis)	Parenteral and intra-rectal	
Roussel-2017 (meta-analysis)	Intravenous and intra-rectal	

El Ket *et al*., 2020

The authors report on an historical cohort of all cases of *Plasmodium falciparum* severe imported malaria reported from 2011 to 2017 via a network of 110 hospitals throughout France. A severe case of malaria was defined by a positive blood smear for the asexual form of *P. falciparum* and the presence of 1 or more severity criteria at admission. All patients who received artesunate or quinine as first-line treatment at the time of diagnosis of severe malaria were eligible. The primary objective was to compare 28-day mortality rates between individuals receiving artesunate and quinine.

The cohort comprised 1544 patients as shown below. The majority of cases followed visits to west and central Africa (92.5%). The most common severity criterion was hyperparasitaemia (59%), followed by cerebral malaria (38%). Among the 1544 reported cases, 50 died: 32 patients (2.9%) in the artesunate group and 18 (3.9%) in the quinine group. Two more patients in the quinine group died at day 36 and day 40; both were censored at day 28 in the survival analysis. Among all deaths, 82% occurred while receiving first-line treatment while 18% had switched treatments. The estimated weighted hazard ratio (HR) after multiple imputation and IPTW based on the propensity score was 1.03 (95% CI 0.47–2.25; P = .923), with no evidence of difference between the 2 groups with regard to mortality rate.

A higher risk of death was observed in non-African patients, patients older than 60 years and patients with a parasitaemia level above 10% in univariable hazard models. Treatment in a centre with >12 malaria cases per year was a protective factor.

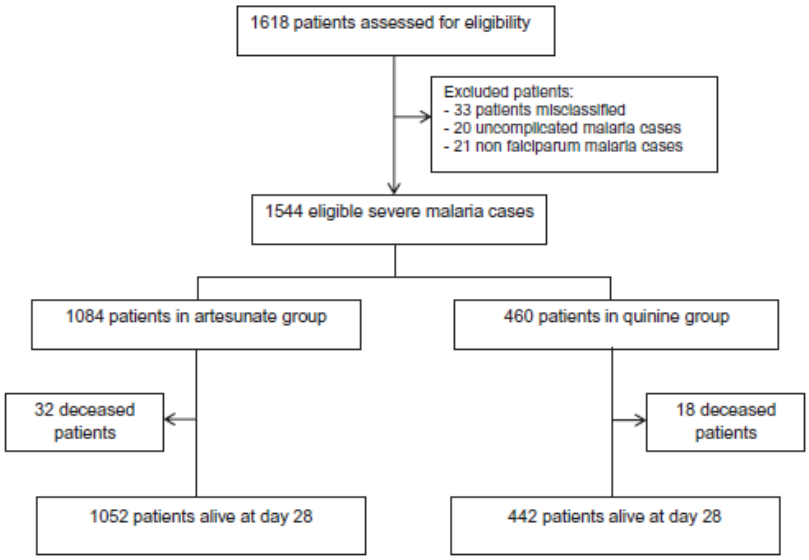


Figure 1. Flowchart of treated patients, French cohort 2011–2017. As a second-line treatment in the artesunate group: 151 patients received nonartemisinin-based combination therapy such as atovaquone + proguanil (Malarone, Glaxosmithkline Group), chloroquine (Nivaquine, Sanofi Aventis), halofantrine (Halfan, Glaxosmithkline Group), doxycycline (Doxypalu, BAILLEUL-BIORGA), mefloquine (Lariam, Hoffmann–La Roche), and quinine per os; 573 received artemisinin-based combination therapy such as artemether + lumefantrine (Coartem, Riamet, Novartis Pharmaceuticals Corp), dihydroartemisinine + piperaquine (Eurartésim, Sigma-tau Industrie Farmaceutiche Riunite), and artemether intramuscular (Paluther, Sanofi Aventis); and 8 patients switched to quinine while 320 did not receive a second-line treatment or data were missing for these patients. In the quinine group, as a second-line treatment, 107 received nonartemisinin-based combination therapy, 44 received artemisinin-based combination therapy, and 140 patients switched to artesunate, whereas 169 did not receive a relay treatment. Two patients died after day 28, thus were censored in survival analysis. Misclassified patients are those who received the 2 treatments but had missing data on criteria and dosing at admission.

Figure 14

Kurth *et al.*, 2015 - The TropNet severe malaria study

The study described 185 cases with severe falciparum malaria that occurred over 9 years (2006-2014) in 28 participating centres across 12 EU countries. The majority of patients were European tourists (106/185 [57%]), followed by patients with a history of migration (68/185 [37%]) and tourists from endemic areas (11/185 [6%]). Most infections occurred in men (71%), 59% were acquired in W. Africa and 22% in Central Africa. Intravenous quinine was the main first-line treatment in 93/185 patients (50%) and IV artesunate was used in 63/185 (35%). The proportion treated with IV artesunate increased from 27% (8/30) in 2006 to 60% (18/30) in 2013. Seven patients (4%) received both agents in combination. Hyperparasitaemia (≥5%) was the most common criterion defining severe disease. Artesunate exhibited a faster PCT99% (median 36 vs. 48 h; P=0.02, n=100) and a faster complete PCT (median 72 h vs. 96 h; P=0.005, n=84) compared with quinine.

Of 151 patients available for comparative analysis, 60 received IV artesunate and 91 received IV quinine as main first-line treatment. The overall 28-day survival rate was 98.4% (182/185). Of the three patients who died, two had been treated with intravenous quinine and one with quinine and artesunate simultaneously. All deaths occurred within the first 3 days after admission and occurred in patients with baseline hyperparasitaemia (9, 10 and 40%) and respiratory distress requiring mechanical ventilation. Two were aged 22 and 34 years and had no underlying co-morbidities while the third was aged 70 years and had chronic cardiomyopathy.

In 70 patients treated with intravenous artesunate, PADH was reported in 19 (27%). Onset of PADH was reported during days 10 to 14 (median 14) and median duration of haemolysis was 14 (IQR 8–18) days. Three patients with PADH received blood transfusions and two were re-hospitalised for 3 or 5 days. One case of PADH was reported after therapy with only oral artemether–lumefantrine.

Eder et al., 2012

The authors report on all subjects >16 years admitted to a UK Infectious Diseases Unit for treatment of severe falciparum malaria from June 2009 to September 2011. Intravenous treatment with artesunate (Guilin) was given for hyperparasitaemia (>2% of red blood cells infected), vomiting or in the presence of severity criteria (impaired consciousness, seizures, circulatory failure or prostration, pulmonary oedema, jaundice [bilirubin >50 mmol/l], renal impairment, metabolic acidosis or spontaneous bleeding. Comparable cases were selected from the departmental database from July 1991 to May 2009 using the same severity criteria, all of whom were all treated with IV quinine.

Artesunate was given at 2.4 mg/kg body weight 12 hourly for 2 doses, then 24 hourly until scanty or no trophozoites were detected on thin films. Oral follow-on treatment was either atovaquone plus proguanil or doxycycline. Quinine was given IV at 10 mg/kg 8 hourly until able to tolerate oral doxycycline. A 20 mg/kg loading dose of quinine was used only for cases of life-threatening hyperparasitaemia not responding to treatment with exchange transfusion.

