

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

213036Orig1s000

MULTI-DISCIPLINE REVIEW

Summary Review

Office Director

Cross Discipline Team Leader Review

Clinical Review

Non-Clinical Review

Statistical Review

Clinical Pharmacology Review

NDA/BLA Multi-Disciplinary Review and Evaluation

Application Type	NDA
Application Number(s)	213036
Priority or Standard	Priority
Submit Date(s)	8/16/2019 (SN001); 9/26/2019 (SN002)
Received Date	9/26/2019
PDUFA Goal Date	5/26/2020
Division/Office	Division of Anti-Infectives/Office of Infectious Diseases
Review Completion Date	5/26/2020
Established/Proper Name	Artesunate for Injection
(Proposed) Trade Name	Artesunate
Pharmacologic Class	Artemisinin derivative
Applicant	Amivas, LLC
Dosage form	Intravenous
Applicant Proposed Dosing Regimen	(b) (4)
Applicant Proposed Indication(s)/Population(s)	Initial treatment of severe (b) (4) malaria in adults and children.
Applicant Proposed SNOMED CT Indication Disease Term for each Proposed Indication	61462000 Malaria (disorder)
Regulatory Action	APPROVAL
Indication(s)/Population(s)	Initial treatment of severe malaria in adult and pediatric patients
SNOMED CT Indication Disease Term for each Indication	61462000 Malaria (disorder)
Recommended Dosing Regimen	2.4 mg/kg administered intravenously at 0 hours, 12 hours and 24 hours, and thereafter, administered once daily until the patient is able to tolerate oral antimalarial therapy

Table of Contents

Table of Tables.....	6
Table of Figures.....	13
Reviewers of Multi-Disciplinary Review and Evaluation.....	15
Glossary	21
1 Executive Summary	23
1.1. Product Introduction	23
1.2. Conclusions on the Substantial Evidence of Effectiveness	23
1.3. Benefit-Risk Assessment	25
1.4. Patient Experience Data.....	30
2 Therapeutic Context.....	31
2.1. Analysis of Condition	31
2.2. Analysis of Current Treatment Options.....	31
3 Regulatory Background.....	34
3.1. U.S. Regulatory Actions and Marketing History.....	34
3.2. Summary of Presubmission/Submission Regulatory Activity.....	34
4 Significant Issues from Other Review Disciplines Pertinent to Clinical Conclusions on Efficacy and Safety.....	35
4.1. Office of Scientific Investigations (OSI)	35
4.2. Product Quality.....	35
4.3. Devices and Companion Diagnostic Issues.....	35
5 Nonclinical Pharmacology/Toxicology	36
5.1. Executive Summary	36
5.2. Referenced NDAs, BLAs, DMFs	40
5.3. Pharmacology.....	40
5.4. ADME/PK.....	42
5.5. Toxicology.....	51
5.5.1. General Toxicology	52
5.5.2. Genetic Toxicology.....	57
5.5.3. Carcinogenicity.....	59
5.5.4. Reproductive and Developmental Toxicology	59
5.5.5. Other Toxicology Studies.....	76
6 Clinical Pharmacology.....	82
6.1. Executive Summary	82

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

6.2.	Summary of Clinical Pharmacology Assessment.....	84
6.2.1.	Pharmacology and Clinical Pharmacokinetics.....	84
6.2.2.	General Dosing and Therapeutic Individualization	84
6.3.	Comprehensive Clinical Pharmacology Review.....	86
6.3.1.	General Pharmacology and Pharmacokinetic Characteristics.....	86
6.3.2.	Clinical Pharmacology Questions.....	88
7	Sources of Clinical Data and Review Strategy.....	93
7.1.	Table of Clinical Studies	93
7.2.	Review Strategy.....	97
8	Statistical and Clinical Efficacy Evaluation.....	98
8.1.	Review of Relevant Individual Trials Used to Support Efficacy	98
8.1.1.	SEAQUAMAT: A multicenter, open, randomized comparison between quinine and artesunate for the treatment of falciparum malaria in SE Asia. (Dondorp et al. 2005)	98
8.1.2.	AQUAMAT: Artesunate versus quinine in the treatment of severe falciparum malaria in African children: an open-label, randomized trial	119
8.1.3.	EDCTP/MMV07-01 Phase II Randomized, Double-Blind Study of the Efficacy, Safety, Tolerability, and Pharmacokinetics of Intravenous Artesunate in Children with Severe Malaria	130
8.2.	Integrated Review of Effectiveness.....	138
8.2.1.	Assessment of Efficacy Across Trials	138
8.2.2.	Integrated Assessment of Effectiveness.....	140
8.3.	Statistical Issues.....	141
8.4.	Conclusions and Recommendations.....	141
9	Clinical Microbiology Review.....	143
9.1.	Nonclinical Microbiology.....	143
9.1.1.	Mechanism of Action.....	143
9.1.2.	<i>In vitro</i> Activity	144
9.1.3.	<i>In vivo</i> Activity (Animal Studies).....	144
9.1.4.	Drug Resistance	145
9.1.5.	Cross-resistance.....	145
9.2.	Clinical Microbiology.....	145
9.2.1.	Parasitological Assessments.....	146
9.2.2.	Interpretive Criteria.....	158
10	Review of Safety.....	159
10.1.	Safety Review Approach.....	159
10.2.	Review of Safety Database	161

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

10.2.1.	Overall Exposure.....	161
10.2.2.	Relevant characteristics of the safety population.....	164
10.2.3.	Adequacy of the safety database.....	165
10.3.	Adequacy of Applicant’s Clinical Safety Assessments.....	165
10.4.	Safety Results.....	166
10.4.1.	Treatment Emergent Adverse Events and Adverse Reactions.....	167
10.4.2.	Deaths.....	174
10.4.3.	Serious Adverse Events.....	176
10.4.4.	Dropouts and/or Discontinuations Due to Adverse Effects.....	179
10.4.5.	Significant Adverse Events.....	180
10.4.6.	Laboratory Findings.....	181
10.4.7.	Vital Signs.....	183
10.4.8.	Electrocardiograms (ECGs).....	183
10.4.9.	QT Interval.....	183
10.4.10.	Immunogenicity.....	184
10.5.	Analysis of Submission-Specific Safety Issues.....	184
10.5.1.	Neurological Events.....	184
10.5.2.	Delayed Hemolysis and Anemia.....	188
10.5.3.	Neutropenia.....	189
10.5.4.	Hypersensitivity.....	190
10.5.5.	Reduced Reticulocyte Count.....	190
10.6.	Clinical Outcome Assessment (COA) Analyses Informing Safety/Tolerability.....	191
10.7.	Safety Analyses by Demographic Subgroups.....	191
10.8.	Specific Safety Studies/Clinical Trials.....	192
10.9.	Additional Safety Explorations.....	192
10.9.1.	Human Carcinogenicity or Tumor Development.....	192
10.9.2.	Human Reproduction and Pregnancy.....	193
10.9.3.	Overdose, Drug Abuse Potential, Withdrawal, and Rebound.....	197
10.9.4.	Dosing in Patients > 100 kg.....	197
10.10.	Safety in the Postmarket Setting.....	198
10.11.	Integrated Assessment of Safety.....	199
10.12.	Conclusions and Recommendations.....	200
11	Advisory Committee Meeting and Other External Consultations.....	202
12	Pediatrics.....	203
12.1.	SEAQUAMAT.....	205

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

12.2.	CDC-060.....	206
12.3.	Dose Regimen and Lower Age Range for Pediatric Indication.....	207
13	Labeling Recommendations.....	212
13.1.	Prescription Drug Labeling.....	212
	5.2 Post Artesunate Delayed Hemolysis.....	213
14	Risk Evaluation and Mitigation Strategies (REMS).....	215
15	Postmarketing Requirements and Commitment.....	216
16	Division Director Comments.....	218
17	Office Director Comments.....	218
18	Appendices.....	219
18.1.	Financial Disclosure.....	219
18.2.	Nonclinical Pharmacology/Toxicology.....	222
18.3.	OCP Appendices (Technical documents supporting OCP recommendations).....	233
18.4.	Pharmacometrics review of the proposed artesunate dosing in pediatric patients.....	266
18.5.	Clinical Microbiology Appendix.....	274
	18.5.1. Mechanism of Action.....	274
	18.5.2. In vitro Activity.....	280
	18.5.3. In vivo Activity (Animal Studies).....	280
	18.5.4. Drug combination.....	298
	18.5.5. Drug Resistance.....	298
	18.5.6. Cross-resistance.....	303
18.6.	Review of Specific Studies/Clinical Trials with IV Artesunate.....	305
	18.6.1. SEAQUAMAT.....	305
	18.6.2. CDC-060.....	310
	18.6.3. WRAIR Study 1168 (Phase 2 Uncomplicated Malaria).....	326
	18.6.4. WRAIR Study 1263ab (Phase 2 Uncomplicated Malaria).....	335
	18.6.5. WRAIR Study 1128.....	347
	18.6.6. WRAIR Study 1142.....	356
	18.6.7. AQUAMAT.....	365
	18.6.8. EDCTP-MMV07-01.....	365
18.7.	References.....	369

Table of Tables

Table 2-1: CDC and WHO Guidelines for the Treatment of Severe Malaria (All Regions).....	32
Table 5-1. Absorption of ¹⁴ C-AS and unchanged AS in plasma and blood following a single IV dose in rats	42
Table 5-2. Absorption of AS and DHA on day 1 after a single IV dose of AS in dogs.....	43
Table 5-3. Mean tissue concentration of radioactivity in rats following single IV dose (5 mg/20 mcgCi/kg) in rats.....	43
Table 5-4. In vitro and in vivo distribution of [¹⁴ C] AS in red blood cells	44
Table 5-5. In vitro plasma protein binding of [¹⁴ C] AS in rat and human	44
Table 5-6. Mean PK parameters of ¹⁴ C-AS following single IV injection (5 mg/147 mcgCi/kg) in the tissue or organs of the pregnant rats by QWBA	44
Table 5-7. Formation of DHA from AS in liver microsomes after 60 min incubation.....	47
Table 5-8. Excretion in male rats Following a Single Dose of ¹⁴ C-AS	48
Table 5-9. Toxicokinetic parameters for 4-week IV study in rats.....	48
Table 5-10. Toxicokinetic parameters for 4-week IV study in dogs.....	49
Table 5-11. Toxicokinetic data following ¹⁴ C-AS 5 mg/kg single IV injection in pregnant rats	50
Table 5-12. Toxicokinetic data after a single oral or IV AS dose in pregnant rats	50
Table 5-13. Toxicokinetic data after single or 12 days of oral AS dosing in pregnant rats.....	50
Table 5-14. Toxicokinetic data for DHA* following oral AS administration.....	51
Table 5-15 Toxicokinetic data after 14 daily oral doses of AS in pregnant monkeys (GD33)	51
Table 5-16. Summary of cesarean section data and body weight gain from rats treated with intravenous AS on Day 11 of gestation	64
Table 5-17. Summary of cesarean section data from rabbits treated with oral AS during organogenesis.....	65
Table 5-18. Rabbit group mean cesarean data following oral AS administration during organogenesis.....	66
Table 5-19. Rabbit fetal malformation data following oral AS administration during organogenesis.....	67
Table 5-20. Summary of cesarean section data from rats treated with oral AS during organogenesis.....	69
Table 5-21. Rat group mean cesarean data following oral AS administration during organogenesis.....	69
Table 5-22. Rat fetal malformation data following oral AS administration during organogenesis	70
Table 5-23. Summary of cesarean section data from rats treated daily with IM artesunate during organogenesis (GD6 to GD 18)	75
Table 5-24. Summary of cesarean section data from rats treated with IM artesunate from Days 6 to 8 of gestation	75
Table 5-25. Summary of cesarean section data from rats treated with 6 mg/kg IM artesunate during different periods of gestation.....	76

Table 5-26. Mean PK parameters of 14C-AS following a single IV injection of 5 mg/kg/147 mcCi/kg in the tissue or organs of the pregnant rats by quantitative whole-body autoradiography.....	77
Table 5-27. PK Parameters Following 14C-AS 5 mg/kg Single IV Injection to Pregnant Rats on GD 11	78
Table 5-28. Establishment of bioequivalence (BE) for two IV formulations in rat.....	80
Table 5-29. Establishment of bioequivalence (BE) for two IV formulations in dog	80
Table 7-1: Clinical Trials Relevant to this NDA.....	93
Table 8-1: SEAQUAMAT Intention to Treat and Severe Malaria Populations.....	102
Table 8-2: SEAQUAMAT Demographic Characteristics (ITT Population).....	103
Table 8-3: SEAQUAMAT Pregnancy Status on Admission (ITT Population).....	104
Table 8-4: SEAQUAMAT Pretreatment with Antimalarial Drugs and Antibiotics (ITT Population)	104
Table 8-5: SEAQUAMAT Malaria Status and Parasite Count on Admission (ITT Population)	105
Table 8-6: SEAQUAMAT Complications on Admission (ITT Population)	106
Table 8-7: SEAQUAMAT Coma Scales on Admission (ITT Population).....	106
Table 8-8: SEAQUAMAT Days of Fever and Coma Prior to Admission (ITT Population)	107
Table 8-9: SEAQUAMAT Laboratory-Defined Conditions on Admission (ITT Population).....	108
Table 8-10: SEAQUAMAT Baseline Vital Signs (ITT Population)	108
Table 8-11: SEAQUAMAT Antimalarial Pretreatment and Quinine Loading Dose in the Quinine Group (ITT Population)	109
Table 8-12. SEAQUAMAT Total Doses of IV Artesunate (%) (ITT Population)	110
Table 8-13. SEAQUAMAT Total Doses of Quinine (Including loading dose)* (ITT Population) .	111
Table 8-14: SEAQUAMAT Outcome Summary Table (ITT Population).....	112
Table 8-15. SEAQUAMAT Mortality Rate (Severe Malaria Population)	113
Table 8-16: SEAQUAMAT Secondary Endpoint Summary Table (ITT Population)	114
Table 8-17: SEAQUAMAT Time to Event Summary (ITT Population).....	115
Table 8-18 SEAQUAMAT In-hospital Mortality Subgroup Analysis (ITT Population)	117
Table 8-19: AQUAMAT Modified Criteria for Severe Malaria	119
Table 8-20. AQUAMAT Demographic Characteristics (ITT Population)	124
Table 8-21. AQUAMAT Other Baseline Characteristics	124
Table 8-22. AQUAMAT In-hospital Mortality and Some Secondary Outcomes.....	126
Table 8-23. AQUAMAT Recovery Times in Surviving Patients	128
Table 8-24. Patient disposition (ITT Population).....	134
Table 8-25. EDCTP/MMV07-01 Age Distribution (ITT Population).....	135
Table 8-26. EDCTP/MMV07-01 Medical History (ITT Population).....	135
Table 8-27. EDCTP/MMV07-01 Asexual Parasite Counts at Baseline (ITT Population).....	136
Table 8-28. EDCTP/MMV07-01 Proportion of Patients with $\geq 99\%$ Parasite Clearance at 24 Hours by Treatment Group (ITT population).....	137
Table 8-29. EDCTP/MMV07-01 Percent Reduction in Asexual Parasites from Baseline at 24 and 48 Hours after Initiation of Randomized Study Drug	138
Table 8-30. SEAQUAMAT and AQUAMAT Primary Endpoint: In-hospital Mortality.....	139
Table 8-31. SEAQUAMAT and AQUAMAT Secondary Endpoints.....	139

Table 8-32. SEAQUAMAT Mortality and AQUAMAT In-hospital Mortality Subgroup Analyses..	139
Table 9-1: Study 1263ab - Follow-up of <i>P. falciparum</i> and <i>P. ovale</i> parasitemia in 2 patients with mixed infection	150
Table 9-2: Study CDC-060 - Survival in patients with <i>P. vivax</i> and <i>P. ovale</i> malaria	151
Table 9-3: Artemisinin resistance among <i>P falciparum</i> samples - CDC (2012-2016)	151
Table 9-4: Literature reports - Intravenous artesunate treatment of patients with severe malaria due to <i>Plasmodium</i> species other than <i>P. falciparum</i>	152
Table 9-5: Time to parasite clearance in patients with severe malaria.....	156
Table 10-1: NDA 213036 Clinical Trials Reviewed for Safety Analysis.....	160
Table 10-2: Overall Exposure to IV Artesunate in Clinical Development Plan (Safety Population)	162
Table 10-3: Exposure to IV Artesunate Across the Studies Submitted, Including Severe and Uncomplicated Malaria (Safety Population).....	163
Table 10-4: Exposure to IV Artesunate in Healthy Volunteers (Safety Population).....	163
Table 10-5: Overview of Adverse Events and Deaths in IV Artesunate Recipients (All Dose Regimens) (Safety Population)	167
Table 10-6: SEAQUAMAT -- Treatment Emergent Adverse Event by PT Incidence >2% (Safety Population)	168
Table 10-7: CDC-060 - TEAE by PT, Incidence > 2% (Safety Population).....	168
Table 10-8: Study 1168 - TEAE by Preferred Term >=5%.....	169
Table 10-9: Phase 2 Study 1263ab - TEAE by SOC/PT >=5% (Safety Population)	170
Table 10-10: Studies 1168 and 1263ab - Severe Adverse Events (Grade 4) in Patients with Uncomplicated Malaria.....	171
Table 10-11: Study 1128 and 1142 (Healthy Volunteers) - TEAE by Preferred Term (Pooled Artesunate > 5%) (Safety Population).....	172
Table 10-12: Study 1128 - TEAE by Study Dose by PT, Incidence >5% (Safety Population).....	172
Table 10-13: Study 1142 - TEAE by Study Dose by PT, in Any Artesunate Group >=5%, by Dose Regimen (Safety Population).....	173
Table 10-14: Overall Mortality in Studies of Severe Malaria (Safety Population)	174
Table 10-15: CDC-060 - Clinical Characteristics of Patients who Died (Safety Population).....	175
Table 10-16: CDC-060 - Serious Adverse Events (SAE), Safety Population*	177
Table 10-17: EDCTP-MMV07-01 Serious Adverse Events by SOC/PT (Safety Population)	179
Table 10-18: SEAQUAMAT - Designated Medical Events (Safety Population)	180
Table 10-19: CDC-060 - Designated Medical Events	181
Table 10-20: SEAQUAMAT – Number of Patients with Neurological Sequelae on Discharge (Safety Population).....	185
Table 10-21: SEAQUAMAT – Incidence of TEAE by Demographic Subgroups ¹ (Safety Population)	191
Table 10-22: CDC-060 -- TEAE by Demographic Subgroups ¹ (Safety Population).....	192
Table 10-23: SEAQUAMAT – Pregnancies, Maternal Deaths, and Still Births and Spontaneous Abortions (Safety Population).....	194
Table 10-24: SEAQUAMAT -- Clinical Events After Admission in Pregnant Patients (Safety Population)	194

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Table 10-25: SEAQUAMAT -- All Pregnancies: Maternal and Fetal Outcomes (Safety Population)	195
Table 10-26: CDC-060 TEAE > 5% by PT According to Weight 100 kg (Safety Population)	198
Table 12-1: NDA 213036 - Size of Pediatric Database < 18 years old for Artesunate Recipients (Safety Population).....	204
Table 12-2: SEAQUAMAT - TEAE (Preferred Term) for Patients < 18 years old, Incidence > 5% in Artesunate Group (Safety Population).....	205
Table 12-3: SEAQUAMAT TEAE (SOC/PT) > 1% in Pediatric ¹ Subjects by Weight 20 kg (Safety Population)	205
Table 12-4: CDC-060 - TEAE in Pediatric Patients < 18 years old (Safety Population)	206
Table 12-5: CDC-060 Pediatric* Enrollment by Weight < or > 20 kg (Safety Population)	207
Table 12-6: CDC-060 - Pediatric Time to Discharge by Weight < 20 kg or > 20 kg (Safety Population)*.....	207
Table 12-7: Case Reports of Infants Less Than 6 Months of Age with Severe Malaria Treated with Artesunate.....	208
Table 12-8 - Information from Published CDC Malaria Surveillance Summaries - Infants with Severe Malaria treated under CDC-060 from 2011- 2016.....	210
Table 13-1: Summary of Significant Labeling Changes.....	212
Table 18-1. Compartmental Analyses of AS by Intravenous Infusion Modeling after Single IV Administrations of 0.5, 1, 2, 4 and 8 mg/kg by Short-term Infusion (2 minutes) in healthy volunteers.....	233
Table 18-2. Pharmacokinetic Parameters of DHA Following Single Doses of IV Artesunate after Single IV Administrations of 0.5, 1, 2, 4 and 8 mg/kg by Short-term Infusion (2 minutes) in healthy volunteers.....	233
Table 18-3. Mean (CV%) PK parameters for AS by Compartmental Modeling: IV AS.....	236
Table 18-4. Mean (CV%) PK parameters of DHA by Noncompartmental Analysis Following Multiple Administrations of IV AS (2 Min Infusion) at Doses of 2, 4, and 8 mg/kg Daily for 3 days in Healthy Volunteers.....	237
Table 18-5. Pharmacokinetic Parameters for artesunate following administration of 2.4 mg/kg once a day for 3 days.	238
Table 18-6. Pharmacokinetic Parameters for DHA following administration of 2.4 mg/kg once a day for 3 days.	238
Table 18-7. Transport rates for 5 and 20 μ M DHA through Caco-2 subclone (CLEFF9) monolayers in both apical-to-basolateral (A $\rightarrow$ B) and basolateral-to-apical directions (B $\rightarrow$ A)	240
Table 18-8: Mefloquine Pharmacokinetic parameters (Mean $\pm$ SD) in healthy volunteers receiving mefloquine alone or mefloquine +artesunate.....	241
Table 18-9: Pharmacokinetic parameters for artesunate and dihydroartemisinin on days 1 and 3 of periods 1 (artesunate alone) and period 2 (artesunate given with mefloquine).....	244
Table 18-10: Mean (SD) pharmacokinetics of atovaquone following the administration of the third dose of either atovaquone plus proguanil or atovaquone plus proguanil plus artesunate in 12 healthy adult volunteers	246
Table 18-11. Mean PK parameters of proguanil and cycloguanil following the administration of the third dose of either atovaquone plus proguanil or atovaquone plus proguanil plus	

artesunate in 12 healthy volunteers. Data from single drugs (Mepron and Paludrine) and the fixed atovaquone-proguanil combination (Malarone) are combined..... 247

Table 18-12: Pharmacokinetic parameters for artesunate and dihydroartemisinin when artesunate was administered alone and with amodiaquine (ACT) 251

Table 18-13. Pharmacokinetic parameters for desethylamodiaquine (DEAQ) when amodiaquine (AQ) was administered alone and with artesunate (ACT) 252

Table 18-14. Pharmacokinetic parameters of artesunate (AS) after a single oral dose of AS (200 mg) alone and in combination with a single dose of 3 sulfadoxine/pyrimethamine (SP) tablets (sulfadoxine 500 mg +pyrimethamine 25 mg/tablet), Sudan* 253

Table 18-15. Pharmacokinetic parameters of dihydroartemisinin (DHA) after a single oral dose of artesunate (AS) (200 mg) alone and in combination with a single dose of 3 sulfadoxine/pyrimethamine (SP) tablets (sulfadoxine 500 mg + pyrimethamine 25 mg/Tablet), Sudan* 254

Table 18-16. Phenotyping metrics in the five different treatment groups [artemisinin (ART), dihydroartemisinin (DHA), arteether (ARE), artemether (ARM) and artesunate (AS)]. The presented quotients (mean, 98.75% CI) are based on anti-logarithms of the contrast for the different occasions. 256

Table 18-17. Pharmacokinetic parameters of pyronaridine in subjects administered pyronaridine/artesunate (PA) +ritonavir (Arm A) or administered PA only. Cmax and area under the curve (AUC) values are normalized to 9.72 mg/kg† 259

Table 18-18. Pharmacokinetic parameters of artesunate and dihydroartemisinin (DHA) in subjects administered pyronaridine/artesunate (PA) + ritonavir (Arm A) or administered PA only*..... 259

Table 18-19: Geometric mean ratios (90% confidence intervals [CI]) for the pharmacokinetic parameters of pyronaridine, artesunate, and DHA after administration with ritonavir (Arm A) or without ritonavir (Arm B)..... 260

Table 18-20. Pharmacokinetic parameters of ritonavir from Day 1 and Day 10*. Day 1 represents Ritonavir alone and Day 10 represents Ritonavir coadministered with PA. 260

Table 18-21. PK parameters of artesunate and dihydroartemisinin following administration of oral artesunate with and without nevirapine..... 262

Table 18-22. Pharmacokinetic parameters of artesunate and dihydroartemisinin following IV administration of 2.4 mg/kg artesunate once a day for 3 days in 14 patients with severe malaria 265

Table 18-23. Population pharmacokinetic studies of artesunate that were reviewed by the pharmacometrics reviewer..... 266

Table 18-24. Proposed body weight–adjusted dosing regimen for IM artesunate. 268

Table 18-25: In vitro activity of artesunate and DHA against the asexual stage parasites (black font) and/or gametocytes (green font) of the laboratory strains of *P. falciparum* 281

Table 18-26: In vitro activity of artesunate against the clinical/field isolates (asexual stage parasites) of *P. falciparum* 284

Table 18-27: In vitro activity of artesunate/DHA against the laboratory strains/clinical isolates (asexual stage parasites) of *P. vivax*, *P. knowlesi*, *P. malariae* and *P. ovale* 288

Table 18-28: Suppressive activity of artesunate and DHA in mice and rats infected with the parasitized erythrocytes of <i>Plasmodium berghei</i>	291
Table 18-29: Suppressive activity of artesunate in mice and nonhuman primates infected with the parasitized erythrocytes of <i>Plasmodium falciparum</i> or <i>Plasmodium knowlesi</i>	297
Table 18-30: SEAQUAMAT – Mortality by Malaria Status on Admission and Age (Safety Population)	307
Table 18-31: SEAQUAMAT – Clinical Events After Admission ¹ (Safety Population).....	307
Table 18-32: SEAQUAMAT – Clinical Events After Admission (Severe Malaria Population).....	308
Table 18-33: CDC-060 – Schedule of Events.....	311
Table 18-34: CDC-060 – Demographics (Safety Population).....	313
Table 18-35: CDC-060 – Age Category of Enrolled Subjects (Safety Analysis).....	314
Table 18-36: CDC-060 – Country of Residence and Potential Exposure (Safety Population)	314
Table 18-37: CDC-060 – Pretreatment with Quinidine or Quinine (Safety Population).....	315
Table 18-38: CDC-060 – Reason for Artesunate Therapy (Safety Population)	315
Table 18-39: CDC-060 – Baseline Malaria Species (Safety Population)	315
Table 18-40: CDC-060 – Baseline Clinical Events (Safety Population)	316
Table 18-41: Follow-on Treatment (Safety Population)	316
Table 18-42: CDC-060 – Number of Artesunate Doses Administered (Safety Population).....	316
Table 18-43: CDC-060 – Disposition of Subjects (Safety Population).....	317
Table 18-44: CDC-060 – Percent Parasitemia by Study Day (Safety Population)	317
Table 18-45: CDC-060 – Clinical Characteristics of Patients who Died (Safety Population).....	318
Table 18-46: CDC-060 SAEs and Fatal Outcomes.....	318
Table 18-47: CDC-060 – Serious Adverse Event by SOC/PT (Safety Population)*	319
Table 18-48: CDC-060 – TEAE (SOC/PT > 2%) by Grade (Safety Population).....	321
Table 18-49: Study 1168 – Schedule of Assessments.....	327
Table 18-50: Study 1168 Demographics (Safety Population).....	329
Table 18-51: Study 1168 – Baseline <i>P. falciparum</i> Asexual Parasites*.....	329
Table 18-52: Study 1168 TEAE by Severity, Incidence >5% (Safety Population).....	331
Table 18-53: Study 1263 ab – Schedule of Events (Thailand).....	335
Table 18-54: Study 1262ab Demographics (Safety Population).....	337
Table 18-55: Study 1263ab Demographics Pediatric Subjects (< 18 years old) (ITT).....	338
Table 18-56: Study 1263ab – Disposition.....	339
Table 18-57: Study 1263ab – TEAE > 5% Total Incidence by SOC/PT According to Treatment Arm (Safety Population).....	340
Table 18-58: Study 1263ab TEAE by SOC/PT Categorized as Severe	341
Table 18-59: Study 1263ab – TEAE Categorized as Severe (Patient Details).....	342
Table 18-60: Study 1128 – Schedule of Events.....	348
Table 18-61: Study 1128 – Demographics Pooled Artesunate vs Placebo (Safety Population)..	350
Table 18-62: Study 1128 – TEAE by Pooled Artesunate vs Placebo by SOC/PT, Incidence PT >5% in Either Group or Total (Safety Population)	351
Table 18-63: Study 1142 Schedule of Events.....	358
Table 18-64: Study 1142 TEAE (SOC/PT), Incidence >5% in Pooled Artesunate (Safety Population)	360

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Table 18-65: Study EDCTP-MMV07-01 – Schedule of Events 366
Table 18-66: EDCTP-MMV07-01 – Number of SAEs per Treatment Group (Safety Population) 367
Table 18-67: EDCTP-MMV07-01 – SAEs by SOC/PT (Safety Population) 367

Table of Figures

Figure 5-1. Metabolic pathway of artesunate	47
Figure 6-1: Simulated exposure (AUC ₀₋₁₂) of DHA in patients with severe malaria after the first dose of IV AS (2.4 mg/kg) across a range of weights or ages (inset) using a PK model accounting for size and organ maturation.....	89
Figure 8-1. SEAQUAMAT Kaplan-Meier Survival Curve (ITT Population).....	113
Figure 8-2. AQUAMAT: Trial Profile	123
Figure 8-3. AQUAMAT Kaplan-Meier Curves.....	128
Figure 8-4. AQUAMAT Treatment Effect in Protocol-specified Subgroups.....	129
Figure 9-1: Diagrammatic representation of the mechanism of action and resistance to qinghaosu/artemisinin.....	143
Figure 10-1- Study 1128 Reticulocytes following IV Artesunate by Study Dose.....	182
Figure 10-2– Study 1128 - Reticulocytes below Lower Limit of Normal (Pooled Artesunate)....	182
Figure 10-3: AQUAMAT – Neuro Sequelae at discharge and 28 days in children with severe falciparum malaria (Per Protocol).....	187
Figure 18-1. Mean plasma concentration-time profiles of AS (top) and DHA (bottom), an active metabolite of AS measured by LC-MS/MS after a single intravenous dosage with a 2-minute short-term infusion of AS at 0.5, 1, 2, 4, and 8 mg/kg in healthy volunteers (N = 6 for each dose cohort).....	234
Figure 18-2. Dose proportional increases of AS and DHA Cmax and AUC Single Doses of IV Artesunate after Single IV Administrations of 0.5, 1, 2, 4 and 8 mg/kg by Short-term Infusion (2 minutes) in healthy volunteers.....	235
Figure 18-3. Mean plasma dihydroartemisinin and artesunate concentrations during days 1 and 3 of periods 1 and 2 in healthy male volunteers.....	243
Figure 18-4. Plasma concentration-time curves (mean ± SEM) of atovaquone in 12 healthy adults following the third daily dose of either 1000 mg atovaquone plus 400 mg proguanil (open circles) or 1000 mg atovaquone plus 400 mg proguanil plus 250 mg artesunate (closed circles)	246
Figure 18-5. Plasma concentration time curves of proguanil in 12 healthy adults following the third daily dose of either 1000 mg atovaquone plus 400 mg proguanil (open circles) or 1000 mg atovaquone plus 400 mg proguanil plus 250 mg artesunate (closed circles). Cycloguanil concentrations in seven volunteers after atovaquone plus proguanil (open squares) and atovaquone plus proguanil plus artesunate (closed squares)	247
Figure 18-6. Profiles of mean drug concentrations over time following monotherapy (<i>mono</i>) and combination (<i>combo</i>) therapy for a) artesunate (AS) and dihydroartemisinin (DHA) and b) amodiaquine (AQ) and desethylamodiaquine (DEAQ).....	250
Figure 18-7. Individual plots of parasite density against time post start of treatment	265
Figure 18-8. 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. (red).....	269

Figure 18-9. Simulated DHA exposure after first AS dose (3 mg/kg in children 2-20 kg and 2.4 mg/kg in children 20-25 kg).....	272
Figure 18-10. Simulated DHA exposure after first AS dose of 2.4 mg/kg regardless of body weight.....	272
Figure 18-11. DHA exposure simulated using a model without maturation effect. The exposure is after an IV AS dose of 2.4 mg/kg regardless of body weight.....	273
Figure 18-12: SEAQUAMAT – Diagram of Study Structure	305
Figure 18-13: CDC-060 Hematocrit and Hemoglobin by Day of Hospitalization.....	323
Figure 18-14: CDC-060 – Platelets by Day of Hospitalization.....	324
Figure 18-15: CDC-060 – Reticulocyte Count by Day of Hospitalization.....	324
Figure 18-16: CDC-060 – Leukocytes by Day of Hospitalization.....	325
Figure 18-17: CDC-060 – Neutrophils by Day of Hospitalization.....	325
Figure 18-18: CDC-060 – Transaminases and Bilirubin by Day of Hospitalization.....	326
Figure 18-19: 1168 Fever Histogram.....	329
Figure 10-12: Study 1168 - Hemoglobin and Hematocrit by Study Visit.....	332
Figure 18-21: Study 1168 – Platelets by Study Visit.....	333
Figure 18-22: Study 1168 – WBC Parameters by Study Visit	333
Figure 10-15: Study 1168 - Transaminases by Study Visit	334
Figure 18-24: Study 1168 - Total Bilirubin by Study Visit.....	334
Figure 18-25: Study 1263ab – Fever Clearance by Study Dose	339
Figure 18-26: Study 1263ab – ALT by Study Visit and Dose.....	344
Figure 18-27: Study 1262ab – Hematocrit and Hemoglobin by Study Visit and Dose.....	345
Figure 18-28: Study 1263ab – Reticulocytes by Study Visit	346
Figure 18-29: Study 1263ab – Neutrophils by Study Visit.....	346
Figure 10-22: Study 1128 - Hemoglobin and Hematocrit by Study Visit (Pooled Artesunate versus Placebo)	353
Figure 18-31: Study 1128 – Reticulocyte Count by Study Visit (Pooled Artesunate versus Placebo).....	354
Figure 18-32: Study 1128 – Reticulocyte Count by Dose Regimen and Study Visit.....	354
Figure 18-33: Study 1128 -- Neutrophils by Study Visit and Dose Regimen versus Placebo.....	355
Figure 18-34: Transaminases by Study Visit (Pooled Artesunate versus Placebo).....	355
Figure 18-35: Study 1142 – Hematocrit by Study Visit (Pooled Artesunate vs. Placebo).....	362
Figure 18-36: Study 1142 – Hemoglobin by Study Visit (Pooled Artesunate vs. Placebo)	362
Figure 18-37: Study 1142 – Platelets by Study Visit (Pooled Artesunate vs. Placebo).....	362
Figure 18-38: Study 1142 – Reticulocytes by Study Visit (Pooled Artesunate vs. Placebo and Dose of Artesunate vs. Placebo).....	363
Figure 18-39: Study 1142 – Neutrophils by Study Visit (Pooled Artesunate vs. Placebo and Dose of Artesunate vs. Placebo	363
Figure 18-40: Study 1142 – ALT by Study Visit (Pooled Artesunate vs. Placebo and Dose of Artesunate vs. Placebo)	364
Figure 10-33: Study 1142 - AST by Study Visit (Pooled Artesunate vs Placebo and Dose of Artesunate vs. Placebo)	364

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Glossary

AC	advisory committee
ADME	absorption, distribution, metabolism, excretion
AE	adverse event
AR	adverse reaction
BLA	biologics license application
BPCA	Best Pharmaceuticals for Children Act
BRF	Benefit Risk Framework
CBER	Center for Biologics Evaluation and Research
CDER	Center for Drug Evaluation and Research
CDRH	Center for Devices and Radiological Health
CDTL	Cross-Discipline Team Leader
CFR	Code of Federal Regulations
CMC	chemistry, manufacturing, and controls
COSTART	Coding Symbols for Thesaurus of Adverse Reaction Terms
CRF	case report form
CRO	contract research organization
CRT	clinical review template
CSR	clinical study report
CSS	Controlled Substance Staff
DHOT	Division of Hematology Oncology Toxicology
DMC	data monitoring committee
ECG	electrocardiogram
eCTD	electronic common technical document
ETASU	elements to assure safe use
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FDASIA	Food and Drug Administration Safety and Innovation Act
GCP	good clinical practice
GRMP	good review management practice
ICH	International Conference on Harmonisation
IND	Investigational New Drug
ISE	integrated summary of effectiveness
ISS	integrated summary of safety
ITT	intent to treat
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent to treat
NCI-CTCAE	National Cancer Institute-Common Terminology Criteria for Adverse Event
NDA	new drug application
NME	new molecular entity
OCS	Office of Computational Science

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

OPQ	Office of Pharmaceutical Quality
OSE	Office of Surveillance and Epidemiology
OSI	Office of Scientific Investigation
PBRER	Periodic Benefit-Risk Evaluation Report
PD	pharmacodynamics
PI	prescribing information
PK	pharmacokinetics
PMC	postmarketing commitment
PMR	postmarketing requirement
PP	per protocol
PPI	patient package insert (also known as Patient Information)
PREA	Pediatric Research Equity Act
PRO	patient reported outcome
PSUR	Periodic Safety Update report
REMS	risk evaluation and mitigation strategy
SAE	serious adverse event
SAP	statistical analysis plan
SGE	special government employee
SOC	standard of care
TEAE	treatment emergent adverse event

1 Executive Summary

1.1 Product Introduction

Artesunate for Injection is a semi-synthetic anti-malarial drug in the class of artemisinins. Each single-dose vial contains 110 mg of artesunate for constitution with sodium phosphate buffer. Artesunate for Injection is to be administered at 2.4 mg/kg/dose as a slow intravenous bolus infusion over 1 to 2 minutes.

Derived from

(b) (4)

(b) (4)

Study of artemisinins for the treatment of severe malaria began over 30 years ago. ([Myint and Shwe 1987](#)) The mode of action involves iron-catalyzed cleavage of the endoperoxide bridge with generation of free radicals and alkylation of malaria-specific proteins, inhibiting growth and replication of *Plasmodium*. ([Kamchonwongpaisan and Meshnick 1996](#)) Artesunate, a succinic acid ester of artemisinin, is water soluble and is rapidly hydrolyzed to dihydroartemisinin (DHA), its active metabolite.

Artesunate and DHA are active against the erythrocytic asexual blood stage *Plasmodium* parasites, affecting rapid clearance of parasitemia generally within 48 to 72 hours.