There were 167 severe malaria cases, of which 24 received IV artesunate and 143 received IV quinine. Demographic and baseline clinical and laboratory data were mostly similar. All 24 in the artesunate group and 140 in the quinine group acquired their malaria in Africa. Five in the quinine group (3%) and none in the artesunate group died in hospital. The median length of stay was 3.5 days in the artesunate group vs. 5 days in the quinine group. Fewer admissions to ICU and shorter length of stay in ICU were seen in the artesunate group and none of the 24 required exchange transfusion. There was a significantly reduced median parasite clearance time with artesunate vs. quinine (65 h vs. 85 h) and a significantly shorter fever clearance time (18 h vs. 54 h). There were no instances of on-treatment hypoglycaemic episodes with artesunate vs. 6 in the quinine group.

Zoller *et al.*, 2011

The authors report a retrospective analysis of 25 patients (one child and 24 adults) from 7 treatment centres in 4 European countries who were hospitalised with *P. falciparum* malaria from January 2006–June 2010. All were classified as severe according to WHO criteria and received IV artesunate as their main antiparasitic therapy. Hyperparasitaemia (range 5%–51%) in 20 patients and cerebral malaria in 8 patients were the most common severe malaria defining criteria observed. Seven had renal failure and 2 required haemodialysis.

All but 3 patients received IV artesunate as first-line therapy, mostly with an initial dose of 2.4 mg/kg, then 1.2 mg/kg every 12 hours and 1.2 mg/kg every 24 hours. Therapy for 19/25 was changed to oral artemether/lumefantrine or atovaquone/proguanil on days 3–4 of treatment.

In all patients with hyperparasitaemia, parasite load was reduced by ≈1 $\log_{10}$ after 24–36 h and 24/25 were free of parasites at 36-134 h from time of first dose. The remaining patient with PCT 158 h was found to have HIV with CD4 count 382 cells/µL. Mean ± SD parasite clearance time was 81.2 ± 35.4 hours for all patients treated with IV artesunate as first-line drug, who had initial parasitaemia levels >1% and for whom data were available.

All patients survived and all complications related to severe malaria resolved at time of hospital discharge except for one patient who had a more severe clinical course (respiratory and renal failure) and required further rehabilitation and physiotherapy because of critical illness (neuropathy) that developed while he received prolonged intensive care and immobilization. Unusual haemolysis in 6 patients resolved spontaneously during weeks 3–6 after the first dose of IV artesunate.

Table 17: Laboratory test results for 6 patients with posttreatment hemolysis who had been treated with intravenous artesunate for severe malaria, Europe, January 2006-June 2010*

Patient no.	Initial parasitemia level, %	Levels at first examination		Treatment duration, d	Parasite clearance, d	Levels at end of treatment		Day of diagnosis of hemolysis†	Levels at diagnosis of hemolysis		Other test results
		Hb, g/dL	LDH, U/L			Hb, g/dL	LDH, U/L		Hb, g/dL	LDH, U/L	
6	30	11.3	765	7	4	7.7	317	15	5.7	1,437	Coombs negative, reticulocytes 10.2%, G6PD deficiency ruled out
7	20	13.2	1,359	7	7	8.2	NA	32‡	6.1	805	None
9	30	13.4	1,033	4	5	7.6	650	19‡	5.3	672	None
11	4	13.4	904	7	2	9.8	311	15	7.8	660	Standard reticulocyte count
23	9	15.5	490	4	2	11.1	571	15	5.7	1,489	Reticulocytes >2× upper reference value, haptoglobin <0.1g/L, Coombs negative
25	10	14.2	570	3	NA	7.8	454	16‡	5.8	444	Reticulocytes 3× upper reference value, haptoglobin <0.08 g/L (day 14), G6PD deficiency ruled out

*Hb, hemoglobin; LDH, lactate dehydrogenase; G6PD, gluose-6-phosphate dehydrogenase; NA, not available.
†After first dose of artesunate.
‡Patients had persistent hemolytic activity after the end of malaria treatment.

Twomey *et al*., 2015

This was a retrospective analysis of US patients who were enrolled into a Treatment IND protocol managed by the US CDC (known as CDC-060) to receive IV US Army artesunate for initial treatment of *P. falciparum* severe malaria. Eligible patients had to meet at least one criterion in each of 3 groups of inclusion criteria:

A malaria confirmed by microscopy

B need for IV treatment

C need for IV artesunate

The dose regimen was 2.4 mg/kg at 0 h, 12 h, 24 h and 48 h for a total of 4 doses. The follow-on oral antimalarial drug was at the discretion of the treating physician and was to be initiated on the last day of IV treatment, starting at least 4 h after the last dose.

Of the 128 patients enrolled, 102 received IV artesunate and 93 (91%) completed treatment. Patients who had concomitant administration of other antimalarial agents during IV artesunate dosing were not excluded from the safety, evaluable or per protocol populations. 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 (met enrolment criteria, received artesunate and had microscopic confirmation of *P falciparum*). In this population, the median time to negative parasitology was 42.7 h. No differences in time to negative parasitology were observed in the subgroup analyses (i.e. baseline level of renal or hepatic impairment or baseline markers for cerebral malaria). Patients who received concurrent antimalarial agents (n=38 evaluable) with IV artesunate did not show a statistically significant difference in time to negative parasitology vs. 49 who did not receive concurrent antimalarial agents (43 h vs. 51 h, respectively; p=0.942). Patients who were also exposed to quinidine (n=43 evaluable) did not show a statistically significant difference in time to negative parasitology (Figure 2) compared with the 44 evaluable patients who had no quinidine exposure (41 h vs. 50 h, respectively; p=0.608).

Analyses of changes in enrolment B Criteria indicated that the clinical signs and symptoms of severe malaria improved or resolved in a majority of patients by Day 3 of artesunate treatment. Exceptions were subjects with ARDS, acute renal failure or abnormal bleeding.

Overall, 7 patients died. All deaths were judged to be associated with the severity of malaria. Five of the 7 had *P. falciparum* infections with baseline hyperparasitaemia from 12% to 90%. One patient had *P. vivax.* All showed significant baseline neurological deterioration (seizures, impaired consciousness, ventilator support) and all had either moderate or severe hepatic injury as assessed by MELD score. In 5/7 cases, death occurred before the full 4-dose regimen of IV artesunate could be completed. 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.

COCHRANE Meta-analysis (Sinclair 2012)

This meta-analysis was conducted over a 10-year period (1992-2012). A total of 8 studies were considered particularly useful for the evaluation of safety because they 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.

Table 18: 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.

Roussel *et al.*, 2016

The authors performed a systematic analysis of the use of artesunate in malaria non-endemic areas for the treatment of imported severe malaria. Treatment regimens varied. Through the published studies on the 574 cases where outcome was reported, 23 patients died (4%) but no death attributed to the treatment was reported.