1.2 Conclusions on the Substantial Evidence of Effectiveness

The Applicant has provided substantial evidence of effectiveness and sufficient safety data to support the approval of Artesunate for Injection for the initial treatment of severe malaria in adult and pediatric patients. IV artesunate treatment resulted in an improved survival over the previous standard of care (quinine) in a single, randomized, active-controlled trial in adults and pediatric patients with severe malaria in Asia (SEAQUAMAT), with supportive evidence from a published, randomized, active-controlled trial in pediatric patients with severe malaria in Africa (AQUAMAT). Both trials were conducted by the Wellcome Trust using parenteral artesunate manufactured by Guilin Pharmaceuticals. The Applicant submitted patient level data from SEAQUAMAT and sufficient information to support the scientific bridge based on comparative Chemistry, Manufacturing and Controls information and comparable pharmacokinetics of the proposed drug product from the Phase 1 and Phase 2 studies (1128, 1142 and 1168) and the Guilin product used in the published clinical trials.

SEAQUAMAT, which enrolled 1461 adults and pediatric patients 2 years of age and older, had a primary efficacy endpoint of in-hospital mortality. The in-hospital mortality in the IV artesunate group (13.2%) was significantly lower than in the comparator group receiving IV quinine (20.5%), OR 0.59 (95% CI: 0.44, 0.79, p=0.0002). AQUAMAT, which enrolled pediatric patients younger than 15 years of age, evaluated IV artesunate at a similar dose and regimen as SEAQUAMAT except that a total of 35.5% of patients received artesunate or quinine

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

intramuscularly. Treatment with parenteral artesunate in AQUAMAT resulted in a similar effect on reducing in-hospital mortality compared to IV quinine as observed in SEAQUAMAT. The in-hospital mortality in the artesunate group (8.5%) was significantly lower than in the comparator group receiving quinine (10.9%), OR 0.75 (95% CI: 0.63, 0.90, p=0.0022).

Although patient-level data were only available from SEAQUAMAT, the data were considered adequate to provide substantial evidence of effectiveness based on the persuasive finding of mortality benefit in this trial with a similarly robust mortality benefit reported in the AQUAMAT publication. Additional supportive data were provided from a study of two artesunate dosing regimens in pediatric patients with severe malaria (EDCPT-MMV07-01).

1.3 Benefit-Risk Assessment

Benefit-Risk Summary and Assessment

In NDA 213036, the Applicant is seeking approval for Artesunate for Injection for intravenous (IV) use for the initial treatment of severe malaria in adults and pediatric patients. Artesunate for Injection is intended to be administered intravenously at 0 hours, 12 hours, 24 hours and thereafter once daily until oral therapy is tolerated and must be followed by a complete course of oral antimalarial therapy.

Severe malaria is a life-threatening infection and is a medical emergency. Most cases are due to *Plasmodium falciparum*. There are currently no approved drugs in the US for treatment of severe malaria. The Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) currently recommend IV artesunate as the first-line treatment of severe malaria in adults, children and pregnant women. IV artesunate is currently available in the US from the CDC under an Investigational New Drug (IND) expanded access treatment protocol.

IV artesunate was superior to IV quinine, the standard of care when the study was conducted, for the treatment of severe malaria in a single, randomized, open-label, active-controlled trial in adults and pediatric patients in Southeast Asia (SEAQUAMAT), with supportive evidence from a published, randomized, open-label, active-controlled trial in pediatric patients in Africa (AQUAMAT). The primary efficacy endpoint for both trials was in-hospital mortality. In SEAQUAMAT, which enrolled patients two years of age and older with severe malaria, in-hospital mortality was 13% in artesunate recipients compared to 21% in quinine recipients [OR 0.59, 95% CI (0.44, 0.79), $p=0.0002$]. AQUAMAT, which enrolled pediatric patients less than 15 years of age, demonstrated a similar significant survival benefit for parenteral artesunate compared to parenteral quinine. The in-hospital mortality in the artesunate group (8.5%) was significantly lower than in the comparator group receiving quinine (10.9%), OR 0.75 (95% CI: 0.63, 0.90, $p=0.0022$).

Safety data for IV artesunate were obtained from SEAQUAMAT and an uncontrolled, open-label, retrospective study in U.S. patients with severe malaria treated with Artesunate for Injection under the IND expanded access treatment protocol (CDC-060). Additional supportive evidence for safety was provided by AQUAMAT, a study of artesunate dosing regimens in pediatric patients with severe malaria, EDCPT-MMV07-01, a phase 2 trial of two dosing regimens of IV artesunate in pediatric patients with severe malaria, two phase 2 studies in patients with uncomplicated malaria, and two phase 1 studies in healthy adults. In SEAQUAMAT, the most common adverse reactions in patients receiving IV artesunate included acute renal failure requiring dialysis, hemoglobinuria, and jaundice. The most frequently observed adverse reactions in CDC-060 were anemia, elevation of transaminases, thrombocytopenia, hyperbilirubinemia, and acute renal failure. In SEAQUAMAT, the incidence of neurological impairment at the time of hospital discharge was higher in the artesunate group compared to the quinine; similar

findings were reported in AQUAMAT.

Post-artemisinin delayed hemolysis (PADH), defined as a 10% decrease in hemoglobin associated with laboratory findings of hemolysis occurring more than one week after treatment initiation, has been reported following IV artesunate therapy and is associated with hyperparasitemia. Because severe PADH may require transfusions, patients should be monitored with weekly blood counts for up to four weeks after treatment.

Hypersensitivity reactions including anaphylaxis has been reported following IV artesunate administration. In the phase 1 clinical development of IV artesunate, one case of urticaria was observed in a healthy volunteer. In a phase 2 study of IV artesunate in uncomplicated malaria, Stevens Johnson Syndrome was documented in a single patient. Transient neutropenia and a decrease in reticulocyte count were observed following Artesunate for Injection in phase 1 studies in healthy volunteers and phase 2 studies in patients with uncomplicated malaria.

In nonclinical studies, embryo-fetal toxicity (embryo lethality and malformations) was observed in three different animal species administered intravenous or oral artesunate in early gestation. However, the clinical relevance of these findings is unclear since extensive experience with oral artesunate and other artemisinin class drugs (via various routes of administration) in pregnant women has not identified a drug-associated risk of adverse maternal or fetal outcomes. The Applicant is required to conduct a postmarketing study to collect data on women exposed to artesunate during pregnancy to assess the risk of maternal and fetal complications.

The Artesunate for Injection labeling includes warnings for post-treatment hemolysis, hypersensitivity, and embryo-fetal toxicity in animals.

In summary, the studies submitted demonstrated a clinically meaningful survival benefit of IV artesunate treatment in patients with severe malaria when compared to quinine the previous standard of care in well-controlled trials. The results were robust and consistent in adults and pediatric patients across different geographic regions. The primary safety concern with artesunate is the risk of delayed hemolysis, though the risk can be mitigated with post-treatment laboratory monitoring.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	- Severe malaria is a life-threatening infection and is a medical emergency. - Most cases of severe malaria are due to <i>P. falciparum</i>, but the condition may occur with other species such as <i>P. vivax</i>. - Approximately 2000 cases of malaria are diagnosed each year in the U.S. with about 300 classified as severe. Most cases occur in travelers and immigrants from malaria-endemic	Severe malaria is associated with significant morbidity and mortality. Higher morbidity and mortality are found in pregnant women, children, and

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	regions of the world. Under the CDC IND expanded access protocol a total of 477 patients have been treated with IV artesunate in the US from 2007 to 2019; 31 deaths have been reported (6.5% of total treated).	immunocompromised patients. Malaria can progress to severe or life-threatening disease if diagnosis is delayed or overlooked; timely access to effective therapy is essential.
Current Treatment Options	- Quinidine, the only FDA-approved drug for severe malaria, is no longer marketed for use in the US, and IV quinine, the prior standard of care treatment worldwide, is not approved for use in the U.S. - Oral antimalarials approved by FDA for uncomplicated malaria such as artemether/lumefantrine (Coartem), mefloquine hydrochloride, atovaquone/proguanil (Malarone), and quinine sulfate are sometimes administered off-label to patients with severe malaria until IV artesunate is available; however, oral therapy is often not tolerated due to the severity of clinical illness and may be associated with treatment failures in patients with marked hyperparasitemia. - The CDC and the WHO currently recommend parenteral artesunate as the first-line treatment of severe malaria in adults, children and pregnant women. - IV artesunate is currently available in the US from the CDC under an IND expanded access protocol.	There are currently no FDA-approved drugs for treatment of severe malaria in the U.S.
Benefit	- IV artesunate was evaluated in a single, randomized, open-label, active-controlled trial in Asia in adults and pediatric patients with severe malaria (SEAQUAMAT), with supportive evidence from a published, randomized, open-label, active-controlled trial in Africa in pediatric patients younger than 15 years of age (AQUAMAT). - The primary efficacy endpoint for both SEAQUAMAT and AQUAMAT was in-hospital mortality. - In SEAQUAMAT, in-hospital mortality in artesunate recipients was 13.2% (96 of 730) compared with 20.5% (150 of 731) in quinine recipients; odds ratio 0.59 (95% CI 0.44, 0.79, p=0.0002). AQUAMAT demonstrated a similar advantage with respect to in-hospital mortality.	Substantial evidence of artesunate's effectiveness for the treatment of severe malaria has been demonstrated in adults and children. Significant survival benefit was observed over quinine, the previous standard of care.

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	• These efficacy findings were robust across age, gender and geographic regions. • Limitations of these studies included limited follow up which ended upon death or hospital discharge, and the absence of data on malarial species other than <i>P. falciparum</i>.	
Risk and Risk Management	• The safety database consisted of 832 patients who received IV artesunate at 2.4 mg/kg/dose in SEQUAMAT and CDC-060, an uncontrolled, open-label, retrospective study from U.S. patients receiving IV artesunate under an expanded access IND, with supportive evidence for safety from published studies AQUAMAT and EDCPT-MMV07-01, a controlled trial in pediatric patients comparing two dose regimens, two phase 2 studies of uncomplicated malaria, and two phase 1 studies in healthy volunteers. • Most common adverse reactions with IV artesunate in SEQUAMAT included acute renal failure requiring dialysis, hemoglobinuria and jaundice. The most frequently observed adverse reactions with Artesunate for Injection in CDC-060 were anemia, elevation of transaminases, thrombocytopenia, hyperbilirubinemia, and acute renal failure. • In SEQUAMAT, a higher incidence of neurological impairment at the time of hospital discharge was reported in artesunate recipients compared to the comparator; similar findings were reported in AQUAMAT. As neurological sequelae can occur following severe malaria, particularly patients with cerebral malaria, the higher incidence in artesunate recipients may be due to improved survival in these artesunate-treated patients. • Delayed hemolysis following administration of artemisinins has been reported in published literature. This condition, defined as a 10% decrease in hemoglobin associated with laboratory findings of hemolysis occurring more than one week after treatment initiation, occurs more frequently in patients hyperparasitemia, and may require transfusions. • Hypersensitivity reactions including anaphylaxis have been reported following IV artesunate administration. • Transient neutropenia and a decrease in reticulocyte count were observed following Artesunate for Injection in phase 1 studies of healthy volunteers and phase 2 studies of patients with uncomplicated malaria.	Artesunate demonstrated an acceptable safety profile for the treatment of severe malaria. As the anemia associated with delayed hemolysis may be significant enough to require transfusions, patients should be monitored with weekly blood counts for 4 weeks after initiating IV artesunate therapy. The labeling includes a warning for delayed hemolysis following Artesunate for Injection. The Applicant is required to conduct postmarketing studies to evaluate the potential development of artesunate resistance and to collect data on women exposed to artesunate during pregnancy to assess the risk of maternal and fetal complications. The labeling includes a warning for hypersensitivity and a contraindication for patients with known serious hypersensitivity to artesunate. The labeling includes a warning for embryo-

NDA Multi-disciplinary Review and Evaluation NDA 213036
 Artesunate for Injection

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	In nonclinical studies, embryo-fetal toxicity was observed in animals administered intravenous or oral artesunate. However, the clinical relevance of this finding is unclear since experience with oral artesunate and other artemisinin class drugs (via various routes of administration) in pregnant women over several decades has not identified a drug-associated risk of major birth defects, miscarriage, or adverse maternal or fetal outcomes.	fetal toxicity in animals that also cautions providers that delaying treatment of severe malaria in pregnancy may result in serious morbidity and mortality to the mother and fetus. The Applicant is required to conduct postmarket nonclinical studies to assess artesunate effects on male and female fertility in rats, in an <i>in vivo</i> Micronucleus Assay and in a bacterial reverse mutation test.

1.4 Patient Experience Data

Patient Experience Data Relevant to this Application (check all that apply)

☐	The patient experience data that were submitted as part of the application include:	Section of review where discussed, if applicable
☐	Clinical outcome assessment (COA) data, such as	
☐	Patient reported outcome (PRO)	
☐	Observer reported outcome (ObsRO)	
☐	Clinician reported outcome (ClinRO)	
☐	Performance outcome (PerfO)	
☐	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel, etc.)	
☐	Patient-focused drug development or other stakeholder meeting summary reports	
☐	Observational survey studies designed to capture patient experience data	
☐	Natural history studies	
☐	Patient preference studies (e.g., submitted studies or scientific publications)	
☐	Other: (Please specify):	
☐	Patient experience data that were not submitted in the application, but were considered in this review:	
☐	Input informed from participation in meetings with patient stakeholders	
☐	Patient-focused drug development or other stakeholder meeting summary reports	
☐	Observational survey studies designed to capture patient experience data	
☐	Other: (Please specify):	
☒	Patient experience data was not submitted as part of this application.	

2 Therapeutic Context

2.1 Analysis of Condition

In 2017, malaria was associated with an estimated 219 million cases and 435,000 deaths worldwide. Approximately 92% to 93% of malaria cases and deaths occurred in Africa. *P. falciparum* was associated with 99.7% of malaria cases in Africa, 62.8% in South-East Asia, 69% in the Eastern Mediterranean, and 71.9% in the Western Pacific. ([World Health Organization 2018](#)) In the U.S., most cases of malaria occur in travelers and immigrants returning from malaria-endemic regions in sub-Saharan Africa or South Asia. In 2016, 2078 cases of confirmed malaria were reported in the U.S. *P. falciparum* was found in 1,419 of 1,853 (76.6%) with an identified species. ([Mace et al. 2019](#))

Patients with malaria caused by *P. falciparum* can progress to severe or life-threatening disease if diagnosis is delayed or overlooked. ([Pasvol 2006](#)) In 2016, 305 of 2078 (14.7%) U.S. cases were classified as severe malaria; of these 7 (2.3%) died. ([Mace et al. 2019](#)) Severe malaria is characterized by hyperparasitemia, destruction of parasitized red cells, and sequestration of red cells in small blood vessels causing end-organ dysfunction or failure. The World Health Organization (WHO) defines severe falciparum malaria as having one or more of the following: impaired consciousness, prostration, multiple seizures, shock, pulmonary edema, recurrent or prolonged bleeding, hypoglycemia, acidosis, severe anemia, hyperparasitemia, renal impairment, and/or jaundice. ([World Health Organization 2015](#))

Following the publication of SEAQUAMAT and AQUAMAT, in 2010, the WHO recommended parenteral artesunate as preferred treatment for severe malaria in adults, children, and pregnant women in the second and third trimester, due to its reported superior efficacy, reduced mortality, and better adverse event profile compared to quinine. ([World Health Organization 2010](#)) In 2015, the WHO extended the preference for parenteral artesunate for the treatment of severe malaria in pregnant women during all trimesters. ([World Health Organization 2015](#))

2.2 Analysis of Current Treatment Options

Parenteral antimalarial therapy is recommended in patients with severe malaria.

The only approved parenteral antimalarial therapy for severe malaria in the US was quinidine gluconate, an anti-arrhythmic drug. ([Centers for Disease Control and Prevention 2019](#)) Parenteral quinidine gluconate is cardiotoxic and may cause ventricular arrhythmia, hypotension, hypoglycemia, and prolongation of the QTc interval; administration in an intensive care setting is recommended. In 2017, the manufacturer discontinued production of intravenous quinidine gluconate and distribution in the U.S. was discontinued in March 2019. ([Centers for Disease Control and Prevention 2017](#)).

Both WHO and CDC recommend parenteral artesunate as the first line therapy for severe malaria. Intravenous artesunate has been available in the U.S. since 2007 through the CDC IND expanded access protocol (IND 076725). ([Paczkowski et al. 2014](#)) Under the CDC IND protocol, a total of 477 patients have been treated with IV artesunate from 2007 to 2018, averaging 48 patients per year with 31 deaths reported (6.5% of total treated). During the last full reporting period from June 2018 to June 2019, a total of 63 patients were enrolled with two deaths documented; all patients received the U.S. Army formulation of IV artesunate.¹ IV artesunate (U.S. Army formulation) is also available to U.S. military personnel under IND 064769.²

IV quinine is not commercially available in the US. FDA-approved oral antimalarials such as artemether/lumefantrine (Coartem), mefloquine hydrochloride, atovaquone/proguanil (Malarone), and quinine sulfate are indicated for treatment of uncomplicated malaria. While these antimalarials may be used off-label for severe malaria or until IV artesunate is available, oral therapy is often not tolerated due to the severity of clinical illness and may be associated with treatment failures in patients with marked hyperparasitemia.

In its 2015 guidelines for treatment of malaria, the WHO revised its pediatric dose recommendation for parenteral artesunate from 2.4 mg/kg to 3 mg/kg ([World Health Organization 2015](#)); CDC's current dosing recommendation in children mirrors the WHO recommendations. ([Centers for Disease Control and Prevention 2019](#)) The WHO based its revision on a review of PK data on artesunate and DHA in published studies of 71 adults and 265 children and simulations of exposures in various age groups which predicted underexposure of younger children. ([Hendriksen et al. 2013](#)) ([Zaloumis et al. 2014](#)) In its review, the WHO noted that parenteral artesunate doses between 1.75 and 4 mg/kg have been evaluated without any toxicity observed. ([World Health Organization 2015](#)) (See Section 12, Pediatrics and 18.4 Pharmacometrics review of the proposed artesunate dosing in pediatric patients, for additional discussion of pediatric dosing.)

Table 2-1: CDC and WHO Guidelines for the Treatment of Severe Malaria (All Regions)

	CDC ¹	WHO ²
Initial treatment with IV artesunate		
Adults and pediatric patients ≥20 kg	IV artesunate 2.4 mg/kg/dose at 0, 12, 24 hours for a total of 3 doses	IV or IM artesunate 2.4 mg/kg/dose for at least 24 hours until patient can tolerate oral medication; If parenteral artesunate is not available, use artemether in preference to quinine.

¹Right of Reference IND 76725 Module 1, Section 1.4.2. IND 76725 Submission Number 48—Annual Report dated August 19, 2019.

² Right of Reference IND 64767 Module 1, Section 1.4.2.

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

	CDC ¹	WHO ²
Pediatric patients <20 kg	IV artesunate 3.0 mg/kg/dose at 0, 12, and 24 hours for a total of three doses	IV artesunate 3.0 mg/kg/dose for at least 24 hours. Where IM artesunate not available, treat children < 6 years with single rectal dose artesunate (10 mg/kg body weight)
Oral follow-on treatments		
Adults and pediatric patients ≥20 kg	If after 24 hours of artesunate parasitemia < 1% and patient able to tolerate oral medications, administer one of the following starting at least four hours after the last dose of artesunate: • Artemether-lumefantrine (Coartem)³ (preferred) • Atovaquone-proguanil (Malarone) • Quinine plus doxycycline • Quinine plus clindamycin If parasitemia > 1% after 24 hours of artesunate, continue until parasitemia ≤1% for a maximum of 7 days⁴ Proceed with full dose oral follow-on treatment as soon as parasitemia ≤1% and patient able to tolerate oral. If parasitemia ≤ 1% but patient not able to take oral, continue IV artesunate at same daily dose of a maximum of 7 days, or switch to IV doxycycline up to 7 days, or clindamycin up to 7 days. Proceed with full dose oral as soon as patient tolerating oral meds. If needed, give interim treatment until IV artesunate arrives. If oral meds not tolerated, consider administration via nasogastric tube or after an anti-emetic: artemether-lumefantrine, atovaquone-proguanil, quinine sulfate, or mefloquine.	Once patient received at least 24 hours of parenteral artesunate and can tolerate oral therapy, complete treatment with 3 days of an artemisinin-based combination therapy (ACT): artesunate plus amodiaquine, artemether plus lumefantrine, or dihydroartemisinin plus piperaquine. If ACT not available, administer artesunate plus clindamycin, artesunate plus doxycycline, quinine plus clindamycin, or quinine plus doxycycline
Pediatric patients <20 kg	Follow on medication recommendations for children < 20 kg are same as children ≥20 kg but at pediatric doses.	

¹ Centers for Disease Control Malaria Treatment Table – Guidelines for Treatment of Malaria in the United States, Based on drugs available for use in the U.S. October 2019. Available at https://www.cdc.gov/malaria/resources/pdf/Malaria_Treatment_Table_120419.pdf

² World Health Organization -- Treatment of Severe Malaria, in Guidelines for the Treatment of Malaria. 3rd edition, 2015. Available at [link](#)

³ Artemether-lumefantrine (Coartem) is the only FDA-approved ACT.

⁴ Per CDC Malaria Treatment Table, doses given at 0, 12, and 24 hours count as one day.

3 Regulatory Background

3.1 U.S. Regulatory Actions and Marketing History

Artesunate for injection (IV artesunate) is being developed for the initial treatment of severe malaria in adult and pediatric patients. IV artesunate is a new molecular entity and is not currently marketed in the United States. The Department of the Army sponsored the IND and Amivas is the applicant of the NDA.

3.2 Summary of Presubmission/Submission Regulatory Activity

The Office of the Surgeon General, Department of the Army (US Army), submitted an IND for the development of IV artesunate for the treatment of severe (b) (4) malaria. While under development multiple submissions were received and meetings held with the Agency and the IND received fast track, breakthrough and orphan designations.

In 2017, the US Army entered into a Cooperative Research and Development Agreement with Amivas, LLC (Amivas). Amivas was designated as the co-development partner and commercial partner responsible for the submission of the New Drug Application for Artesunate for Injection for the immediate treatment of malaria. The US Army also provided Amivas with a letter of authorization to allow the US Food and Drug Administration review of all pertinent information in their IND in support of the NDA.

During the Pre-NDA discussions in 2019, both the US Army and Amivas participated in multiple meetings with the Agency, and a request for NDA rolling review was submitted and granted. On August 12, 2019, Amivas, LLC, began their rolling review submission of NDA 213036 for Artesunate for Injection for the initial treatment of severe (b) (4) malaria in adults and children; NDA submission was completed on September 26, 2019. The submission included a request for Priority Review and for issuance of a Tropical Disease Priority Review Voucher at the time of approval.

4 Significant Issues from Other Review Disciplines Pertinent to Clinical Conclusions on Efficacy and Safety

4.1 Office of Scientific Investigations (OSI)

Pre-approval inspections of clinical sites from OSI were not requested because source data were unavailable for audit. The Applicant did not sponsor or conduct pivotal trial SEAQUAMAT and supportive study AQUAMAT. While the Applicant obtained patient level datasets for SEAQUAMAT, the sponsor Wellcome Trust confirmed in multiple queries from the Applicant that the only source documentation used was the standardized case report forms and no additional patient level data were available.

4.2 Product Quality

The NDA, as amended, has provided adequate CMC information to assure the identity, strength, purity, and quality of the proposed drug product. The CMC Integrated Quality Assessment submitted to DARRTS on 2/26/2020 recommended the NDA for a CR due to outstanding microbiology, drug product and facilities issues. The microbiology review had identified deficiencies with the Container Closure Integrity Test (CCIT) and the drug product review identified a deficiency with the analytical method used in the leachables study. Following review of the IR responses, both the microbiology and drug product reviews recommended this NDA for approval; however, an overall “Withhold” recommendation for the facilities was entered into Panorama on 04/17/2020. For further details, refer to the CMC Integrated Quality Assessment submitted to DARRTS on 4/27/2020. Following the receipt of additional CMC information on 4/27/2020 and 5/13/2020, this NDA was recommended for approval. An overall “Approve” recommendation for the facilities was entered into Panorama on 05/19/2020. For further details, refer to the CMC Integrated Quality Assessment submitted to DARRTS on 5/21/2020.

This NDA is recommended for approval from a CMC perspective.

4.3 Devices and Companion Diagnostic Issues

Not applicable

5 Nonclinical Pharmacology/Toxicology

5.1 Executive Summary

Artesunate is a semi-synthetic derivative of artemisinin, (b) (4). The nonclinical safety profile consists of pharmacology, pharmacokinetics and toxicology studies *in vitro* and *in vivo* in rats, dogs, rabbits and monkeys. Pivotal studies with artesunate discussed in this review include repeated-dose IV studies (up to 28 days), embryo-fetal development studies conducted via oral administration, and genetic toxicology studies.

Although the subject of this NDA is IV artesunate, some of the submitted supporting literature and nonclinical studies were conducted using artemisinin and other artemisinin derivatives (i.e., arteether and artemether) to illustrate how artesunate compares across areas of known artemisinin class effects. Oral artemether, the only FDA approved artemisinin, is part of a fixed oral combination therapy with lumefantrine (Coartem[®], approved 2009; Novartis Pharmaceuticals Corp) for treatment of uncomplicated malaria. Studies early in the drug development phase with artesunate were conducted primarily using a non-GMP formulation of IV artesunate produced in China by Guilin Pharmaceutical Co., Ltd. More recent nonclinical studies were conducted using a GMP Walter Reed Army Institute of Research (WRAIR) formulation of artesunate (b) (4). The pivotal repeated-dose toxicity studies in rat and dog were conducted using the to-be-marketed formulation of semi-synthetic artesunate in 0.3 M phosphate buffer. No nonclinical bioequivalence studies have been conducted between the semi-synthetic formulation and the (b) (4) WRAIR or Guilin formulations. In general, animal studies conducted using the (b) (4) WRAIR and semi-synthetic IV artesunate formulations show a consistent pattern of toxicity across species (described below).

Pharmacokinetics

Artesunate (AS) is rapidly hydrolyzed to its metabolite dihydroartemisin (DHA) following intravenous administration, with a half-life of less than 0.5 hr for AS and DHA across all species. A mass balance study in rats indicate artesunate has a biphasic pattern of distribution, with an initial peak concentration of radioactivity present in plasma and whole blood 5 min after dosing, with a secondary drug plasma peak concentration occurring 3 to 4 hr after dosing, suggesting enterohepatic circulation or the presence of a second absorption phase. In rats, artesunate is present in whole blood at concentrations 2 to 4-fold that of plasma, with plasma protein binding capacities of 66 to 81% (comparable to human plasma protein binding of 63 to 75%). The rate and extent of artesunate metabolism to DHA varies by species, with extent of DHA formation in liver microsomes following artesunate incubation greatest in humans (85%), followed by rat (76%), cynomolgus monkey (59%), then rhesus monkey and beagle dog (both at 44%). Glucuronidation is a major metabolic pathway for both DHA, mainly via UGT1A9 and

UGT2B7. After IV administration, AS was mainly excreted via the urine (56%), with approximately 38% of the administered dose excreted via the feces. No nonclinical studies evaluating the excretion of artesunate in milk have been conducted.

Safety Pharmacology

No studies have been conducted with AS on the inhibition of the tail current in human embryonic kidney 293 cells (transfected with hERG cDNA). The IC₅₀ for DHA on hERG current was 7.7 ± 0.9 mcM. No adverse effects on cardiovascular function were observed in conscious beagle dogs following single or repeated (up to 27 days) IV dosing up to 40 mg/kg, approximately nine times the clinical dose based on body surface area comparison.

Neurobehavioral assessments in rats conducted after 26 days of repeated IV dosing of AS (3, 10, or 30 mg/kg) showed no biologically significant effects on parameters evaluated in a standard functional observational battery (e.g., gait and posture, motor movements, piloerection, etc.). Neurotoxicity is a class effect of artemisinins, with artemisinin and artemisinin-derived compounds (i.e., artemether and arteether) displaying a pattern of damage to specific brainstem nuclei in experimental animals when administered in intoxication or lethal doses. The extent of neurotoxicity seen following administration of artemisinins appears to be dependent on the specific compound, dose, vehicle, and route of administration. Intramuscular administration of arteether causes widespread neuronal necrosis and gliosis in monkey and degenerative lesions in the trapezoid nucleus in rat. Brain histopathologic evaluations were unremarkable in monkeys and rats following repeated IV artesunate exposure (7 days) at doses that were 4.3 and 10-fold clinically relevant doses based on body surface area comparisons. Additional comparative studies reported in the literature report no behavioral or histopathological changes in brainstem nuclei of rats administered artesunate IM daily for seven days whereas rats receiving an equivalent molar concentration of arteether intramuscularly displayed significant behavioral deficits with histopathological evidence of chromatolysis, pyknosis and gliosis in brainstem nuclei.

Repeat dose Toxicology Studies

The hematopoietic system was the primary target organ of toxicity in rats and dogs. A dose-dependent anemia characterized by reductions in hematocrit and red blood cells, increased mean corpuscular volume, reticulocyte count and percentage, and a moderate to marked leukocytosis was observed after 28 days of repeated IV dosing in rats at doses 0.7 to 2-fold the clinical dose based on body surface area comparisons. Hematologic changes were accompanied by erythroid hyperplasia in the spleen, bone marrow and kidneys. Reductions in body weight were also observed, with deaths occurring in 1 animal each from the mid and high dose groups. Histopathologic and hematologic changes were comparable to controls at the end of recovery, indicating reversibility of effects. The NOAEL was 3 mg/kg/day, a dose equivalent to 0.2 that of the clinical dose based on body surface area comparisons. Differences in IV artesunate formulation (i.e., (b) (4) versus semi-synthetic) are not likely to impact toxicity given similar findings were observed in dogs administered the WRAIR formulation and the

semi-synthetic formulation. Repeated intravenous administration of 20 to 50 mg/kg IV artesunate to dogs for 14 days resulted in a reduction of red blood cells, hemoglobin, hematocrit and absolute reticulocyte count with no changes in histopathology. The NOAEL for this study was 10 mg/kg (approximately 2-fold the clinical dose based on body surface area comparison). Artesunate hematologic toxicities were dose dependent in the 28-day study, with anemia occurring at lower doses (5 mg/kg) compared with the 14-day study. In addition to reductions in red blood cells and hemoglobin, animals also had increased leukocytes and fibrinogen, indicating an inflammatory response. Histopathologic changes included extramedullary hematopoiesis in liver and spleen and erythropoietic hyperplasia in the bone marrow of the sternum and femur. Reversibility of hematologic and histopathologic changes was evident following a 28-day recovery period. No NOAEL was determined given hematologic and histopathologic changes were observed in all dose groups in the 28-day dog study. The lack of a NOAEL in the 28-day study is not of appreciable concern since this product is indicated for short duration of use (3 to 7 days) and hematologic changes observed were characteristic of artemisinin antimalarial drugs and findings were reversible.

Genetic Toxicology and Carcinogenicity

In studies conducted by the Applicant, artesunate was negative in the Ames test, mammalian chromosome aberration assay (CHO), and in vivo micronucleus assay (up to 500 mg/kg, highest concentration tested). Conflicting findings reported in the published literature showed artesunate induced DNA damage in human lymphocytes and Hep2G liver cells in a Comet assay and increased micronuclei formation in human lymphocytes. The published literature also indicates that in vivo, artesunate is positive for micronucleus formation but negative for DNA damage in peripheral blood cells in mice following oral administration. No carcinogenicity studies have been conducted with artesunate. Given the short duration of treatment (up to 7 days) carcinogenicity studies are not indicated.

Reproductive Toxicology

No fertility studies with IV artesunate have been conducted. No significant changes in testicular histology, sperm motility, counts or morphology were observed in rats and dogs following 28 days of repeated dosing with IV artesunate. Histopathological changes of the seminiferous tubules and altered spermatogenesis (increased percentage of abnormal sperm and/or decreased sperm motility and viability) in rats and mice have been reported in the literature following repeated oral or intraperitoneal artesunate administration (3 days to 6 weeks) at less than clinically relevant doses. Given the conflicting findings in animals and lack of any formal fertility study(ies), the risk that IV artesunate administration poses to human fertility is uncertain.

Pivotal GLP embryofetal developmental toxicity studies have not been conducted with IV artesunate. A single intravenous administration of artesunate to pregnant rats on day 11 of pregnancy displayed a steep dose response curve for embryotoxicity, with a NOAEL of 0.75 mg/kg and the next highest dose tested of 1.5 mg/kg (0.1 times the recommended human dose based on body surface area comparison) inducing complete embryoletality in the absence of

maternal toxicity. A mass balance study conducted in pregnant rats demonstrated accumulation of artesunate in feto-placental tissues following a single administration of [¹⁴C]-artesunate on day 11 of pregnancy at a dose 0.3 times the recommended human dose based on body surface area comparison. When given intramuscularly during organogenesis in rat, artesunate induced high levels of postimplantation loss, with complete resorption of litters seen at 0.75 mg/kg, 0.05-fold the clinical dose based on body surface area comparisons (fetal LD₅₀ = 0.61 mg/kg/day; 95% CI: 0.59-0.63). Given the extent of embryoletality observed following a single administration, embryo-fetal development studies conducted with IV AS cannot feasibly be conducted at doses that would be clinically relevant. Embryo-fetal development studies have been conducted using oral artesunate, which has a bioavailability of less than 1% in the pregnant rat. Oral administration of artesunate during organogenesis caused a significant increase in post-implantation loss in pregnant rats, rabbits and cynomolgus monkeys at significantly less than clinically relevant doses based on body surface area comparisons. Surviving rat and rabbit fetuses displayed a spectrum of cardiovascular (ventricular septal defects, retro-esophageal right subclavian artery arising from the descending aorta) and skeletal malformations (e.g., ribs, long bone, and scapulae). Rabbit fetuses also displayed an increased incidence of brain abnormalities, including severely dilated brain ventricles and pons with absent areas. Embryoletality and/or fetal malformations following oral artesunate administration occurred in the absence of maternal toxicity. In rats and monkeys, the critical window for oral artesunate exposure associated embryoletality and developmental toxicity occurs early during organogenesis, at time periods roughly equivalent to the human first trimester.

Pre- and postnatal developmental toxicity studies have not been conducted with IV artesunate. The extent of embryoletality and fetal malformations observed in the oral embryo-fetal development studies indicate that pre- and postnatal development study(ies) conducted with artesunate, by any route of administration route, would not yield any clinically meaningful information.

Excipients/Impurities

Artesunate for Injection contains no excipients and there are no significant differences between the (b) (4) and semi-synthetic batches (see Quality Assessment review). The drug product is reconstituted with 0.3 M sodium phosphate buffer (b) (4)

The same sodium phosphate buffer in the to be marketed formulation has been used in the PK, safety and efficacy studies conducted under IND 64769. The use of the 0.3 M phosphate buffer is considered to be qualified from a Pharmacology/Toxicology perspective based on prior extensive clinical use. The drug product specification includes an acceptance criteria of NMT (b) (4) % for artemisinin and (b) (4) % for (b) (4). The proposed acceptance criteria for (b) (4) % artemisinin and (b) (4) % (b) (4) are adequately qualified in the 28-day toxicity study in dog.

Leachables:

Per communication with the CMC review team, there are no leachables associated with IV AS present that warrant Pharmacology/Toxicology involvement; all leachables were below the safety concern threshold of (b)(4) mcg/day. (For details of product quality assessment refer to Office of Product Quality review by Dr. George Lunn in Panorama).

Recommendation

The submitted nonclinical safety information on IV artesunate lacks adequate characterization of the in vivo genotoxic potential and the potential risks on female fertility. However, given the severity of the disease and the unmet need for drugs intended to treat severe malaria the current nonclinical package supports approval for marketing in the U.S. The Applicant will be conducting the following nonclinical studies as postmarketing requirements: a GLP-in vivo micronucleus test and a female fertility study with IV artesunate.

5.2 Referenced INDs, NDAs, BLAs, DMFs

IND 64769

5.3 Pharmacology

Primary Pharmacology

No formal studies investigating the mechanism of action of artesunate have been performed. The endoperoxide bridge in artesunate is thought to confer its antimalarial properties through a two-step process: activation involving an iron-mediated cleavage of the endoperoxide bridge to generate an unstable organic free radical followed by alkylation of malarial proteins. Details of the primary pharmacology studies conducted with Artesunate, including efficacy studies can be found in Nonclinical Microbiology Section 9.1.

Secondary Pharmacology

No secondary pharmacology studies with artesunate have been performed.

Safety Pharmacology

Cardiovascular and Respiratory Safety

Artesunate has not been evaluated in GLP studies for effects on human ether-a-go-go-related gene (hERG) tail currents. The Applicant was not requested to conduct a hERG study using artesunate as no significant QTc prolongation of artesunate was detected in the QT assessment (see Section 10.4.9).

The Applicant provided the following information on cardiovascular effects with the artesunate active metabolite, DHA:

In Vitro Cardiovascular Effects of Dihydroartemisinin-Piperaquine Combination Compared with Other Antimalarials. *Antimicrobial Agents and Chemotherapy* 2012. 56(6): 3261-3271. DHA was assessed for effects on hERG blockage and trafficking in HEK-293 cells stably transfected with the hERG clone and effects on electrophysiological properties of the isolated rabbit ventricular wedge preparation (including I_{Na} and I_K ion current interference). The IC_{50} for DHA on hERG current was 7.7 ± 0.9 mcM. DHA also produced a significant inhibition of hERG trafficking, but only at a concentration that caused >75% cell death (i.e., 100 mcM). DHA was without effect on QT, T_{p-e} (interval between the end and peak of the T wave), endocardial action potential duration measured at 90% repolarization (ENDO APD₉₀) and QRS in rabbit heart ventricular wedge preparations at concentrations up to 30 mcM.

Reviewer comment: Other antimalarials (e.g., chloroquine, lumefantrine, DHA in combination with piperaquine) were tested but only results for assays in which DHA was tested alone are described. The authors of the paper indicate the IC_{50} for DHA on hERG current was approximately 30 times the reported mean therapeutic C_{max} of free DHA (0.265 mcM) in patients receiving Eurartesim, an oral piperaquine phosphate and DHA fixed-combination antimalarial treatment. The estimated C_{max} of DHA following 3 days of IV artesunate is approximately 9.8 mcM (2,789.6 ng/mL), levels that are comparable to the IC_{50} value reported for DHA on hERG current in this study but without effect on QT in rabbit heart ventricular wedge preparations. Although the GLP status is unknown, there is sufficient detail regarding methodology, mean $\pm$ SEM values, and statistical analysis to have confidence in the reported findings.

Cardiovascular Safety Pharmacology Study of Artesunate in Beagle Dogs/Study No. 1466-103

Telemetered beagle dogs aged 6-7 months (2 each per sex) were administered sterile 0.3M phosphate buffer (vehicle control) via intravenous injection on study day 1. Beginning study day 2, animals received a dose of 10, 20, and 50 mg/kg via a single slow intravenous dose (i.e., 3 min bolus) and 50 mg/kg administered via a single fast intravenous dose (i.e., 1 min bolus) with at least a one-week wash out period between each escalating dose level. Artesunate (single slow IV dose of 0, 10, 20 and 50 mg/kg or single fast IV dose of 50 mg/kg) did not have any effects on mortality, clinical observations, body weights, ECGs, QT intervals, heart rate, blood pressure, or respiratory rate.