Kreeftmeijer-Vegter *et al*., 2012 - European travellers from NL and BE

This study was not listed in the applicant's tabulation, but it is included in Module 5. Artesunate (Guilin) became available in the Netherlands and Belgium through a named patient programme. Hospitalised patients treated with IV artesunate (2.4 mg/kg on admission and 12 and 24 h followed by daily dosing until they could receive oral Malarone or Riamet) between November 2007 and December 2010 were retrospectively evaluated.

Of the 68 treated patients, 65 were adults and 39 were considered non-immune with 29 considered partially immune. In 66/68 cases malaria had been acquired in Africa and 65/68 had *P. falciparum* (one also had *P. malariae*), 2 had *P. vivax* and one had *P. ovale*. Median parasitaemia was 5%. Overall, 55 had severe malaria. The prior, concomitant and follow-on treatment in these patients was variable.

Two patients died (2/55=3.6%) but both deaths occurred after parasite clearance. The mean times to 50% parasite clearance, 90% clearance and 99% clearance were 4.4 h, 14.8 h and 29.5 h, respectively.

Table 19: Outcome

Endpoint	Number of patients (n = 68)
Mortality, n (%)	
among severe malaria,% (n = 55)	2* (3.6)
Mean parasite clearance times (95% CI), hours†	
$PCT_{50\%}$	4.4 (3.9 - 5.2)
$PCT_{90\%}$	14.8 (13.0 - 17.2)
$PCT_{99\%}$	29.5 (25.9 - 34.4)
ICU admittance, n (%)	42 (62)
Median duration of ICU stay (range), days	2 (1-35)
Median duration of hospital stay (range), days	4.5 (1-76)

PCT50%, PCT90%, and PCT99% are the parasite clearance times, defined as the time (in hours) to obtain a 50%, 90%, and 99% reduction in parasite count after start of artesunate treatment, respectively

* One patient still suffered from a delirious state and in an unobserved moment he inadvertently removed his haemofiltration catheter from the femoral vein. He died due to a loss of blood. A second patient died shortly after sudden collapse during defecation and pulmonary embolism was suspected but not confirmed as autopsy was not permitted. It occurred while the patient was treated with subcutaneous low molecular weight heparin

† Calculated from 57 patients with the following conditions:

- the limit of detection was set at 10 parasites/μL. Non detectable parasite counts were not included in the log linear effects model
- when more than one slide was made before artesunate treatment was given, the oldest slides were excluded from analysis. The value nearest to T_0 was used
- for repeated parasite counts around the detection limit, only the first value was included in the model
- Malaria cases caused by other species than *P. falciparum* were excluded for estimation of parasite clearance rates
- In most patients (88%) parasite counts were performed at least twice in the first 24 hours (range 1-7 times)

Post-artemisinin delayed severe haemolysis was seen in 7 patients. Haemolysis was seen only in those with very high parasitaemia (11-37%) and was not consistently associated with a positive Coomb's test but one patient was heterozygous for G6PD deficiency and three received exchange transfusions.

3.3.6. Discussion on clinical efficacy

Design and conduct of clinical studies

Randomised controlled studies - *SEAQUAMAT* and *AQUAMAT*

These were both prospective, randomised trials that compared initial treatment with parenteral artesunate obtained from Guilin 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. AQUAMAT required that 24 h parenteral treatment was given before an oral switch. There was no 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, the initial impact of the two parenteral treatments on parasite density was not 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 studies and the 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 *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.

Other studies

There were two dose and regimen-finding studies in African children reported by Kremsner et al. that had a randomised design. These studies were included in the dossier but not mentioned by the applicant in Module 2. The study conducted by the Medicines for Malaria Venture in children 6 months-10 years 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). In the later and larger study, 5 IM injections of 2.4 mg/kg (at 0, 12, 24, 48, and 72 h) were compared with 3 injections of 4 mg/kg (at 0, 24, and 48 h) IM or 3 injections of 4 mg/kg (at 0, 24, and 48 h) IV. The primary endpoint was the proportion of children with >99% reduction in parasitaemia at 24 h.

The other studies referred to by the applicant were descriptive in nature, based on serial case reports in returning travellers. Any comparisons made were with historical controls. Thus, these studies have very limited value although they do describe outcomes (death rates) in persons not likely to have any or, at least, any substantial pre-existing naturally acquired immunity to malaria.

Efficacy data and additional analyses

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. 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. 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 20% with artesunate compared to 28% 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. While the two treatment groups seem to have been very similar overall in terms of host and disease characteristics, it is unclear whether there were differences between countries in host or disease features that may have affected the observed mortality rates.

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.

According to Figure 3, 1779 and 1724 children in the artesunate and quinine groups, respectively, were treated IV and 933 vs. 989 were treated IM based on study site policy (rather than on actual documented route). The mortality rates with IV treatment were 8% for artesunate and 10% for quinine while those for IM treatment were 10% and 13%, respectively.

Other studies

In children with severe malaria, the MMV study indicated a numerical advantage for the 3x4 mg/kg dose group over the 5x2.4 mg/kg 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. It was concluded from the overall results and the age subgroup analyses that both IV regimens were suitable for the initial treatment of severe malaria in the age range studied.

In the second study in African children published 4 years later, non-inferiority for PC99 was concluded between the two IM dose groups based on the margin set at 10%.

In the 3-dose IV arm, 246/333 (74%) children had >99% reduction in parasitaemia at 24 h. Non-inferiority was not shown when comparing IM administration of artesunate in a 3-dose regimen at a dose of 4 mg/kg with a 3-dose IV regimen at a dose of 4 mg/kg.

Both these studies were conducted after AQUAMAT and neither has led to a change in the standard dose of 2.4 mg/kg. These studies did not use the alternative 3 mg/kg dose for children <20 kg and the applicant provides no publications on the safety and efficacy of this dose.

The studies in returning travellers were not randomised. Most are uncontrolled case series, with or without an attempt to put the data into context using historical controls.

3.3.7. Conclusions on clinical efficacy

Efficacy in the initial treatment of severe malaria

In the EU, a switch from initial use of IV quinine to use of IV artesunate occurred over several years following the publication of the two prospective randomised and active controlled trials (SEAQUAMAT and AQUAMAT) conducted in populations that differed in age range and geographical distribution. Different approaches were taken to the oral follow-on treatment in these studies 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 2.4 mg/kg IV artesunate dose regimen for the initial treatment of severe *P. falciparum* malaria in children aged from ~18 months, adolescents and adults.

There are no outstanding issues.

3.3.8. Clinical safety

The applicant's *Summary of safety* consists of a 63-page tabulation of information from a range of published studies. Of these, only SEAQUAMAT and AQUAMAT provide information from randomised, prospective and active controlled trials. The other publications range from single case reports to series of cases, including instances of treatment of uncomplicated or complicated malaria using a range of doses and routes of administration. A second *Summary of safety* document consists of a single PSUR derived from information collected from November 2016-November 2019.

Patient exposure

In SEAQUAMAT, 730 patients received at least one dose of IV artesunate and 731 received at least one IV dose of quinine.
In AQUAMAT, 2712 patients received at least one dose of IV artesunate and 2713 received at least one IV dose of quinine.

Adverse events

SEAQUAMAT
There is no description of the overall safety profile of IV artesunate in the publication. Ten patients were discharged from hospital with residual neurological sequelae - 7 in the artesunate group and 3 in the quinine group. Five individuals had psychiatric sequelae, four had persisting problems with balance (one of whom had both psychiatric sequelae and a tremor) and two had a hemiparesis.

There was a significant excess of hypoglycaemia after study entry in the quinine group compared with the artesunate group (RR 3.2, 95% CI 1.3–7.8). 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). With the exception of hypoglycaemia there were no serious adverse effects that could be attributed to either treatment.

AQUAMAT
There were 497 children (224 artesunate, 273 quinine) with convulsions that either developed after admission, irrespective of duration, or were present on admission and persisted for more than 6 h. There were 156 patients (65 artesunate, 91 quinine) who developed coma or had a deterioration of their coma score after starting antimalarial treatment. 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 the 4898 survivors, 170 (99 artesunate, 71 quinine) had not yet made full neurological recoveries at discharge. Of these patients, 129 (76 artesunate, 53 quinine) were followed up between 3 and 8 weeks after enrolment. At this first follow-up assessment 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 incidence of any persistent neurological sequelae in assessed survivors at 28 days after cerebral malaria was 3.2% (24/706 artesunate, 23/737 quinine) and of 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, 7 quinine), 7 had multiple convulsions (3 quinine, 4 artesunate) and all had severe prostration on admission.

There were no severe adverse effects that could be attributed directly to drug toxicity.

Although one patient treated with artesunate developed a mild urticarial rash, no severe type 1 hypersensitivity reactions were recorded. Another patient treated with artesunate developed peripheral gangrene of toes and fingers, which was attributed to the disease and not the drug. One patient given quinine developed severe stridor after administration of ampicillin and died. This death was attributed to ampicillin rather than quinine.

Hypoglycaemia after starting antimalarial treatment was significantly less frequent in patients who received artesunate than in those who received quinine. Blackwater fever was rare in both groups.

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

Other literature reports of note

Jaureguibery (2015) reported on returning French travellers with severe malaria treated with IV artesunate. Safety data were available for days 0–8 and 9–28 for 123 and 78 patients, respectively.

Six (4.9%) patients died due to multi-organ failure within 3 days of receiving artesunate. No deaths were related to any AEs. Overall, 97 AEs occurred, and all resolved during follow-up. Among the 78 patients who received follow-up for >8 days after treatment initiation, 76 (97%) had anaemia, and 21 (27%) of the 78 cases were recorded as PADH. The median drop in haemoglobin was 1.3 g/dL and 15% with PADH had haemoglobin <7 g/dL of which one required transfusion. Despite the high incidence of PADH, the resulting anaemia remained mild in 85% of cases. For AEs considered possibly related to artesunate, the severity was reported as shown:

Adverse event	Grade 1	Grade 2	Grade 3	Grade 4	Total
Cutaneous	2[b]	1[c]	0	0	3
Ataxia, tremor, CNS ischemia	0	2	1	1	4
Tinnitus	1	0	0	0	1
Cardiac and arterial ischemia	1	1	1	1	4
Hypertension	0	0	1[d]	0	1
Elevated level of ALT	2	2	3	1	8
Hyperkalaemia	1	0	0	0	1
Anaemia[e]	20	11	27	17	75
Total	**27**	**17**	**33**	**20**	**97**

Grade 1 = mild; Grade 2 = moderate; Grade 3 = severe; Grade 4 = life-threatening.
[a] Adverse events were obtained from medical charts and graded by using the National Institutes of Health classification for side effects
[b] Mild pruritus and rash
[c] Telogen effluvium
[d] Regressive retinopathy
[e] Grading was done during the follow-up by using available nadir of haemoglobin. For HIV-negative patients, the severity of anaemia was graded as follows: Grade 1, 10.0-10.9 g/dL; Grade 2, 9.0-9.9 g/dL; Grade 3, 7.0-8.9 g/dL; Grade 4, <7.0 g/dL. For HIV-positive patients, the severity of anaemia was graded as follows: Grade 1, 8.5-10.0 g/dL; Grade 2, 7.5-8.4 g/dL; Grade 3, 6.5-7.4 g/dL; Grade 4, <6.5 g/dL.

Kreeftmeijer-Vegter (2012) reported safety for 65 adults and 3 children treated with IV artesunate. Two deaths were assessed as not directly related to malaria and/or treatment with artesunate. Haemolytic anaemia was observed in 7 patients. Most recorded complications were compatible with severe malaria and already present before initiation of IV artesunate. Late onset haemolytic anaemia occurred in 6 patients, characterized by increased reticulocyte counts, unconjugated bilirubin and LDH and decreased haptoglobin and haemoglobin. The Hb nadir occurred between 7 to 31 days after treatment initiation. Complications observed in >5% of patients are summarized in the table.

Complication	Number of patients	% of patients
Anaemia	32	47
Thrombocytopenia	16	24
Elevated liver enzymes (LDH>2xURL)	31	46
Acute renal insufficiency	7	10
Elevated creatinine levels (>20% above URL)	9	13
Pneumonia	4	6
Respiratory insufficiency	4	6

Kurth (2017) reported safety from 70 patients treated with IV artesunate of which 19/70 (27%) had PADH. Onset of PADH was reported during days 10 to 14 (median 14) days and median duration of haemolysis was reported to be 14 (IQR 8–18) days. Three patients (15%) with PADH received blood transfusions, with 2 patients (10%) re-hospitalised (for 3–5 days, respectively). In one patient, PADH was reported after therapy with only oral artemether–lumefantrine. Three patients died but only one had received IV artesunate (with quinine).

Twomey (2015) – CDC-060 study

A total of 278 AEs were reported, with 92.2% of patients experiencing at least 1 AE. The most frequently reported AEs (≥5%) are shown below by MedDRA SOC and PT.

System Organ Class/ Preferred Term, n (%)	N (%) in Safety Population (N=102)
At Least One Adverse Event	94 (92)
Blood and lymphatic system disorders	75 (74)
Anaemia	66 (65)
Thrombocytopenia	18 (18)
Leukocytosis	10 (10)
Lymphopenia	7 (7)
Neutropenia	5 (5)
Investigations	**52 (51)**
Both hepatic transaminases increased or aspartate aminotransferase (AST) increased	50 (49)
Both hepatic transaminases increased	28 (27)
Aspartate aminotransferase (AST) increased	22 (22)
Respiratory, thoracic and mediastinal disorders	**20 (20)**
Acute respiratory distress syndrome	8 (8)
Hepatobiliary disorders	**17 (17)**
Hyperbilirubinaemia	14 (14)
Renal and urinary disorders	**13 (13)**
Renal failure acute	10 (10)
Vascular disorders	**8 (8)**
Gastrointestinal disorders	**8 (8)**
Cardiac disorders	**6 (6)**
Infections and infestations	**7 (7)**
Metabolism and nutrition disorders	**7 (7)**

The most commonly reported AEs were anaemia and increased hepatic transaminases. The majority of AEs (55%) were assessed as mild or moderate in severity, 25% were severe and 20% were life-threatening. No Grade 4 AE was considered "definitely" related to artesunate. Most AEs were attributed to the severity of malaria rather than to artesunate.