A 28-day Repeated Intravenous Dose Toxicity and Toxicokinetic Study of Artesunate in Beagle Dogs Followed by a 28-day Recovery Period/Study No. 352638

Artesunate was administered intravenously to dogs (3/sex/group at 0(vehicle), 5, 15, or 40 mg/kg/day for 28 days). Electrocardiograms (6 limb leads) and indirect blood pressure measurements were obtained for all animals (1 hr $\pm$ 30 min after end of dosing) on Day 1 and Day 27 and on recovery animals on Day 56. Systolic pressure, diastolic pressure, heart rate, P-duration, PR-interval, QRS duration, and QT-interval were reported. All ECG parameters remained within normal physiologic limits during the measured study periods and there were no significant pathologic changes in the development of arrhythmias for artesunate at the dosing intervals tested.

Central Nervous System

A 28-day repeated intravenous dose toxicity and toxicokinetic study of artesunate in Sprague Dawley rats followed by a 28-day recovery period/Study No. 351852

Neurological examinations performed in rats after approximately 26 days of repeated IV dosing found no effects of artesunate on behavior (gait and posture, clonic/toxin involuntary movement, vocalization, motor activity, etc) or functional observational battery parameters.

Dose Range Finding Study of Injectable Artelinate and Artesunate in Rats/Study No. SR01-1

Artesunate was administered intravenously to Sprague Dawley rats daily for 7 days with 0, 9.4, 18.8, 37.5, 75, or 150 mg/kg artesunate or intramuscular injection of 25 mg/kg arteether daily for seven days followed by histopathological examination of hind brain tissues. No macroscopic or microscopic lesions observed in hindbrain from artesunate-treated animals were observed at any dose level, whereas degenerative lesions in trapezoid nucleus were noted in animals treated with arteether (see Nonclinical Appendix for full study review).

Toxicity of Subacute, 7-day Dosing of Candidate Intravenous Artemisinins in Rhesus Monkeys/Study Nos. AQ0008_02_AF and PN02-01.

Animals administered IV Artesunate (Guilin formulation) at doses of 4, 8, 16, 32, and 128 mg/kg/day for 7 days displayed depressed motor activity (≥ 32 mg/kg) lasting up to 6h post-dosing. Three out of 4 animals treated with a higher artesunate dose (128 mg/kg/day) died prematurely or were humanely euthanized, displaying persistent depressed motor activity, slight truncal ataxia, and altered urinalysis parameters prior to death. The surviving animal showed neuronal chromatolysis in the cranial nerve nucleus. Necropsies in the control, 4, 8, 16 and 32 mg/kg group were unremarkable for brain histological findings. In a separate group, 2 animals treated with 16 mg/kg IM arteether for 14 days (positive control) displayed motor depression and/or piloerection with foot and arm tremor and exhibited histological findings consistent with widespread neuronal necrosis and gliosis in brain stem nuclei.

5.4 ADME/PK

Type of Study	Major Findings				
Absorption					
Tissue distribution, mass balance, protein binding, metabolic profile, and whole-body autoradiography of [¹⁴ C]-artesunate followingsingle intravenous injection in rats. Study No. AQ0007_04_WR	Table 5-1. Absorption of ¹⁴C-AS and unchanged AS in plasma and blood following a single IV dose in rats				
		14C-Artesunate (5 mg/20 mcgCi/kg)		Unchanged Artesunate in Plasma	Unchanged DHA in Plasma
		Plasma	Blood		
	C _{max} (ng/mL)	4271 ± 1482	4527 ± 1016	1628 ± 415	468 ± 92
	t _{max} (h)	0	0	0	0.27 ± 0.04
	AUC _∞ (ng·h/mL)	21702 ± 2949	66213 ± 5918	392.2 ± 63.3	376.1 ± 53.8
Ratio of DHA/AS			0.98 ± 0.22		

14-Day Toxicity Study of Artesunate in Beagle Dogs. Study No. 1466-102	C_{max} at 1 st peak (ng/mL)	2631 ± 692	2999 ± 789	1026 ± 183	468 ± 92																																																																																												
	t_{max} at 1 st peak (h)	0.083	0.083	0.083	0.27 ± 0.04																																																																																												
	C_{max} at 2 nd peak (ng/mL)	744.4 ± 200.2	1316 ± 178	-	-																																																																																												
	t_{max} at 2 nd peak (h)	3.25 ± 1.47	3.75 ± 1.84	-	-																																																																																												
	<i>Blood was sampled at 5, 10, 30 min and 1, 1.5, 3, 6, 12, 48, 72, and 96 hr after dosing.</i>																																																																																																
Table 5-2. Absorption of AS and DHA on day 1 after a single IV dose of AS in dogs																																																																																																	
<table border="1"> <thead> <tr> <th colspan="5">Artesunate</th> </tr> <tr> <th>Dose</th> <th>Sex</th> <th>C_{max} [ng/mL]</th> <th>t_{max} (min)</th> <th>AUC_{∞} (ng·h/mL)</th> </tr> </thead> <tbody> <tr> <td rowspan="2">10</td> <td>M</td> <td>9795</td> <td>0</td> <td>1150</td> </tr> <tr> <td>F</td> <td>4902</td> <td>0</td> <td>471</td> </tr> <tr> <td rowspan="2">20</td> <td>M</td> <td>24696</td> <td>0</td> <td>2254</td> </tr> <tr> <td>F</td> <td>15844</td> <td>0</td> <td>2282</td> </tr> <tr> <td rowspan="2">35</td> <td>M</td> <td>67098</td> <td>0</td> <td>7351</td> </tr> <tr> <td>F</td> <td>52789</td> <td>0</td> <td>5903</td> </tr> <tr> <td rowspan="2">50</td> <td>M</td> <td>112065</td> <td>0</td> <td>12733</td> </tr> <tr> <td>F</td> <td>130249</td> <td>0</td> <td>12755</td> </tr> <tr> <th colspan="5">DHA</th> </tr> <tr> <th>Dose</th> <th>Sex</th> <th>C_{max} [ng/mL]</th> <th>t_{max} (min)</th> <th>AUC_{∞} (ng·h/mL)</th> </tr> <tr> <td rowspan="2">10</td> <td>M</td> <td>3690</td> <td>0.03</td> <td>1181</td> </tr> <tr> <td>F</td> <td>2139</td> <td>0.04</td> <td>739</td> </tr> <tr> <td rowspan="2">20</td> <td>M</td> <td>3611</td> <td>0.03</td> <td>2126</td> </tr> <tr> <td>F</td> <td>3910</td> <td>0.05</td> <td>2129</td> </tr> <tr> <td rowspan="2">35</td> <td>M</td> <td>5172</td> <td>0.14</td> <td>3010</td> </tr> <tr> <td>F</td> <td>4604</td> <td>0.09</td> <td>2553</td> </tr> <tr> <td rowspan="2">50</td> <td>M</td> <td>7179</td> <td>0.11</td> <td>4518</td> </tr> <tr> <td>F</td> <td>7971</td> <td>0.07</td> <td>4137</td> </tr> </tbody> </table>						Artesunate					Dose	Sex	C_{max} [ng/mL]	t_{max} (min)	AUC_{∞} (ng·h/mL)	10	M	9795	0	1150	F	4902	0	471	20	M	24696	0	2254	F	15844	0	2282	35	M	67098	0	7351	F	52789	0	5903	50	M	112065	0	12733	F	130249	0	12755	DHA					Dose	Sex	C_{max} [ng/mL]	t_{max} (min)	AUC_{∞} (ng·h/mL)	10	M	3690	0.03	1181	F	2139	0.04	739	20	M	3611	0.03	2126	F	3910	0.05	2129	35	M	5172	0.14	3010	F	4604	0.09	2553	50	M	7179	0.11	4518	F	7971	0.07	4137
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Tissue distribution, mass balance, elimination, pharmacokinetics, protein binding, metabolic profile, and whole-body autoradiography of [¹⁴ C]-artesunate following single intravenous injection in rats.	Table 5-3. Mean tissue concentration of radioactivity in rats following single IV dose (5 mg/20 mcgCi/kg) in rats																																																																																																
	Tissue type	Radioactivity (ng equivalent/g tissue)																																																																																															
		1 hr	6 hr	24 hr	192 hr	$t_{1/2}$ (hr)																																																																																											
	Blood	687.93	990.04	868.38	51.17	98.8																																																																																											
	Plasma	499.06	740.54	317.41	22.97	63.7																																																																																											
	Brain	691.86	591.37	735.46	123.52	94.2																																																																																											
Eyes	1373.05	560.03	461.82	60	62.1																																																																																												
Adrenals	2688.05	3122.01	1874.82	754.01	109.6																																																																																												

Study No. AQ0007_04_WR	Fat	473.38	501.38	96.45	57.48	115.5																		
	Muscle	404.29	815.55	242.76	100.12	105.8																		
	Lungs	612.84	1568.24	2579.44	191.87	123.8																		
	Heart	811.25	1967.07	4313.07	338.66	108.6																		
	Spleen	3707.27	6448.14	5282.39	3524.37	154.3																		
	Kidneys	2401.76	7234.39	4975.95	1372.32	94.2																		
	Liver	3638.56	5417.10	2088.16	544.39	78.9																		
	Stomach	756.99	1753.69	782.51	209.3	93.5																		
	Stomach content	327.62	2171.11	213.63	56.83	77.9																		
	Large Intestine	1429.32	6006.64	2548.93	193.98	69.5																		
	Large Intestinal Content	1186.58	9421.07	7601.61	255.77	65.6																		
	Small Intestine	11463.61	7453.89	1484.20	202.4	74.9																		
	Small Intestinal Content	95906.52	79448.03	24192.94	57.47	29.4																		
	Bone Marrow	1441.56		617.65		40.0																		
Table 5-4. In vitro and in vivo distribution of [¹⁴C] AS in red blood cells <table border="1"> <thead> <tr> <th></th> <th>Ratio blood:plasma</th> </tr> </thead> <tbody> <tr> <td>Rat</td> <td></td> </tr> <tr> <td> In vivo</td> <td>2.1-4.3</td> </tr> <tr> <td> In vitro</td> <td>1.07-1.73</td> </tr> <tr> <td>Human</td> <td></td> </tr> <tr> <td> In vitro</td> <td>0.79-0.94</td> </tr> </tbody> </table> Table 5-5. In vitro plasma protein binding of [¹⁴C] AS in rat and human <table border="1"> <thead> <tr> <th>Species</th> <th>% bound</th> </tr> </thead> <tbody> <tr> <td>Rat</td> <td>66-81</td> </tr> <tr> <td>Human</td> <td>63-75</td> </tr> </tbody> </table>								Ratio blood:plasma	Rat		In vivo	2.1-4.3	In vitro	1.07-1.73	Human		In vitro	0.79-0.94	Species	% bound	Rat	66-81	Human	63-75
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Tissue distribution, of [¹⁴ C]-AS in pregnant rats Study No. (b) (4) 132N-0501	Table 5-6. Mean PK parameters of ¹⁴C-AS following single IV injection (5 mg/147 mcgCi/kg) in the tissue or organs of the pregnant rats by QWBA <table border="1"> <thead> <tr> <th>Tissue type</th> <th>Tissue</th> <th>C_{max} (mcg/g)</th> <th>t_{1/2} (h)</th> <th>AUC_{0-192h} mcg·h/g</th> <th>% total AUC_{0-192h}</th> </tr> </thead> <tbody> <tr> <td>Adipose</td> <td>Adipose (brown & white)</td> <td>6.49</td> <td>118.72</td> <td>806.67</td> <td>3.53</td> </tr> <tr> <td colspan="5" style="text-align: right;">% Total</td> <td>3.53</td> </tr> </tbody> </table>						Tissue type	Tissue	C _{max} (mcg/g)	t _{1/2} (h)	AUC _{0-192h} mcg·h/g	% total AUC _{0-192h}	Adipose	Adipose (brown & white)	6.49	118.72	806.67	3.53	% Total					3.53
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NDA Multi-disciplinary Review and Evaluation NDA 213036
 Artesunate for Injection

	Alimentary Canal	Large Intestine	1.82	99.08	173.41	0.76
		Large Intestine (contents)	7.34	49.00	617.83	2.70
		Stomach & contents	3.44	141.53	361.46	1.58
		Small Intestine	9.47	68.61	204.78	0.90
		Small Intestine (contents)	120.35	27.55	3172.53	13.87
	% Total 19.81					
	CNS	Brain	2.11	155.21	88.84	0.39
		Spinal Cord	2.72	168.31	127.65	0.56
	% Total 0.95					
	Dermal	Skin	1.71	100.77	111.97	0.49
	% Total 0.49					
	Endocrine	Adrenal Gland	7.59	207.79	1145.58	5.01
		Pituitary Gland	2.93	172.50	213.82	0.93
		Thyroid Gland	2.58	139.81	323.57	1.41
	% Total 7.35					
	Excretory/ Metabolic	Bile (in duct)	16.84	153.87	1387.64	6.07
		Renal Cortex	6.71	179.10	1490.90	6.52
		Renal Medulla	6.19	117.97	326.73	1.43
		Liver	9.06	160.42	903.65	3.95
		Urinary Bladder	23.66	74.39	674.17	2.95
		Urinary Bladder (contents)	44.27	41.79	592.24	2.59
	% Total 23.51					
	Ocular	Eye Uveal Tract	1.08	71.83	45.09	0.20
Eye Lens		0.29	70.75	17.22	0.08	
% Total 0.28						
Reproductive	Amnion & Amniotic Fluid	1.56	76.91	64.32	0.28	
	Fetal Body	2.26	83.63	80.91	0.35	
	Mammary	2.99	120.76	100.03	0.44	
	Ovary	5.84	159.90	555.13	2.43	
	Placenta	3.21	200.55	366.77	1.60	

NDA Multi-disciplinary Review and Evaluation NDA 213036
 Artesunate for Injection

	Uterus	2.19	152.60	215.58	0.94
	Vagina	1.46	88.96	96.98	0.42
% Total					6.46
Respiratory Tract	Lung	2.13	93.92	135.31	0.59
	Nasal Turbinates	4.27	155.33	311.18	1.36
% Total					1.95
Secretory	Harderian Gland	4.34	78.01	157.18	0.69
	Pancreas	2.96	142.56	235.57	1.03
	Salivary Gland	2.34	206.18	313.67	1.37
% Total					3.09
Skeletal/Muscular	Bone	0.32	171.66	25.79	0.11
	Heart	2.96	166.21	405.69	1.77
	Skeletal Muscle	1.91	158.88	135.96	0.59
% Total					2.47
Vascular/Lymphatic	Blood (cardiac)	1.57	97.73	133.50	0.58
	Bone Marrow	9.76	359.31	2822.62	12.34
	Lymph Nodes	2.27	200.63	357.48	1.56
	Spleen	8.68	246.06	3359.66	14.68
	Thymus	1.75	175.93	219.16	0.96
Total %					30.12
			Mean	Total AUC	Total %
			131.69	22879.22	100
Metabolism					

In Vitro Biotransformation and Drug Interaction	Figure 5-1. Metabolic pathway of artesunate												
(b) (4)	APPEARS THIS WAY ON ORIGINAL												
	Table 5-7. Formation of DHA from AS in liver microsomes after 60 min incubation <table border="1"> <thead> <tr> <th>Species</th> <th>DHA (% [AS])</th> </tr> </thead> <tbody> <tr> <td>Human</td> <td>85</td> </tr> <tr> <td>Rat (SD)</td> <td>76</td> </tr> <tr> <td>Cynomolgus monkey</td> <td>59</td> </tr> <tr> <td>Rhesus monkey</td> <td>44</td> </tr> <tr> <td>Beagle Dog</td> <td>44</td> </tr> </tbody> </table> DHA is subject to rapid Phase 2 glucuronidation via UGT1A9 and UGT2B7.	Species	DHA (% [AS])	Human	85	Rat (SD)	76	Cynomolgus monkey	59	Rhesus monkey	44	Beagle Dog	44
Species	DHA (% [AS])												
Human	85												
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Excretion													

Elimination of ¹⁴C-AS following a Single IV administration of 5 mg/kg [¹⁴C]-artesianate to male rats through 192 hr following injection	Table 5-8. Excretion in male rats Following a Single Dose of ¹⁴C-AS <table border="1"> <thead> <tr> <th>Excretion route</th> <th>Recovery of Radioactivity (% Administered Dose)</th> </tr> </thead> <tbody> <tr> <td>Urine</td> <td>56</td> </tr> <tr> <td>Feces</td> <td>38</td> </tr> <tr> <td>Cage rinses</td> <td>1.6</td> </tr> </tbody> </table>	Excretion route	Recovery of Radioactivity (% Administered Dose)	Urine	56	Feces	38	Cage rinses	1.6																																																																																																																																																																																																
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TK data from general toxicology studies Rat: A 4-week IV study in rats to investigate the pharmacokinetics of and general toxicity of artesunate 0, 3, 10, and 30 mg/kg/day Samples collected pre-dose, 5 and 10 min, 0.5, 1, 2, 3, and 4 hrs postdose Study No. 35182	<u>Rat</u> T_{LAST}: AS < 0.5 hr, DHA < 2h Dose proportionality: Could not be assessed; comparison of 5-min plasma AS concentrations indicate higher plasma levels with increased dose levels were observed on Day 1 and Day 28 of dosing. Table 5-9. Toxicokinetic parameters for 4-week IV study in rats <table border="1"> <thead> <tr> <th colspan="8">Artesunate*</th> </tr> <tr> <th>Day</th> <th>Dose</th> <th>Sex</th> <th>C₀ [ng/mL]</th> <th>[AS] at 5 min (ng/mL)</th> <th>AUC_(0-T_{last}) (ng.h/mL)</th> <th>T_{last} (h)</th> <th>MRT_{last} (h)</th> </tr> </thead> <tbody> <tr> <td rowspan="2">1</td> <td rowspan="2">3</td> <td>M</td> <td>NR</td> <td>141.83</td> <td>NR</td> <td>0.17</td> <td>NR</td> </tr> <tr> <td>F</td> <td>NR</td> <td>335.43</td> <td>NR</td> <td>0.17</td> <td>NR</td> </tr> <tr> <td rowspan="2"></td> <td rowspan="2">10</td> <td>M</td> <td>NR</td> <td>1611.52</td> <td>NR</td> <td>0.17</td> <td>NR</td> </tr> <tr> <td>F</td> <td>NR</td> <td>1330.19</td> <td>NR</td> <td>0.17</td> <td>NR</td> </tr> <tr> <td rowspan="2"></td> <td rowspan="2">30</td> <td>M</td> <td>112137</td> <td>10395.33</td> <td>5794</td> <td>0.5</td> <td>0.02</td> </tr> <tr> <td>F</td> <td>40909</td> <td>7675.31</td> <td>2687</td> <td>0.5</td> <td>0.05</td> </tr> <tr> <td rowspan="2">28</td> <td rowspan="2">3</td> <td>M</td> <td>NV</td> <td>NV</td> <td>NV</td> <td>NV</td> <td>NV</td> </tr> <tr> <td>F</td> <td>533.05</td> <td>NV</td> <td>NV</td> <td>NV</td> <td>NV</td> </tr> <tr> <td rowspan="2"></td> <td rowspan="2">10</td> <td>M</td> <td>NR</td> <td>2217.17</td> <td>NR</td> <td>0.17</td> <td>NR</td> </tr> <tr> <td>F</td> <td>NR</td> <td>1805.62</td> <td>NR</td> <td>0.17</td> <td>NR</td> </tr> <tr> <td rowspan="2"></td> <td rowspan="2">30</td> <td>M</td> <td>373103</td> <td>17118.95</td> <td>17107</td> <td>0.5</td> <td>0.01</td> </tr> <tr> <td>F</td> <td>112918</td> <td>11833.32</td> <td>5936</td> <td>0.5</td> <td>0.02</td> </tr> <tr> <th colspan="8">DHA</th> </tr> <tr> <th>Day</th> <th>Dose</th> <th>Sex</th> <th>C_{max} [ng/mL]</th> <th>AUC_(0-T_{last}) (ng.h/mL)</th> <th>T_{max} (h)</th> <th>T_{last} (h)</th> <th>MRT_{last} (h)</th> </tr> <tr> <td rowspan="2">1</td> <td rowspan="2">3</td> <td>M</td> <td>352.18</td> <td>82</td> <td>0.083</td> <td>0.5</td> <td>0.2</td> </tr> <tr> <td>F</td> <td>604.21</td> <td>108</td> <td>0.083</td> <td>0.5</td> <td>0.2</td> </tr> <tr> <td rowspan="2"></td> <td rowspan="2">10</td> <td>M</td> <td>1974.47</td> <td>677</td> <td>0.083</td> <td>1.0</td> <td>0.33</td> </tr> <tr> <td>F</td> <td>2412.07</td> <td>661</td> <td>0.083</td> <td>1.0</td> <td>0.3</td> </tr> <tr> <td rowspan="2"></td> <td rowspan="2">30</td> <td>M</td> <td>5403.69</td> <td>2990</td> <td>0.083</td> <td>2.0</td> <td>0.39</td> </tr> <tr> <td>F</td> <td>7750.31</td> <td>3703</td> <td>0.17</td> <td>2.0</td> <td>0.41</td> </tr> <tr> <td rowspan="2">28</td> <td rowspan="2">3</td> <td>M</td> <td>NV</td> <td>NV</td> <td>NV</td> <td>NV</td> <td>NV</td> </tr> <tr> <td>F</td> <td>385.96</td> <td>NR</td> <td>0.083</td> <td>0.17</td> <td>0.1</td> </tr> <tr> <td rowspan="2"></td> <td rowspan="2">10</td> <td>M</td> <td>1524.66</td> <td>397</td> <td>0.083</td> <td>0.5</td> <td>0.17</td> </tr> <tr> <td>F</td> <td>2046.29</td> <td>386</td> <td>0.083</td> <td>0.5</td> <td>0.16</td> </tr> <tr> <td rowspan="2"></td> <td rowspan="2">30</td> <td>M</td> <td>6307.75</td> <td>1388</td> <td>0.083</td> <td>0.5</td> <td>0.19</td> </tr> <tr> <td>F</td> <td>6577.21</td> <td>2348</td> <td>0.083</td> <td>1.0</td> <td>0.23</td> </tr> </tbody> </table> *Rapid drop in plasma concentrations of artesunate and DHA below LLOQ prevented characterization of the terminal phase of elimination. NR: not reported, too few plasma concentration time points available. NV: no value calculated. MRT: Mean residence time	Artesunate*								Day	Dose	Sex	C ₀ [ng/mL]	[AS] at 5 min (ng/mL)	AUC _(0-T_{last}) (ng.h/mL)	T _{last} (h)	MRT _{last} (h)	1	3	M	NR	141.83	NR	0.17	NR	F	NR	335.43	NR	0.17	NR		10	M	NR	1611.52	NR	0.17	NR	F	NR	1330.19	NR	0.17	NR		30	M	112137	10395.33	5794	0.5	0.02	F	40909	7675.31	2687	0.5	0.05	28	3	M	NV	NV	NV	NV	NV	F	533.05	NV	NV	NV	NV		10	M	NR	2217.17	NR	0.17	NR	F	NR	1805.62	NR	0.17	NR		30	M	373103	17118.95	17107	0.5	0.01	F	112918	11833.32	5936	0.5	0.02	DHA								Day	Dose	Sex	C _{max} [ng/mL]	AUC _(0-T_{last}) (ng.h/mL)	T _{max} (h)	T _{last} (h)	MRT _{last} (h)	1	3	M	352.18	82	0.083	0.5	0.2	F	604.21	108	0.083	0.5	0.2		10	M	1974.47	677	0.083	1.0	0.33	F	2412.07	661	0.083	1.0	0.3		30	M	5403.69	2990	0.083	2.0	0.39	F	7750.31	3703	0.17	2.0	0.41	28	3	M	NV	NV	NV	NV	NV	F	385.96	NR	0.083	0.17	0.1		10	M	1524.66	397	0.083	0.5	0.17	F	2046.29	386	0.083	0.5	0.16		30	M	6307.75	1388	0.083	0.5	0.19	F	6577.21	2348	0.083	1.0	0.23
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Dog: A 28-day IV study in rats to investigate the pharmacokinetics and general toxicity of artesunate. 0, 5, 15, 40 mg/kg/day Samples collected pre-dose, 5 and 10 min, 0.5, 1, 2, 3, and 4 hrs postdose Study No. 352638

Reviewer comment: Study report indicates several plasma samples were hemolyzed. As such, determined plasma concentrations of AS and DHA were likely lower than the actual concentrations and may have contributed to variability of measured AS and DHA plasma concentrations.

Dog

$t_{1/2}$: < 0.5h (DHA)

Accumulation: No dose accumulation of artesunate or metabolite DHA with repeated dosing over 28 days

Dose proportionality: For artesunate, AUC_{0-Tlast} appeared slightly greater than dose proportional. For DHA, C_{max} and AUC_{0-Tlast} were approximately dose proportional

Table 5-10. Toxicokinetic parameters for 4-week IV study in dogs

Artesunate*							
Day	Dose	Sex	C ₀ [ng/mL]	[AS] at 5 min (ng/mL)	AUC _(0-Tlast) (ng.h/mL)	T _{Last} (h)	MRT _{Last} (h)
1	5	M	7399.18 ^a	4783.22	924 ^a	0.24	0.09 ^a
		F	NR	3512.68	NR	0.17	NR
	15	M	91464.39	30823.80	8659	0.5	0.07
		F	56126.11 ^b	23421.66	6036 ^b	0.3	0.08 ^b
	40	M	152870.39 ^c	90304.23 ^c	25677 ^c	0.88 ^c	0.11 ^c
		F	163850.59	87572.28	24975	0.7	0.11
28	5	M	NR	5769.65	NR	0.17	NR
		F	NR	5456.05	NR	0.17	NR
	15	M	53545.01	17320.29	5015	0.5	0.08
		F	63070.0 ^a	18659.81	5548 ^a	0.43	0.07 ^a
	40	M	137379.20	86595.08	24749	0.7	0.11
		F	140063.74	76537.21	21268	0.5	0.1
DHA							
Day	Dose	Sex	C _{max} [ng/mL]	t _{max} (h)	AUC _(0-TLAST) (ng.h/mL)	T _{Last} (h)	MRT _{Last} (h)
1	5	M	1080.21	0.12	478	0.90	0.29
		F	1097.8	0.1	499	1.0	0.29
	15	M	3921.28	0.14	2203	1.20	0.37
		F	3790.78	0.14	1930 ^c	0.83	0.26 ^c
	40	M	7396.98 ^c	0.17 ^c	6313 ^c	3 ^c	0.61 ^c
		F	6885.60	0.17	5458	2.2	0.55
28	5	M	1242.32	0.12	590	1.0	0.31
		F	1173.31	0.14	485	1.0	0.28
	15	M	3222.39	0.17	1588	1.0	0.33
		F	2837.34	0.17	1198	0.9	0.27
	40	M	7255.79	0.17	5063	2.0	0.49
		F	6526.08	0.17	4150	2.0	0.45

*Rapid drop in plasma concentrations of artesunate below LLOQ prevented characterization of the elimination phase TK parameters. NR: Not reported, too few plasma concentrations for reliable determination. ^an=1, ^bn=2, ^cn=4

Reviewer comment: Study report indicates several plasma samples were hemolyzed. As such, determined plasma concentrations of AS and DHA were likely lower than the

	<i>actual concentrations and may have contributed to variability of measured AS and DHA plasma concentrations</i>																																																													
TK data from reproductive toxicology studies Quantitative tissue distribution of drug-related material using whole body autoradiography (WBA) following a single intravenous dose of ¹⁴C artesunate (5 mg/kg) to 11-day pregnant Sprague Dawley rats Report ^{(b) (4)}-132N-0501 (Study No. AQ0004_04_WR)	Table 5-11. Toxicokinetic data following ¹⁴C-AS 5 mg/kg single IV injection in pregnant rats <table border="1" data-bbox="532 428 984 600"> <tr> <td>T_{max} (h)</td> <td>1.15 ± 0.24</td> </tr> <tr> <td>C_{max} (h)</td> <td>1.77 ± 0.12</td> </tr> <tr> <td>AUC (mcg·hr/mL)</td> <td>142.92 ± 60.39</td> </tr> <tr> <td>t_{1/2} (alpha, hr)</td> <td>7.05 ± 2.43</td> </tr> <tr> <td>t_{1/2} (beta, hr)</td> <td>115.52 ± 0.004</td> </tr> </table> <i>Samples collected at 0.5, 3, 8, 24, 48, 96, and 192 hr postdose</i>	T _{max} (h)	1.15 ± 0.24	C _{max} (h)	1.77 ± 0.12	AUC (mcg·hr/mL)	142.92 ± 60.39	t _{1/2} (alpha, hr)	7.05 ± 2.43	t _{1/2} (beta, hr)	115.52 ± 0.004																																																			
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Single dose intravenous or oral artesunate to pregnant rats on day 11 of gestation Study No. CD2006/01570/00	Table 5-12. Toxicokinetic data after a single oral or IV AS dose in pregnant rats <table border="1" data-bbox="477 947 1304 1430"> <thead> <tr> <th rowspan="3">Compound</th> <th rowspan="3">Parameter</th> <th colspan="2">Oral</th> <th colspan="2">IV</th> </tr> <tr> <th colspan="4">Dose (mg/kg)</th> </tr> <tr> <th>10 (n=4)</th> <th>17 (n=4)</th> <th>0.75 (n=4)</th> <th>1.5 (n=4)</th> </tr> </thead> <tbody> <tr> <td rowspan="5">Artesunate</td> <td>AUC_{0-24h} (ng.h/mL)</td> <td>8.27</td> <td>19.4</td> <td>160</td> <td>466</td> </tr> <tr> <td>T (h)</td> <td>1</td> <td>1</td> <td>1</td> <td>3</td> </tr> <tr> <td>C_{max} (ng/mL)</td> <td>24.6</td> <td>58.9</td> <td>727</td> <td>2300</td> </tr> <tr> <td>T_{max} (h)</td> <td>0.3</td> <td>0.27</td> <td>0.09</td> <td>0.12</td> </tr> <tr> <td>F_{po} (%)</td> <td>0.4</td> <td>0.5</td> <td>n/a</td> <td>n/a</td> </tr> <tr> <td rowspan="4">DHA</td> <td>AUC_{0-8h} (ng.h/mL)</td> <td>30.6</td> <td>147</td> <td>119</td> <td>219</td> </tr> <tr> <td>T (h)</td> <td>1</td> <td>3</td> <td>1</td> <td>1</td> </tr> <tr> <td>C_{max} (ng/mL)</td> <td>49.0</td> <td>179</td> <td>218</td> <td>498</td> </tr> <tr> <td>T_{max} (h)</td> <td>0.52</td> <td>0.54</td> <td>0.09</td> <td>0.12</td> </tr> </tbody> </table> <i>Samples collected at 0.25, 0.5, 1, 3, 8, and 24 hr (oral) and 0.083, 0.25, 0.5, 1, 3, and 8 hr (IV)</i>	Compound	Parameter	Oral		IV		Dose (mg/kg)				10 (n=4)	17 (n=4)	0.75 (n=4)	1.5 (n=4)	Artesunate	AUC _{0-24h} (ng.h/mL)	8.27	19.4	160	466	T (h)	1	1	1	3	C _{max} (ng/mL)	24.6	58.9	727	2300	T _{max} (h)	0.3	0.27	0.09	0.12	F _{po} (%)	0.4	0.5	n/a	n/a	DHA	AUC _{0-8h} (ng.h/mL)	30.6	147	119	219	T (h)	1	3	1	1	C _{max} (ng/mL)	49.0	179	218	498	T _{max} (h)	0.52	0.54	0.09	0.12
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Definitive EFD Study with Oral Artesunate in Rats Study No. CD2005/00559/00	<u>Rat</u> Table 5-13. Toxicokinetic data after single or 12 days of oral AS dosing in pregnant rats <table border="1" data-bbox="477 1661 1317 1890"> <thead> <tr> <th colspan="2">Dose (mg/kg)</th> <th>6</th> <th>10</th> <th>16.7</th> </tr> </thead> <tbody> <tr> <td colspan="5" style="text-align: center;"><i>Artesunate</i></td> </tr> <tr> <td rowspan="2">AUC_(0-4h) (ng.h/mL)</td> <td>GD6</td> <td>-</td> <td>-</td> <td>-</td> </tr> <tr> <td>GD17</td> <td>-</td> <td>-</td> <td>-</td> </tr> <tr> <td rowspan="2">C_{max} (ng/mL)</td> <td>GD6</td> <td>-</td> <td>7.53</td> <td>19.53</td> </tr> <tr> <td>GD17</td> <td>-</td> <td>-</td> <td>6.8</td> </tr> </tbody> </table>	Dose (mg/kg)		6	10	16.7	<i>Artesunate</i>					AUC _(0-4h) (ng.h/mL)	GD6	-	-	-	GD17	-	-	-	C _{max} (ng/mL)	GD6	-	7.53	19.53	GD17	-	-	6.8																																	
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	GD17	-	-	6.8																																																										

	T _{max} (h)	GD6	-	0.25	0.25
		GD17	-	-	0.25
	<i>DHA</i>				
	AUC _(0-4h) (ng.h/mL)	GD6	-	49.94	116.76
		GD17	-	-	-
Definitive EFD Study with Oral Artesunate in Rabbits Study No. CD2005/00560/00	C _{max} (ng/mL)	GD6	17.59	49.77	119.10
		GD17	-	35.07	26.31
	T _{max} (h)	GD6	0.50	0.25	0.50
		GD17	-	1.00	1.00
	<i>Samples collected pre-dose, 15 and 30 min, 1, 2, and 4 hrs postdose</i>				
Definitive EFD Study with Oral Artesunate in Monkeys CD2006/00794/00 Toxicokinetic parameters following dosing on Day 33 of gestation (14 th dose). Samples collected pre-dose, 0.25, 0.5, 1 and 3 h postdose	<u>Rabbit</u>				
	Table 5-14. Toxicokinetic data for DHA* following oral AS administration				
	Dose (mg/kg)		5	7	12
	AUC _(0-4h) (ng.h/mL)	GD7	-	-	92.30
		GD19	-	-	67.99
C _{max} (ng/mL)	GD7	45.46	66.23	179.95	
	GD19	30.77	42.60	97.03	
T _{max} (h)	GD7	0.25	0.25	0.25	
	GD19	0.5	0.5	0.5	
*Study did not quantify artesunate given the reported t _{1/2} of artesunate in rabbits is ~1.7 min. <i>Samples collected pre-dose, 15 and 30 min, 1, 2, and 4 hrs postdose.</i>					
	<u>Monkey</u>				
	Table 5-15 Toxicokinetic data after 14 daily oral doses of AS in pregnant monkeys (GD33)				
	Dose (mg/kg)	4	12	30	
	<i>Artesunate</i>				
	AUC _(0-t) (ng.h/mL)	3.69	9.84	NC	
	C _{max} (ng/mL)	8.14	15.5	77.1	
	T _{max} (h)	0.25	0.25	0.25	
	<i>DHA</i>				
	AUC _(0-t) (ng.h/mL)	6.93	16.4	NC	
	C _{max} (ng/mL)	3.94	40.2	158	
T _{max} (h)	1.0	0.5	0.25		
NC, not calculated – dosing in the 30 mg/kg/day group discontinued due to high incidence of abortion/embryonic death. <i>Samples collected pre-dose, 0.25, 0.5 and 1hr postdose (12 mg/kg/day) or pre-dose, 0.25, 0.5, 1 and 3 hr postdose (4 mg/kg/day).</i>					

5.5 Toxicology

5.5.1 General Toxicology

A 28-day repeated intravenous dose toxicity and toxicokinetic study of artesunate in Sprague Dawley rats followed by a 28-day recovery period/Study No. 351852

- Artesunate treatment caused a moderate to marked regenerative anemia, with a dose-dependent decrease in hemoglobin, RBC, and MCH with increase in MCV and reticulocytes. This was accompanied by splenomegaly and erythroid hyperplasia in spleen, liver, and bone marrow in all animals from the high dose group (30 mg/kg). While evidence of persistent anemia was present at the of recovery in the high dose animals, the noted histopathological changes were comparable to controls at the end of recovery.
- Artesunate was rapidly converted to DHA, such that plasma concentrations were < LOQ (55 ng/mL) at 1 hr post dosing in the high dose group, and earlier for the low and mid dose groups. Maximum plasma concentrations for DHA peaked at 5 min post dosing for most dose groups and the AUC_{0-Tlast} for DHA decreased with repeated dosing, suggesting alterations in artesunate metabolism and/or tissue distribution.
- The NOAEL for this study was 3 mg/kg, approximately 0.2-fold the clinical dose based on body surface area comparisons.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes (study was conducted in accordance with 21 CFR Part 58 and OECD Principles of Good Laboratory Practice).

Methods

Dose and frequency of dosing:	3, 10, and 30 mg/kg/day for 28 days with 28-day recovery period
Route of administration:	Intravenous (slow injection over 2-3 min)
Formulation/Vehicle:	0.3 M phosphate buffer, pH 7.4 (dose formulations within ± 15% nominal values)
Species/Strain:	Rat/CD® [CrI:CD® (SD)] (Sprague-Dawley)
Number/Sex/Group:	10M + 10F/group main study animals; 5M + 5F/group recovery animals
Age:	7-8 weeks
Satellite groups/ unique design:	TK: 6M + 6F/group (3M + 3F/control)
Deviation from study protocol affecting interpretation of results:	No

Observations and Results: changes from control

Parameters	Major findings
Mortality	2 animals found dead during study: MD: 1 male on day 16 HD: 1 female day 21 Gross findings included congestion of lung lobes, soft consistency of kidney (M) and hyperemia of mandibular lymph nodes and hemorrhagic ovaries (F).
Clinical Signs	MD and HD: Pallor on the ears of animals during last week of treatment (41% of animals in MD and 100% of animals in HD)
Body Weights	MD: decrease in weight gain HD: decrease in weight gain, mean body weight (males only), food consumption (females only)
Ophthalmoscopy	No treatment-related findings
Hematology	Dose-dependent anemia as indicated by decreased hemoglobin and RBC (up to 47% in HD), decreased MCHC (up 17% in HD), and increased MCV and reticulocytes (up to 29% and 1569%, respectively, in HD). Anemia persisted through recovery in HD males (evidenced by higher MCV and MCH). MD and HD: Increased platelet concentration and volume, moderate to marked leukocytosis. The hematological findings were comparable to controls at the end of the recovery period.
Clinical Chemistry	LD, MD and HD (males only): Increased albumin levels (up to 11%), Increased ALT (23%) and AST (up to 44%) MD and HD (males and females): Increased total bilirubin (up to 145%) and increased phosphorus levels (up to 15%).
Urinalysis	No treatment-related changes
Gross Pathology	Enlarged spleen in all MD and HD females and all treated males One HD males had hydronephrosis in the right kidney and another HD male had enlarged left seminal vesicle
Organ Weights	Increased mean spleen weight and spleen/brain weight ratios in MD and HD males and females. Increased spleen weight in HD animals compared with controls in HD animals was greater in males (448% increase organ/body weight in males vs. 270% increase organ/body weight in females). Increased mean heart weight and heart/brain weight ratios in MD females (10 and 15%, respectively) and 13% in heart/brain weight ratios in HD males and females (13-14%).
Histopathology Adequate battery: Yes	HD (all animals): splenomegaly and erythroid hyperplasia in spleen, liver, and bone marrow. Erythroid proliferation indistinguishable from control at end of recovery. Findings related to dosing procedure observed in control and treated groups included minimal to mild mixed cell perivenous infiltrates in and/or around the caudal veins associated with active or resolving thrombosis.

Neurological assessment	A complete functional observation battery in Main Study male rats found no treatment-related findings.
Male reproductive assessment	No significant changes in cauda epididymalsperm motility, counts, and morphology.

LD: low dose; MD: mid dose; HD: high dose.

A 28-day repeated intravenous dose toxicity and toxicokinetic study of artesunate in Beagle Dogs followed by a 28-day recovery period. Study No. 352638

Key findings:

- Artesunate treatment induced a dose-dependent regenerative anemia associated with erythroid hyperplasia in the spleen, liver and bone marrow. Changes in measured hematologic parameters and erythroid hyperplasia had resolved by the end of the recovery period. Since changes in hematological parameters and evidence of extramedullary hematopoiesis in the spleen were observed in the low dose (5 mg/kg), a NOAEL could not be determined.
- Artesunate quickly converted to DHA following IV dosing, resulting in a rapid drop of plasma concentrations after first sample collection. DHA plasma levels also dropped rapidly post dosing, as evidenced by short half-life (<0.5 hr). Measured TK parameters were not markedly different between males and females on Day 1 and 28 of dosing. Plasma exposure to DHA was 20-24% that of artesunate following administration at the high dose (40 mg/kg). For DHA, a linear relationship was observed between dose and C_{max} or AUC_{0-Tlast} which was either dose proportional or close to dose proportional.
- The LOAEL of this study was 5 mg/kg, equivalent to the clinical dose based on body surface area comparisons.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes (study was conducted in accordance with 21 CFR Part 58 and OECD Principles of Good Laboratory Practice).

Methods

Dose and frequency of dosing: 5, 15, and 40 mg/kg/day for 28 days with 28-day recovery period

Route of administration: Intravenous; slow bolus injection over 2-3 min via cephalic or saphenous vein (IV catheter).

Formulation/Vehicle: 0.3 M phosphate buffer, pH 8.1(dose formulations within ± 15% nominal values)

Species/Strain: Dog/Beagle

Number/Sex/Group: 3M + 3F/group main study animals; 2M + 2F/group recovery animals

Age: 11-12 months (M) and 7-8 months (F)

Satellite groups/ unique design: None

Deviation from study protocol affecting interpretation of results: No

Observations and Results: changes from control

Parameters	Major findings
Mortality	None. All animals completed the 28-day treatment period and survived until scheduled termination for necropsy.
Clinical Signs	LD and MD: soft/mucoid feces in one male each dose group, one female (MD) exhibited mild vomiting HD: mild vomiting, soft feces, swelling at injection site, pale mucous membranes and prolonged capillary refill time, which resolved during the recovery period.
Body Weights	No treatment-related changes
Ophthalmoscopy	No abnormalities observed
ECG and indirect blood pressure	None. PR interval, wave duration, QRS duration, and QT interval were within normal limits for all groups and no pathologic arrhythmias were noted at any of the time points. No treatment-related changes in systolic blood pressure, diastolic blood pressure, and mean heart rate during the study.
Hematology	Decreased MCV (2-5%) and increased leukocytes (100-1800%) in all treatment groups Dose-dependent reduction in RBC, hemoglobin concentration and hematocrit (up to 35% in HD) MD and HD: Increased platelet concentration (26-65%) Increased fibrinogen concentration (up to 174%) Anemia and leukocyte changes resolved by end of recovery period.
Clinical Chemistry	Increased LDH in MD (females) and HD (males and females), though still within normal range. LDH activity was reduced by end of recovery.
Urinalysis	No treatment-related changes
Gross Pathology	Pale bone marrow in femoral metaphysis in one MD male. Injection site related findings of perivascular hemorrhage in one HD male and one LD female.
Organ Weights	LD males: 14% reduction in heart weight as a ratio to body weight.

Histopathology Adequate battery: Yes	Increased amounts of extramedullary hematopoiesis in the spleen and erythropoietic hyperplasia with increased megakaryocytes and overall cellularity in bone marrow of sternum and femur were observed in animals from all treatment groups. A few foci of erythroid extramedullary hematopoiesis were also observed in the liver of treated animals. These findings returned to control level by the end of the recovery period. Minimal or mild increases in hemosiderin deposits were noted in histiocytes in the splenic red pulp of LD (1 of 4), MD (3 of 4) and HD (4 of 4) recovery animals, likely indicative of increased residual iron deposition after erythrocyte degradation during treatment. Findings related to dosing procedure observed in control and treated groups included perivascular inflammation characterized by focal fibrosis and mixed cell infiltrates at site of injection.
Male reproductive assessment	No treatment-related changes observed following histological evaluation of cellular stages of spermatogenesis. No significant changes in cauda epididymal sperm motility, counts, and morphology.

LD: low dose; MD: mid dose; HD: high dose.

Reviewer comment: In Module 2.6.6, Applicant notes that while the low dose is not a no-effect dose, the effects are considered to be mild and non-adverse in nature and that 5 mg/kg could be considered a NOAEL. However, based on the changes in hematological parameters observed at all doses (including 5 mg/kg), a NOAEL cannot be determined for this study.

General toxicology; additional studies

Additional repeated-dose toxicity studies previously reviewed under IND 64769 can be found below. A similar pattern of toxicity was observed in dogs following a 14-day repeated IV dosing with the WRAIR formulation (Study No. 1466-102) and the semi-synthetic artesunate formulation in the 28-day toxicity study (352638) albeit toxicity was more pronounced following the longer duration of exposure. Similarly, the findings in rat following 28-day repeated IV dosing using the semi-synthetic formulation are consistent with findings from a 7-day dose-range finding study conducted using the WRAIR formulation (Study No. SR01-01; see Nonclinical Appendix), again with extent of toxicity greater following the longer duration of exposure. Unless otherwise indicated, the additional nonclinical studies reviewed below were conducted with the WRAIR formulation.

14-Day Toxicity Study of Artesunate in Beagle Dogs/Study No. 1466-102

IV administration of Artesunate at 10, 20, 35, or 50 mg/kg/day for 14 days resulted in changes in hematological parameters including significant reductions in RBC, HCT, and RETIC at mid- and high-doses that resolved after a 14-day recovery period. Changes in clinical observations, body weight, gross pathology and histopathology were unremarkable. The NOAEL for this study was 10 mg/kg (approximately 2.25-times the clinical dose based on body surface area comparison)

Toxicity of Subacute, 7-Day Dosing of Candidate Intravenous Artemisinins in Rhesus Monkey/Study No. AQ0008-02-AF and PN02-01

IV administration of Artesunate 4, 8, 16, 32 and 128 mg/kg/day for 7 days resulted in hematological changes (leukopenia, neutropenia, RBC loss, and reticulocytopenia), decreased body weight (5-10%), and microscopic hemoglobinuria that resolved after a 7-day recovery period. Depressed motor activity was observed in mid- and high-dose groups was observed that lasted up to 6h post-dosing. Three out of 4 animals treated with a higher artesunate dose (128 mg/kg/day) died prematurely or were humanely euthanized. Animals in the 128 mg/kg group displayed persistent depressed motor activity, slight truncal ataxia, altered urinalysis parameters (ketonuria, hemoglobinuria, pyuria and proteinuria), and hepatic vacuolation prior to death/at necropsy. Animals who died prematurely also had hypocellular marrow consistent with the peripheral neutropenia and reticulocytopenia. Hepatic vacuolation was observed the 3 animals that died but was not observed in the surviving animal. Moderate-to-severe congestion and hemorrhage of the stomach and intestines were also observed, and 2 of the 3 animals that died had moderate adrenocortical congestion and hemorrhage. The males in the 128 mg/kg dose group had noted spermatid degeneration. The surviving animal in the 128 mg/kg dose group showed neuronal chromatolysis in the cranial nerve nucleus. Necropsies in the control, 4, 8, 16 and 32 mg/kg were unremarkable for brain or other organ abnormalities. A separate group of monkeys were administered IM with 16 mg/kg arteether for 14 days as a positive control for the neurotoxicity assessment. Animals treated with arteether exhibited histological findings consistent with widespread neuronal necrosis and gliosis in brain stem nuclei.

Reviewer comment: Study was not conducted in compliance with GLP regulations.

5.5.2 Genetic Toxicology

In Vitro Reverse Mutation Assay in Bacterial Cells (Ames)

Evaluation of Artesunic Acid in the *Salmonella-Escherichia coli*/microsome plate incorporation assay/Study No. G305-08

Key Study Findings:

- Artesunic acid was not mutagenic under the conditions of this study.

GLP compliance: Yes

Test system: *S. typhimurium* TA1535, TA1537, TA98, TA100 and *E. coli* WP2 (*uvrA*).

Concentrations in definitive study up to 2500 mcg/plate $\pm$ S9, as cytotoxicity was observed at 5000 mcg/plate.

Study is valid: Yes

In Vitro Assays in Mammalian Cells

Evaluation of Artesunic Acid in the Chinese Hamster Ovary (CHO) Chromosome Aberration Assay/ Study No. G306-08

Key Study Findings:

- Artesunic acid was not clastogenic under the conditions of this study.

GLP compliance: Yes

Test system: Chinese hamster ovary (CHO) cells. Concentrations in definitive study up to 80 mcg/ml (+/- S9) for 3h and up to 2.5 mcg/ml (-S9) for 21 h.
Study is valid: Yes

In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)

Evaluation of Artesunic Acid in the Mouse Bone Marrow Micronucleus Assay/Study No. G307-08

Key Study Findings:

- Artesunic Acid did not induce micronucleated erythrocytes in the bone marrow of mice that received single oral doses of up to 500 mg/kg under the conditions of this study

GLP compliance: Yes

Test system: Mice (male Swiss-Webster) were exposed to a single oral (gavage) dose of Artesunic Acid (125, 250 and 500 mg/kg) or 0.5% methyl cellulose (vehicle control). Positive control was urethane (300 mg/kg). Femoral bone marrow was harvested at 24 and 48 hours post dosing and evaluated for presence of micronuclei.

Study is valid: Yes*

**Reviewer comment: The in vivo micronucleus assay appeared to be well conducted. However, the plasma levels of artesunate and DHA were not evaluated in this study and the oral bioavailability of artesunate in mice is unknown, limiting the interpretation of the negative findings. It is of note that in pregnant rats and nonpregnant female cynomolgus monkeys the oral bioavailability of artesunate is < 1-2%. In addition, no significant suppression of polychromatic erythrocytes among red blood cells in mice of either sex at any dose level were observed in this study. It is therefore unlikely that sufficient artesunate and DHA exposures were achieved at the target tissues.*

Other Genetic Toxicity Studies

The literature reports that artesunate is clastogenic in vitro and in vivo:

In vitro evaluation of the genotoxic and cytotoxic effects of artesunate, an antimalarial drug, in human lymphocytes. *Environmental and Molecular Mutagenesis*. 2011;52(7):590-4.

Key Study Findings:

- Artesunate increased the frequency of micronuclei and induced DNA damage in a concentration-dependent manner in primary human blood lymphocytes under conditions of this study. Significant increases in DNA damage and MN formation were observed at the lowest concentration tested (0.5 mcg/mL).

GLP compliance: unknown

Test system: Primary human blood lymphocytes. Cells were exposed for 52 h (MN test) or 20h (Comet Assay) to 0.5, 1 and 2 mcg/mL artesunate or 20 ng/ml doxorubicin (positive control).

Study is valid: Yes

Genotoxic evaluation of the antimalarial drugs artemisinin and artesunate in human HepG2 cells and effects on CASP3 and SOD1 gene expressions. *Genetics and Molecular Research*. 2013;12(3):2517-2527.

Key Study Findings:

- Artesunate induced DNA damage in Hep2G cells under conditions of this study. Results of COMET assay showed Class 1 and 2 DNA damage at non-cytotoxic concentrations of artesunate (2.5 and 5 mcg/mL).

GLP compliance: unknown

Test system: Human hepatoma cells (HepG2). Cells were exposed for 3h to 2.5, 5, or 10 mcg/mL artesunate or 2 mcg/mL doxorubicin (positive control).

Study is valid: Yes

Genotoxicity assessment of the antimalarial compound artesunate in somatic cells of mice. *Food and Chemical Toxicology*. 2011;49(6):1335-9.

Key Study Findings:

- Oral artesunate induced mild DNA damage (Class 1) in liver cells at 5 and 50 mg/kg along with a dose-dependent increase in the number of micronucleated polychromatic erythrocytes (MNPCE) in bone marrow cells at ≥ 50 mg/kg, approximately 1.6 times the clinical dose based on body surface area comparisons, without evidence of cytotoxicity.

GLP compliance: Unknown

Test system: Mice (male Swiss albino) were administered oral (gavage) artesunate at 5, 50, and 100 mg/kg or 80 mg/kg doxorubicin (positive control). Bone marrow (MN assay), peripheral blood and liver cells (comet assay) were collected 24h after treatment.

Study is valid: Yes

Reviewer comment: Although GLP status of the literature reports are unknown, the publications contain mean $\pm$ SD and/or individual animal data across dose groups for the endpoints evaluated and included concurrent positive controls and statistical analysis that allow for confidence in reported findings. There have been no in vivo micronucleus studies conducted with intravenously administrated artesunate.

5.5.3 Carcinogenicity

Not applicable. No carcinogenicity studies were conducted with IV artesunate.

5.5.4 Reproductive and Developmental Toxicology

Fertility and Early Embryonic Development

Fertility studies have not been conducted with artesunate. No histopathologic findings of reproductive organs were observed following 28 days of repeated IV dosing of artesunate in rats and dogs, nor were there any artesunate-related changes in sperm motility, sperm count or morphology (See review of Study Nos. 351852 and 352638 in Section 5.5.1 above). Available literature evaluating the effects of artesunate on fertility and spermatogenesis in mice and rats

following oral or intraperitoneal administration are summarized below and in the Nonclinical Appendix.

Long-Term Administration of Artesunate Induces Reproductive Toxicity in Male Rats (J Reprod Infertil. 2011; 12(4):249-260)

Key Study Findings

- A significant reduction sperm motility and viability was observed in rats following a 5-day and 6-week treatment with artesunate. At 6 weeks, this was accompanied by an increase in the percentage of abnormal sperm and decreases in daily sperm production, body weight and relative weight of the testes and epididymis. Histopathological findings included duration-dependent degenerative changes in the testes and epididymis, with noted disorganization of the seminiferous tubules, degeneration of the plasmalemma and arrest of spermatogenesis. Artesunate-induced changes in sperm production and histopathology showed evidence of recovery at 6 weeks.
- No changes in serum LH, FSH or testosterone were observed in animals exposed to artesunate following a 5-day or 6-week treatment period.
- No significant changes were reported in the litter size of females cohabitated with male rats that were treated with artesunate for 6 weeks.

Conducting laboratory and location:



GLP compliance:

Unknown

Methods

Dose and frequency of dosing:	2.9 mg/kg daily for 5 days or 6 weeks
Route of administration:	Oral (gavage)
Formulation/Vehicle:	Distilled water
Species/Strain:	Rat/Wistar
Number/Sex/Group:	6 males/group
Study design:	Adult male rats were administered 2.9 mg/kg/day artesunate for 5 days or 6 weeks followed by a 6-week recovery period. Sperm characteristics, serum FSH, LH, testosterone, testicular and epididymal histology were assessed. Males treated for 6 weeks were cohabited with untreated females at a 1:2 ratio to evaluate effects of artesunate of fertility.
Deviation from study protocol affecting interpretation of results:	Unknown

Reviewer comment: The study is limited in that only one dose level of artesunate was tested using a route that is not clinically relevant and fertility assessment only included effects on litter size. How the findings of abnormal/decreased sperm production and adverse histopathological changes following oral artesunate treatment translate to a risk of adverse effects on male fertility in humans following IV artesunate is uncertain.

Effects of Short- and Medium-Term use of Artesunate on Fertility in Male Rats (Nigerian Journal of Health and Biomedical Sciences. 2008. Vol 7(2):18-21)

Key Study Findings: A significant reduction in sperm density (~60%) with approximate 2-fold increase in the percentage of morphologically abnormal spermatozoa was observed in rats following a 4-week treatment with 2 mg/kg oral artesunate. Following a shorter duration of treatment, increases in percentage of abnormal spermatozoa were observed in animals from the 2 mg/kg group whereas no changes were not noted between the 1 mg/kg group and controls. The findings in the 2 mg/kg group were accompanied by histopathological evidence of damage to the seminiferous epithelium (i.e., destruction of cells in adluminal compartment and exfoliation of cells from seminiferous epithelium).

Conducting laboratory and location

(b) (4)

GLP compliance:

unknown

Methods

Dose and frequency of dosing:

1 and 2 mg/kg

Route of administration:

Oral (gavage)

Formulation/Vehicle:

Water

Species/Strain:

Rat/Wistar

Number/Sex/Group:

5/group

Satellite groups:

None

Study design:

Animals were dosed with 1 or 2 mg/kg/day for 2 weeks or 2 mg/kg/day for 4 weeks. Testicular histopathology and sperm evaluation were performed 4 weeks after the last administered dose.

Deviation from study protocol

Unknown

affecting interpretation of results:

Reviewer note: It is unknown whether the histopathology findings were peer reviewed or if pathologist was blinded to the data. While publication does not contain individual animal data, group means $\pm$ SD of evaluated sperm parameters across treatment groups were provided with statistical analysis.

The antimalarial agent artesunate causes sperm DNA damage and hepatic antioxidant defense in mice (Mutation Research 777 (2015) 1-6)

Key Study Findings

- Artesunate induced a significant increase in % abnormal sperm and decrease in total sperm count/epididymis 24 hours following a single acute dose or 3-day repeated fractionated dosing administered via intraperitoneal injection. The abnormal sperm findings persisted out to 35 days.
- Artesunate induced significant DNA damage as evidenced by increase in % of tail DNA, olive tail movement, and DNA damage index.

Conducting laboratory and location
GLP compliance:

(b) (4)
Unknown

Methods

Dose and frequency of dosing:	Single dose of 40 mg/kg or 13.4 mg/kg/day for 3 consecutive days
Route of administration:	Intraperitoneal injection
Formulation/Vehicle:	Normal saline
Species/Strain:	Mouse/Swiss albino
Number/Sex/Group:	5 males/group
Satellite groups:	None
Study design:	Animals were given with a single dose of 40 mg/kg artesunate or 3 doses of 13.4 mg/kg at 24h intervals. Sperm head abnormalities were assessed at days 1 and 35 post-treatment and DNA damage was analyzed using comet assay 35 days after treatment.
Deviation from study protocol affecting interpretation of results:	Unknown

Reviewer note: Although GLP status is unknown, the article provided sufficient detail on study methods, group means $\pm$ SD of evaluated sperm parameters across treatment and time points and statistical analysis to have confidence in reported findings.

Embryo-Fetal Development

Reviewer comment: The oral bioavailability of artesunate in the pregnant rat is < 1% and the extent to which artesunate and its metabolites distribute to gestational tissues following oral administration is unknown. Given the extent of embryoletality observed following a single IV AS administration in the pregnant rat, embryo-fetal development studies conducted with IV AS cannot feasibly be conducted at doses that would be clinically relevant. It is likely that the findings described below following oral exposure to artesunate during organogenesis

underestimate the risk of IV artesunate on embryofetal toxicity. Given that there are noted species-specific differences in the rate of conversion to DHA by the different artemisinin derivatives (i.e., arteether, artemether, and artesunate) and potential differences in the bioavailability of DHA and its distribution to gestational tissues following oral versus intravenous exposure, the findings of embryofetal development studies conducted using oral artemisinin derivatives in evaluating the risk for IV artesunate use in human pregnancy is of uncertain value.

SB-714702 (Artesunate): Single Dose Intravenous Embryo-Fetal Development Study in Rats/CD2006/01142/00 ((b) (4) Study No. I06318)

Key Study Findings

- Significant post-implantation loss occurred in all animals administered a single intravenous dose of either 1.5 or 3 mg/kg artesunate on Day 11 post coitum (pc) (0.1 to 0.2-fold clinical dose based on body surface area comparison). A reduction in mean maternal body weight gain of up to 62% was also observed, likely due to total litter loss. No effects on embryo-fetal survival were noted in animals receiving a single dose of 0.75 mg/kg on Day 11 pc.

Conducting laboratory and location:

(b) (4)

GLP compliance:

No; report notes study was conducted in facility that maintains GLP compliance.

Methods

Dose and frequency of dosing:	0.75, 1.5 or 3 mg/kg, single dose
Route of administration:	Intravenous
Formulation/Vehicle:	Phosphate buffered saline
Species/Strain:	Rat/CD™ (Sprague-Dawley)
Number/Sex/Group:	4 pregnant females/group; 3 pregnant females/control
Satellite groups:	None
Study design:	Animals were administered a single dose of 0.75, 1.5 or 3 mg/kg artesunate on Day 11 pc. C-sections were performed on Day 21 pc and uterine contents examined.
Deviation from study protocol affecting interpretation of results:	No.

Table 5-16. Summary of cesarean section data and body weight gain from rats treated with intravenous AS on Day 11 of gestation

Dose (mg/kg)	0	0.75	1.5	3
Females pregnant/on study (n)	3/3	4/4	4/4	4/4
Died or euthanized moribund	0	0	0	0
Number of females with 100% post implantation loss (all early resorptions)	0	0	4	4
F ₀ body weight, mean (SD)				
Day 11	274.1 (17.01)	276.0 (16.87)	271.5 (19.0)	269.8 (21.33)
Day 18	342.7 (23.59)	348.0 (24.43)	305.3 (28.63)	307.8 (29.88)
Day 21	396.0 (25.53)	408.5 (35.59)	317.0 (32.89)	319.0 (27.77)

A mass balance study conducted in pregnant rats demonstrated accumulation of artesunate in fetoplacental tissues (e.g., ovary, placenta and uterus) following a single administration of [¹⁴C]-artesunate on day 11 of pregnancy at a dose 0.3 times the recommended human dose based on body surface area comparison. Approximately 8% of total radioactivity was found in reproductive system, suggesting that ¹⁴C-AS and related drug material can readily cross the placenta and distribute to the developing conceptus (See Section 5.5.5 Study (b) (4) -132N-0501).

Artesunate: Oral (Gavage) Study of Embryo-Foetal Development in the Rabbit/Study No. CD2005/00560/00 (b) (4) Report Number 1552/94-D6154)

Key Study Findings

- No NOAEL could be established given that artesunate increased the incidence of fetal cardiovascular, rib, and brain malformations at all doses tested, doses which were to 0.7 to 1.6-fold the clinical dose based on body surface area comparisons.
- At the highest dose tested (12 mg/kg), artesunate was embryofetal toxic, inducing abortion, total embryo-fetal loss, and increased incidences of intrauterine deaths and lower fetal weight.
- No maternal toxicity was observed at any of the doses tested.
- C_{max} values for DHA increased with increasing administered dose with higher C_{max} and AUC_(0-4h) DHA values on Day 7 than on Day 19 (see Section 5.4 ADME/PK above).

Conducting laboratory and location:



GLP compliance:

Yes

Methods

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Dose and frequency of dosing: 5, 7, or 12 mg/kg/day
 Route of administration: Oral (gavage)
 Formulation/Vehicle: 1% w/v methyl cellulose
 Species/Strain: Rabbit/NZW/Kbl BR
 Number/Sex/Group: 24/mated females/group
 Satellite groups: None
 Study design: Groups of 24 mated female rabbits were administered artesunate at 0 (vehicle), 5, 7, or 12 mg/kg/day from Day 7 to Day 19 of gestation, inclusive. Dams were killed on Day 29 of gestation and uterine contents examined.

Deviation from study protocol affecting interpretation of results: No

Observations and Results

Parameters	Major findings
Mortality	None
Clinical Signs	None
Body Weights	None
Necropsy findings Cesarean Section Data	Increased incidence of pre-implantation loss in all treatment groups compared with control (12.3 to 14.6% compared with 9.4%, respectively) HD: 3 females aborted and were killed, 2 females showed total embryo-fetal loss, increased early- and late-intrauterine deaths and post-implantation loss compared with control.
Necropsy findings Offspring	A statistically significant increase in both fetal and litter incidence of skeletal malformations and variations (displaced and/or fused ribs and sternbrae) and cardiovascular malformations and variations (branches from the left subclavian artery arising from the aorta and retro-esophageal right subclavian artery arising from descending aorta) were observed. Incidence of fetal malformation per dose group were as follows: LD: 22 fetuses from 10 litters MD: 16 fetuses from 7 litters HD: 11 fetuses from 7 litters

LD: low dose; MD: mid dose; HD: high dose

Table 5-17. Summary of cesarean section data from rabbits treated with oral AS during organogenesis

Dose (mg/kg)	0	5	7	12
Females pregnant/on study (n)	22/24	24/24	23/24	21/24
Died or euthanized moribund	0	0	0	0
Aborted and euthanized	0	0	0	3
Number of females with total embryofetal loss	1	0	0	2

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Dose (mg/kg)	0	5	7	12
Number of females with live fetuses on Day 29 gestation	21	24	23	16
Pre implantation loss (%)/number of dams affected	9.9/14	12.3/17	13.6/13	13.5/14
Post implantation loss (%)/number of dams affected*	6.3/9	9.6/13	9.6/13	14.2/12
Mean number of fetuses per female	9.2	9.5	9.3	8.4

* Significant dose response (Cochran-Armitage test)

Table 5-18. Rabbit group mean cesarean data following oral AS administration during organogenesis

	Artesunate Dose (mg/kg/day)			
	0	5	7	12
<i>External/Visceral Defects^a</i>				
No. fetuses examined	203	229	215	151
No. litters examined	21	24	23	16
No. showing malformations	2	19	8	8
Mean % fetuses examined	1.2	7.5	4.6	5.1
No. of litters affected	2	10*	4	6
No. showing variations	59	82	74	76
Mean % fetuses examined	28.4	36.7	34.2	53.3
No. of litters affected	19	23	21	16
<i>Skeletal Defects</i>				
No. fetuses examined	203	229	215	151
No. litters examined	21	24	23	16
No. showing malformations	0	6	9	5
Mean % fetuses examined	0	2.9	4.2	3.8
No. of litters affected	0	5*	4	5*
No. showing variations	185	210	207	134
Mean % fetuses examined	89.9	91.9	96.7	89.5
No. of litters affected	21	24	23	16
Total no. fetuses showing malformations	2	22	16	11
% fetuses examined	1	9.6	7.4	7.3
No. litters affected	2	10*	7	7*

^aNo notable treatment-related external malformations or variations were observed, grouped data largely reflects visceral observations. *, p<0.05

Table 5-19. Rabbit fetal malformation data following oral AS administration during organogenesis

Malformation	# of litters with malformations (% litter incidence)				Historical control % Litter Incidence ^a (# litters with malformation/total # litters)
	Artesunate dose (mg/kg/day)				
	0	5	7	12	
Cardiac System					
Total malformations to CV system	1 (5)	8 (33)	3 (13)	4 (25)	
Accessory vessel to descending aorta/aortic arch present	1 (5)	3 (13)	2(9)	0 (0)	Unk
Incomplete ventricular septum	0 (0)	3 (13)	0 (0)	0 (0)	Unk
Enlarged left heart ventricle	0 (0)	1 (4)	0 (0)	0 (0)	Unk
Narrowed pulmonary trunk; dilated aortic arch; and heart ventricles disorganized	0 (0)	1 (4)	0 (0)	0 (0)	Unk
Interrupted aortic arch; right subclavian artery arising from descending aorta; and left subclavian artery arising from pulmonary trunk	0 (0)	1 (4)	0 (0)	0 (0)	3.3 (4/122)
Retro-esophageal right subclavian artery arising from descending aorta	0 (0)	2 (8)	1 (4)	4 (25)	3.3 (4/122)
Branches from left subclavian artery arising from ductus arteriosus	0 (0)	1 (4)	0 (0)	0 (0)	Unk
Persistence of primitive venous vessels	0 (0)	0 (0)	0 (0)	1 (6)	Unk
Skeletal System					
Total malformations to skeleton	0	5 (21)	4 (17)	4 (25)	
Fused ribs	0 (0)	3 (13)	0 (0)	0 (0)	0.8 (1/122)
Displaced or shortened, or misshapen rib	0 (0)	1 (4)	2 (9)	0 (0)	1.6 (2/122)
Lateral curvature of spinal column	0 (0)	2 (8)	0 (0)	0 (0)	Unk
Fused sternebrae; cleft sternebrae; displaced sternebrae or widened sternebrae	0 (0)	1 (4)	2 (9)	2 (13)	2.5 (3/122)
Brain					
Absent areas of the brain (pons)	0 (0)	1 (4)	1 (4)	2 (13)	Unk
Severely dilated brain ventricles	0 (0)	2 (8)	0 (0)	0 (0)	Unk

^aWhere available, the incidence of malformations in historical control NZW rabbits from 6 studies (n=122 litters total) performed by the same laboratory were calculated and listed in the table above. Unk: Unknown, no historical control data available on % litter incidence.

Source: Tables 4 and 6 from Applicant's response to IR submitted 3/4/2020.

Artesunate: Oral (Gavage) Study of Embryo-Foetal Development in the Rat/Study No. CD2005/00559/00 ((b) (4) Report Number 1552/93-D6154)

Key Study Findings

- No NOAEL could be established given that artesunate was embryotoxic with total embryo-fetal loss, increased incidence of intrauterine death and decreased fetal weights observed at all doses tested (0.4 to 1.1-times the clinical dose based on body surface area comparisons).
- Increased incidence in cardiovascular and skeletal malformations were observed in fetuses from the low and mid-dose groups. Long bone and/or scapular malformations and ventricular septal defects were not observed in fetuses from the high dose group, likely due to the small number of surviving fetuses (mean of 1.4 per female).
- No maternal toxicity was observed at any of the doses tested.
- C_{max} values for Artesunate and C_{max} and $AUC_{(0-4h)}$ values for DHA increased with increasing administered dose and were higher on Day 6 than on Day 17; T_{max} for dihydroartemisinin occurred later on Day 17 of gestation than on Day 6 (see Section 5.4 ADME/PK above).

Conducting laboratory and location:



GLP compliance:

Yes

Methods

Dose and frequency of dosing: 6, 10, or 16.7 mg/kg/day
Route of administration: Oral (gavage)
Formulation/Vehicle: 1% w/v methyl cellulose
Species/Strain: Rat/CD(Sprague-Dawley)IGSBR
Number/Sex/Group: 24/mated females/group
Satellite groups: None
Study design: Groups of 24 mated female rats were administered artesunate at 0 (vehicle), 6, 10, or 16.7 mg/kg/day from Day 6 to Day 17 of gestation, inclusive. Dams were killed on Day 20 of gestation and uterine contents examined.

Deviation from study protocol affecting interpretation of results: No

Observations and Results

Parameters	Major findings

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Mortality	None
Clinical Signs	None
Body Weights	None
Necropsy findings Cesarean Section Data	A dose-related increase in total embryo-fetal loss (up to 21/24 observed in HD females) and numbers of litters with late intrauterine deaths were observed. No treatment-related effects on mean numbers of corpora lutea, implantations or % pre-implantation loss were observed.
Necropsy findings Offspring	Significant dose-related decrease in mean fetal weight. Increased incidence of litters with treatment-related limb and heart malformations (ventricular septal defects, retro-esophageal subclavian artery arising from descending aorta), long bone and/or scapular malformations in LD (10 fetuses from 6 litters) and MD (5 fetuses in 4 litters). The lack of increased incidence of malformations in HD attributed to small number of surviving litters (n=3).

LD: low dose; MD: mid dose; HD: high dose

Table 5-20. Summary of cesarean section data from rats treated with oral AS during organogenesis

Dose (mg/kg)	0	6	10	16.7
Females pregnant/on study (n)	23/24	24/24	23/24	24/24
Died or euthanized moribund	0	0	0	0
Number of females with total embryofetal loss	0	4	14	21
Number of females with live fetuses on Day 20 gestation	23	20	9	3
Pre implantation loss (%) / number of dams affected	18/17	13.5/19	14.0/15	12.2/13
Post implantation loss (%) / number of dams affected	4.8/9	28.2/16	74.0/21***	91.4/22***
Mean number of fetuses per female	12.3	10.4	3.2***	1.4***

***p<0.001

Table 5-21. Rat group mean cesarean data following oral AS administration during organogenesis

	Artesunate Dose (mg/kg/day)			
	0	6	10	16.7
<i>External/Visceral Defects^a</i>				
No. fetuses examined	284	250	73	33
No. litters examined	23	20	9	3

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

No. showing malformations	6	8	3	0	^a No notable treatment-related external malformations or variations were observed, grouped data largely reflects visceral observations.	
Mean % fetuses examined	2	5.8	10.2	0		
No. of litters affected	4	4	2	0		
No. showing variations	30	38	9	3		
Mean % fetuses examined	10.5	18.1	20.9	6.1		
No. of litters affected	15	18	6	2		
<i>Skeletal defects</i>						
No. fetuses examined	142	125	36	18		
No. litters examined	23	20	9	3		
No. showing malformations	7	3	3	0		
Mean % fetuses examined	4.3	4.9	13.3	0	Table 5-22. Rat fetal	
No. of litters affected	3	3	3	0		
No. showing variations	133	118	35	17		
Mean % fetuses examined	93.8	95.2	97.8	66.7		
No. of litters affected	23	20	9	2		
Total no. fetuses showing malformations	12	10	5	0		
% fetuses examined	4.2	4	6.8	0		
No. litters affected	6	6	4	0		

malformation data following oral AS administration during organogenesis

Malformation	# of litters with malformations (%)				Historical control % Litter Incidence ^a (# litters with malformation/total # litters)
	Artesunate dose (mg/kg/day)				
	0	6	10*	16.7*	
Cardiac System					
Incomplete interventricular septum	1 (4)	5 (25)	1 (11)	0 (0)	Unk
Retro-esophageal subclavian artery arising from descending aorta	0 (0)	1 (5)	1 (11)	0 (0)	Unk
Thickened walls of right atrium	0 (0)	0 (0)	1 (11)	0 (0)	Unk
Interrupted aortic arch	0 (0)	0 (0)	1 (11)	0 (0)	Unk
Skeletal System					
Multiple limb and/or pectoral skeletal malformations ^b	1 (4)	2 (10)	3 (33)	0 (0)	1.4 (2/137)
Fused ribs	0 (0)	1 (5)	0 (0)	0 (0)	Unk

*The lack of malformations in the 10 and 16.7 mg/kg group is due to small number of litters/fetuses available for evaluation (n=9 and 3 surviving litters, respectively). Unk: Unknown, no historical control data available on % litter incidence.

^aThe historical control data provided in the study report does not contain sufficient details to calculate the incidence of malformations on a percent litter basis. Where possible, the incidence of malformations in historical

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

control rats of the same strain from 6 studies (n=137 litters total) performed by the same laboratory were calculated and presented in the table above.

^bShortened and/or bent scapulae, humerii, fibulae, tibiae, femurs and ulnae. For summary statistics, when individual fetuses had multiple skeletal malformations, they were counted once in this category. If a fetus had a separate skeletal malformation, it was counted separately and is shown in the grouping under this system.

Source: Tables 1 and 3 from Applicant's response to IR submitted 3/4/2020.

A Study for the Effect of SB-714702 (Artesunate) on Embryo-Foetal Development in Cynomolgus Monkeys by Oral Administration/CD2006/00794/00 ((b) (4) Study No. G04361)

Key Study Findings

- A NOAEL of 4 mg/kg/day was determined based on lack of any treatment-related effects in dams and fetuses in this dose group. Treatment-related decreases in reticulocyte count, embryonic deaths and abortions were observed between gestation days 30 and 40 in dams administered 12 and 30 mg/kg/day oral artesunate, approximately 1.6 to 4-times the clinical dose based on body surface area comparisons.
- A significant decrease in the length of ossification of the ulna was observed in the 12 mg/kg group. Dosing in the 30 mg/kg group was discontinued due to high incidence of abortion/embryonic death prior to Day 40 of gestation.
- Systemic exposure to artesunate and DHA increased in an approximately dose-proportional manner between 4 and 12 mg/kg/day. Lack of data precluded any TK assessments from the 30 mg/kg/day dose.

Conducting laboratory and location:

(b) (4)

GLP compliance:

Yes

Methods

Dose and frequency of dosing:	4, 12, or 30 mg/kg/day
Route of administration:	Oral (naso-gastric catheter)
Formulation/Vehicle:	1% w/v methyl cellulose
Species/Strain:	Monkey/Cynomolgus
Number/Sex/Group:	9-15 pregnant females/group
Satellite groups:	None
Study design:	Animals were administered artesunate at dose levels of 0, 4, 12, and 30 mg/kg/day during organogenesis (from Day 20 to Day 50 of gestation). Fetal viability was determined by ultrasonography on Days 25, 30, 40, 51, 60, 70,

80, and 90 of gestation. Cesarean sectioning was performed on Day 100 of gestation and fetuses were removed and examined.

Deviation from study protocol affecting interpretation of results: No

Observations and Results

Parameters	Major findings
Mortality	None
Clinical Signs	None
Body Weights	None; significant decrease in food consumption was observed in HD on Days 23 and 27.
Hematologic findings	A decrease in reticulocyte count (0.0-0.2% of blood cells) was observed in 1/11 dams in MD group and 4/9 dams in HD group at Day 31 of gestation and/or day of embryonic death, abortion or cesarean sectioning compared with pre-treatment values (reticulocytes 0.8-2.1% of blood cells). A decrease in erythrocyte count, leukocyte count, and hematocrit value and increase in platelet count was noted in the MD and HD on Day 31 of gestation and/or day of embryonic death, abortion or cesarean sectioning compared with before treatment. No treatment-related effects were observed in fetal hematological parameters (nucleated and non-nucleated RBC or leukocytes) on Day 100 of gestation.
Necropsy findings Cesarean Section Data	MD: Embryonic death was observed in 7 of 11 dams between Day 30 and 45 of gestation. HD: abortion/embryonic death occurred in 4 dams on Day 37 or 40 of gestation. Administration to the remaining 5 dams was discontinued on Day 39, 37, 36, 32, or 26 of gestation. Of these, embryonic death occurred in 1 dam on Day 39 of gestation and 1 dam aborted on Day 37 gestation.
Necropsy findings Offspring	MD: Significant decrease in length of ossification of the ulna No visceral abnormalities or variations were observed in any fetus.

LD: low dose; MD: mid dose; HD: high dose

A histopathological evaluation of surviving embryos from the 30 mg/kg treatment group indicate an overall reduction in erythroblasts, with evidence of cardiovascular defects in 2 embryos. Both artesunate and DHA were shown to be cytotoxic in vitro to maternal and fetal blood cells isolated from pregnant Cynomolgus monkeys. (see review of Study Nos. CD2006/01492/00 and CD2006/00795/00 in Nonclinical Appendix).