There are also several case reports in the literature of anaphylactic reactions as well as case reports of rash and pruritus.

Serious adverse events and deaths

Deaths
In the SEAQUAMAT trial the death rate for patients with severe malaria who received Guilin artesunate was 15% compared to 22% for quinine. In the AQUAMAT trial the death rate with Guilin artesunate was 8.5% compared to 10.9% for quinine. Mortality in CDC-060 (6.9%) was lower than observed in the published comparative trials. Similarly, rates reported from various case series reports in returning European travelers have been lower than reported from the RCTs in endemic regions.

SAEs
Specific information on SAEs is not available.

Laboratory findings
Specific information is not available from the published RCTs. Data are reported sporadically from other publications and cannot be put into context due to lack of adequate control groups and the very marked effects on severe malaria on multiple laboratory readouts during the acute phase followed by rapid changes in response to successful treatment.

Post marketing experience
The applicant provides a PSUR covering 11/2016-11/2019. By end 2019, Guilin artesunate was marketed in 35 countries in Africa and Asia. Taking into account the WHO defined daily dose of 280 mg, the report states that approximately 18,566,995 patients were exposed to Guilin artesunate during the reporting interval.

Cumulative and interval data of spontaneous serious and non-serious reactions from marketing experience (including reports from healthcare professionals, consumers, competent authorities), scientific literature and serious reactions from non-interventional studies and other non-interventional solicited sources are listed by MedDRA (version 22.1) PTs in the following summary table.

Table 20: Numbers of adverse drug reactions by preferred term from marketing sources

SOC MedDRA PT	Spontaneous, including regulatory authority and literature					Non-interventional PMS and Other solicited sources	
	Serious		Non-serious		Total Spontaneous	Serious	
	Interval	Cumulative	Interval	Cumulative	Cumulative	Interval	Cumulative
Infections and infestations (10021881) [3]							
Babesiosis	0	1	0	0	1	0	0
Injection site abscess	0	1	0	0	1	0	0
Salpingitis	0	0	0	0	0	0	1
Subtotal	*0*	*2*	*0*	*0*	*2*	*0*	*1*
Blood and lymphatic system disorders (10005329) [6]							
Anaemia	0	6	0	0	6	0	3
Autoimmune haemolytic anaemia	0	1	0	0	1	0	0
Haemolysis	1	6	0	1	7	0	0
Haemolytic anaemia	1	3	0	0	3	0	0
Intravascular haemolysis	0	1	0	0	1	0	0
Sickle cell anaemia with crisis	0	0	1	1	1	0	0
Subtotal	*2*	*17*	*1*	*2*	*19*	*0*	*3*
Immune system disorders (10021428) [1]							
Urticaria	0	0	1	1	1	0	0
Subtotal	*0*	*0*	*1*	*1*	*1*	*0*	*0*
Vascular disorders (10047065) [1]							
Anaphylactic shock	1	1	0	0	1	0	0
Subtotal	*1*	*1*	*0*	*0*	*1*	*0*	*0*
Skin and subcutaneous tissue disorders (10040785) [2]							
Pruritus	2	2	1	1	3	0	0
Urticaria	0	0	1	1	1	0	0
Subtotal	*2*	*2*	*2*	*2*	*4*	*0*	*0*
Renal and urinary disorders (10038359) [1]							
Acute kidney injury	0	2	0	0	2	0	0
Subtotal	*0*	*2*	*0*	*0*	*2*	*0*	*0*
General disorders and administration site conditions (10018065) [5]							
Drug ineffective	1	1	2	2	3	0	0
Hyperthermia	0	0	1	1	1	0	0
Pyrexia	0	0	1	1	1	0	1
Treatment failure	1	1	0	0	1	0	0
Death	0	0	0	0	0	0	1
Subtotal	*2*	*2*	*4*	*4*	*6*	*0*	*2*
Injury, poisoning and procedural complications (10022117) [1]							
Exposure during pregnancy	1	1	0	0	1	0	0
Subtotal	*1*	*1*	*0*	*0*	*1*	*0*	*0*
Gastrointestinal disorders (10017947) [1]							
Abdominal distension	0	0	0	0	0	0	1
Subtotal	*0*	*0*	*0*	*0*	*0*	*0*	*1*
TOTAL	***8***	***27***	***8***	***9***	***36***	***0***	***7***

The PSUR reports detailed information from a cohort study - the *Modified Cohort Event Monitoring of Injectable Artesunate, Artemether And Quinine in Ethiopia, Ghana, Malawi And Uganda* (CEMISA) – which was a prospective cohort study sponsored by the African Collaborating Centre for Pharmacovigilance. Under this protocol, 1103 patients were treated with Guilin artesunate in Ghana and Uganda between May and December 2016 for severe/complicated malaria.

The 6 serious ADRs reported to the pharmacovigilance (PV) unit (4 fatal) are included in Table 20.

The 4 fatal cases were as follows:

A 7-month-old female patient (7 kg) presented with cough, anaemia (haemoglobin 2.3 g/dL on 12-Jun-2016) and fever. She received artesunate 21 mg 3 times in 12-h intervals from 12-Jun-2016 to 13-

Jun-2016 for severe malaria. Concomitant drugs included penicillin and transfusion but died on 15-Jun-2016 from unspecified aetiology.

An 11-month-old female patient (8 kg) with cough, abdominal upset, vomiting diarrhoea and convulsion received artesunate 3 times in 12-hour intervals from 26-Jun-2016 to 27-Jun-2016 for

severe malaria. Concomitant drugs included Trixone and Panadol. She improved and was discharged. The patient was readmitted 2 weeks later, on 14-Jul-2016, with severe anaemia (haemoglobin 4.9 g/dL) and was transfused but died on 16-Jul-2016 from severe anaemia.

A 1-year-old female patient (10 kg) with flu, cough, loose stools, vomiting, fever and rash received artesunate 3 times in 12-h intervals from 27-Jun-2016 to 28-Jun-2016. She improved and was discharged. On 09-Jul-2016 severe anaemia was detected leading to hospital admission on 11-Jul-2016 with Hb 5.6 g/dL. She received IV quinine 100 mg 6 times at 8-h intervals for severe malaria on 11-Jul-2016 and 12-Jul-2016. She died on 12-Jul-2016.

A 16-month-old male patient was abandoned by his mother. On admission he presented with cough, mouth ulcer, fever, excretions around the genitals, pneumonia and severe acute malnutrition. He received artesunate for severe malaria diagnosed on admission 3 times at 12-h intervals from 24-Jun-

2016 to 25-Jun-2016. Following treatment, the abdomen grossly enlarged and the family took the patient out of the hospital. He died 5 days later on 30-Jun-2016. Possible causes other than the investigational product are medical conditions, severe acute malnutrition and pneumonia.