The critical window for embryo lethality and developmental malformations associated with oral artesunate exposure during pregnancy was subsequently evaluated in rats and cynomolgus monkeys in study Nos. CD2004/00133/00, CD2004/00353/00 and CD2007/00245/00. In rats,

the critical window for oral artesunate exposure associated embryoletality and developmental toxicity was determined to be between days 9 and 14 of pregnancy. The critical window for artesunate-induced embryoletality in cynomolgus monkeys following oral administration is estimated to fall between days 20 and 27 of pregnancy (see Nonclinical Appendix for review).

A separate study (CD2004/00606/01) conducted in rats comparing the embryo-fetal toxicity following a single oral administration of artesunate, its active metabolite DHA, or other artemisinin-derived compounds (i.e., artemether and arteether) to rats on day 10 of pregnancy found similar treatment-related effects on embryoletality and cardiovascular and skeletal malformations, suggesting the DHA metabolite plays a significant role in mediating the developmental toxicity associated with artesunate exposure. This is further supported by in vitro studies demonstrating concentration-dependent cytotoxic effects of DHA on fetal blood cells isolated from pregnant monkeys during gestation (see Nonclinical Appendix for review).

Reviewer comment: Similar to the gestation-dependent sensitivity of cynomolgus fetal blood to artesunate and DHA (reported in Study No. 2006/00795/00), a pattern of gestation-dependent sensitivity to artesunate has been reported in the published literature for rat embryos, both in vitro and in vivo. Laffan et al. (2005)³ report that rat embryonic blood cells are more sensitive than adult erythrocytes to artesunate-induced cytotoxicity, with an apparent enhanced sensitivity of blood cells isolated from GD 12 embryos compared with blood cells isolated from GD 14 embryos. White et al. (2006)⁴ report that following a single oral dose of 17 mg/kg artesunate to rats on GD 10, GD 10.5 or GD 11, depletion of embryonic erythroblasts preceded malformations (i.e., thin atrial and ventricular walls and thin/underdeveloped trabeculae) and embryo death. In addition, Longo et al. (2006)⁵ found that addition of DHA to rat whole embryo cultures cultured from GD 9.5 to GD 11.5 resulted in yolk sac anemia and disorganization of yolk sac vessels. Use of oral artesunate and other oral artemisinin class drugs in pregnant women has not identified a drug-associated risk of major birth defects, miscarriage, or adverse maternal or fetal outcomes. The apparent discrepancy between the nonclinical and clinical data regarding the safety of oral artemisinin use during pregnancy has been hypothesized to be due, in part, to the period of time during which the embryonic erythroblast cells are sensitive to artesunate-induced cytotoxicity. As maturation of embryonic RBC occurs, the embryo becomes less sensitive to artesunate-induced developmental toxicity. In humans, this 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 any teratogenic effects. However, there is insufficient information regarding the risk associated with the use of intravenous artesunate during pregnancy. As noted above, a single exposure to intravenous artesunate during pregnancy in rats at 0.1-times

³ Laffan, S.B., et al. (2005) "In vitro sensitivity of rat embryonic blood cells to the anti-malarial drug artesunate" Birth Defects Res (Part A) **73**:13

⁴ White, T., et al. (2006) "Artesunate-induced depletion of embryonic erythroblasts precedes embryoletality and teratogenicity in vivo" Birth Defects Res (Part B) **77**:413-429

⁵ Longo M., et al. (2006) "Effects of the antimalarial drug dihydroartemisinin (DHA) on rat embryos in vitro" Reprod Toxicol **21**(1): 83-93

the clinical dose based on body surface area comparison resulted in complete embryoletality. Given that the bioavailability of oral artesunate is expected to be significantly lower than intravenous artesunate, the clinical relevance of studies involving oral exposure to artesunate and other oral artemisinin class drugs is uncertain.

The Assessment of Embryonic Toxicity of Artesunate with Multiple Injective Doses in Rats/MIDRP (AQ0004_04_WR)

Key Study Findings

- Intramuscular administration of artesunate during organogenesis induced high levels of postimplantation loss, with complete resorption of litters seen at 0.75 mg/kg, 0.05-times the clinical dose based on body surface area comparisons. The fetal LD₅₀ of AS was 0.61 mg/kg/day (95% CI: 0.59-0.63).
- The critical window for AS-induced embryoletality following IM administration occurs between Days 11-15, though significant post-implantation loss is also observed when administered late in gestation, between Days 15 and 20.

Conducting laboratory and location:

GLP compliance: No

Methods

Dose and frequency of dosing: 0.5 to 13.5 mg/kg daily for 3 to 13 days during different periods of gestation

Route of administration: Intramuscular

Formulation/Vehicle: 0.3M PBS, pH 8.1

Species/Strain: Rat/Sprague-Dawley

Number/Sex/Group: 3-12 mated females/group

Study design: *Dose-response portion:* Groups of 3 mated female rats were administered artesunate at 0 (vehicle), 0.5, 0.55, 0.6, 0.65, 0.75, 1.0, 1.25, 1.5, 4.5, or 13.5 mg/kg/day from Day 6 to Day 18 of gestation, inclusive.
Early embryonic development study: Groups of 5 mated female rats were administered artesunate at 0, 2, 4, or 6 mg/kg/day from Days 6 to 8 of gestation.
Critical window study: Groups of 5 mated female rats were administered artesunate at 6 mg/kg/day from Days 6 to 10, Days 11-15, or Days 16-20 of gestation.
Dams were killed on Day 20 of gestation and uterine contents examined.

Deviation from study protocol affecting interpretation of results: No

Observations and Results

No treatment-related effects in clinical observation, hematology, clinical chemistry or pathology were observed in dams administered IM artesunate from GD6 to GD 18. Reductions in body weight increases observed in dams administered ≥ 0.75 mg/kg IM artesunate are likely reflective of litter loss.

Table 5-23. Summary of cesarean section data from rats treated daily with IM artesunate during organogenesis (GD6 to GD 18)

Dose (mg/kg)	0	0.5	0.55	0.6	0.65	0.75	1.0	1.25	1.5	4.5	13.5
Dams	7	2	3	3	3	3	3	2	2	2	2
No. Pregnant	6	2	3	3	3	3	3	2	1	0	2
Died or euthanized moribund	0	0	0	0	0	0	0	0	0	0	0
No. aborted	0	0	0	0	0	0	0	0	0	0	0
Total resorption of Litter	0	0	0	0	1*	3**	3**	2**	1	0	2**
Body Weight Increase (%)	37.6	33.1	41.0	34.8	37.8	23.6**	22.1**	22.8*	18.32	15.98**	23.9*
Mean % Preimplantation Loss	21.8	3.8	24.5	2.2	10.4	6.3	7.2	9.2	53.5	100*	3.5
Mean % Postimplantation Loss	1.3	0	12.8	41.6	92.8*	100**	100**	100**	100**	0	100**

*p<0.05, ** p<0.01

Table 5-24. Summary of cesarean section data from rats treated with IM artesunate from Days 6 to 8 of gestation

Dose (mg/kg)	0	2	4	6
Dams	5	5	5	5
No. Pregnant	4	5	5	5
Died or euthanized moribund	0	0	0	0
No. aborted	0	0	0	0
Total resorption of Litter	0	0	0	0
Body Weight Increase (%)	55.1	55.5	57.3	48.8
Mean % Preimplantation Loss	6.4	9.1	15.3	18.7
Mean % Postimplantation Loss	1.67	6.04	2.86	10.30

Table 5-25. Summary of cesarean section data from rats treated with 6 mg/kg IM artesunate during different periods of gestation

Dose (mg/kg)	0	GD6-10	GD11-15	GD16-20
Dams	5	12	12	12
No. Pregnant	5	12	12	12
Died or euthanized moribund	0	0	0	0
No. aborted	0	0	0	0
Total resorption of Litter	0	0	12**	1
Body Weight Increase (%)	41.7	45.2	24.1*	40
Mean % Preimplantation Loss	6.3	2.5	0.7	7.4
Mean % Postimplantation Loss	0	11.4	100**	92.2**

*p<0.05, ** p<0.01

Prenatal and Postnatal Development

No pre- and post-natal development studies have been conducted with artesunate, nor are there any publications that evaluate the pre- and post-natal developmental toxicity of artemisinins.

Reviewer comment: The Applicant cited a WHO 2003 publication "Assessment of the safety of artemisinin compounds in pregnancy" which indicates that GLP studies on pre- and postnatal dosing and subsequent development of pups were conducted according to requirements of major regulatory agencies found no effect on postnatal development and maturation of offspring of dosed dams for any artemisinin derivatives, including artesunate. However, the WHO publication does not provide any information regarding study design (species, route of administration, dose) or study findings. Given the available data on the embryofetal toxicity and fetal malformations associated with artesunate in rat, rabbit and monkey, it is unlikely that the doses used in the negative pre- and postnatal study(ies) referenced in the WHO document achieved clinically relevant exposures.

5.5.5 Other Toxicology Studies

Quantitative tissue distribution of drug-related material using whole body autoradiography (WBA) following a single intravenous dose of [¹⁴C] artesunate (5 mg/kg) to 11-day pregnant Sprague Dawley rats/ (b) (4) -132N-0501

Key findings:

- The highest concentrations of artesunate in tissues following intravenous administration in rat were observed at 0.5 hr post dosing, with ~41% of radioactivity amassed in small intestine and its contents and ~6% in bile of the bile duct. At 8 hr post dosing, high levels of radioactivity were present in small intestine (28.2%) and bladder (25.8%).

- Approximately 8% of total radioactivity was found in reproductive tissues (e.g., uterus and placenta), suggesting that ¹⁴C-AS and related drug material can readily cross the placenta and distribute to the developing conceptus. A greater amount of radiolabeled AS distributed in the ovary, placenta and uterus compared with blood (~2 to 4-fold). The total AUC_{0-192h} of the radioactivity was 22879 mcg equivalents·h/g in all measured tissues, with 6.5% (1480 mcg equivalents·h/g) of total radioactivity present in feto-reproductive tissues. The half-life of radioactivity in blood was also shorter compared with feto-reproductive tissues, 97.7 hr in blood compared with 159.9, 200.5, and 152.6 hr in ovary, placenta, and uterus, respectively.

Conducting laboratory and location: [REDACTED] (b) (4)

GLP compliance: No

Methods

Dose and frequency of dosing: Single administration of [¹⁴C] AS (5 mg/kg) to rats on Day 11 of pregnancy

Route of administration: Intravenous

Formulation/Vehicle:

Species/Strain: Rat/Sprague-Dawley

Number/Sex/Group: 2/time point

Age:

Study design: A single IV injection of AS was administered on Day 11 of pregnancy. At each of 0.5, 3, 8, 24, 48, 96 and 192 hr after dosing, 2 rats were euthanized, and sagittal sections obtained for whole-body autoradiography.

Observations and Results:

Table 5-26. Mean PK parameters of ¹⁴C-AS following a single IV injection of 5 mg/kg/147 mCi/kg in the tissue or organs of the pregnant rats by quantitative whole-body autoradiography.

Tissue type	Tissue	C _{max} (mcg/g)	t _{1/2} (h)	AUC _{0-192h} mcg·h/g	% total AUC _{0-192h}
Adipose	Adipose (brown & white)	6.49	118.72	806.67	3.53
Alimentary Canal	Large Intestine	1.82	99.08	173.41	0.76
	Large Intestine (contents)	7.34	49.00	617.83	2.70
	Stomach & contents	3.44	141.53	361.46	1.58
	Small Intestine	9.47	68.61	204.78	0.90
	Small Intestine (contents)	120.35	27.55	3172.53	13.87
	Brain	2.11	155.21	88.84	0.39

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Tissue type	Tissue	C _{max} (mcg/g)	t _{1/2} (h)	AUC _{0-192h} mcg·h/g	% total AUC _{0-192h}
	Spinal Cord	2.72	168.31	127.65	0.56
Dermal	Skin	1.71	100.77	111.97	0.49
Endocrine	Adrenal Gland	7.59	207.79	1145.58	5.01
	Pituitary Gland	2.93	172.50	213.82	0.93
	Thyroid Gland	2.58	139.81	323.57	1.41
Excretory/Metabolic	Bile (in duct)	16.84	153.87	1387.64	6.07
	Renal Cortex	6.71	179.10	1490.90	6.52
	Renal Medulla	6.19	117.97	326.73	1.43
	Liver	9.06	160.42	903.65	3.95
	Urinary Bladder	23.66	74.39	674.17	2.95
	Urinary Bladder (contents)	44.27	41.79	592.24	2.59
Ocular	Eye Uveal Tract	1.08	71.83	45.09	0.20
	Eye Lens	0.29	70.75	17.22	0.08
Reproductive	Amnion & Amniotic Fluid	1.56	76.91	64.32	0.28
	Fetal Body	2.26	83.63	80.91	0.35
	Mammary	2.99	120.76	100.03	0.44
	Ovary	5.84	159.90	555.13	2.43
	Placenta	3.21	200.55	366.77	1.60
	Uterus	2.19	152.60	215.58	0.94
	Vagina	1.46	88.96	96.98	0.42
Respiratory Tract	Lung	2.13	93.92	135.31	0.59
	Nasal Turbinates	4.27	155.33	311.18	1.36
Secretory	Harderian Gland	4.34	78.01	157.18	0.69
	Pancreas	2.96	142.56	235.57	1.03
	Salivary Gland	2.34	206.18	313.67	1.37
Skeletal/Muscular	Bone	0.32	171.66	25.79	0.11
	Heart	2.96	166.21	405.69	1.77
	Skeletal Muscle	1.91	158.88	135.96	0.59
Vascular/Lymphatic	Blood (cardiac)	1.57	97.73	133.50	0.58
	Bone Marrow	9.76	359.31	2822.62	12.34
	Lymph Nodes	2.27	200.63	357.48	1.56
	Spleen	8.68	246.06	3359.66	14.68
	Thymus	1.75	175.93	219.16	0.96
			Mean	Total AUC	Total %
			131.69	22879.22	100

Table 5-27. PK Parameters Following 14C-AS 5 mg/kg Single IV Injection to Pregnant Rats on GD 11

Parameter	Blood	Fetal Body	Placenta	Amniotic Fluid	Amnion
T _{max} (h) (ratio)	1.15 ± 0.24 (1)	1.09 ± 0.15 (0.94)	1.10 ± 0.60 (0.95)	3.30 ± 1.06 (2.86)	0.81 ± 0.53 (0.70)
C _{max} (h) (ratio)	1.77 ± 0.12 (1)	2.37 ± 0.68 (1.33)	3.41 ± 0.35 (1.92)	0.68 ± 0.005 (0.38)	0.96 ± 0.10 (0.54)

Parameter	Blood	Fetal Body	Placenta	Amniotic Fluid	Amnion
AUC (mcg·hr/mL) (ratio)	142.92 ± 60.39 (1)	91.5 ± 41.93 (0.64)	353.67 ± 160.90 (2.47)	12.04 ± 2.33 (0.08)	29.48 ± 3.75 (0.20)
t _{1/2} (alpha, hr) (ratio)	7.05 ± 2.43 (1)	6.49 ± 1.70 (0.92)	4.65 ± 2.32 (0.66)	2.80 ± 0.45 (0.39)	3.46 ± 5.20 (0.49)
t _{1/2} (beta, hr) (ratio)	115.52 ± 0.004 (1)	234 ± 149.76 (2.02)	579.18 ± 149.76 (2.41)	99.94 ± 116.62 (0.86)	69.31 ± 0.03 (0.6)

Pharmacokinetics and bioequivalence of new artesunate intravenous formulation compared to Chinese drug product in rats and beagle dogs/Study No. ET-05003

Key Study Findings:

- In rats, pharmacokinetic analysis indicated that the 90% confidence interval (CI) of the difference between the Guilin (vehicle, 5% NaHCO₃) and WRAIR (b) (4) formulations following daily intravenous dosing for 3 days were contained within 80 to 125% of the geometric mean of C_{max} and AUC. Bioequivalence was fulfilled with AS (82.3-117.7% for all parameters) and partially satisfied for DHA in rats following single or multiple injections.
- In dogs, pharmacokinetic analysis also indicated that the 90% CI of the difference between the Guilin and WRAIR formulations following a single intravenous dose were contained within 80 to 125% of the geometric mean of C_{max} and AUC. Following multiple injections, bioequivalence was fulfilled with regards to AUC but not C_{max}.

Conducting laboratory and location:

(b) (4)

GLP compliance:

No

Methods

Species/Strain:

Rat/Sprague-Dawley
 Dog/Beagle

Study design:

Twenty-four male rats received 2 IV formulations of AS (b) (4) at 20 mg/kg once daily for 3 days in a two-sequence, two-period crossover design with a two-week washout between periods. Blood samples were collected at 0, 5, 15, 30, 45 min, 1, 2, 3, 6, 24.25, 48.167, 48.25, 48.75, 49, 50, 51, and 54 hr post-dosing.

Twelve male dogs received 2 IV formulations of AS (b) (4) at 20 mg/kg once daily for 3 days in a two-sequence, two-period crossover design with a two-week washout between periods. Blood samples were collected prior to dosing (0 hr), and at 0.083, 0.25, 0.5, 0.75,

1, 1.5, 2, 4, and 6r h post-dosing (measured from the end of the injection) after the first and last treatments.

Deviation from study protocol affecting interpretation of results: None

Table 5-28. Establishment of bioequivalence (BE)⁶ for two IV formulations in rat

PK Parameters	Artesunate 90% CI for T/R		BE	DHA 90% CI for T/R		BE
	Lower (%)	Upper (%)		Lower (%)	Upper (%)	
Single Dose						
C _{maxD1}	90.21	109.79	Yes	75.17	124.78	No
AUC _{D1}	88.72	111.28	Yes	82.04	117.96	Yes
Multiple Doses						
C _{maxD3}	83.68	116.32	Yes	81.94	118.06	Yes
AUC _{D3}	82.30	117.69	Yes	71.32	128.67	No
AUC _{D1-3}	87.65	112.35	Yes	79.11	120.89	No
C _{av}	89.54	106.31	Yes	82.16	93.09	Yes
DF	98.46	100.87	Yes	106.55	99.09	Yes

BE= bioequivalence, T=test article (WRAIR formulation), R=reference article (China formulation), C_{av}=Concentration average=AUC_{0-3/3}, DF=Degree of fluctuation=100% X (C_{max}-C_{min})/C_{av}

Source: Study No. ET05003-Rat

Table 5-29. Establishment of bioequivalence (BE) for two IV formulations in dog

PK Parameters	Artesunate 90% CI for T/R		BE	DHA 90% CI for T/R		BE
	Lower (%)	Upper (%)		Lower (%)	Upper (%)	
Single Dose						
C _{maxD1}	86.87	118.88	Yes	84.24	100.97	Yes
AUC _{D1}	84.38	116.56	Yes	92.27	119.62	Yes
Multiple Doses						
C _{maxD3}	68.83	125.55	No	76.01	103.30	No
AUC _{D3}	86.49	123.22	Yes	86.70	112.22	Yes
AUC _{D1-3}	90.14	117.10	Yes	88.61	113.47	Yes
C _{av}	81.95	130.40	No	86.77	112.62	Yes
DF	75.69	102.67	No	73.59	106.75	No

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

BE= bioequivalence, T=test article (WRAIR formulation), R=reference article (China formulation),
 C_{av} =Concentration average= $AUC_{0-3/3}$, DF=Degree of fluctuation= $100\% \times (C_{max}-C_{min})/C_{av}$

Source: Study No. ET05003-Dog

Evaluation of a Test Article to Induce Hemolysis in Human, Rat, Dog and Monkey Blood/Study No. 0725XW16.001

Key Study Findings:

- Hemolysis of dog blood was observed following incubation with 0.3M phosphate buffer, pH 8.1 (OD₄₅₀ of 1.481 compared with an OD₄₅₀ of 0.028 in saline). Human, rat and monkey blood were found to be compatible with 0.3M phosphate buffer, pH 8.1.
- Artesunate caused hemolysis in human (68.74%), rat (54.38%), and monkey (58.87%) blood at 10 mg/mL, with no hemolysis observed at 30 or 60 mcM artesunate. In dog blood, 100% hemolysis was observed following exposure to 30 mcM and 60 mcM and ~44% hemolysis was observed at 10 mg/mL artesunate.

GLP compliance: Yes

Test system: Artesunate (30 mcM, 60 mcM and 10 mg/ml), vehicle control (0.3M phosphate buffer, pH 8.1), saline, and water were mixed with whole blood from human, rat, dog and monkey and incubated for 4 hr. Red cells were removed from samples via centrifugation, and Hb in supernatant measured spectrophotometrically (OD at ^{(b) (4)} nm).

6 Clinical Pharmacology

6.1 Executive Summary

The Office of Clinical Pharmacology reviewed the relevant information contained in NDA 213036. The clinical pharmacology information submitted in the NDA supports the approval of Artesunate for Injection (IV AS) for the initial treatment of severe (b) (4) malaria in adult and pediatric patients. The dosing regimen for IV AS is proposed as 2.4 mg/kg administered (b) (4) at time 0, then at 12, 24 (b) (4) hours (b) (4) (b) (4)

The PK of AS and DHA following IV administration of artesunate was investigated in healthy subjects (Studies 1128 and 1142) and in patients with uncomplicated malaria (Study 1168). The PK of AS and DHA following administration of IV AS was evaluated in patients with severe malaria in 3 published studies. The in vivo drug-drug interactions (DDI) between AS and DHA and various concomitant medications was evaluated in 9 published studies. These studies were deemed to be acceptable for review. See Sections 6.2.2 and Appendix 18.3 below for more details of the PK and DDIs.

The Applicant is relying on the efficacy and safety information from two published pivotal randomized clinical efficacy trials of intravenous artesunate compared with intravenous quinine in severe malaria: SEAQUAMAT, which evaluated 730 patients aged 2 to 87 years who received IV artesunate compared with 731 patients who received IV quinine ([Dondorp et al. 2005](#)); and AQUAMAT, which studied 2715 pediatric patients administered IV artesunate compared with 2713 who received IV quinine ([Dondorp et al. 2010](#)). A Phase 2 study, EDCTP/MMV07-01, which compared a 5-dose regimen of IV artesunate with a 3-dose regimen of IV artesunate with the same total dose, provided *P. falciparum* parasite clearance information at 24 hours in children with severe malaria. See sections 7,8, and 9 for the Clinical and Statistical reviews of the safety and efficacy of IV AS for the initial treatment of severe malaria.

The Applicant proposed the same dose of 2.4 mg/kg IV artesunate for both adults and pediatric patients based on the results of the SEAQUAMAT and AQUAMAT trials. It is to be noted that the WHO recommends a higher dose of 3 mg/kg for pediatric patients <20 kg body weight. During the clinical pharmacology review, the Pharmacometrics review team evaluated the appropriateness of the WHO recommended dose of 3 mg/kg for patients <20 kg body weight by reviewing a publication by Zaloumis, et al⁷ that described the population PK of IV artesunate in adults and pediatric patients with severe malaria. Additionally, adult and pediatric patient data were obtained from 6 different studies with diverse study designs and dosing scheme. The Pharmacometrics team reviewed the data and performed simulations to evaluate the appropriateness of the 2.4 mg/kg dosing regimen proposed by the Applicant in pediatric patients. The PK data were pooled from the different studies for

⁷ Zaloumis, CPT Pharmacometrics Syst. Pharmacol. (2014) 3, e145

pharmacokinetic analysis. Patients received either IM, intra-rectal (IR), or IV artesunate depending on study specific designs and dosing schemes. PK data following IV AS administration were used for population PK modeling; this included IV AS data collected in cross-over trials.

Based on the simulations, it was concluded that the 2.4 mg/kg dosing regimen is appropriate for all patients with severe malaria regardless of body weight or age. This dosing regimen is predicted to result in comparable or greater steady-state DHA exposure (AUC_{0-12}) in neonates and infants to that observed in older children and adults. For example, the average predicted steady-state AUC_{0-12} was up to 90% greater in pediatric patients with severe malaria weighing less than 6 kg (neonates and infants less than 3 months old) compared to older children and adults weighing 21 kg to 25 kg administered 2.4 mg/kg IV AS. Since no exposure-response relationship for safety was able to be evaluated, the safety risk of this higher DHA AUC_{0-12} cannot be determined. However, given the severity of severe malaria in pediatric patients, the clinical pharmacology review team believes that the benefits of Artesunate for Injection outweigh this unknown risk.

6.2 Summary of Clinical Pharmacology Assessment

6.2.1 Pharmacology and Clinical Pharmacokinetics

Table 6-1. Summary of the Clinical Pharmacokinetics of IV Artesunate

Pharmacokinetics (PK)	Following repeat administration of 2.4 mg/kg IV Artesunate 4 times over 3 days at 0, 12, 24, and 48 hrs, the Cmax and AUC of AS in patients with severe malaria are approximately 3.3 µg/mL and 0.7 µg-h/mL, respectively. The Cmax and AUC of DHA are approximately 3.1 µg/mL and 3.5 µg-h/mL respectively.
Distribution	Human plasma protein binding of AS and DHA is approximately 73% and 93%, respectively. The volume of distribution of AS and DHA is approximately 70 L and 60 L, respectively.
Elimination	Mean terminal half-life of AS and DHA is approximately 0.2 hours and 1.2 hours, respectively. The clearance of AS and DHA is approximately 180 L/h and 32.3 L/h, respectively.
	Metabolism Artesunate is rapidly hydrolyzed to DHA by plasma esterases. DHA is not metabolized by CYP 450 oxidation or by cytosolic sulfotransferases. A glucuronide metabolite, α-DHA-G, is a major metabolite of DHA in humans and its formation is catalyzed by UGT1A9 and UGT2B7
	Excretion A radiolabeled mass balance study was not conducted and so the disposition and extent of elimination and/or excretion pathways of AS and DHA are unknown.

6.2.2 General Dosing and Therapeutic Individualization

General Dosing

The Applicant's proposed dosing regimen for severe malaria is supported by efficacy and safety data from the published clinical trials submitted in the application. The proposed dosing regimen for IV AS is 2.4 mg/kg administered (b) (4) at time 0, then at 12, 24 (b) (4) hours (b) (4)



Therapeutic Individualization

Intrinsic Factors

No dedicated PK studies were conducted to evaluate the effect of age and gender on the PK of AS and DHA. Both children and women were included in the pivotal clinical trials.

Extrinsic factors

No clinically significant drug interactions were reported with co-administration of oral artesunate with atovaquone/proguanil, mefloquine, amodiaquine and sulfadoxine/pyrimethamine. Therefore, drug interactions between IV AS and these drugs are not expected. There are two scenarios described below, where coadministration of IV AS should be avoided with nevirapine, ritonavir and other strong UGT inducers.

Drug Interaction Studies from the Literature

Effect of Other Drugs on IV Artesunate:

Nevirapine:

Co-administration of **oral** Artesunate with nevirapine⁸ resulted in a decrease in C_{max} and AUC of DHA by 59% and 68%, respectively. However, it is to be noted that the authors evaluated oral artesunate in this study, which is not approved in the US. This reduction in systemic exposure is may result in the loss of efficacy of IV Artesunate. Based on this reduction of systemic exposure observed in this study, IV Artesunate should not to be given with nevirapine. Thus, co-administration of IV Artesunate with nevirapine should be avoided.

Ritonavir

Co-administration of **oral** Artesunate with ritonavir⁹ resulted in a 27% and 38% decrease in C_{max} and AUC, respectively, of DHA. However, it is to be noted that the authors evaluated oral artesunate in this study, which is not approved in the US. This effect of reduced DHA exposure following coadministration of oral artesunate with ritonavir maybe due to induction effect of ritonavir on DHA metabolism via CYP3A, 1A2, 2C9, 2C19 and 2B6. It is expected that co-administration of ritonavir with IV artesunate may also decrease the C_{max} and AUC of DHA. Thus, co-administration of IV Artesunate with ritonavir should be avoided.

Outstanding Issues

A multiple-dose safety, tolerability, PK study in pediatric patients with severe malaria from 0 to 12 months of age receiving 2.4 mg/kg IV AS is recommended by the clinical pharmacology review team to confirm the adequacy of the PK extrapolation approach in pediatric patients. Given the scarcity of PK data from infants younger than 6 months, the Applicant will be asked to place additional focus on this age group so that the majority of pediatric patients enrolled in the study will be 6 months of age and younger. For the PK assessment, a random sparse sampling population approach to DHA PK can be conducted with post-infusion PK sample(s) collected after any dose during treatment with 2.4 mg/kg of Artesunate for Injection.

⁸ Fehintola, AIDS Research and Treatment Volume 2012, Article ID 703604

⁹ Morris, Am. J. Trop. Med. Hyg., 86(3), 2012, pp. 489–495

6.3 Comprehensive Clinical Pharmacology Review

6.3.1 General Pharmacology and Pharmacokinetic Characteristics

Summary of Pharmacologic Activity and Clinical Pharmacology

Mechanism of action:

Artemisinin is an endoperoxide derived from artemisinin, for which activation of the endoperoxide bridge is essential for antimalarial activity. Semisynthetic lactol derivatives such as DHA improve bioavailability and efficacy.

Active Moiety:

DHA is the active in vivo metabolite of artesunate.

QT prolongation:

The QT-IRT group provided an assessment of the QTc prolongation effect of IV AS. Based on the group's conclusion, no significant QTc prolongation effect of artesunate or DHA was detected.

The effect of artesunate on the QT interval was evaluated in Studies 1128 and 1142. Study 1128 was randomized, double-blind, placebo-controlled, single ascending dose study assessing the safety, tolerability, and pharmacokinetics of artesunate following intravenous infusion (dose levels - 0.5, 1, 2, 4, and 8.0 mg/kg; 10 mg/mL) in healthy subjects (n=40). Study 1142 was a randomized, double-blind, placebo-controlled, multiple ascending dose (3- days) study assessing the safety, tolerability, and pharmacokinetics and pharmacodynamics of artesunate following intravenous infusion (dose levels - 2, 4, and 8 mg/kg; 10 mg/mL) in healthy subjects (n=26/40). In both studies, the highest dose evaluated was 8 mg/kg (administered as intravenous infusion over 2 min), which is the maximum studied dose and covers the expected therapeutic exposures (C_{2min}: 17650 ng/mL; end-of-infusion). Since the first ECG was collected at 5 min and the infusion duration was 2 min, the peak concentrations of artesunate and DHA were predicted based on the linear pharmacokinetics.

The data were analyzed using exposure-response analysis as the primary analysis, which did not suggest that artesunate or DHA are associated with a significant QTc prolonging effect and are presented in Table 6-2.

Table 6-2: The Point Estimates and the 90% CIs (FDA Analysis)

ECG parameter	Treatment	Concentration (ng/mL)	$\Delta\Delta\text{QTcF}$ (msec)	90% CI (msec)
QTc	Artesunate 0.5 mg/kg	141.4	0.2	(-3.7 to 4.1)
QTc	Artesunate 1.0 mg/kg	2908.8	0.3	(-3.5 to 4.2)
QTc	Artesunate 2.0 mg/kg	5052.5	0.4	(-3.5 to 4.4)
QTc	Artesunate 4.0 mg/kg	9920.0	0.7	(-3.6 to 5.0)

ECG parameter	Treatment	Concentration (ng/mL)	$\Delta\Delta QTcF$ (msec)	90% CI (msec)
QTc	Artesunate 8.0 mg/kg	20603.8	1.2	(-4.8 to 7.3)

Pharmacokinetics of AS and DHA

Bioanalysis:

Validated HPLC/MS/MS methods were used to determine the concentrations of AS and DHA, and co-administered drugs in various biological matrices as applicable to individual studies.

Reviewer Comment: The validation and performance of the bioanalytical assay methods were deemed to be acceptable.

PK in Patients:

Drug exposure at steady state (SS) following administration of the proposed 2.4 mg/kg once a day for 3 days dose regimen in patients with severe malaria are given below:

Table 6-3. Pharmacokinetic parameters of artesunate and dihydroartemisinin following IV administration of 2.4 mg/kg artesunate once a day for 3 days in 14 patients with severe malaria

Parameter	Artesunate	Dihydroartemisinin
	Median (range)	Median (range)
C_{max} (ng/ml)	3260 (1020-164000)	3140 (1670-9530)
AUC_{0-last} (ng·h/ml)	727 (290-111256)	3492 (2183-6338)
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)

PK in Healthy Subjects A single dose PK, safety and tolerability study was conducted over the dose range of 0.5 – 8 mg/kg. The PK parameter values of artesunate and DHA following single IV Artesunate doses of 0.5 mg/kg to 8 mg/kg in healthy subjects are summarized in Table 6-4 and 6-5.

Table 6-4. PK parameters of artesunate following single IV administrations of 0.5, 1, 2, 4 and 8 mg/kg in healthy subjects

PK parameters	Mean (% CV)				
	0.5 mg/kg (N = 1)	1 mg/kg (N = 5)	2 mg/kg (N = 6)	4 mg/kg (N = 6)	8 mg/kg (N = 6)
C_{max} (ng/mL)	4.8	6.1 (28)	19.4 (19)	36.1 (24)	83.3 (73)
T_{max} (min)	2	2	2	2	2
AUC_{inf} (ng·h/mL)	386	593 (37)	1595 (16)	3038 (25)	6994 (60)
$t_{1/2}$ elimination (h)	0.1	0.1 (22)	0.2 (89)	0.2 (7)	0.2 (29)
CL (mL/min/kg)	21.6	30.6 (38)	21.4 (17)	23.5 (31)	26.2 (57)
Vd (mL/min/kg)	92	187 (46)	106 (35)	109 (26)	165 (75)

Table 6-5. PK parameters of DHA following single IV administrations of 0.5, 1, 2, 4 and 8 mg/kg in healthy subjects

PK parameters	Mean (%CV)				
	0.5 mg/kg (n = 6)	1 mg/kg (n = 6)	2 mg/kg (n = 6)	4 mg/kg (n = 6)	8 mg/kg (n = 6)
NCA modeling					
C _{max} (ng/mL)	428 (15)	802 (22)	1286 (20)	3,148 (30)	4744 (36)
T _{max} (min)	0.16 (66)	0.25 (56)	0.16 (63)	0.12 (0)	0.40 (65)
AUC ₀₋₆ (ng·h/mL)	379 (19)	1008 (29)	1801 (17)	4645 (24)	10057 (28)
AUC _{inf} (ng·h/mL)	385 (18)	1082 (28)	1850 (17)	4886 (24)	10410 (26)
t _½ elimination (h)	0.96 (26)	1.54 (41)	1.15 (23)	1.37 (9)	2.14 (38)
CL (mL/min/kg)	22.2 (17)	16.4 (26)	18.5 (19)	14.4 (27)	13.6 (28)
Vd (mL/kg)	1734 (25)	2201 (49)	1860 (37)	1701 (27)	2403 (34)

Following administration of single IV doses of artesunate to healthy subjects, across the range of 1.0 mg/kg - 8.0 mg/kg, both AS and DHA AUC and C_{max} estimates showed approximate dose proportional increases. After multiple doses of 2, 4 and 8 mg/kg IV injection of AS, the C_{max} and AUC values of AS and DHA increased proportionally to the AS dose. No plasma accumulation of AS and DHA was observed upon repeat administration of IV Artesunate. This is to be expected given the short half-lives of AS and DHA.

6.3.2 Clinical Pharmacology Questions

Does the clinical pharmacology program provide supportive evidence of effectiveness?

The pivotal clinical safety and efficacy studies conducted in support of this application did not include PK and PD assessments and thus, exposure-response analyses were not conducted.

Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?

From a clinical pharmacology perspective, the proposed IV Artesunate dosing regimen of 2.4 mg/kg (b) (4) is acceptable for adult and pediatric patients with severe malaria. The assessment of the IV Artesunate dosing regimen is based on two published clinical studies mentioned previously. No dose-response data exist for IV Artesunate and the Applicant did not conduct systematic dose-finding studies. Based on a published report¹⁰ in the literature, the systemic PK exposure (C_{max} and AUC) of AS and DHA following administration of IV Artesunate is similar in pregnant women and post-partum female patients.

¹⁰ Mcgready R, Phyo AP, Rijken MJ, et al. Artesunate/dihydroartemisinin pharmacokinetics in acute falciparum malaria in pregnancy: absorption, bioavailability, disposition and disease effects. Br J Clin Pharmacol. 2012;73(3):467-77.

Is an alternative dosing regimen or management strategy required for subpopulations based on intrinsic patient factors?

From a clinical pharmacology perspective, no alternative dosing regimens for IV AS are deemed necessary based on intrinsic patient factors. No clinically significant differences in the effectiveness of IV AS were observed based age, on sex and race.

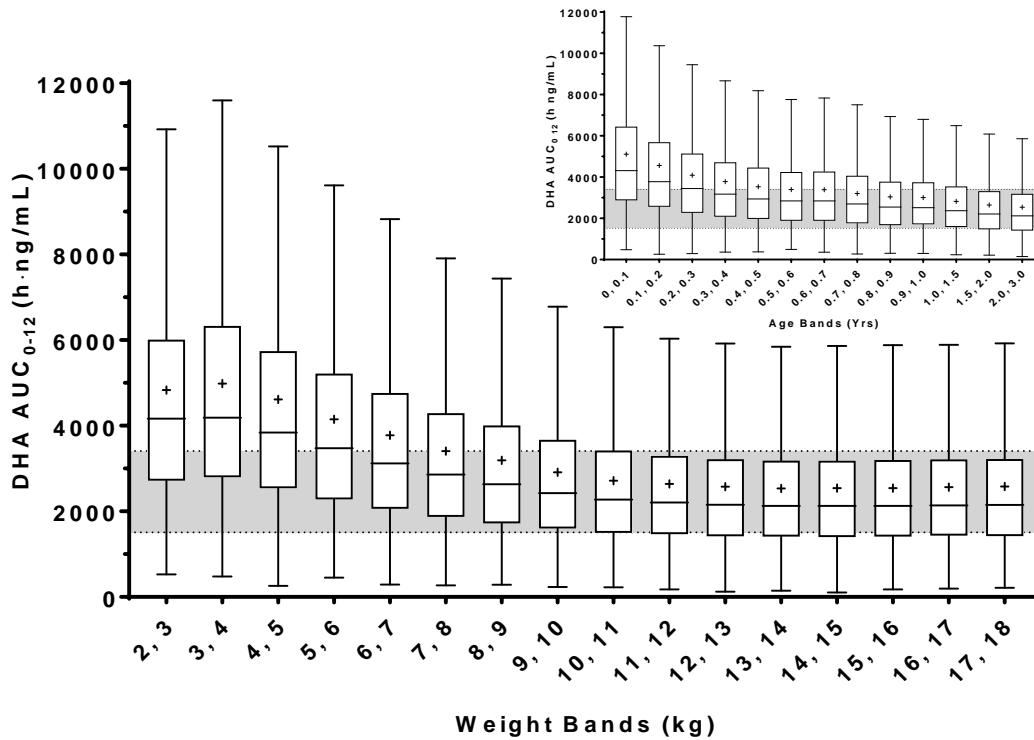
Pediatric Patients

The Applicant proposed the same dose of 2.4 mg/kg IV Artesunate for both adults and pediatric patients based on the results of the SEAQUAMAT and AQUAMAT trials. It is to be noted that the WHO recommends a higher dose of 3 mg/kg for pediatric patients <20 kg body weight. During the clinical pharmacology review, the Pharmacometrics review team evaluated the appropriateness of the WHO recommended dose of 3 mg/kg for patients <20 kg body weight by reviewing a publication by Zaloumis, et al¹¹ that described the population PK of IV artesunate in adults and pediatric patients with severe malaria. Additionally, patient data (adult and pediatric) were obtained from 6 different studies with diverse study designs and dosing scheme. The Pharmacometrics team reviewed the data and performed simulations to evaluate the appropriateness of the 2.4 mg/kg dosing regimen proposed by the Applicant in pediatric patients. The PK data were pooled from the different studies for pharmacokinetic analysis. Patients received either IM, intra-rectal (IR), or IV artesunate depending on study specific designs and dosing schemes. PK data following IV AS were used for population PK modeling; this included IV AS data collected in second periods for cross-over trials.