The two non-fatal cases were as follows:

An 8-year-old male patient (20 kg) with sickle cell disease, cough, vomiting, bloody urine and fever received artesunate 3 times at 12-h intervals from 05-Jul-2016 to 06-Jul-2016. On 05-Jul-2016 the Hb was 10 g/dL and on 06-Jul-2016 the patient was detected with severe anaemia and fever. On 09-Jul-2016, the Hb was 5.8 g/dL and anaemia was assessed as life-threatening. Anaemia and fever were not resolved at the time of the report (on 12-Aug-2016). Severe anaemia was assessed as possibly related to artesunate.

A 41-year-old female patient, exposed to HIV, was put on antiretroviral agents on 21-Jun-2016 (TDF/3TC/EFV) until 20-Jul-2016. Four days later, she developed headache, palpitations, diarrhoea, vomiting, sore mouth, appetite lost, fever, and dizziness diagnosed as malaria. She received artesunate 3 times at 12-hour intervals from 24-Jun-2016 to 25-Jun-2016, leading to improvement. On 18-Jul-2016 she developed severe lower abdominal pain on the left side, considered as medically significant. The patient was treated for salpingitis.

Literature reports

A literature survey during the reporting period identified 8 articles, which are summarised as follows:

Use of artesunate in patients with sickle cell anaemia

A case report of cardiac arrest and ventricular tachycardia in a 19-year-old male with sickle cell anaemia. He had fever, nausea, headache and yellowish discoloration of sclera. Haemoglobin was 7.3 g/dL. *Plasmodium falciparum* was positive with a parasitaemia index of 10%. He was treated with IV artesunate 2.4 mg/kg at 0 h, 12 h and 24 h and every 24 h thereafter for 5 days. At 4 h after the third dose he had a cardiac arrest. ECG showed wide complex ventricular tachycardia and QTc was 516 msec. Electrolytes were within normal limits. Tachycardia was managed by administration of amiodarone (150 mg IV for 10 minutes). The patient recovered and artesunate was continued. It is presumed that artesunate induced the ventricular arrhythmia.

Convulsions

Byakika-Kibwika presented the results of a randomised single blind clinical trial conducted in Tororo District Hospital situated in Eastern Uganda. This clinical trial assessed the 42-day parasitological outcomes of severe malaria treatment with intravenous artesunate or intravenous quinine followed by oral artemisinin-based combination therapy in children living in a high malaria transmission setting in

Eastern Uganda. One SAE was reported in this study concerning repeated convulsions after IV artesunate with death within 2 h post drug administration.

Adverse events reported in published studies
The top five adverse events (AEs) in the CEMISA study recorded among patients treated with artesunate were pyrexia (3.5%), abdominal pain (2.5%), diarrhoea (1.7%), cough (1.5%) and asthenia (1.5%). Regarding the relatedness of these AEs to artesunate, 78.9% of pyrexia (30/38), 63% of abdominal pain (17/27), 68.4% of diarrhoea (13/19), 85.5% of cough (14/16) and 75% of asthenia (12/16) were assessed as possibly related. Seventeen SAEs included 13 deaths of which 4 involved death outside the hospital with little information on follow-up.

Post Artesunate Delayed Haemolysis
Six articles were found on PADH, all of which were case reports or literature reviews. Salehi *et al*. 2018 described a case report of PADH in a 27 year old patient with a recent travel history to Ghana. The patient received 2 doses of artesunate at dose 2.4 mg/kg and developed haemolytic anaemia with the lowest haemoglobin level at 4.7 g/dl. From 2010 to 2012, the CDC in Iran described 19 cases of PADH plus 30 other cases from the literature. Although most of the cases were adult returning travellers (n = 37), the publication also reported on patients with no travelling history who were living in endemic areas. Among previously published reports, 29 cases required transfusion as supportive care for PADH. Some of them received corticosteroids and all of them recovered without complications.

A retrospective study included 21 patients with severe malaria due to *Plasmodium falciparum* treated with intravenous artesunate between August 2013 and July 2015 in Barcelona. Four developed PDAH, 2 required red blood cell transfusion for low haemoglobin level (8.6 g/dL and 6.7 g/dL) and 2 other patients did not require any specific treatment.

Metabolism information
One article reported that CYP2A6 is known to be involved in the metabolism of artesunate to its active metabolite dihydroartemisinin *in vitro*. CYP2A6 has large inter-individual and inter-ethnic variability which is thought to be primarily due to genetic polymorphisms. There are 13 genetic variants in CYP2A6 and a majority of them have been shown to alter CYP2A6 enzyme activity. Yusof and Hua completed a study assessing the influence of CYP2A6 and CYP2C8 genes on the incidence of adverse effects in 24 healthy Malaysian volunteers receiving single doses of artesunate and amodiaquine. Frequencies of CYP2A6*1B, CYP2A6*4, CYP2A6*8, and CYP2A6*9 alleles were 54, 17, 4, and 10%, respectively. A total of 22 subjects were included in the final analysis. Adverse effects reported by participants included flushing (n = 7), giddiness (n = 6), nausea (n = 3), raised liver enzymes (n = 3), headache (n = 2), and epigastric discomfort (n = 1).

When adverse effects were analysed according to CYP2A6 variant alleles, significantly more adverse effects were noted in those patients with CYP2A6*1B variants. This variant is known to be responsible for ultra-metabolism of artesunate and this suggests that these patients may be at an increased risk of experiencing an adverse event due to accumulation of DHA.

3.3.9. Discussion on clinical safety

Based on SEAQUAMAT and AQUAMAT the majority of the safety data come from children aged from ~18 months, adolescents and adults. The adult data come mainly from subjects aged < 50 years. 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 by investigators was confined to deteriorations and development of complications.

In many of the publications, the doses used do not seem to have been 2.4 mg/kg given as recommended in the applicant's SmPC and in some cases it seems that dosing was not mg/kg but was perhaps based on weight banding. This complicates understanding the safety profile of the applicant's product when used in accordance with the SmPC.

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. Most cases have been described in returning travellers, with very variable estimated rates for PADH in the literature ranging from 0.9%-27.8% but even higher rates have been reported from small case series. It is unclear if all publications reporting PADH used the same definition, such that true delayed haemolysis was adequately distinguished from the normal response to successful treatment. Nevertheless, PADH is clearly a substantial risk after artesunate treatment, it occurs very commonly, and it may require transfusion in a high proportion of patients.

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.

In SEAQUAMAT, ~10% of artesunate-treated patients and 13% treated with quinine developed neurological defects after admission but <1.5% 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. 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 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
The final content of 4.8 reflects multiple data sources and is based on best available estimates.

3.3.10. Conclusions on clinical safety

The appraisal of the safety of the applicant's IV artesunate is not straightforward due to variable reports in the literature and inconsistent methods for collection and reporting of data.

The overall opinion is that IV artesunate itself is probably not associated with major safety concerns but it clearly carries a risk for hypersensitivity reactions, which may sometimes be severe. There is a clear risk of PADH with onset after successful treatment. PADH occurs very commonly according to most publications based on returning travellers with severe falciparum malaria, even when considering that the definition applied has not been consistent. PADH often requires transfusion.

In summary, there are no remaining issues.