Based on the simulations (Figure 6-1), it was concluded that the 2.4 mg/kg dosing regimen is appropriate for all patients with severe malaria regardless of body weight or age. This dosing regimen is predicted to result in comparable or greater DHA exposure (AUC_{0-12}) in neonates and infants to that observed in older children and adults. For example, the average predicted AUC_{0-12} was up to 90% greater in pediatric patients with severe malaria weighing less than 6 kg (neonates and infants less than 3 months old) compared to children weighing 21 kg to 25 kg administered 2.4 mg/kg IV AS. The safety risk of this higher DHA AUC_{0-12} cannot be determined. However, given the severity of the disease, the clinical pharmacology review team believes that the benefits outweigh this unknown risk.

Figure 6-1: Simulated exposure (AUC_{0-12}) of DHA in patients with severe malaria after the first dose of IV AS (2.4 mg/kg) across a range of weights or ages (inset) using a PK model accounting for size and organ maturation.

¹¹ Zaloumis, CPT Pharmacometrics Syst. Pharmacol. (2014) 3, e145



Each box represents the 25th and 75th percentile of the DHA exposure measure. The bar and cross inside the box represents the median and mean respectively, whiskers represent 1.5 times the interquartile range. The gray band represents the interquartile range for patients weighing 20 to 25 kg or 8 to 10 years (inset). Clearance was estimated using a combination of allometric weight scaling with a sigmoid function to account for organ maturation.
Data source: Pharmacometrics reviewer's independent simulation of Zaloumis et al¹

A multiple-dose pediatric DHA PK and safety post-marketing commitment study is recommended by the clinical pharmacology review team to confirm the adequacy of the PK extrapolation approach in pediatric patients with severe malaria 1 month of age and older. For the PK assessment, a random sparse sampling population PK approach can be conducted with post-infusion PK sample(s) collected after any dose during treatment with 2.4 mg/kg IV AS. in pediatric patients <20 kg body weight. See section 18.4 for additional details of the Pharmacometric review.

Renal or Hepatic Impairment

The effect of renal or hepatic impairment on the pharmacokinetics of IV Artesunate was not studied by the Applicant. Given that patients with severe malaria present with some degree of hepatic and renal impairment and given that the adverse event profiles in patients with renal and hepatic impairment were similar to the adverse event profile in patients with normal hepatic and renal function, no dosage adjustments are deemed necessary in patients with any degree of hepatic and renal impairment

Are there clinically relevant drug-drug interactions, and what is the appropriate management strategy?

Yes, there are PK drug-drug interactions (DDIs) that potentially pose a clinically significant risk (efficacy loss) for IV AS.

Clinical DDI Studies

The Applicant did not conduct any dedicated DDI studies to evaluate whether AS and DHA are substrates/inhibitors/inducers of the CYP enzymes. However, the Applicant provided several published papers that evaluated interactions between oral artesunate and other medications such as mefloquine, malarone, amodiaquine, nevirapine, pyronaridine/ritonavir and sulfadoxine/pyrimethamine. Oral artesunate is not approved in the US and thus, these DDI studies should be interpreted with caution. Based on review of the literature papers, it was concluded that no significant interactions have been observed for most of the above-mentioned drugs. However, coadministration of IV AS with nevirapine and ritonavir should be avoided. A description of the results of these two published interaction studies between oral artesunate and nevirapine and ritonavir is given below:

Nevirapine

Co-administration of 200 mg oral artesunate with nevirapine¹² administered at a dose of 200 mg resulted in a decrease in C_{max} and AUC of DHA by 59% and 68%, respectively. However, it is to be noted that the authors evaluated oral artesunate in this study, which is not approved in the US. This reduction in systemic exposure is likely to result in the loss of efficacy of IV Artesunate. Based on this reduction of systemic exposure observed in this study, IV Artesunate should not to be given with NVP. Thus, co-administration of IV Artesunate with nevirapine and other CYP3A4 inducers should be avoided.

Ritonavir

Co-administration of 60 mg oral Artesunate with ritonavir¹³ administered at a dose of 100 mg twice daily for 17 days resulted in a 27% and 38% decrease in C_{max} and AUC of DHA. However, it is to be noted that the authors evaluated oral artesunate in this study, which is not approved in the US. This effect of reduced DHA exposure oral artesunate following coadministration with ritonavir could be due to induction effect of ritonavir on DHA metabolism via CYP3A, 1A2, 2C9, 2C19 and 2B6. It is expected that co-administration of ritonavir with IV AS will also decrease the C_{max} and AUC of DHA. Thus, co-administration of IV AS with ritonavir should be avoided.

Evaluation of Potential DDIs from In Vitro Studies

In vitro AS is not an inhibitor of the major drug metabolizing CYP enzymes CYP 1A2, 2B6, 2C9, 2C19, 2D6, or 3A4/5 based on maximal inhibition no greater than 4% at AS concentrations exceeding those observed in healthy volunteers by approximately 2-fold (see Appendix 21.4).

¹² Fehintola, AIDS Research and Treatment Volume 2012, Article ID 703604,

¹³ Morris, Am. J. Trop. Med. Hyg., 86(3), 2012, pp. 489–495

In vitro DHA is deemed to be a weak inhibitor of CYP 1A2 and 2C19 based on inhibition assays¹⁴. Additionally, a static mechanistic model (AUCR) using DHA's maximum concentration after IV administration of the proposed 2.4 mg/kg dose in patients with severe malaria and worst-case unbound fraction (39%) predicts an in vivo potential interaction with the values slightly past the threshold value (i.e., $R > 1.02$). However, when the typical unbound fraction (10%) is considered, no potential in vivo interaction is predicted ($R < 1.25$ threshold value). Taken together, the data suggest a low in vivo potential interaction for DHA.

¹⁴ Ericsson, Xenobiotica, 2014; 44(7): 615–626

7 Sources of Clinical Data and Review Strategy

7.1 Table of Clinical Studies

Table 7-1: Clinical Trials Relevant to this NDA

Trial Identity (Publication) [NDA submissions]	NCT no.	Trial Design (Objectives)	Regimen/ schedule/ route (IV AS formulation)	Study Endpoints (Primary and Key Secondary)	Treatment Duration/ Follow Up	No. of Patients Enrolled/ Randomized*	Study Population	No. of Centers and Countries
Controlled Studies to Support Efficacy and Safety								
SEAQUAMAT (Dondorp et al. 2005) [CSR reanalysis, protocol, CRF, datasets]	-	Ph 3 OL, R, active comparator (Efficacy and Safety)	IV AS 2.4 mg/kg 0, 12, 24 hr, then daily until po; or IV quinine 20 mg/kg over 4 hr, then 10 mg/kg over 2-8 hr 3x per day until po Concomitant doxycycline x 7 days except in children <8y and pregnant women Follow on: oral artesunate or quinine (Guilin)	1°: Mortality	Up to 7 days/Until death or discharge	730 731 N= 1461 total	Severe <i>P. falciparum</i> (<i>P.f.</i>) malaria (adults and children ≥ 2y)	Myanmar, Bangladesh, India, Indonesia (10 sites)
AQUAMAT (Dondorp et al. 2010) [publication only]	-	Ph3 OL, R, active comparator (Efficacy and Safety in children)	IV or IM AS 2.4 mg/kg at 0, 12, 24 hr, then daily until po; or IV or IM quinine 20 mg/kg over 4 hr, then 10 mg/kg over 2-8 hr 3x per day until po	1°: Mortality; 2°: Severe neuro sequelae at 28 days; combined death and neuro seq.	Until discharge; if incomplete neuro recovery at discharge, followed up to 12 mo.	2712 2713 N=5425 total	Severe <i>P.f.</i> malaria (children < 15 y)	9 African countries (11 sites)

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Trial Identity (Publication) [NDA submissions]	NCT no.	Trial Design (Objectives)	Regimen/ schedule/ route (IV AS formulation)	Study Endpoints (Primary and Key Secondary)	Treatment Duration/ Follow Up	No. of Patients Enrolled/ Randomized*	Study Population	No. of Centers and Countries
			Follow on: artemether/lumefantrine x 3 days (Guilin)					
EDCTP-MMV07-01 (Kremsner et al. 2012) [CSR, protocol, CRF] datasets, including PK data, not available for review	0052 213	Ph 2 DB, MC, R, parallel-group (Safety and efficacy of two dose IV AS regimens, PK)	IV AS 2.4 mg/kg at 0, 12, 24, 48 and 72 hr; or IV AS 4.0 mg/kg at 0, 24 and 48 hr Follow on: Sulfadoxine-pyrimethamine (Gabon); artemether/lumefantrine (Malawi) (US Army)	1°: % of ≥99% clearance parasitemia at 24 hr; 2°: Clinical response and parasite clearance on day 28; Safety: AEs or sign changes in labs, mortality, neuro seq.	72 hrs/28 days	100 94 N=194 (Total receiving at least one dose)	Severe <i>P.f.</i> malaria (children 6 mo to 10 y)	Gabon and Malawi (3 sites)
Studies to Support Safety								
R-CDC-060 (Twomey et al. 2015) [CSR, protocol, CRF, SAP, datasets]		Retrospective analysis of IND treatment study (Safety, tolerability, clinical benefits)	IV AS 2.4 mg/kg at 0, 12, 24, and 48 hr, then daily until tolerated oral med (up to max 7 days) Follow on: Not specified (US Army)	Safety: PE, VS, labs, AEs Efficacy: Time to neg parasitology; Time to follow on po	Up to 7 days	Enrolled Safety analysis pop n=102 (at least 1 dose)	Severe malaria (adults and children 1 – 72 y)	U.S.
Phase 2 Studies in Uncomplicated Malaria								
1168	0029	Ph 2 non-R, OL	IV AS 2.4 mg/kg x 3 days	1°: 99% parasite	3 days IV	30	Uncomplicated	Kenya

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Trial Identity (Publication) [NDA submissions]	NCT no.	Trial Design (Objectives)	Regimen/ schedule/ route (IV AS formulation)	Study Endpoints (Primary and Key Secondary)	Treatment Duration/ Follow Up	No. of Patients Enrolled/ Randomized*	Study Population	No. of Centers and Countries
(Li et al. 2014) [CSR, CRF, datasets]	8610	(safety, efficacy, PK) in uncomplicated malaria	Follow on: atovaquone/proguanil (US Army)	reduction at 48 hr, 2°: AEs and SAEs; Parasite clearance time, fever clearance time PK: Day 0 at 0, 5, 10, 20, 40 min; 1, 2, 4, 6 h	AS; 5 days with atovaquone /proguanil -Smears days 1-3, 7, 14 -Safety labs Day 3, repeat Day 7 if abnl	(enrolled and randomized) n=30 IV AS	<i>P.f.</i> malaria (adults males and nonpregnant females 18-65 y)	
1263ab [CSR, CRF, datasets]	0045 9615	Ph 2 R, OL (dose-ranging) in acute uncomplicated <i>P.f.</i> malaria	IV AS 1.2 mg/kg x 3 days IV AS 2.4 mg/kg x 3 days IV AS 2.4 mg/kg at 0, 12, 24, and 48 hrs IV AS 4.8 mg/kg x 3 days Follow on: mefloquine (Thailand) or atovaquone/proguanil (Kenya) (US Army)	1°: 90% parasite clearance at 48 hr, time to 20% and 100% parasite clearance. 2°: No. and severity of AEs	Hosp x 3 days: Out pt safety visits Days 7, 14 and 28 (VS, PE and labs); malaria smear Day 7)	25 25 25 n=100 IV AS	Uncomplicated <i>P.f.</i> malaria (<u>Thailand:</u> 18 - 65 y; Kenya: 5-65 y)	Thailand, Kenya
Other studies pertinent to the review of efficacy or safety (e.g., clinical pharmacological studies)								
(Li et al. 2009)	0029 2929	Ph 1 R, DB, PC single dose, dose escalation (safety, tolerability, ECG, PK)	IV AS 0.5 mg/kg IV AS 1.0 mg/kg IV AS 2.0 mg/kg IV AS 4.0 mg/kg IV AS 8.0 mg/kg Placebo	1°: Safety; AEs, HR, BP, ECG (0, 5, 20, 40 min; 1, 2, 4, 6, 24 hr)	1 injection; follow up - to Day 15 (safety, incl. hem, VS, ECG, PE)	6 6 6 6 10	Healthy adults (males and females 18-55 y)	U.S.

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Trial Identity (Publication) [NDA submissions]	NCT no.	Trial Design (Objectives)	Regimen/ schedule/ route (IV AS formulation)	Study Endpoints (Primary and Key Secondary)	Treatment Duration/ Follow Up	No. of Patients Enrolled/ Randomized*	Study Population	No. of Centers and Countries
[CSR, CRF, datasets, pdf ECGs]			(US Army)	2 ^o : PK (0, 5, 20, 40 min; 1, 2, 4, 6, 24 hr)		n=30 IV AS		
1142 (Miller et al. 2012) [CSR, CRF, datasets, pdf ECGs]	0029 2942	Ph 1 R, DB, PC multiple dose, dose escalation (safety, tolerability, ECG, PK)	IV AS 2.0 mg/kg x 3 days IV AS 4.0 mg/kg x 3 days IV AS 8.0 mg/kg x 3 days Placebo (US Army)	1 ^o : Safety; AEs, HR, BP, ECGs 2 ^o : PK (0, 5, 20, 40 min; 1, 2, 4, 6, 24 hr)	Treatment x 3 days: Safety follow up Day 15 (safety, incl. hem, VS, ECG, HCG, PE)	6 7 7 6 N=20 IV AS	Healthy adults (males and females 18-55y)	U.S.
Total IV AS exposure --Severe <i>P.f.</i> malaria indication --US Army formulation – (all doses and regimens)						--3,918 --3,718 --476		

Abbreviations: AS, artesunate; BID, twice daily; DB, double-blind; LTE, long-term extension study; MC, multi-center; N, number of subjects; OL, open-label; PC, placebo-controlled; PG, parallel group; R, randomized.

*N reflects number enrolled if non-randomized trial.

7.2 Review Strategy

In this NDA, evidence of clinical efficacy is based primarily on a single randomized, open-label clinical efficacy trial in patients 2 to 87 years of age with severe malaria, (South East Asian Quinine Artesunate Malaria Trial [SEAQUAMAT]), which evaluated 730 patients randomized to receive IV artesunate compared with 731 patients randomized to IV quinine ([Dondorp et al. 2005](#)).

Supportive evidence for efficacy was obtained from a randomized open-label trial (African Quinine Artesunate Malaria Trial [AQUAMAT], which studied 2715 pediatric patients administered IV artesunate compared with 2713 who received IV quinine ([Dondorp et al. 2010](#)) and a randomized dose-finding study in Africa (EDCTP-MMV07-01) which evaluated 194 pediatric patients with severe malaria receiving one of two dose regimens of IV artesunate. ([Kremsner et al. 2012](#))

For SEAQUAMAT, this application included a protocol dated June 30, 2003, an annotated and sample case report form (CRF), CSR (a re-analysis of dataset from SEAQUAMAT by U.S. Army and the (b) (4) Corporation in 2010), analysis datasets, and publication ([Dondorp et al. 2005](#)). According to the SEAQUAMAT Analysis Data Reviewer's Guide, the analysis files were derived from a single legacy dataset file provided by the Wellcome Trust. Review of AQUAMAT relied on the publication ([Dondorp et al. 2010](#)) only; no protocol, CRF, CSR, or datasets were provided by the Applicant. A SAP for a re-analysis of the data was written on 10/15/2008 by the (b) (4) Corporation after the study was conducted and results were published in 2005.

A Phase 2 study, EDCTP/MMV07-01, which compared a 5-dose regimen of artesunate at 2.4 mg/kg/dose with a 3-dose regimen of artesunate at 4.0 mg/kg/dose, each with the same total dose of 12 mg/kg, provided *P. falciparum* parasite clearance information at 24 hours in pediatric patients with severe malaria. The U.S. Army (WRAIR) formulation of IV artesunate was used in the study and was claimed to be equivalent to the Guilin formulation in the Clinical Study Report (CSR) by Medicines for Malaria Venture. Protocol, sample CRF, and CSR were provided for EDCTP/MMV07-01, with no datasets submitted.

None of the 3 trials were conducted by the Applicant.

8 Statistical and Clinical Efficacy Evaluation

8.1 Review of Relevant Individual Trials Used to Support Efficacy

8.1.1 SEAQUAMAT: A multicenter, open, randomized comparison between quinine and artesunate for the treatment of falciparum malaria in SE Asia. ([Dondorp et al. 2005](#))

8.1.1.1 Study Design

This was a multicenter, open-label, randomized, active comparator trial conducted between June 2003 and May 2005. The trial was conducted at 10 sites in 4 countries in Southeast Asia: Myanmar (7 sites), Bangladesh (1 site), India (1 site), and Indonesia (1 site). It was sponsored by the Wellcome Trust, with a study coordinating center at the Wellcome Trust Unit/Mahidol University in Thailand.

The study objectives were to compare the mortality of severe *falciparum* malaria treated with parenteral quinine and artesunate (primary) as well as significant sequelae, recovery times, and development of complications of severe malaria.

Patients with suspected malaria were admitted and examined. Malaria diagnosis was confirmed using HRP-2 (histidine rich protein 2) immunochromatographic test (Paracheck, Orchid Biosystems, Goa India), designed to detect *P. falciparum*; confirmatory microscopy was performed after randomization.

Key eligibility criteria included the following:

- Inclusion criteria
 - Clinical diagnosis of severe malaria
 - Positive test result for Paracheck
 - Age 2 years or older
 - Written informed consent by patient or relative
- Exclusion criteria
 - Known allergy to artemisinin derivatives or quinine
 - Convincing history of artesunate, mefloquine or significant quinine (>2g) intake in the previous 24 hours.

Eligible patients were randomized 1:1 to the parenteral artesunate or quinine group. The original randomization method was planned to use blocks of size 10 or 20, but unrestricted randomization was implemented according to the Statistical Analysis Plan (SAP).

Study drug (artesunate or quinine) was started with dosage based on body weight, as follows:

- **Artesunate:** Intravenous artesunate (Guilin No 2 Pharmaceutical Factory, Guangxi, China) 2.4 mg/kg on admission, 12 hours after admission, and thereafter once daily until able to take po.

Oral artesunate at 2 mg/kg/day was started once a patient was able to take tablets, to complete a total course (including parenteral) of 7 days; with total cumulative dose between 17 and 18 mg/kg over 7 days.

- **Quinine:** Intravenous quinine dihydrochloride (Government Pharmaceutical Organization, Thailand) 20 mg salt/kg loading dose infused over 4 hours (in 666 ml 5% dextrose water) over 2 hours three times a day until the start of oral therapy. Loading dose of quinine was not to be given if patient received partial pretreatment with quinine or mefloquine.

Oral quinine at 10 mg/kg every 8 hours was started once a patient was able to take tablets, to complete a total quinine course of 7 days.

During the study, supportive treatment was given to patient in line with guidelines provided ([World Health Organization 2000](#)). At the time of hospital discharge or death in the hospital, information on outcome was recorded. Complications and significant neurological sequelae were assessed in discharged patients.

Under the protocol, investigators were to replace any study center that enrolled primarily patients without severe malaria, with another center in the same country. Severe malaria was defined on study entry, with a single criterion listed below, in addition to asexual *P. falciparum* parasites on the peripheral blood smear ([Dondorp et al. 2005](#)):

- Glasgow coma scale <11/15 in adults, or Blantyre coma scale $\leq$ 3/5 in children.
- Shock, as assessed by the admitting physician (low blood pressure and cool peripheries)
- Blood bicarbonate <15 mmol/L
- Hematocrit < 20% and *P. falciparum* parasitemia >100,000/ μ L
- Visible jaundice and *P. falciparum* parasitemia > 100 000/ μ L
- Blood (BUN) > 17 mmol/L
- Asexual *P. falciparum* parasitemia >10%
- Plasma glucose <2.2 mmol/L
- Respiratory distress (>32 breaths/min)

The trial was open-label. Local investigators were only blinded until after patients were randomized. The trial center, microscopists assessing blood slides, and data analysts remained unaware of treatment allocation until the end of the study. An external data safety monitoring committee assessed the progress of the trial. The study monitors inspected randomization, drug boxes, and patient records. External monitors regularly inspected the trial sites. From the publication, it is not clear if study monitors and external monitors were the same individuals.

There was no information on compliance with Good Clinical Practices (GCP).

8.1.1.2 Study Endpoints

The following primary and secondary endpoints were defined in the protocol.

- Primary efficacy endpoint was in-hospital all-cause mortality measured to time of hospital discharge (approximately one week)

The protocol specified the main outcome measure as in-hospital mortality, but mortality, including both in hospital and after discharge were included as “in-hospital death” in the publication. In this review, we refer to in-hospital mortality as only deaths occurring in hospital and overall mortality as deaths occurring both in hospital and out of hospital.

- Secondary efficacy endpoints (in order of assigned priority):
 1. Combined incidence of in-hospital death or significant neurological sequelae
 2. Significant neurological sequelae (cortical blindness, cranial nerve palsies, ataxia, loss of balance, extrapyramidal rigidity, hemiplegia/paresis, neuropsychiatric, e.g. psychosis, other)
 3. Other significant sequelae
 4. Complications after hospital entry that were not present on admission (admission to ICU, development of coma, development of convulsions, development of shock, need for vasopressors, need for mechanical ventilation, need for dialysis, development of severe anemia, bleeding, blackwater fever, hypoglycemia, jaundice, sepsis, abortion during admission (pregnant patients), use of antibiotics, other)
 5. Fetal death as pregnancy outcome (pregnant patients)
 6. Time to death in days (with discharge at any time as additional category)
 7. Time to discharge in days (with death at any time as additional category)
 8. Time to eat in days (unaided)
 9. Time to sit in days (unaided)
- Safety: Drug exposure

8.1.1.3 Statistical Analysis Plan

A protocol was submitted, with very few statistical details. A SAP for a re-analysis of the data was written on 10/15/2008 (Version 2 by the (b) (4)) after the study results were published in 2005.

The intent-to-treat population (ITT) included all patients randomized in the study. The severe malaria population consisted of randomized patients having parasitemia on blood smear

fulfilling the criteria for severe malaria on post-hoc analysis using modified definitions by Hien et al. (1996), as previously listed.

In the protocol, Kaplan-Meier plots and Cox's regression analysis were specified for the primary and secondary outcome variables. In the SAP, Cochran-Mantel-Haenszel (CMH) method was specified for the primary efficacy analysis in the ITT population, stratified by site (hospital). The relative risk and odds ratios with 95% confidence intervals (CIs) would be calculated. We will report the results from both survival and binary outcomes.

According to the SAP, for sensitivity analyses, the primary efficacy endpoint in the ITT population would be analyzed using the logistic regression method. According to the study report dated June 2010, covariates used in the sensitivity analyses included age (child <15 years versus ≥ 15), gender, and severe malaria (yes/no).

Primary efficacy endpoint would be analyzed in the severe malaria population and non-severe malaria populations using the CMH method. Analyses of mortality would be conducted in the three populations using Pearson's Chi-squared test (with no stratification).

Secondary efficacy endpoint analyses of the incidence of significant sequelae, the overall incidence of significant sequelae, the combined incidence of mortality and any significant sequelae after hospital admission, the incidence of specific complications would be conducted using the logistic regression if there were sufficient incidence data to permit reliable statistical testing.

Fetal death or abortion as a pregnancy outcome would be summarized for the pregnant ITT patients. Besides time to death, time to discharge, to eat, and to sit were analyzed using survival analysis.

A sample size of 2000 was calculated for a detection of a 33% reduction in mortality from 12% to 8% with a statistical power of 80% and a two-sided Type I error of 5%, allowing a drop-out rate of 5%. The mortality estimate was approximately half the mortality rate of 24% from patients meeting the modified WHO criteria for severe malaria.

According to the CSR, the protocol specified an interim analysis by an external Data Safety Monitoring Committee (DSMC) after inclusion of 500 patients and subsequently at time points chosen by the committee. The protocol did not provide a formal pre-specified plan to control the Type 1 error and there were no formal stopping rules pre-specified. The DSMC was advised that the clinical practice should only be changed if there was compelling evidence of at least a 25% treatment effect for artesunate compared with quinine. The DSMC was chaired by Prof. T. Peto, Oxford University, Oxford, UK.

The first interim analysis was conducted after 659 patients completed the study. A second interim analysis was conducted when 1294 patients (about two thirds of the planned sample size) completed the trial with 244 reported deaths, which exceeded the number of the deaths projected for a completed trial. The p-value from the interim analysis was 0.0007. The trial was stopped after the second interim analysis. After termination of the trial, unblinded analysis results of 1461 patients were published. The estimated treatment effect in the publication was slightly smaller than that observed in the second (also the final) interim analysis.

The primary endpoint was planned to be analyzed with a two-tailed test with a Type I error =0.05 at the final analysis with the planned sample size. There was no consideration for Type I error control due to the interim analyses. The method used to control the Type I error should have been prespecified in the analysis plan of the study. The Applicant considered this for the interim analyses and claimed that the test statistic would have crossed the stopping boundary had either of two commonly used stopping criteria been proposed, the O'Brien-Fleming or the Haybittle-Peto procedure with 3 to 5 interim analyses planned. Using either method, the study would have stopped early with the results seen at the second interim analysis. Additional discussion of this can be found in Statistical Issues 8.3.

8.1.1.4 Study Results

Compliance with Good Clinical Practices

This information is not available.

8.1.1.5 Financial Disclosure

This trial was supported by the Wellcome Trust. No information is available for financial disclosure from the Applicant.

Patient Disposition

A total of 1461 patients were enrolled and randomized in the trial, as shown in the following table. All patients received the assigned treatment and were included in the ITT population. About 72% of the patients had severe malaria. There were 10 hospitals (sites) in the study, enrolling from 2 to 288 patients per hospital. Although the study report states that two sites from Myanmar were replaced, the data sets and analyses did not exclude these two sites (155 patients).

Table 8-1: SEAQUAMAT Intention to Treat and Severe Malaria Populations

	Artesunate (N=730)	Quinine (N=731)	Totals (N=1461)
Enrolled/ Randomized/treated/ITT population	730	731	1461
Severe malaria population	509 (69.7%)	541 (74.0%)	1050 (71.9%)

Protocol Violations/Deviations

The original plan was to assign a center to a route of administration (IV or IM) for the study drugs. However, all centers administered the study drugs IV. Additionally, a few centers (Chittagong Hospital in Bangladesh, the Ispat Hospital in Orissa, India) did not administer doxycycline as part of the sequential therapy with oral study drug as planned.

A total of 143 patients in the quinine group did not receive a loading dose. Of these, 105 received no pre-treatment with quinine, which appears to be a deviation from the assigned treatment protocol. Specific information on protocol deviations was not captured in the database.

Demographic Characteristics

Demographic characteristics are shown in the following table. About 13.8% of subjects were under the age of 15 years and 2.6% were 65 years old or older. The majority of the subjects were male. Age, sex, and body weight were well balanced between the two treatment groups. The proportion of subjects with severe malaria or cerebral malaria was slightly lower in the artesunate group than in the quinine group. Therefore, efficacy analysis by these two diagnoses will be conducted.

Table 8-2: SEAQUAMAT Demographic Characteristics (ITT Population)

	Artesunate (N=730)	Quinine (N=731)	All (N=1461)
Age (years)			
Mean [SD]	27.9 [14.9]	27.9 [15.3]	27.9 [15.1]
Median	25	25	25
Range	2-85	2-87	2-87
2 to <15	97 (13.3%)	105 (14.4%)	202 (13.8%)
2 to <5	37 (5.1%)	33 (4.5%)	70 (4.8%)
5 to <10	20 (2.7%)	33 (4.5%)	53 (3.6%)
10 to <15	40 (5.5%)	39 (5.3%)	79 (5.4%)
≥15	633 (86.7%)	626 (85.6%)	1259 (86.2%)
≥65	17 (2.3%)	21 (2.9%)	38 (2.6%)
Body weight (kg)			
Number of subjects with data	729	729	1358
Mean [SD]	47.1 [13.11]	47.6 [14.13]	47.3 [13.63]
Median	50	50	50
Range	9-85	9-100	9-100
<20 kg	52 (7.1%)	57 (7.8%)	109 (7.5%)
≥20 kg	677 (92.7%)	672 (91.9%)	1349 (92.3%)
Missing	1 (0.14%)	2 (0.27%)	3 (0.21%)
Sex			
Male	546 (74.8%)	529 (72.4%)	1075 (73.6%)
Female	184 (25.2%)	202 (27.6%)	386 (26.4%)

	Artesunate (N=730)	Quinine (N=731)	All (N=1461)
Country*			
Myanmar	295 (40.4%)	282 (38.6%)	577 (39.5%)
Bangladesh	222 (30.4%)	231 (31.6%)	453 (31.0%)
Indonesia	143 (19.6%)	146 (20.0%)	289 (19.8%)
India	70 (9.6%)	72 (9.8%)	142 (9.7%)
Severe Malaria Population Flag	509 (69.7%)	541 (74.0%)	1050 (71.9%)
Cerebral Malaria Flag	284 (38.9%)	304 (41.6%)	588 (40.2%)

*Data on Race and Ethnicity not collected.

Source: Clinical Reviewer (J Review)

The following table reports the number of pregnant women in the trial and the gestation time. In general, the two treatment groups were comparable. The artesunate group had a slightly shorter mean and median gestation time at baseline than the quinine group.

Table 8-3: SEAQUAMAT Pregnancy Status on Admission (ITT Population)

	Artesunate (N=730)	Quinine (N=731)	All (N=1461)
Number of Female Subjects	184	202	386
Pregnant	23 (13%)	26 (13%)	49
Estimated Gestation Time (Weeks)			
Number of subjects with data	23	26	49
Mean [SD]	24.35 [6.46]	27.38 [7.94]	25.96 [7.37]
Median	24	30	26
Range	8-36	8-40	8-40

Source: Clinical Reviewer (J Review 13.1) and Statistical Reviewer

Other Baseline Characteristics

Pre-treatment was the treatment received prior to admission, which included antimalarial drugs, such as quinine, artemisinin derivative, chloroquine, and sulphadoxine-pyrimethamine. About 21% of the patients were pre-treated with an antimalarial drug and 16.6% with an effective antimalarial drug (quinine, artemisinin derivative, or mefloquine). About 13% of the patients were pre-treated with quinine, the most commonly used antimalarial drug. The artesunate group had a slightly higher proportion of pre-treatment with quinine. As chloroquine was not considered as an effective antimalarial drug in this trial, the slightly higher proportion of patients using chloroquine in the artesunate group should not be a concern.

Table 8-4: SEAQUAMAT Pretreatment with Antimalarial Drugs and Antibiotics (ITT Population)

	Artesunate (N=730)	Quinine (N=731)	All (N=1461)
Antimalarial Drug	167 (22.9%)	142 (19.4%)	309 (21.1%)

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

	Artesunate (N=730)	Quinine (N=731)	All (N=1461)
Effective Antimalarial drug*	125 (17.1%)	118 (16.1%)	243 (16.6%)
Quinine	103 (14.1%)	84 (11.5%)	187 (12.8%)
Chloroquine	43 (5.9%)	21 (2.9%)	64 (4.4%)
Artemisinin Derivative	25 (3.4%)	42 (5.7%)	67 (4.6%)
Antibiotics	15 (2.1%)	31 (4.2%)	46 (3.1%)
Sulfadoxine Pyrimethamine	9 (1.2%)	10 (1.4%)	19 (1.3%)
Doxycycline	2 (0.3%)	1 (0.1%)	3 (0.2%)
Mefloquine	0 (0.0%)	5 (0.7%)	5 (0.3%)

Source: Clinical Reviewer (J Review 13.1)

*Effective antimalarial drugs included quinine, artemisinin derivative, or mefloquine.

Malaria status on admission is shown in the following table. The first row reports the results from Paracheck, a rapid diagnostic test. The second row reports the results from the malaria smear for *P. falciparum*. There was a high correlation between the two and the results were balanced between the treatment arms. The 3rd row reports any malaria parasites. The percentage of subjects with hyperparasitemia (>10% infected erythrocytes) and the mean of parasitemia percentage [of erythrocytes] were slightly numerically higher in the artesunate group, which is not a concern in the evaluation of the efficacy of artesunate. Baseline parasite count and parasitemia percentage were slightly higher in the artesunate group than in the quinine group. Again, this was not a concern for evaluating the efficacy of artesunate.

Table 8-5: SEAQUAMAT Malaria Status and Parasite Count on Admission (ITT Population)

	Artesunate (N=730)	Quinine (N=731)	All (N=1461)
Paracheck result: <i>P. falciparum</i> , n(%)	691 (94.7%)	699 (95.6%)	1390 (95.1%)
Positive Blood Smear Flag*, n(%)	689 (94.4%)	693 (94.8%)	1382 (94.6%)
Malaria Parasites on Blood Film Flag, n(%)	708 (97.0%)	716 (97.9%)	1424 (97.5%)
Hyperparasitemia (>10%) Flag, n(%)	121 (16.6%)	108 (14.8%)	229 (15.7%)
Baseline Parasite Count			
Number of subjects with data	729	730	1459
Mean [SD]	180446.81 [300068.95]	170454.78 [339752.33]	175447.37 [320467.99]
Median	52500.8	49738.0	51119.2
Range	0-2481856	0-3661365.5	0-3661365.5
Parasitemia Percentage			
Number of subjects with data	730	731	1461
Mean [SD]	5.28 [8.67]	4.87 [8.97]	5.08 [8.82]
Median	1.60	1.40	1.50
Range	0-61.2	0-80.22	0-80.22

Source: Clinical Reviewer (J Review) and Statistical Reviewer (SAS)

*The diagnosis of *falciparum* malaria based on the presence of asexual *P. falciparum* parasites on the peripheral blood smear

Complications on admission are shown in the following table. These complications were well balanced between the two treatment groups. Note the variable severe anemia was from the severe malaria flag (trial inclusion criteria), rather than the laboratory value (severe malaria [Hb < 5 g/dL]).

Table 8-6: SEAQUAMAT Complications on Admission (ITT Population)

Reported Term for Clinical Event	Artesunate (N=730)	Quinine (N=731)	All (N=1461)
Clinical Jaundice	355 (48.6%)	349 (47.7%)	704 (48.2%)
Suspected Severe Metabolic Acidosis	308 (42.2%)	334 (45.7%)	642 (43.9%)
Coma at Admission	308 (42.2%)	321 (43.9%)	629 (43.1%)
Coma (GCS<11 or BCS <3)	284 (38.9%)	304 (41.6%)	588 (40.2%)
Severe Prostration	263 (36.0%)	270 (36.9%)	533 (36.5%)
Severe Anemia (from Inclusion Criteria)	174/703 (24.8%)	178/699 (25.5%)	352/1402 (25.1%)
Anuria/oliguria	99 (13.6%)	135 (18.5%)	234 (16.0%)
Convulsions	89 (12.2%)	87 (11.9%)	176 (12.0%)
Respiratory Insufficiency	79 (10.8%)	96 (13.1%)	175 (12.0%)
Shock (Clinical)	78 (10.7%)	92 (12.6%)	170 (11.6%)
Hypotension	37 (5.1%)	59 (8.1%)	96 (6.6%)
Other Reasons	25 (3.4%)	26 (3.6%)	51 (3.5%)
Black Water Fever	20 (2.7%)	16 (2.2%)	36 (2.5%)
Hypoglycemia	8/701 (1.1%)	17/693 (2.5%)	25/1394 (1.8%)
Suspected Severe Metabolic Acidosis	308/662 (46.5%)	334/648 (51.5%)	642/1310 (49.0%)

Source: Clinical Reviewer (J Review) and Statistical Reviewer

Regarding coma scales on admission, the two treatment groups were comparable, except for a numerically higher proportion of Blantyre Coma Score < 3 in the quinine group (but the sample sizes were too small to make a reliable conclusion).

Table 8-7: SEAQUAMAT Coma Scales on Admission (ITT Population)

Coma Scales on Admission	Artesunate (N=730)	Quinine (N=731)	All (N=1461)
Total Glasgow Score on Admission (GCSAD)			
Number of subjects with data	711	715	1426
Mean [SD]	11.44 [3.64]	11.26 [3.71]	11.35 [3.67]
Median	12	12	12
Range	3-15	3-15	3-15
GCSAD <11	281/711 (39.5%)	300/715 (42.0%)	581/1426 (40.7%)
Glasgow Scores on Admission			
GCSAD Eye Opening			
Mean [SD]	3.28 [1.00]	3.17 [1.02]	3.22 [1.01]
Median	4	4	4

Coma Scales on Admission	Artesunate (N=730)	Quinine (N=731)	All (N=1461)
Range	1-4	1-4	1-4
GCSAD Motor			
Mean [SD]	4.77 [1.45]	4.69 [1.47]	4.73 [1.46]
Median	5	5	5
Range	1-6	1-6	1-6
GCSAD Verbal			
Mean [SD]	3.40 [1.58]	3.40 [1.55]	3.40 [1.57]
Median	4	4	4
Range	1-4	1-4	1-4
Total Blantyre Coma Score on Admission (BCSAD)			
Number of subjects with data	19	17	36
Mean [SD]	3.89 [1.37]	3.53 [1.50]	3.72 [1.43]
Median	0	0	0
Range	1-5	0-5	0-5
BCSAD <3	3/19 (15.8%)	4/17 (23.5%)	7/36 (19.5%)
Blantyre Coma Scores on Admission			
BCSAD Eye Opening			
Mean [SD]	0.74 [0.45]	0.47 [0.51]	0.61 [0.49]
Median	1	1	1
Range	0-1	0-1	0-1
BCSAD Motor			
Mean [SD]	1.58 [0.69]	1.65 [0.61]	1.61 [0.64]
Median	2	2	2
Range	0-2	0-2	0-2
BCSAD Verbal			
Mean [SD]	1.58 [0.61]	1.41 [0.71]	1.50 [0.65]
Median	2	2	2
Range	0-2	0-2	0-2

Source: Clinical Reviewer [J Review] with Statistical Reviewer's edits

The days of fever and coma prior to admission were comparable between the two treatment groups, as the following table shows.

Table 8-8: SEAQUAMAT Days of Fever and Coma Prior to Admission (ITT Population)

	Artesunate (N=730)	Quinine (N=731)	All (N=1461)
Days of Fever Prior Admission			
Number of subjects with data	728	729	1457
Mean [SD]	6.43 [8.01]	7.00 [8.25]	6.71 [8.13]
Median	5	5	5
Range	0-120	0-120	0-120
Days of Coma Prior Admission			

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

	Artesunate (N=730)	Quinine (N=731)	All (N=1461)
Number of subjects with data	611	601	1212
Mean [SD]	0.40 [0.62]	0.51 [0.81]	0.46 [0.72]
Median	0	0	0
Range	0-4	0-7	0-7

Source: Clinical Reviewer (J Review) with Statistical Reviewer's edits

The following table shows some laboratory measurements. Hemoglobin, glucose, and blood urea nitrogen were comparable between the two treatment groups.

Table 8-9: SEAQUAMAT Laboratory-Defined Conditions on Admission (ITT Population)

	Artesunate (N=730)	Quinine (N=731)	All (N=1461)
Hemoglobin			
Number of subjects with data	683	675	1358
Mean [SD]	9.97 [3.24]	9.83 [3.45]	9.90 [3.35]
Median	10.0	10.0	10.0
Range	2.6-20.0	0.8-20.0	0.8-20.0
Hemoglobin <5 g/dL (severe anemia)	40/683 (5.9%)	54/675 (8.0%)	94/1358 (6.9%)
Glucose (mg/dL)			
Number of subjects with data	701	693	1394
Mean [SD]	136.85 [78.99]	136.52 [83.97]	136.69 [81.47]
Median	117.0	118.0	118.0
Range	0, 700.0	0, 700.0	0, 700.0
Blood Urea Nitrogen (mg/dL)			
Number of subjects with data	692	680	692
Mean [SD]	40.14 [37.29]	43.94 [39.14]	40.14 [37.29]
Median	26.0	29.0	26.0
Range	3.0, 292.0	3.0, 243.0	3.0, 292.0

Source: Clinical Reviewer [J Review] with Statistical Reviewer's edits

Baseline vital signs are shown in the following table. The vital signs were well balanced between the two treatment groups.

Table 8-10: SEAQUAMAT Baseline Vital Signs (ITT Population)

	Artesunate (N=730)	Quinine (N=731)	All (N=1461)
Baseline Temperature			
Number of subjects with data	729	727	1456
Mean [SD]	38.05 [1.15]	38.07 [1.20]	38.06 [1.17]
Median	38	37.90	38
Range	35-45	33.8-41.5	33.8-45

	Artesunate (N=730)	Quinine (N=731)	All (N=1461)
Baseline Systolic Blood Pressure			
Number of subjects with data	717	724	1441
Mean [SD]	103.14 [19.07]	102.38 [19.24]	102.76 [19.16]
Median	100	100	100
Range	30-200	30-180	30-200
Baseline Diastolic Blood Pressure			
Number of subjects with data	716	724	1440
Mean [SD]	63.94 [14.31]	62.90 [15.01]	63.42 [14.67]
Median	60	60	60
Range	0-120	0-100	0-120
Baseline Respiratory Rate			
Number of subjects with data	728	730	1458
Mean [SD]	28.54 [11.21]	29.30 [11.15]	28.92 [11.18]
Median	24	28	26
Range	0-106	12-88	0-106
Baseline Weight			
Number of subjects with data	729	729	1458
Mean [SD]	47.05 [13.11]	47.63 [14.13]	47.34 [13.63]
Median	50	50	50
Range	9-85	9-100	9-100

Source: Clinical Reviewer (J Review) with Statistical Reviewer's edits

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

Treatment compliance was not evaluated in the study since treatment was administered by nurses in a hospital setting.

A total of 588 subjects in the quinine group (80.4%) received the loading dose, 93 of whom were pre-treated with antimalarial drugs (46 with quinine and 47 with other). A total of 143 subjects (19.6%) in the quinine group did not receive the loading dose of quinine, 94 of whom were not pretreated with antimalarial drugs, and 49 of whom were pretreated with antimalarial drugs (38 with quinine and 11 with other drugs). It is not clear why some subjects did not receive the loading dose.

Table 8-11: SEAQUAMAT Antimalarial Pretreatment and Quinine Loading Dose in the Quinine Group (ITT Population)

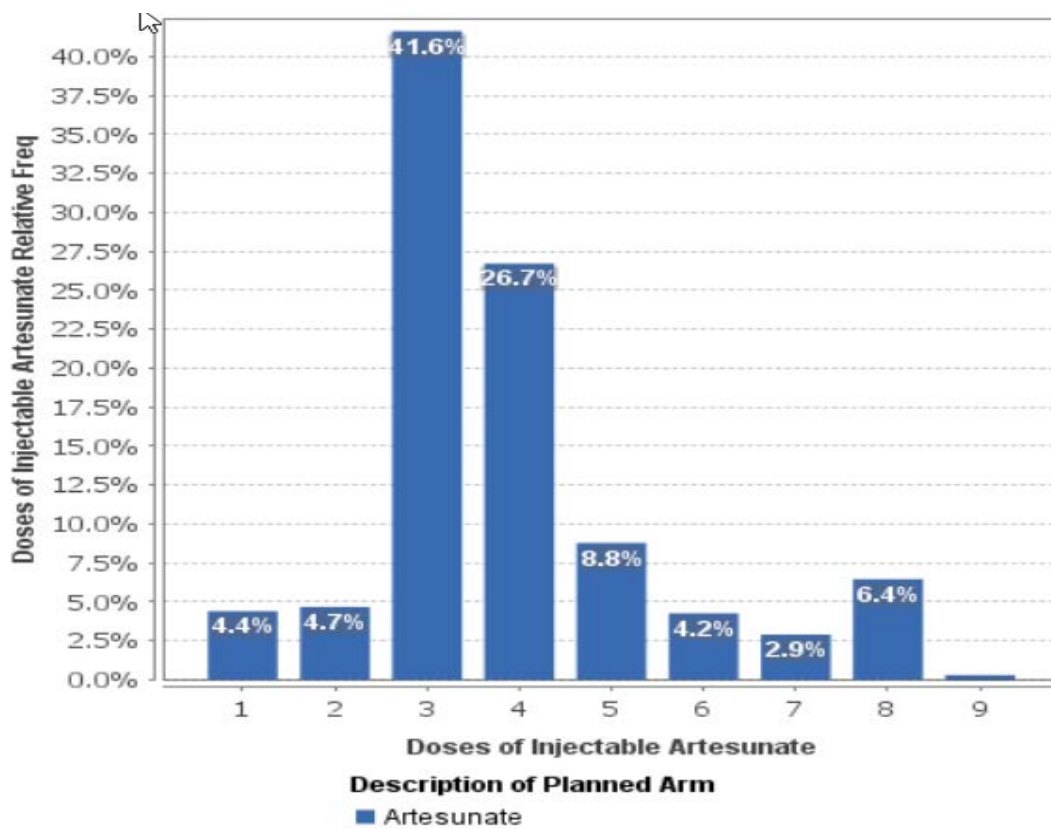
Pretreated with antimalarial drug	Pretreated with quinine	Loading dose of quinine
Yes: 142	Yes: 84	Yes: 46
		No: 38
	No: 58	Yes: 47
		No: 11
No: 589	No: 589	Yes: 495
		No: 94*

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

*One subject (b) (6) from Myanmar died on day 1 and received no quinine.

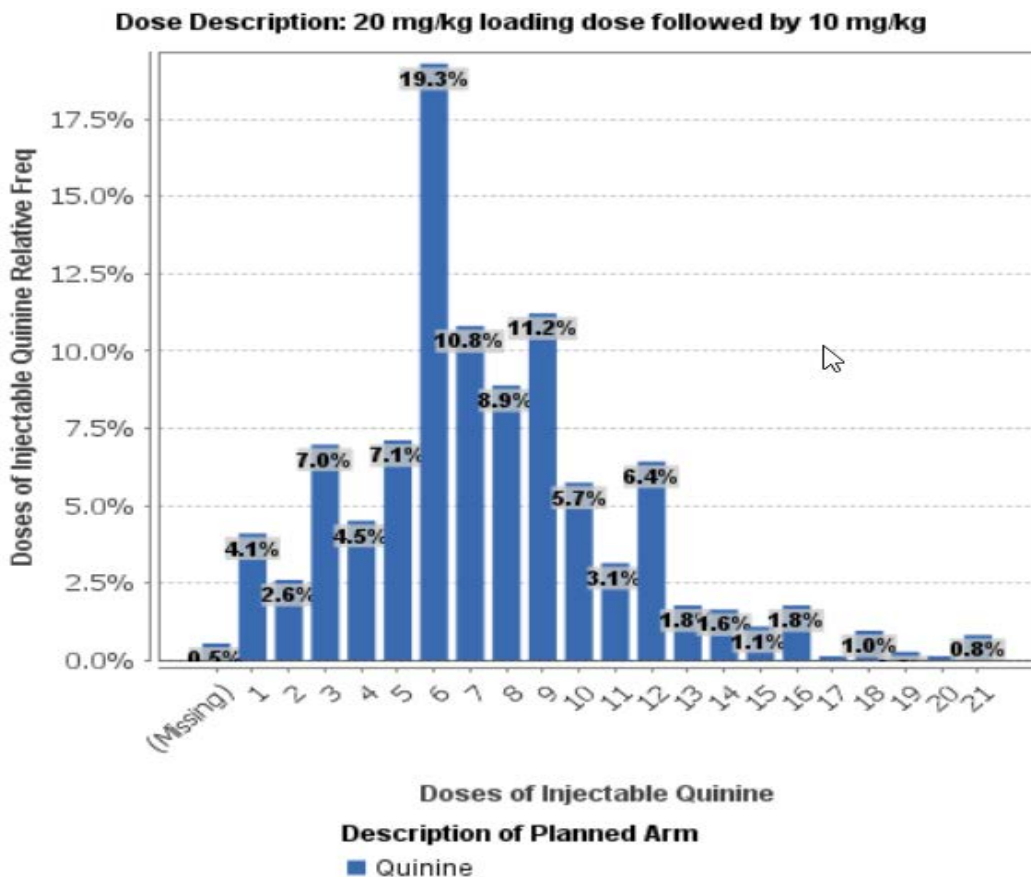
The total doses of injectable artesunate (dosed once a day) or quinine (dosed three times a day) are summarized in the following figures. It is noted that 4 subjects in the quinine group had missing doses. The majority of the subjects in the artesunate group were administered 3 (41.6%) or 4 (26.7%) doses of IV artesunate. It was not clear how many oral doses of follow-on were taken by the subjects as this information was not captured in the datasets.

Table 8-12. SEAQUAMAT Total Doses of IV Artesunate (%) (ITT Population)



Source: Clinical Reviewer (J Review)

Table 8-13. SEAQUAMAT Total Doses of Quinine (Including loading dose)* (ITT Population)



Source: Clinical Reviewer (J Review 13.1)
 *Does not include doses of quinine prior to admission.

About 26% and 31% of subjects in the two groups received concomitant medications. The most common ones were ceftriaxone and ampicillin.

Efficacy Results – Primary Endpoint

The primary efficacy endpoint, in-hospital mortality, was significantly lower in the artesunate group (Table 8-14), based on the statistical reviewer’s analysis. The odds ratio (OR) from the stratified CMH analysis was 0.59 [95% CI: 0.44, 0.79], stratified by study site (hospital) with a p-value of 0.0002. The Breslow-Day test for homogeneity of the ORs across strata for in-hospital mortality did not detect a difference across sites (p-value 0.28). The OR from unstratified analysis of in-hospital mortality was 0.59 [95% CI: 0.44, 0.78] with a p-value of 0.0002. The overall mortality demonstrated a similar result as the in-hospital mortality (OR=0.60, 95% CI [0.45, 0.79], stratified by study site (hospital) with a p-value of 0.0002). The Breslow-Day test for homogeneity of the ORs across strata for overall mortality did not detect a difference across sites (p-value 0.34). The results for overall mortality were reported in the publication and CSR.

The OR from unstratified analysis of overall mortality was 0.59 [95% CI: 0.45, 0.78] with a p-value of 0.0001, based on the statistical reviewer’s analysis.

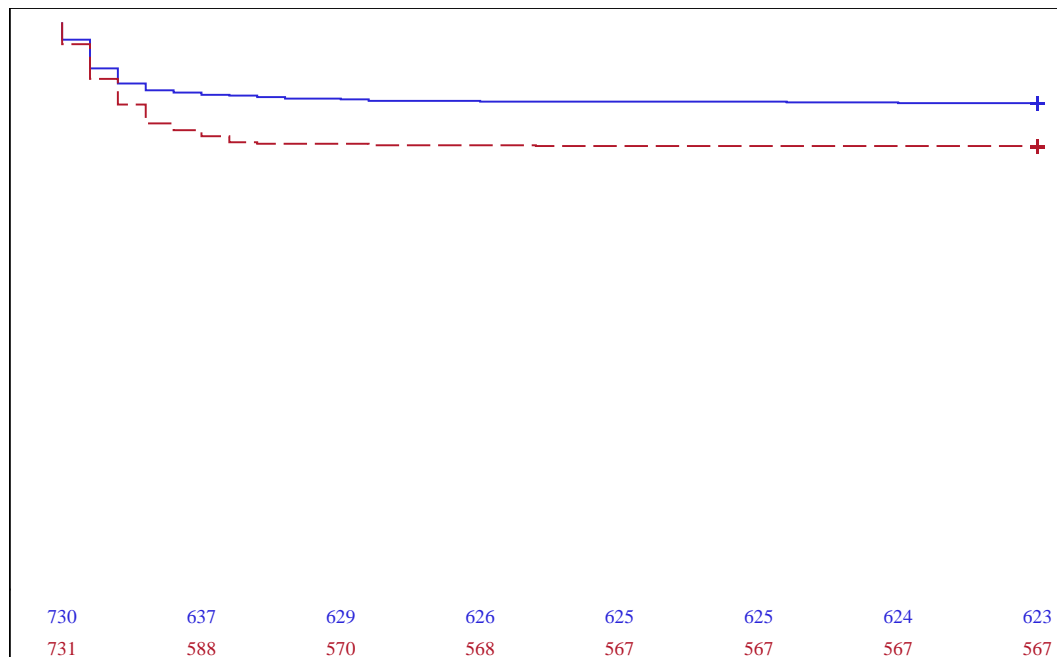
Table 8-14: SEAQUAMAT Outcome Summary Table (ITT Population)

Outcome	Artesunate (N=730)	Quinine (N=731)	OR [95% CI] p-value
In-hospital mortality	96 (13.2%)	150 (20.5%)	0.59 [0.44, 0.79] 0.0002
Overall mortality	107 (14.7%)	164 (22.4%)	0.60 [0.45, 0.79] 0.0002
Death within 48 hours of entry	61 (8.4%)	75 (10.3%)	
Death after 48 hours of entry	46 (6.3%)	89 (12.2%)	
Death on Day0	23 (3.2%)	29 (4.0%)	
Death on Day1	38 (5.2%)	46 (6.3%)	
Death on Day2	20 (2.7%)	34 (4.7%)	
Death on Day3	9 (1.2%)	25 (3.4%)	
Death after Day 3	17 (2.3%)	30 (4.1%)	

Source: Statistical Reviewer (SAS). The odds ratio was from CMH method, stratified by study site. OR: odds ratio.

The Kaplan-Meier survival curves of overall mortality are shown in the following figure. Subjects who did not die were censored at Day 35. The numbers of patients at risk are shown above the x-axis. The p-value from a Log-rank test was 0.0002 from the statistical reviewer’s analysis, indicating a statistically significant difference in the time to death between the two groups.

Figure 8-1. SEAQUAMAT Kaplan-Meier Survival Curve (ITT Population)



Source: Statistical Reviewer (SAS)

Although the objective of the trial was to assesses the effect of IV artesunate in severe malaria, approximately 28% of the patients enrolled were not considered as having severe malaria. The following table contains the results in the sub-population with severe malaria at baseline. The mortality rates in this population were higher than in the full analysis population, as expected, but the treatment effect was similar to that seen with the full analysis population and artesunate demonstrated a statistically significant treatment effect.

Table 8-15. SEAQUAMAT Mortality Rate (Severe Malaria Population)

	Artesunate	Quinine	OR [95% CI]	P-value
In-hospital Mortality	92/509 (18.1%)	139/541 (25.7%)	0.65 [0.48, 0.88]	0.0045
Overall Mortality	101/509 (19.8%)	152/541 (28.1%)	0.64 [0.48, 0.87]	0.0031

P-value was from the CMH test, stratified by site. Breslow-Day test of heterogeneity p-values were 0.08 and 0.122 for in-hospital mortality and overall mortality, respectively. The p-values from an unstratified Chi-squared test for in-hospital mortality and overall mortality by the statistical reviewer were 0.0029 and 0.0018, respectively.

Efficacy Results – Secondary and other relevant endpoints

Results from selected secondary endpoints are presented in Table 8-16. The combined incidence of overall death or significant neurological sequelae was statistically significantly lower in the artesunate group than in the quinine group. The OR was 0.63 with a 95% CI of [0.48, 0.82] and p-value of 0.0007 from the CMH test, stratified by study site (hospital) (Breslow-Day test for homogeneity of the ORs was 0.31).

Neurological sequelae in the CRF included cortical blindness, cranial nerve palsies, ataxia, loss of balance, extrapyramidal rigidity, hemiplegia/paresis, neuropsychiatric, e.g. psychosis, other. Ten patients were discharged with significant neurological sequelae as captured in the CRF with the overall question on significant neurological sequelae (yes/no), 7 in the artesunate group and 3 in the quinine group. The listed sequelae for those subjects were identified from each sequela question. An additional 5 and 4 subjects had specific neurological sequelae on discharge, but not captured in the CRF overall question.

Table 8-16: SEAQUAMAT Secondary Endpoint Summary Table (ITT Population)

Outcome	Artesunate (N=730)	Quinine (N=731)	All (N=1461)
Combined outcome (overall death or significant neurological outcome)	114 (15.6%)	167 (22.8%)	281 (19.2%)
Neurological sequelae on discharge based on overall question	7* (1.0%)	3 (0.4%)	10 (0.7%)
Ataxia	1 (0.1%)	0	1 (0.1%)
Hemiplegia/paresis	1 (0.1%)	0	1 (0.1%)
Loss of balance	3 (0.4%)	1 (0.1%)	4 (0.3%)
Neuropsychiatric (like psychosis)	3 (0.4%)	2 (0.3%)	5 (0.3%)
Other [†]	2 (0.3%)	0	2 (0.1%)
Number of subjects with neurological sequelae on discharge based on each sequela question	12 (1.6%)*§	7 (1.0%)	19 (1.3%)
Ataxia	2 (0.3%)	0 (0%)	2 (0.1%)
Loss of balance	4 (0.5%)	3 (0.4%)	7 (0.5%)
Paresis	2 (0.3%)	0 (0%)	2 (0.1%)
Neuropsychiatric (like psychosis)	4 (0.5%)	4 (0.5%)	8 (0.5%)
Other [‡]	4 (0.5%)	0 (0%)	4 (0.3%)

Source: Clinical Reviewer (J Review) and Statistical Reviewer Event numbers were by subject.

*One subject had 4 events (ataxia, loss of balance, neuropsychiatric [like psychosis], and other [tremor]).

§One subject had 2 events (ataxia and loss of balance). †Including tremor, mono paresis (left upper limb).

‡Including tremor, mono paresis (left upper limb), generalized myopathic weakness, confused and restlessness.

Additional time-to-event variables (death, discharge, eating independently, sitting, and speaking) are summarized in the following table as descriptive statistics for subjects with data available. The number of subjects with non-missing data is included in the table. The means and medians were comparable between the treatment groups. The comparable time to

discharge was likely to indicate that the two groups had comparable observation time for the assessment of the in-hospital mortality, minimizing the possibility that the reduced mortality in the artesunate group was due to short in-hospital observation time. Note that a subject's death can cause a subject to have a missing time to discharge, speak, sit and eat. As these data were supposed to be collected for discharged patients based on the CRF, we summarized the data excluding in-hospital deaths. The results indicated that the artesunate group had slightly shorter time to sit and to eat and almost the same time to speak.

Table 8-17: SEAQUAMAT Time to Event Summary (ITT Population)

	Artesunate (N=730)	Quinine (N=731)	All (N=1461)
Time to death in days			
Number of subjects	107	164	271
Mean [SD]	2.53 [4.39]	2.23 [2.29]	2.35 [3.28]
Median	0	0	0
Range	0-30	0-17	0-30
Time to discharge in days			
Number of subjects with data	623	565	1188
Mean [SD]	6.88 [5.18]	6.78 [5.03]	6.83 [5.11]
Median	5	5	5
Range	0-54	0-45	0-54
Time to eat independently in days			
Number of subjects with data	653	634	1287
Mean [SD]	2.36 [2.87]	2.38 [3.32]	2.37 [3.10]
Median	1	1	1
Range	0-21	0-47	0-47
<i>Excluding in-hospital deaths</i>			
Number of subjects with data	601	554	1155
Mean [SD]	2.57 [2.90]	2.72 [3.42]	2.64 [3.16]
Median	2	2	2
Range	0-21	0-47	0-47
Time to sit independently in days			
Number of subjects with data	660	636	1296
Mean [SD]	2.26 [2.87]	2.19 [3.03]	2.23 [2.95]
Median	1.59	1	1
Range	0-30	0-45	0-45
<i>Excluding in-hospital deaths</i>			
Number of subjects with data	608	556	1164
Mean [SD]	2.44 [2.91]	2.50 [3.13]	2.47 [3.01]
Median	2	2	2
Range	0-30	0-45	0-45
Time to speak in days			
Number of subjects with data	652	631	1283

	Artesunate (N=730)	Quinine (N=731)	All (N=1461)
Mean [SD]	1.43 [2.21]	1.36 [2.01]	1.40 [2.12]
Median	1	0.25	0.50
Range	0-35	0-21	0-35
<i>Excluding in-hospital deaths</i>			
Number of subjects with data	600	549	1149
Mean [SD]	1.55 [2.26]	1.54 [2.09]	1.55 [2.18]
Median	1	1	1
Range	0-35	0-21	0-35

Source: Clinical Reviewer (J Review) and Statistical Reviewer

Subgroup Analyses

The following table displays the in-hospital mortality subgroup analysis results by age, sex, and selected baseline disease characteristics. The artesunate group had a consistently lower mortality than the quinine group for all subgroups except in children less than 20 kg body weight. However, due to the small sample sizes in children < 20 kg, it is difficult to draw conclusions based on these results. Artesunate reduced mortality in subgroups of cerebral malaria, pre-treatment with any or effective antimalarial treatment, parasite smear or count result, pregnancy, and hypoglycemia. For cerebral malaria, the dataset and publication provide different numbers, but these differences did not impact the treatment effect across different subgroups.

It is noted that in subjects in the quinine group not pretreated with quinine, the mortality rate in subjects who did not receive the quinine loading dose was lower compared to those subjects receiving the loading dose. As this was an open-label study, receiving the loading dose may have been dependent on the severity of the disease; the interpretation of this result is difficult. In the artesunate group, pretreatment with quinine did not affect the mortality significantly.

In a logistic regression conducted by the statistical reviewer, the impact of pretreatment antimalarial therapy was explored. The logistic regression contained the following variables: pretreatment with antimalarials, severe malaria, cerebral malaria, treatment group, and age group (<15 years or not). All were significantly associated with in-hospital mortality. In a regression to test if there was an interaction between pretreatment and treatment group, the interaction was not significant. From this analysis, pretreatment and treatment with artesunate had independent effects on the outcome after controlling for severe malaria, cerebral malaria, and age. As the following table shows, it appears that artesunate was more efficacious in subjects with severe malaria, especially those without cerebral malaria, who were pretreated with antimalarials [mortality: 2/49 (4.1%) in the artesunate group versus 8/31 (25.8%) in the quinine group], although the trial could not reach a definitive conclusion on this effect and there were no reasons identified.

Table 8-18 SEAQUAMAT In-hospital Mortality Subgroup Analysis (ITT Population)

	Artesunate (N=730)	Quinine (N=731)
Age (years)		
< 5	1/37 (2.7%)	2/33 (6.1%)
5 to <15	2/60 (3.3%)	8/72 (11.1%)
15 to <65	88/616 (14.3%)	131/605 (21.7%)
≥65	5/17 (29.4%)	9/21 (42.9%)
≥15	93/633 (14.7%)	140/626 (23.4%)
Sex		
Male	71/546 (13.0%)	111/529 (21.0%)
Female	29/184 (15.8%)	41/202 (20.3%)
Body Weight (kg)		
<20	3/52 (5.8%)	2/57 (3.51%)
≥20 to <75	92/672 (13.6%)	161/672 (24.0%)
≥75	1/5 (20.0%)	3/7 (42.9%)
Missing	0/1 (0%)	1/2 (50%)
Body weight and age		
Weight ≥20 kg & age <18 yrs.	8/109 (7.3%)	15/95 (15.8%)
Severe Malaria Flag (SEVMALFL)		
Yes	92/509 (18.1%)	139/541 (25.7%)
No	4/221 (1.8%)	11/190 (5.8%)
Body weight (kg) and severe malaria		
<20 / Yes	3/41 (7.3%)	2/50 (4.0%)
<20 / No	0/11 (0%)	0/7 (0%)
≥20 / Yes	89/468 (19.0%)	136/489 (27.8%)
≥20 / No	4/209 (1.9%)	11/183 (6.0%)
Missing/Yes	0	1/2 (50%)
Missing/No	0/1	0
Cerebral Malaria Flag (CEBRMLFL)		
From data set: Yes	75/284 (26.4%)	103/304 (33.9%)
No	21/446 (4.7%)	47/427 (11.0%)
Pre-treatment with any antimalarial treatment		
Yes	17/167 (10.2%)	34/142 (23.9%)
Severe malaria: Yes	15/101 (14.9%)	32/97 (36.1%)
Cerebral malaria: Yes	13/52 (25.0%)	24/66 (36.4%)
No	2/49 (4.1%)	8/31 (25.8%)
Severe malaria: No	2/66 (3.0%)	2/45 (4.4%)
Cerebral malaria: Yes	0/2 (0%)	1/5 (20.0%)
No	2/64 (3.1%)	1/40 (2.5%)
No	79/563 (14.3%)	116/589 (19.7%)
Severe malaria: Yes	77/408 (20.6%)	107/444 (24.1%)
Cerebral malaria: Yes	62/220 (28.2%)	75/225 (33.3%)
No	15/188 (8.0%)	32/219 (14.6%)

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

	Artesunate (N=730)	Quinine (N=731)
Severe malaria: No	2/155 (1.3%)	9/145 (6.2%)
Cerebral malaria: Yes	0/10 (0%)	3/8 (37.5%)
No	2/145 (1.4%)	6/137 (4.4%)
Pre-treatment with effective antimalarial		
Yes	14/125 (11.2%)	30/118 (25.4%)
No	82/605 (13.6%)	120/613 (19.6%)
Pretreated with quinine	12/103 (11.7%)	21/84 (25.0%)
Quinine loading dose: Yes	Not applicable	11/46 (23.9%)
No	Not applicable	10/38 (23.3%)
Not pretreated with quinine	84/627 (13.4%)	129/647 (19.4%)
Quinine loading dose: Yes	Not applicable	114/542 (21.0%)
No	Not applicable	15/105 (14.3%)
Malaria parasites on blood film		
Yes	96/708 (13.5%)	147/716 (20.5%)
No	0/22 (0%)	3/15 (20.0%)
Hyperparasitemia (>10%)		
Yes	26/121 (21.4%)	52/108 (48.2%)
No	70/609 (11.5%)	98/623 (15.7%)
Positive blood smear for <i>P. falciparum</i>		
Yes	96/689 (13.9%)	143/693 (20.6%)
No	0/41 (0%)	7/38 (18.4%)
Paracheck <i>P. falciparum</i> smear result		
Yes	95/691 (13.8%)	144/699 (20.6%)
No	0/28 (0%)	4/21 (19.0%)
Missing data	1/11 (9.1%)	2/11 (18.2%)
Hypoglycemia on admission		
Yes	3/8 (37.5%)	10/17 (58.8%)
No	89/693 (12.8%)	129/676 (19.1%)
Missing	4/29 (13.8%)	11/38 (29.0%)
Pregnancy flag		
Yes	2/23 (8.7%)	3/26 (11.5%)
No	23/161 (14.3%)	36/176 (20.5%)
Missing	71/546 (13.0%)	111/529 (21.0%)
Acidosis (base excess < -3.3 mmol/L)		
Yes	70/308 (22.7%)	117/334 (35.0%)
No	12/354 (3.7%)	13/314 (4.1%)
Missing	12/68 (19.1%)	20/83 (24.1%)
Severe anemia (hemoglobin <50 g/L)		
Yes	5/40 (12.5%)	9/54 (16.7%)
No	86/643 (13.4%)	124/621 (20%)
Missing	5/47 (10.6%)	17/56 (30.4%)
Shock		

	Artesunate (N=730)	Quinine (N=731)
Yes	11/78 (14.1%)	23/92 (25.0%)
No	85/652 (13%)	127/639 (19.9%)

Source: Statistical Reviewer's analysis

8.1.2 AQUAMAT: Artesunate versus quinine in the treatment of severe *falciparum* malaria in African children: an open-label, randomized trial

8.1.2.1 Study Design

This was a multicenter, randomized, open-label trial conducted between October 2005 and July 2010 at 11 centers in nine African countries (Mozambique, The Gambia, Ghana, Kenya, Tanzania, Nigeria, Uganda, Rwanda, and Democratic Republic of the Congo). This study was funded by the Wellcome Trust and coordinated by the Wellcome Trust Mahidol-Oxford Tropical Medicine Search Unit in Bangkok, Thailand. Data, protocol, and study report were not submitted for this study. All information is obtained from the trial publication ([Dondorp et al. 2010](#)).

This study compared the mortality of severe *falciparum* malaria treated with artesunate versus quinine. Key eligibility criteria and exclusion criteria are as follows:

- Major inclusion criteria
 - Children younger than 15 years of age
 - Positive rapid diagnostic test for *P. falciparum* (Optimal)
 - Severe malaria in the opinion of the admitting physician
 - Informed consent from patient or guardian
- Major exclusion criteria
 - Convincing history of full treatment with parenteral quinine or an artemisinin derivative for more than 24 h before admission
 - Age criteria varied slightly at different sites according to the request of the respective ethics review boards

Severe malaria was defined as in the following table.

Table 8-19: AQUAMAT Modified Criteria for Severe Malaria

At least one of:
• Plasma base excess < -3.3 mmol/L
• Glasgow coma scale < 11 of 15, or Blantyre coma scale < 3 of 5 in preverbal children
• Hemoglobin < 50 g/L and parasitemia greater than 100,000 per μ L
• BUN > 10 mmol/L
• Compensated shock (capillary refill $\geq$ 3 sec or temp gradient on legs, but no hypotension)

• Decompensated shock; systolic BP < 70 mm Hg and cool peripheries
• Asexual parasitemia more than 10%
• Visible jaundice and > 100,000 parasites per μ L
• Plasma glucose < 3 mmol/L
• Respiratory distress, defined as costal indrawing, use of accessory muscles, nasal flaring, deep breathing, or severe tachypnea.

Source: Panel 1 of the publication

Eligible patients were randomized to treatment with either intravenous or intramuscular artesunate or quinine (blocks of 20, with sealed envelopes associated with a study box containing the study drug, CRF and other items needed for study). Treatment was to be started immediately.

- **Artesunate** (Guilin Pharmaceutical Factory, Guangxi): 2.4 mg/kg IV on admission, at 12 hours, 24 hours and thereafter once daily until oral medication could be taken reliably.
 - 60 mg vial was dissolved in 1 mL 5% sodium bicarbonate (provided with study drug), then diluted with 5% dextrose before injection either as a bolus IV or via deep **intramuscular (IM) injection in the anterior thigh**.
- **Quinine** (Indus Pharma, Pakistan) was given as 20 mg salt per kg loading dose infused over 4 h in 5-10 mL/kg 5% dextrose, followed by 10 mg/kg infusion over 2-8 hours three times daily until starting oral therapy.
 - IM doses were the same as IV doses but quinine was diluted in normal saline for a concentration of 60 mg/mL. Loading dose was a split dose in each thigh.
- When a patient was able to take po and after a minimum of 24-hour parenteral treatment, **oral artemether-lumefantrine (Coartem)** was administered at a standard dose (1.5 mg artemether/9 mg lumefantrine/kg) for three days with milk or fat, to complete the treatment.

Patients were discharged at the discretion of the physician; a discharge assessment was completed. Active follow-up after discharge was instituted. Children who had not made a full neurological recovery by discharge were followed at regular intervals for 12 months or until full recovery. Full neurological assessments were completed at every visit.

Two committees provided assessment and classifications of outcomes before study unblinding:

- Neurological outcome committee (two pediatricians and one malariologist) assessed four domains: motor function, visual function, hearing, and speech. This committee also evaluated whether the patient developed epilepsy. Sequelae persisting at follow-up were graded as mild, moderate, or severe in each domain according to the extent of functional impairment.

- Endpoint review committee (one pediatrician and one clinical malariologist) identified cases in which a pathological change other than malaria or its acute complications was likely to be the main cause of death.

Trial was open-label at each site but none of the investigators or trialists, apart from the statistician, had access to the summaries of treatment allocations. When notes or CRFs were reviewed, study drug details were removed to preserve masking. All case reviews and laboratory assessments were blinded.

Sites were monitored regularly. Investigators provided reports to coordinating center in Bangkok every two weeks and met every year to review study progress. DSMB made three interim analyses during the trial. Though, there did not appear to be any procedures for controlling the Type I error due to the interim analyses, it does not appear that the study stopped early based on an interim analysis.

8.1.2.2 Study Endpoints

The primary endpoint was in-hospital mortality. Secondary endpoints included incidence of severe neurological complications assessed at 28 days (range 3-8 weeks) and a combined outcome measure of death and severe persistent neurological sequelae. The publication notes that initially neurological outcomes were assessed only at discharge from the hospital, but the authors state that this led to a substantial overestimation of the neurological deficit, especially in young children. The protocol was changed in April 2007 after 11% of patients were enrolled such that children who had not yet fully recovered at discharge were assessed 28 days after enrollment, with active follow-up thereafter.

8.1.2.3 Statistical Analysis Plan

The sample size of 5306 was planned to demonstrate a 25% reduction in mortality from 8% to 6% with an 80% statistical power and a Type I error of 0.05. Mortality estimate was based on an estimated severe malaria mortality of 16% with about half the enrolled patients anticipated to fulfill the criteria for severe malaria.

Primary analysis was intention-to-treat (ITT), although it was not defined in the publication. Based on the publication, it appears to have included all randomized subjects.

Per-protocol analysis was also conducted, excluding patients:

- Not fulfilling entry criteria;
- With negative or missing malaria smear for *P. falciparum*;
- Who died before receiving study drug; or
- Who were missing a study drug dose on the first day of treatment.

Subgroup analyses included those fulfilling the criteria of severe malaria (all centers) and HIV status (some centers).

The sample size of 5306 was planned to demonstrate a 25% reduction in mortality from 8% to 6% with an 80% statistical power and a type I error of 0.05. Mortality estimate was based on an estimated severe malaria mortality of 16% with about half the enrolled patients anticipated to fulfill the criteria for severe malaria.

There was no statistical analysis plan submitted. For binary outcomes, the ORs between the treatment groups were estimated using the CMH method, stratified by study site. Heterogeneity between sites was examined with the Breslow-Day test. Time to events was compared with the Log-rank test, and hazard ratios (HRs) were estimated by a Cox proportional hazards model, stratified by study site.

8.1.2.4 Protocol Amendments

This information was not provided. As discussed above, initially neurological outcomes were assessed only at discharge from hospital. Due to overestimation of the outcomes, especially in young children, patients who had not fully recovered at discharge were instead assessed 28 days after enrolment when 11% patients had been enrolled.

8.1.2.5 Study Results

Compliance with Good Clinical Practices

This information was not available.

Financial Disclosure

This trial was supported by the Wellcome Trust. No information is available for financial disclosure from the Applicant.

Data Quality and Integrity

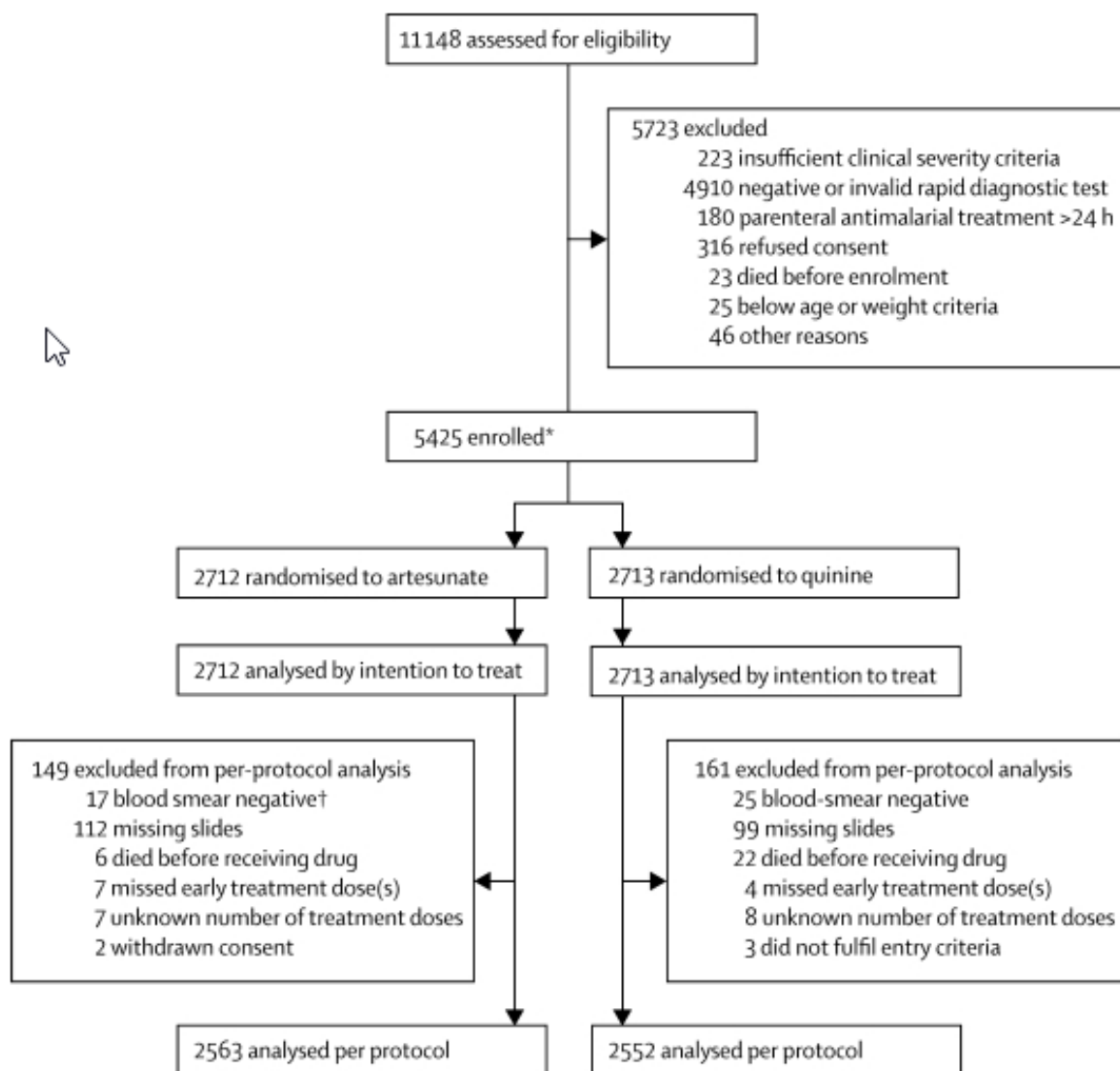
It is not applicable as there were no data submitted.