3.4. Risk management plan

3.4.1. Safety Specification

Summary of safety concerns

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

3.4.2. Discussion on safety specification

Having considered the data in the safety specification the Rapporteur agrees with the applicant's revised proposals.

3.4.3. Conclusions on the safety specification

This is acceptable.

3.4.4. Pharmacovigilance plan

3.4.4.1 Routine pharmacovigilance activities

No routine pharmacovigilance activities beyond adverse reactions reporting and signal detection will be conducted.

3.4.4.2 Summary of additional PhV activities

III.3 Summary Table of additional Pharmacovigilance activities

Part III.1: On-going and planned additional pharmacovigilance activities

Study **Status**	**Summary of objectives**	**Safety concerns addressed**	**Milestones**	**Due dates**
Category 1 - Imposed mandatory additional pharmacovigilance activities which are conditions of the marketing authorisation				
Not applicable				
Category 2 – Imposed mandatory additional pharmacovigilance activities which are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances				
Not applicable				
Category 3 - Required additional pharmacovigilance activities				
Category 3 study: A Pregnancy Registry to Assess the Safety of Antimalarial Use in Pregnancy (Antimalarial pregnancy registry) Status: planned	Pregnancy registry to collect information on pregnancy outcomes in women treated with artesunate especially during the first trimester	Reproductive toxicity (especially in the first trimester)	Full study protocol	15 February 2022
			Regular updates	Data will be reviewed on an on-going basis and reported within PSURs

B&O Pharm contacted MMV/LSTM regarding participation in the prospective "Africa pregnancy registry". The parties agreed to extend the territory scope of the Africa pregnancy registry network to Europe, through the development of a case report form ("CRF") to systematically collect data on pregnant women with severe malaria treated with Garsun in the EU. In this way, data on Garsun use during pregnancy, will be collected both from women exposed and followed up in Africa, as well as in Europe.

The expansion of the Africa pregnancy registry to EU countries, including the update of the registry study protocol and the establishment of the CRF, will be completed within 6 months of the date of signing the Letter of Intent.

The applicant confirms that a two-stage CRF will be implemented, with one CRF completed as early as possible after information about exposure during pregnancy and a second one to collect information after the birth of the child, with follow up until 1 year of age. This kind of collection would allow the distinction between prospective and retrospective pregnancy outcomes, as well as the determination of lost to follow-up with missing information on pregnancy outcome. Examples of the content of the CRFs have been submitted. The final CRFs should be submitted along with the study protocol if possible. Final content of the CRFs will then be adjusted in coordination with the EMA/Rapporteurs along with the study protocol assessment.

In addition, the applicant ensured that he has comprehensive information about the most relevant sites in European countries and that any physician ordering artesunate will be made aware of the pregnancy registry via written communication. In addition, a website will be launched. This approach is supported.

The Rapporteur would appreciate it if the content of the written communication to the physicians would be coordinated with the EMA/Rapporteurs.

3.4.4.3 Overall conclusions on the PhV Plan

The PRAC Rapporteur, having considered the data submitted, is of the opinion that the proposed post-authorisation PhV development plan is sufficient to identify and characterise the risks of the product.

3.4.5. Plans for post-authorisation efficacy studies

Not applicable. No post-authorisation efficacy studies are planned.

3.4.6. Risk minimisation measures

3.4.6.1 Routine Risk Minimisation Measures

Part V.1: Description of routine risk minimisation measures by safety concern

Safety concern	Routine risk minimisation activities
Safety concern 1: Reproductive toxicity (especially in the first trimester)	Routine risk communication: SmPC sections 4.6, 5.3, PL section 2 Routine risk minimisation activities recommending specific clinical measures to address the risk: None Other routine risk minimisation measures beyond the Product Information: Legal status: prescription only medicine

3.4.6.2 Summary of additional risk minimisation measures

V.3: Summary table of pharmacovigilance activities and risk minimisation activities by safety concern

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Safety concern 1: Reproductive toxicity (especially in the first trimester)	Routine risk minimisation measures: SmPC sections 4.6, 5.3, PL section 2 Additional risk minimisation measures: None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None Additional pharmacovigilance activities: Antimalarial pregnancy registry

3.4.6.3 Overall conclusions on risk minimisation measures

The PRAC Rapporteur having considered the data submitted was of the opinion that:

The proposed risk minimisation measures are sufficient to minimise the risks of the product in the proposed indication(s).

3.4.7. Summary of the RMP

The public summary of the RMP is considered appropriate.

3.4.8. PRAC Outcome (July 2021)

During the July 2021 PRAC plenary meeting, the PRAC supported the summary of safety concerns as proposed by the CHMP Rapporteur. PRAC agreed on the need to propose pharmacovigilance activities to address the important potential risk of reproductive toxicity via a pregnancy registry as the most appropriated tool to collect these data. Overall, PRAC supported the PRAC Rapporteur Assessment and list of questions.

3.4.9. Conclusion on the RMP

The CHMP and PRAC considered that the risk management plan version 0.4 could be acceptable if the applicant implements the minor changes to the RMP as detailed in the list of questions.

3.5. Pharmacovigilance

3.5.1. Pharmacovigilance system

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

3.5.2. Periodic Safety Update Reports submission requirements

The active substance artesunate i.v. is not included in the EURD list and a new entry will be required. The new EURD list entry uses the {EBD} or {IBD} to determine the forthcoming Data Lock Points. 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 an alignment of the PSUR cycle with the European birth date (EBD).

4. Benefit risk assessment

4.1. Therapeutic Context

Artesunate is a semi-synthetic artemisinin derivative that can be given parenterally for the initial monotherapy of severe malaria until such time that the patient can start an oral definitive treatment regimen. The applicant proposed that Garsun can be given IV or IM for *treatment of severe malaria caused by Plasmodium falciparum in adults and children*.

4.1.1. 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 death 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.

4.1.2. 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 artesunate to IV quinine for severe malaria in 1461 (mostly adult) Asian patients and found that 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 parenteral quinine in preventing mortality. Based mainly on these trials, the WHO issued a strong recommendation for the use of IV or IM artesunate in patients with severe malaria for at least 24 h and until oral medication is possible. The WHO-recommended oral follow-on treatment consists of 3 days of an artemisinin-based combination therapy (ACT).

In the EU, Artesunate Amivas (artesunate) was granted a licence for the treatment of severe malaria in adults and children on 22 November 2021, while the assessment of Garsun was ongoing. Artesunate Amivas has ODD for the same indication as Garsun. No derogation has been granted.

4.1.3. Main clinical studies

The demonstration of efficacy of IV artesunate is essentially based on two prospective, randomised, controlled trials that used artesunate from Guilin:
- 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 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).

4.2. Favourable effects

Randomised controlled studies - *SEAQUAMAT* and *AQUAMAT*

These were both open label, prospective, randomised and active controlled trials that compared initial treatment with parenteral artesunate with widely recommended and well-established comparative regimens for treatment of severe *P. falciparum* malaria. 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 doses of 2.4 mg/kg given at 0 and 12 h and then once daily dosing with 2.4 mg/kg until oral medication was possible. This is the posology recommended in the applicant's SmPC for subjects ≥ 20 kg. AQUAMAT allowed IM dosing with the same regimen as an alternative to IV administration.

Both studies 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. While AQUAMAT required that 24 h parenteral treatment was given before an oral switch, there was no 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 are no published data on parasite counts over time, 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 of 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, the difference in mortality rates detected between the two randomised treatment arms likely reflects the impact of 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 publication describes results including 271 deaths among the 1461 patients enrolled when the study was stopped on recommendation of the DSMB. 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 and the analysis of efficacy in the PP population essentially reflects adults with complicated *P. falciparum* malaria. 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 20% with artesunate compared to 28% 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. While the two treatment groups seem to have been very similar overall in terms of host and disease characteristics, it is unclear whether there were differences between countries in host or disease features that may have affected the observed mortality rates.

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.

4.3. 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 apparent incentive that would have led the investigators or the persons who conducted the analyses to falsify the data.

With a primary endpoint of in-hospital death, the focus of SEAQUAMAT and AQUAMAT was on mortality ascribable to the acute malaria episode. However, both studies also report the total death and sequelae rates at 28 days and both studies 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 severe malaria subsets as defined in the two studies. The definitions are considered acceptable although not identical to current WHO criteria.

4.4. Unfavourable effects

In CDC-060, the most frequently reported AEs were anaemia, LFTs increased and thrombocytopenia. Neutropenia and lymphopenia were also reported. Of the 66 total reports of anaemia, 13 (20%) were Grade 4. The AEs reported in the SEAQUAMAT and AQUAMAT trials showed little comparability to those reported in CDC-060, suggesting that investigators did not necessarily report changes in the course of disease and complication of malaria as AEs/ADRs.

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 published cases have concerned returning travellers, with rates ranging from 0.9-27.8% or higher.

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.

4.5. Uncertainties and limitations about unfavourable effects

Based mainly on data generated in SEAQUAMAT and AQUAMAT, there is some information on safety for several thousand patients with malaria, many of whom had severe malaria. 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 risk of SAEs cannot be determined from available information.

The limited published information on safety and apparent differences in attitudes to reporting make it difficult to determine which ADRs merit mention in section 4.8 of the SmPC, including differentiation of true ADRs from AEs. The proposed table for section 4.8 takes into account a broad range of data sources.

4.6. Effects Table

Table 21: Effects Table for Artesunate

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

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
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		Uncontrolled study but relevant patient population of returning travellers	CDC-060
Anaemia			66/102 (65%)			
Thrombocytopenia			18/102 (18%)			
LFTs increased			28/102 (27%)			
AST increased			22/102 (22%)			
Bilirubin increased			14/102 (14%)			
Renal failure			10/102 (10%)			
ARDS			8/102 (8%)			

4.7. Benefit-risk assessment and discussion

Efficacy in the initial treatment of severe malaria

This application rests on well-established use of parenteral artesunate for initial treatment of severe falciparum malaria in the EU. Essentially, such usage in the EU followed on from two prospective randomised and active controlled trials SEAQUAMAT and AQAUAMAT that used Guilin artesunate.

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.

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.

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.

These studies support initial treatment of severe *P. falciparum* malaria with IV artesunate.

Safety of IV artesunate

The appraisal of the safety of the applicant's IV artesunate is not straightforward. The most relevant and complete data come from the CDC-060 study reported by Twomey. 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. The data reported from SEAQUAMAT and AQUAMAT using IV or IM Guilin artesunate cover much larger numbers, but very limited information is available and it seems investigators tended to report only AEs not ascribed to the course of the disease.

The assessor's 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. Also, there is a clear risk for PADH, which is very common and which often requires transfusion but is generally manageable.

4.7.1. Importance of favourable and unfavourable effects

Garsun is intended only for use in patients who require parenteral treatment for severe falciparum 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 inclusion of reference to use in accordance with official guidance and recommendation that the product is used only under appropriate supervision, it is anticipated that only those patients who need IV artesunate will receive it and they will follow parenteral treatment with an appropriate follow-on oral regimen.

4.7.2. Balance of benefits and risks

Severe falciparum malaria carries a considerable mortality rate, even in returning travellers who are diagnosed and treated promptly and with access to intensive care. 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 malaria and that the recommended posology has an acceptable safety profile.

4.7.3. Additional considerations on the benefit-risk balance

The overall B/R is negative. The Major Objections on the need for a valid GMP certificate, similarity assessment with authorised orphan product designated for a condition related to the indication being applied, and the NAS claim made by the applicant must be resolved.

5. Biosimilarity assessment

N/A

6. Proposed CHMP list of outstanding issues to be addressed List of outstanding issues to be addressed in an oral explanation and/or in writing

6.1. Quality aspects

Major Objection

1. Valid proof of GMP compliance issued by an EEA national competent authority inspectorate (e.g. a GMP Certificate) for the proposed drug product manufacturer in China' must be provided (from MO1)

6.2. Non clinical aspects

6.3. Clinical aspects

Other Concerns

2. There are two outstanding issues that need to be addressed in the Product Information. Please see the clinical assessment report and the appended PI document.

6.4. Risk management plan

Other Concerns

Pharmacovigilance plan

3. **Formal aspect (see clinical AR):** The RMP now includes the following in Part III.2.11: "[…] included also infant follow up to 6-12 months to assess congenital anomalies not detected at birth including congenital heart defects and neurodevelopment." As the applicant now confirms that the second CRF will collect information after the birth of the child, with a follow-up until the age of 1 year, this period should also be designated in the RMP: …included also infant follow up to ~~6~~ 12 months to assess…

6.5. Pharmacovigilance

None

6.6. Orphan similarity and derogations

Major Objection

The applicant should provide a similarity report against the orphan medicinal product Artesunate Amivas, which is designated for a condition related to the indication being applied for, and which received Commission Decision on 22 November 2021, and if applicable provide ground(s) requesting derogation allowing a Marketing Authorisation in accordance with Point 3 of Article 8 of the Orphan Regulation (EC) No 141/2000.

6.7. New active substance status

Major Objection

In view of the Marketing Authorisation of Artesunate Amivas, the applicant should submit a new report on the claim regarding the NAS status for Garsun.

7. Recommended conditions for marketing authorisation and product information in case of a positive opinion

7.1. Conditions for the marketing authorisation

Post-authorisation measure(s)	Motivation
Proposed post-authorisation measure 1 with proposed classification: category 3	*Motivation/Background information on measure, including due date:*
A pregnancy registry to assess the safety of antimalarial use in pregnancy (antimalarial pregnancy registry)	Pregnancy registry to collect information on pregnancy outcomes in women treated with artesunate especially during the first trimester Full study protocol: date TBC

* Classification: category 1= Annex II D condition; category 2= Annex II E specific obligations; category 3 = All other studies reflected only in the RMP (PASS)

7.2. Summary of product characteristics (SmPC)

(Please refer to separate document.)

7.3. Labelling

(Please refer to separate document.)

7.4. Package leaflet (PL)

(Please refer to separate document.)

User consultation

The applicant submitted a User Testing report with the responses to the D120 LOQ. This was acceptable. See section 10 for the full appraisal.