Patient Disposition

A total 11148 patients were assessed for eligibility and 5425 patients were enrolled. Enrolled subjects were randomized (1:1) to the artesunate group or quinine group. About 65% of randomized subjects received treatment by IV (1779/2712 on artesunate and 1724/2713 on quinine) and 35% by IM. All enrolled patients were included in the ITT population. A total of 148 and 161 ITT patients were excluded from the per-protocol population. The numbers of patients for each exclusion reason were comparable, except for the imbalance of deaths before receiving the assigned treatment (6 on artesunate versus 22 on quinine). According to the

publication, this imbalance of deaths was indicative of the difficulties in administering parenteral quinine promptly and safely, as IV quinine needs a careful rate-controlled infusion and continuous or three-time daily administration to avoid dangerous hypotension. Note that a delay in treatment likely increased mortality in the quinine arm. This appears to have impacted mortality prior to receipt of randomized treatment, but it might have also increased mortality in the quinine group if quinine administration was delayed in the treated patients as well. This should be taken into account when interpreting the study and will be discussed in the result section.

Figure 8-2. AQUAMAT: Trial Profile



Source: Figure 1 of the publication.

Protocol Violations/Deviations

This information is not available from the publication.

Demographic Characteristics

Only a few demographic characteristics were available in the publication and are shown in the following table. Sex and age were similar between the two treatment groups.

Table 8-20. AQUAMAT Demographic Characteristics (ITT Population)

	Artesunate (N=2712)	Quinine (N=2713)
Sex		
Female	1315 (48%)	1295 (48%)
Age		
Median (interquartile range)	2.8 (1.6–4.2)	2.9 (1.7–4.3)

Source: Table 1 of the publication

Other Baseline Characteristics (e.g., disease characteristics, important concomitant drugs)

The following table displays other baseline characteristics. These characteristics were well balanced between the two treatment groups.

Table 8-21. AQUAMAT Other Baseline Characteristics

	Artesunate (N=2712)	Quinine (N=2713)
Weight (kg)	12.4 (4.8)	12.6 (4.6)
Temperature (°C)	38.0 (1.1)	38.0 (1.1)
Blood pressure (mmHg)		
Systolic	95 (16)	95 (16)
Diastolic	56 (14)	56 (14)
Fever before enrollment (days)	3 (2–4)	3 (2–4)
Pretreatment with antimalarials		
None	1281 (47%)	1270 (47%)
Ineffective	387 (15%)	371 (14%)
Effective*	938 (36%)	959 (37%)
Coma depth (total N, median [IQR])		
Blantyre coma score	1713, 4 (2–5)	1704, 4 (2–5)
Glasgow coma score	999, 11 (8–15)	1005, 11 (8–15)
Coma before enrollment (hours)	5.0 (2.0–9.5)	5.0 (2.5–10)
Complications on admission		
Coma	880 (32%)	945 (35%)
Convulsions	811 (30%)	879 (32%)

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

	Artesunate (N=2712)	Quinine (N=2713)
Jaundice	55 (2%)	59 (2%)
Severe anemia (hemoglobin <50 g/L)	737 (30%)	692 (29%)
Shock	323 (12%)	339 (12%)
Decompensated shock	90 (39%)	88 (35%)
Severe acidosis (base excess < -8 mmol/L)	1009 (44%)	975 (43%)
Hypoglycemia (<3 mmol/L)	277 (10%)	278 (10%)
Respiratory distress‡	439 (16%)	428 (16%)
Severe prostration§	1683 (62%)	1668 (61%)
Blackwater fever	121 (4%)	116 (4%)
Hyperparasitemia (>10%)	584 (25%)	573 (24%)
Comorbidity		
Immune compromised (from history)	45 (2%)	49 (2%)
Severe malnutrition	54 (2%)	43 (2%)
Suspected pneumonia	227 (8%)	226 (8%)
Confirmed by radiograph	29 (13%)	29 (13%)
Clinical sepsis	302 (11%)	355 (13%)
Confirmed by culture	32 (11%)	33 (9%)
Suspected meningitis	169 (6%)	166 (6%)
Confirmed meningitis	6 (4%)	3 (2%)
Other significant comorbidities	80 (3%)	71 (3%)
Laboratory assessments		
Parasitemia (parasites/mL; geometric mean, range)	47922 (0–1494640)	49110 (0–1858880)
Sodium (mmol/L)	131 (6.5)	132 (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 (4.6)	6.1 (4.9)
Hemoglobin (g/L)	68 (29)	70 (31)
pH	7.36 (0.14)	7.36 (0.14)

Source: Table 1 of the publication

Data are number (%), median (IQR), or mean (SD), unless otherwise stated

*Defined in the Web Appendix page 12 of the publication

‡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

Reviewer's comment: it is difficult to determine the denominators for some reported percentages, for example, decompensated shock.

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

This information is not available in the article.

Efficacy Results – Primary Endpoint

The following table shows mortality results from the study. In the ITT analysis, there was a

significantly lower mortality rate in the artesunate group than in the quinine group (OR=0.75, 95% CI [0.63–0.90]; p=0.0022). There was no significant heterogeneity in treatment effect between study sites (p=0.99). An unstratified Chi-squared test conducted by the reviewer provided the same results as the reported stratified analysis.

In the per protocol analysis, the treatment effect of artesunate remained highly statistically significant. As mentioned above, there was an imbalance of deaths after randomization but prior to treatment and these subjects were excluded from this per protocol analysis. Nevertheless, this is concerning in that the control group was potentially weakened by delays of treatment, impacting not only these patients but potentially a larger proportion of patients in the quinine group. Therefore, the reported results might have been less of a comparison between IV artesunate and IV quinine and more between IV artesunate and delayed IV quinine.

Eight subjects (5 on artesunate, 3 on quinine) were taken from hospital against medical advice and could not be followed up further. If they were considered as deaths in the primary analyses, the treatment effect still remained highly significant.

Efficacy Results – Secondary and other relevant endpoints

Some secondary endpoints are presented in the following table. Death or sequelae at 28 days occurred less frequently in the artesunate group (OR=0.78, 95% CI [0.65–0.93], p=0.0056).

A lower proportion of children in the artesunate group had convulsions that either developed after admission, or were present on admission and persisted for more than 5 hours (OR=0.80, 95% CI [0.66–0.97]; p=0.0199) or had a deterioration of coma score after starting antimalarial treatment (OR=0.78, 95% CI [0.64–0.97]; p=0.0245).

In the HIV positive children, mortality was numerically lower in the artesunate group than in the quinine group.

Development of coma, deterioration of coma score, convulsions developing or persisting >6 hours after admission, and hypoglycemia occurred statistically significantly less frequently in the artesunate group than in the quinine group.

The incidence of blackwater fever was numerically higher and the incidence of severe anemia was numerically lower in the artesunate group, but not statistically significantly different between the two treatment groups.

Table 8-22. AQUAMAT In-hospital Mortality and Some Secondary Outcomes

	Artesunate (N=2712)	Quinine (N=2713)	OR (95% CI)	P value
Mortality, ITT analysis	230/2712 (8.5%)	297/2713 (10.9%)	0.75 (0.63–0.90)	0.0022
Mortality, per-protocol analysis	208/2563 (8.1%)	260/2552 (10.2%)	0.78 (0.64–0.94)	0.0099

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

	Artesunate (N=2712)	Quinine (N=2713)	OR (95% CI)	P value
Death or sequelae at 28 days	253/2689 (9.4%)	316/2695 (11.7%)	0.78 (0.65–0.93)	0.0056
Malaria-attributable mortality*	223/2705 (8.2%)	288/2704 (10.7%)	0.75 (0.63–0.91)	0.0025
Mortality in strictly defined severe malaria†	226/2280 (9.9%)	291/2338 (12.4%)	0.77 (0.64–0.93)	0.0055
Case fatality in HIV-positive children†	16/64 (25%)	19/61 (31%)	0.74 (0.33–1.62)	0.45
Development of coma§	65/1832 (3.5%)	91/1768 (5.1%)	0.69 (0.49–0.95)	0.0231
Deterioration of coma score	166/2712 (6.1%)	208/2713 (7.7%)	0.78 (0.64–0.97)	0.0245
Convulsions developing or persisting >6 h after admission	224/2712 (8.3%)	273/2713 (10.1%)	0.80 (0.66–0.97)	0.0199
Hypoglycemia	48/2712 (1.8%)	75/2713 (2.8%)	0.63 (0.43–0.91)	0.0134
Severe anemia (Hemoglobin<50 g/L) after admission§	78/1696 (4.6%)	98/1734 (5.7%)	0.81 (0.59–1.11)	0.18
Blackwater fever§	30/2591 (1.2%)	18/2597 (0.7%)	1.69 (0.94–3.05)	0.076

Source: Table 2 of the publication

ITT: intention to treat.

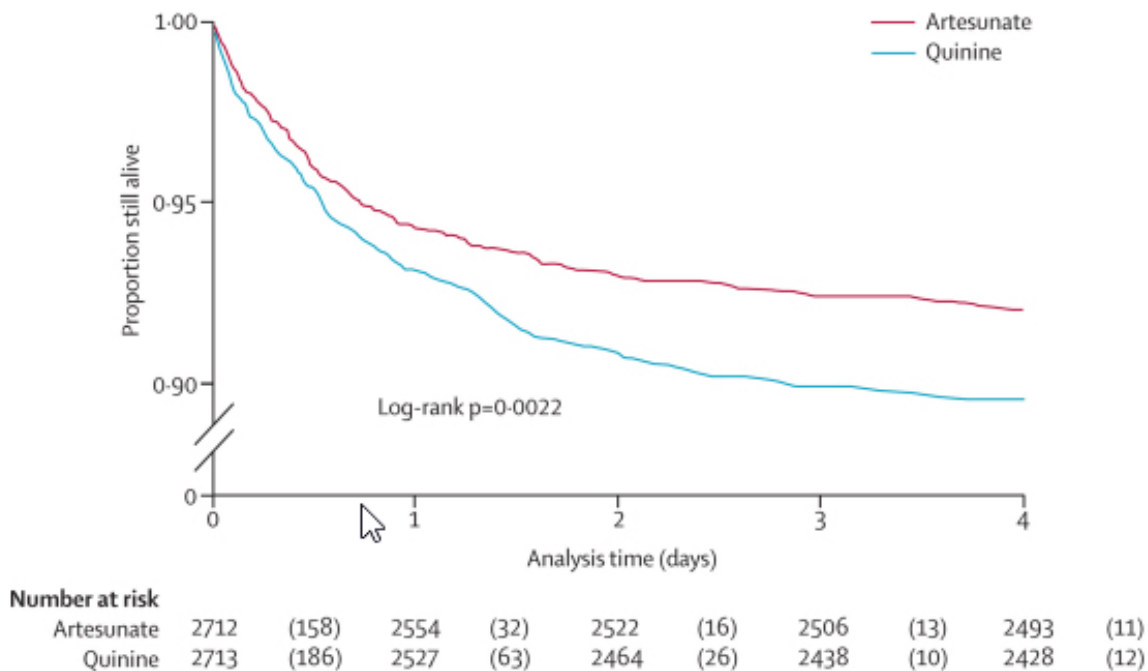
*The likelihood that malaria contributed to or directly caused the death was assessed by an independent endpoint review committee blinded to the treatment allocation.

†HIV status was assessed only in Beira, Muheza, and Kilifi (n=2095).

§Development of coma, anemia, and blackwater fever was assessed only in patients without these disorders on admission.

The following graph shows the Kaplan-Meier survival curves. There was a statistically significant difference between the two treatment groups (p-value from the Log-rank test was 0.0022).

Figure 8-3. AQUAMAT Kaplan-Meier Curves



Source: Figure 2 of the publication

Based on survival analysis, there was no difference detected in time to discharge, to eat, or to sit unsupported; in cerebral malaria survivors, the time to localize a painful stimulus or to speak was slightly longer in the artesunate group than in the quinine group. The median time to discharge was much shorter than in SEAQUAMAT (6.8 days). The reason is not clear. The comparability of the time to discharge made the comparison of in-hospital mortality relevant, as the in-hospital observation time for each group was likely to be comparable.

Table 8-23. AQUAMAT Recovery Times in Surviving Patients

	Artesunate (N=2712)		Quinine (N=2713)		HR (95% CI)	p value
	N	Median (IQR)	N	Median (IQR)		
Time to discharge (days)	2478	3.0 (2.0–5.0)	2412	3.0 (2.0–5.0)	1.04 (0.99–1.10)	0.059
Time to eat (h)	2358	9 (0–24)	2269	12 (2–24)	0.99 (0.93–1.06)	0.74
Time to sit unsupported (h)	2373	18 (6–42)	2312	22 (6–44)	1.02 (0.95–1.08)	0.60
Time to localize pain (h)*	698	12 (6–24)	726	12 (6–24)	0.87 (0.78–0.98)	0.0093
Time to speak(h)*	664	20 (8–42)	695	18 (11–36)	0.88 (0.79–0.99)	0.016

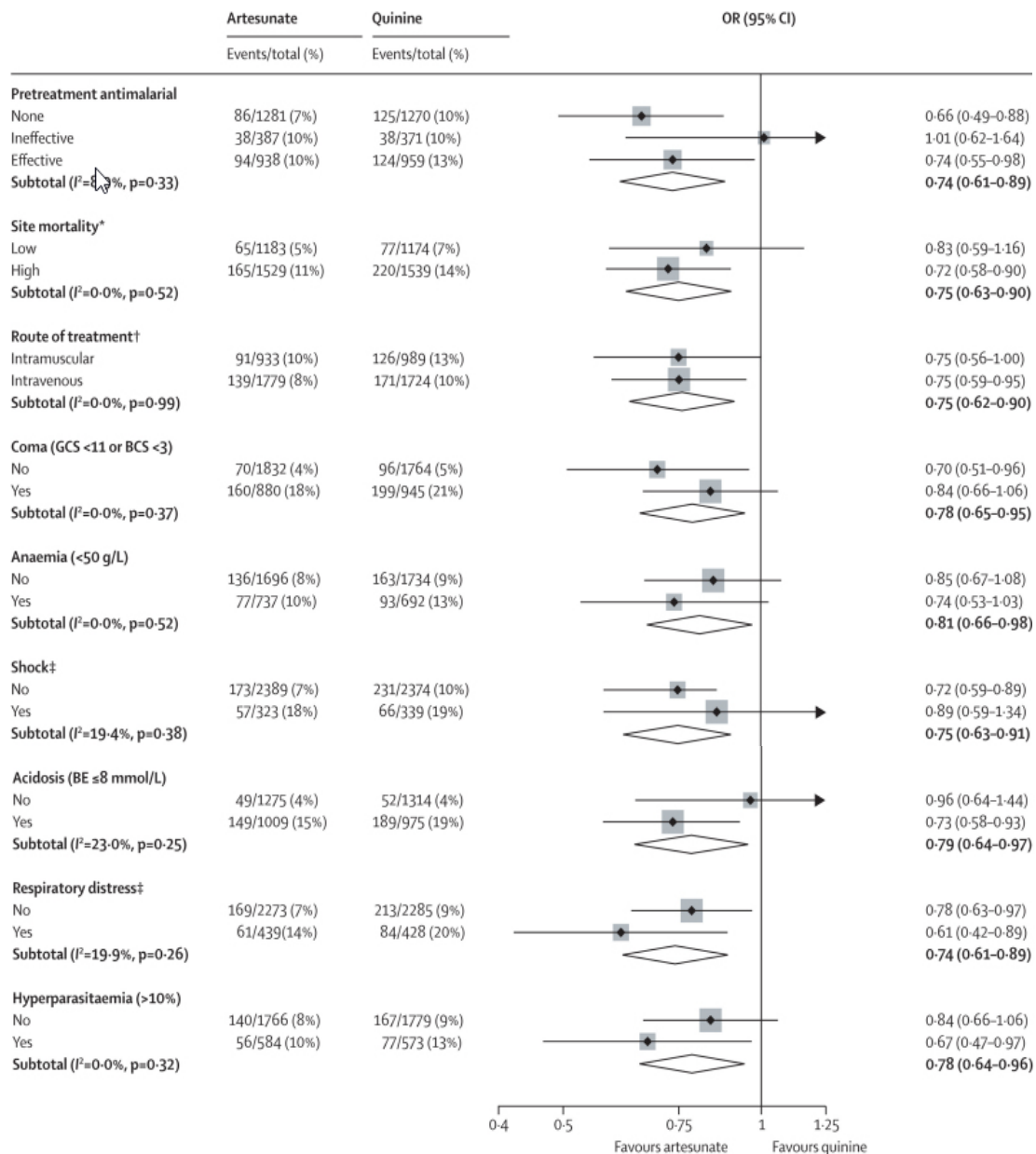
Source: Table 3 of the publication

*Time to localize pain and time to speak was assessed only for surviving patient with coma on admission

Additional Analyses Conducted on the Individual Trial

Subgroup analysis results are presented in the following figure and table. In all subgroups, except for the ineffective anti-malarial treatment group, artesunate demonstrated a statistically significantly or numerically reduced mortality. Subgroup analyses by age and gender are not available.

Figure 8-4. AQUAMAT 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. Hyperparasitemia means greater than 10% of red cells parasitized. OR=odds ratio. GCS=Glasgow coma scale. BCS=Blantyre coma scale. BE=base excess. *Site mortality classified as low if the site mortality rate was lower than the overall study mortality rate, and high if the site mortality rate was higher than the overall study mortality rate. †Classified according to

NDA Multi-disciplinary Review and Evaluation NDA 213036 Artesunate for Injection

center policy (ten sites); classified according to individual data (one site). #Decompensated or compensated shock. I² denotes the percentage of total variation across subgroups resulting from heterogeneity rather than chance, with the p value of significance.

Source: Figure 3 of the publication

8.1.3 EDCTP/MMV07-01 Phase II Randomized, Double-Blind Study of the Efficacy, Safety, Tolerability, and Pharmacokinetics of Intravenous Artesunate in Children with Severe Malaria

8.1.3.1 Study Design

This was a double-blind, multicenter, randomized, parallel-group study, conducted by Medicines for Malaria Venture in Switzerland from October 2007 to July 2008. Only the protocol, sample CRF, and study report was submitted, with no data sets. The review is based on these submitted materials.

There were three centers in this study:

- Center 1001: Albert Schweitzer Hospital, Lambarene, Gabon
- Center 2001: Universite de Medecin et Science de la Sante, Libreville, Gabon
- Center 3001: Queen Elizabeth Central Hospital, Blantyre, Malawi

The primary objective was to evaluate the effectiveness of two IV artesunate dosing regimens in clearing *P. falciparum* parasites in pediatric patients with severe malaria. The secondary objectives were 1) to compare the tolerability and safety of the 2 IV artesunate dosing regimens and 2) to evaluate differences in the pharmacokinetic profile of IV artesunate by patient age and clinical presentation.

We consider that the censoring was non-informative, a strong assumption for survival analysis.

Patients (children with severe malaria) would be block randomized to 1 of 2 regimens, with 6 patients per block and a randomization ratio of 1:1. Doses for the two regimens were as follows:

- 5-dose regimen: 2.4 mg/kg initially and at 12, 24, 48, and 72 hours
- 3-dose regimen: 4.0 mg/kg initially and at 24 and 48 hours (normal saline at 12 and 72 hours to maintain the study blind)

As soon as the patient was able to receive oral medication and no signs and symptoms of severe malaria were present, but not before the last pharmacokinetic sample was taken (approximately 50 hours after the start of therapy), a single dose of sulfadoxine/pyrimethamine based on body weight was to be administered to ensure parasitological cure. Sites 1001 and 2001 used oral sulfadoxine/pyrimethamine. Site 3001 used oral artemether/lumefantrine.

We noted that the 2.4 mg/kg regimen matched the doses of the previous two trials up to 72 hours and the 4.0 mg/kg regimen was at a higher dose and did not include the dose at 12 hours,

although the total dose was the same for the two regimens. Based on the proposed dose for this product, the 5-dose regimen is the focus of this review. Due to a possible use of other single-dose antimalarial drug around 50 hours, only the doses at 0, 12, and 24 hours in the 5-dose regimen could be used for the evaluation of efficacy. After 48 hours, the effect could be the combined effect of artesunate and the other antimalarial drug.

In this double-blind study, the patient and research team (except for the study pharmacist/designee) were kept blinded as to the assigned treatment.

Patients were hospitalized for at least 4 days (Days 0, 1, 2, and 3) and returned to the study site for study visits on Days 7, 14, and 28. Patients with unresolved adverse events by Day 28 would be followed for an additional 30 days or until resolution of the event or no further medical management was deemed necessary. If any untoward event occurred within 30 days of completing the study drug, the patient was instructed to return to the study site.

Inclusion criteria were as follows:

1. Male or female children aged 6 months and ≥ 5 kg to 10 years, inclusive.
2. Clinical diagnosis of severe *P. falciparum* malaria requiring hospitalization.
3. Parasitemia ($\geq 5,000$ parasites/ μ L on initial blood smear).
4. Availability of child's parent/guardian and their willingness to provide written informed consent in accordance to local practice.
5. Willingness and ability to comply with the study protocol for the duration of the study.
6. Willingness to remain in the hospital for 4 days.

Exclusion criteria included:

1. Known serious adverse reaction or hypersensitivity to artemisinins, including artesunate, artemether, dihydroartemisinins or Coartem (artemether/lumefantrine).
2. Any underlying disease that may compromise the diagnosis and the evaluation of the response to the study medication (including concomitant infection, concomitant neurological disease and malnutrition)
3. Participation in any investigational drug study during the 30 days prior to screening
4. Adequate anti-malarial treatment within 24 hours prior to admission

The primary efficacy endpoint was the proportion of patients with parasite clearance ($\geq 99\%$ reduction from the baseline asexual parasite count) at 24 hours after initiation of study drug.

Secondary efficacy endpoints included:

- Time to total clearance of asexual parasites (PC₁₀₀)
- Time to 99% reduction of asexual parasites (PC₉₉)

- Time to 90% reduction of asexual parasites (PC₉₀)
- Time to 50% reduction of asexual parasites (PC₅₀)
- Adequate clinical and parasitological response on day 28
- PCR corrected adequate clinical and parasitological response on day 28
- Parasitological cure rate on day 28
- Percent reduction in asexual parasites from baseline at 24 hours after initiation of randomized study drug
- Percent reduction in asexual parasites from baseline at 48 hours after initiation of randomized study drug
- Percent reduction in gametocytes from baseline at 24 hours after initiation of randomized study drug
- Percent reduction in gametocytes from baseline at 48 hours after initiation of randomized study drug

PC₁₀₀ was defined as no asexual parasites for 2 consecutive negative readings ≥ 6 hours apart, with a confirmed negative reading 24 hours after the first negative slide. Time to PC₁₀₀ was defined as the time from the first dose of study drug to the first negative reading. Patients for whom PC₁₀₀ could not be calculated because they did not have parasite clearance by 72 hours after randomization or who were withdrawn for treatment failure before 72 hours were censored at 72 hours. Patients who terminated early without achieving parasite clearance for reasons other than treatment failure (e.g., adverse event, patient request, intercurrent illness) were censored at the last available parasite count.

8.1.3.2 Statistical Analysis Plan

The planned sample size was 200 in total. Assuming a 0.68 proportion of patients achieving PC₉₉ in both treatment groups and 100 patients per treatment group, the 95% CI (based on the normal approximation) for the observed treatment group difference of zero would be -0.13 to 0.13. The 95% CI for the estimated PC₉₉ for each treatment group would be 0.59 to 0.77.

Intent-to-treat (ITT) population included all patients from the safety population who had parasitologically confirmed infection with *P. falciparum* prior to treatment had an evaluable primary endpoint and no serious protocol violation or withdrawal of consent.

We do not agree with the condition of having an evaluable primary endpoint and no serious protocol violation or withdrawal of consent. The ITT population should include all randomized subjects, regardless of having evaluable primary endpoint, serious protocol violation or withdrawal of consent, or other post-randomization information. These post-randomization exclusions could be impacted by the study treatment, making the two treatment arms being analyzed differently, greatly impacting the benefits of randomization. We will conduct additional analyses including all randomized subjects.

Per Protocol (PP) population included all patients from the ITT population who received at least two doses of randomized study drug and reached the primary efficacy endpoint (or reached an efficacy endpoint such as non-response, relapse, etc.). No antimalarial agent other than the protocol-specified treatment regimens was administered during the period from 72 hours prior to start of randomized study drug until after Day 28 unless the subject is considered a study treatment failure. Inclusion in the PP population was determined before breaking the study blind. This was a supportive analysis population for efficacy.

Analysis Methods

Chi-squared test was used to assess treatment group differences in parasite clearance for the PP and ITT populations. Two-sided 95% CIs were provided for the estimated parasite clearance rates in each treatment group and for the difference between treatment groups.

In addition to the planned analysis, in the study report, a noninferiority test was performed. Two-sided 90% CI was provided in order to have one-sided test at a Type I error of 5% to test the 3-dose group was not inferior to the 5-dose group, using a relative risk of 0.8 as the margin. Given that both groups are considered test groups and the noninferiority margin was not justified, we will not cover the results of the noninferiority test.

PC₁₀₀ was summarized using Kaplan-Meier estimates. Patients for whom PC₁₀₀ could not be calculated because they did not have parasite clearance by 72 hours after randomization or who were withdrawn for treatment failure before 72 hours were noted as having a censored PC₁₀₀ of 72 hours.

Patients who terminated early for reasons other than treatment failure (e.g., adverse event, patient request, intercurrent illness) were noted as having a censored PC₁₀₀ as of the last available information on parasite counts. PC₉₉, PC₉₀, and PC₅₀ were similarly defined and analyzed. Treatment group comparisons for percent reduction in asexual parasites from baseline at 24 hours and 48 hours after initiation of randomized study drug were made with the Wilcoxon rank sum test.

Percent reduction in parasites was imputed with last observation carried forward for those patients who prematurely discontinued before the scheduled evaluation.

Protocol Amendments

This information is not available.

8.1.3.3 Study Results

Compliance with Good Clinical Practices

This study complied with Good Clinical Practice.

Financial Disclosure

The study was sponsored by Medicines for Malaria Venture, International Center Coitrin, Switzerland. The Applicant does not have financial disclosures for this study.

Protocol Violations/Deviations

A few serious protocol violations for which the subjects were not included in the Safety or ITT populations as described below were unknown. Dosing error, parasitemia <5000 on day 0 and administration of quinine/amodiaquine prior to first dose of study drug were reported as the major protocol deviations. The analyses of secondary endpoints Adequate Clinical and Parasitological Response on Day 28 (genotype-corrected, genotype-uncorrected) were not performed, for unknown reasons.

Data Quality and Integrity

There were no data submitted for FDA review. Per CSR, the study monitors were verifying that the data were recorded correctly and completely. The CSR by Medicines for Malaria Venture stated that all data collected in the CRF were double entered into the data management system for the analyses.

Patient Disposition

Of all 197 patients randomized, 194 were included in the safety population and 3 were excluded for no dosing done, withdrawal of consent, or serious protocol violation. Due to serious protocol violation (3), withdrawal of consent (1) or aparasitemia on day 0 (1), and missing time for parasite clearance at 24 hours (7), 12 subjects in the safety population were excluded from the ITT population, leaving 182 subjects in the ITT population. We do not know the assigned treatment for these excluded subjects as no data were available. It is even more difficult to rely on the results of the defined ITT because we cannot assess how subjects were excluded between the arms. Eleven ITT subjects were excluded from the PP population due to the protocol deviations described below. Two patients died; one for unknown reason on Day 2 and the death was classified as possibly related to the study drug. The second patient died on day 1 following grand mal convulsion which was classified as an event not related to the study drug. Both patients were from the 3-dose group.

Table 8-24. Patient disposition (ITT Population)

	5-dose Artesunate	3-dose Artesunate	All
Randomized	101	96	197
Completed study	77/101 (76.2%)	79/96 (82.3%)	156/197 (79.2%)

	5-dose Artesunate	3-dose Artesunate	All
Discontinued	24 (23.8%)	17 (17.7%)	41 (20.8%)
Reason for discontinuation			
Death	0	2 (2.0%)	2 (1.0%)
Withdrawal of consent	2 (2.0%)	1 (1.0%)	3 (1.5%)
Serious protocol violation	4 (4.0%)	0	4 (2.0%)
Loss of follow-up	15 (14.9%)	11 (11.5%)	26 (13.2%)
Other	3 (3.0%)	3 (3.1%)	6 (3.0%)
Safety population	100	94	194
ITT population	93	89	182
PP population	86	85	171
Major protocol deviations	7	4	11
Error dosing	2	2	4
Parasitemia < 5000	3	1	4
Quinine/amodiaquine on Day 0	2	1	3

Source: Chart 6.1-1, Tables 6.1-1, 6.1-2, 6.2-1 of Study Report

Demographic Characteristics

All subjects were African. Those in the 5-dose group were slightly younger, but the difference in median between the two groups was not statistically significant. Sex and other demographic factors were not reported.

Table 8-25. EDCTP/MMV07-01 Age Distribution (ITT Population)

	5-dose Artesunate (N=93)	3-dose Artesunate (N=89)	All (N=182)	Wilcoxon test p-value
Age				
Mean (SD)	3.6 (2.23)	3.9 (2.27)	3.7 (2.25)	
Median	3.1	3.5	3.4	0.344
Min, Max	0.6, 9.3	0.6, 9.1	0.6, 9.3	

Source: Table 7.2-1 of Study Report

Other Baseline Characteristics (e.g., disease characteristics, important concomitant drugs)

Disease characteristics between the two treatment groups were comparable. No information on the comparison of concomitant drugs between the two treatment groups is available in the study report.

Table 8-26. EDCTP/MMV07-01 Medical History (ITT Population)

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

	5-dose Artesunate (N=93)	3-dose Artesunate (N=89)	All (N=182)
Immunologic/Allergy	0	0	0
Head Eyes Ears Nose Throat	3 (3.2%)	2 (2.2%)	5 (2.7%)
Respiratory	11 (11.8%)	7 (7.9%)	18 (9.9%)
Cardiovascular	1 (1.1%)	0 (0.0%)	1 (0.5%)
Gastrointestinal	6 (6.5%)	10 (11.2%)	16 (8.8%)
Urogenital	0 (0.0%)	1 (1.1%)	1 (0.5%)
Musculoskeletal	1 (1.1%)	2 (2.2%)	3 (1.6%)
Dermatological	6 (6.5%)	2 (2.2%)	8 (4.4%)
Hematological/lymphatic	16 (17.2%)	13 (14.6%)	29 (15.9%)
Endocrine	0	0	0
Neurological	6 (6.5%)	6 (6.7%)	12 (6.6%)
Psychiatric	0	0	0
Other	7 (7.5%)	5 (5.6%)	12 (6.6%)

Source: Table 7.2-2 of Study Report

Comparable symptoms of current infection (headache, chest pain, vomiting, nausea, chills, abdominal pain, fever, coma seizures, prostration, etc.) at Day 0 were reported in the study report. The results of complete physical examination data Day 0 were comparable too. Clinical signs and symptoms were balanced in general. There was no significant difference in asexual parasite counts at the baseline, as the following table shows.

Table 8-27. EDCTP/MMV07-01 Asexual Parasite Counts at Baseline (ITT Population)

	5-dose Artesunate (N=93)	3-dose Artesunate (N=89)	Wilcoxon test P-value
Mean (SD)	341469.9 (406762.7)	244171.5 (295049.9)	
Median	195200	150500.0	0.343
Min, max	869.0, 1870264.0	1127.0, 1800860.0	

Source: Table 7.2-6 of Study Report

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

Study drug was administered by authorized staff only and amount was recorded. Compliance with administration according to the protocol was achieved. Adjunctive therapy, including fluids, glucose, and blood (according to SMAC standards and WHO guidelines for the treatment of severe malaria) was given. In case of initial treatment failure with IV artesunate or a severe drug reaction to artesunate, parenteral quinine was administered. However, this information was not included in the study report.

Efficacy Results – Primary Endpoint

The primary efficacy endpoint, the proportion of patients with parasite clearance ($\geq 99\%$ reduction from the baseline asexual parasite count) at 24 hours after initiation of study drug, is shown in the following table. At the time of assessment of the primary endpoint (24 hours), since the dose administered at 24 hours would have had no time to act, the comparison of the 2 arms compares the effect of the first 2 doses of 2.4 mg/kg at 0 and 12 hours in the 5-dose regimen to the first dose (4.0 mg/kg) at 0 hours of the 3-dose regimen. If we assume that the 4.0 mg/kg would have had the same or stronger effect than the 2.4 mg/kg dose at time 0, the difference seen can be considered a conservative estimate of the effect of the 2.4 mg/kg dose given at 12 hours. The difference of 8.5% (95% CI: [-2.7%, 19.7%]) in PC₉₉ was indicative of an effect of the 12-hour dose although it was not statistically significant.

As a sensitivity analysis, we assumed the randomized subjects excluded from the ITT subjects as failures, or as failures on 5-dose regimen and successes on 3-dose regimen. The difference was similar to the result from the ITT population and was close to 0 for two assumptions, respectively.

Table 8-28. EDCTP/MMV07-01 Proportion of Patients with $\geq 99\%$ Parasite Clearance at 24 Hours by Treatment Group (ITT population)

	5-dose Artesunate (N=93)	3-dose Artesunate (N=89)	All (N=182)	Difference [95% CI] Chi-squared p-value
PC ₉₉ reached in 24 hours	80 (86.0%)	69 (77.5%)	149 (81.9%)	8.5% [-2.7%, 19.7%] 0.137
95% CI	79.0%, 93.1%	68.9%, 86.2%	76.3%, 87.5%	
Sensitivity analysis including all randomized subjects by the reviewer				
Assuming all non-ITT subjects were failures	80/101 (79.2%)	69/96 (71.9%)	149/197 (75.6%)	7.3% [-4.7%, 19.3%] 0.231
Assuming all non-ITT subjects in 5-dose group were failures And all non-ITT subjects in 3-dose group were successes	80/101 (79.2%)	76/96 (79.2%)	156/197 (79.2%)	0.04% [-11.3%, 11.4%] 0.994

Source: Table 7.4-1 of Study Report.

Efficacy Results – Secondary and other relevant endpoints

Time to total, 99%, 90%, and 50% clearance of asexual parasite (PC₁₀₀, PC₉₉, PC₉₀, PC₅₀)

The median time to PC₁₀₀ was 36 hours for both groups. There was no statistically significant difference in the survival curves between the two groups (p-value=0.902 from the Log-rank test). The median time to PC₉₉ or PC₉₀ was numerically longer in the 5-dose group, but not statistically significantly longer than that in the 3-dose group.

A summary of percent reduction in asexual parasites from baseline at 24 and 48 hours is shown in the following table. Although there was no difference between the two groups based on the Wilcoxon test, the 5-dose regimen was numerically better.

Table 8-29. EDCTP/MMV07-01 Percent Reduction in Asexual Parasites from Baseline at 24 and 48 Hours after Initiation of Randomized Study Drug

	5-dose Artesunate (N=93)	3-dose Artesunate (N=89)	P-value
% reduction at 24 hours			
N	93	88	
Mean (SD)	99.2 (1.92)	97.6 (8.99)	
Min, Max	88.3, 100.0	32.7, 100.0	
Median	99.9	99.9	Wilcoxon 0.544
% reduction at 48 hours			
N	89	87	
Mean (SD)	100.0 (0.02)	98.8 (9.98)	
Min, Max	99.8, 100.0	7.8, 100.0	
Median	100	100	Wilcoxon 0.610

Source: Table 7.4-7 and Table 7.4-8 of Study Report

Additional Analyses Conducted on the Individual Trial

No subgroup analysis results by age and sex are available in the report and therefore the results could not be reported or assessed.

8.2 Integrated Review of Effectiveness

8.2.1 Assessment of Efficacy Across Trials

The SEAQUAMAT and AQUAMAT trials were supported by the same sponsor, the Wellcome Trust, with similar study designs (open-label, randomized) to compare the efficacy and safety of parenteral artesunate versus quinine. However, there were some differences. The study population in SEAQUAMAT was Asian with adults (86.2%) and pediatric patients aged 2-15 years (13.8%) while in AQUAMAT, African pediatric patients younger than 15 years old (exact range not reported) were enrolled. The administration route in SEAQUAMAT was IV and in AQUAMAT was IV (64.5%) and IM (35.5%). The third study, which had a different study design and primary endpoint and for which no data were submitted, will not be covered in this section.

Primary Endpoint

In the first two trials, the primary endpoint was in-hospital mortality. Artesunate demonstrated a significant reduction in mortality compared with quinine, as the following table shows. AQUAMAT had a higher overall mortality rate; however, given the differences in these trials, including location and patient age, differences in mortality rates between the trials are not unexpected.

Table 8-30. SEAQUAMAT and AQUAMAT Primary Endpoint: In-hospital Mortality

Primary Endpoint	SEAQUAMAT			AQUAMAT		
	Artesunate (N=730)	Quinine (N=731)	OR (95% CI) P-value	Artesunate (N=2712)	Quinine (N=2713)	OR (95% CI) P-value
In-hospital mortality	96 (13.2%)	150 (20.5%)	0.59 (0.44, 0.79) 0.0002	230 (8.5%)	297 (10.9%)	0.75 (0.63–0.90) 0.0022

Secondary and Other Endpoints

In-hospital mortality in patients with severe malaria is displayed in the following table. Although the criteria of severe malaria were different in the two trials, artesunate demonstrated a consistent and significant treatment effect in this study subpopulation.

Sequelae were assessed on discharge in SEAQUAMAT and at Day 28 in AQUAMAT. In each trial, artesunate demonstrated a significant treatment effect in the combined outcome (in-hospital death or sequelae).

Table 8-31. SEAQUAMAT and AQUAMAT Secondary Endpoints

Secondary Endpoint	SEAQUAMAT			AQUAMAT		
	Artesunate	Quinine	OR (95% CI) P-value	Artesunate	Quinine	OR (95% CI) P-value
In-hospital mortality in severe malaria	92/509 (18.1%)	139/541 (25.7%)	0.65 (0.48, 0.88) 0.0045	236/2280 (9.9%)	291/2338 (12.4%)	0.77 (0.64–0.93) 0.0055
In-hospital death or significant neurological outcome on discharge	103/730 (14.1%)	153/731 (29.9%)	0.63 (0.47-0.83) 0.0009	253/2689 (9.4%)	316/2695 (11.7%)	0.78 (0.65–0.93) 0.0056

*SEAQUAMAT results were based on the statistical reviewer’s analysis. AQUAMAT results were based on the reported results from the publication.

Subpopulations

The following table displays the results of in-hospital mortality in selected subpopulations. The efficacy results were generally similar in various subpopulations.

Table 8-32. SEAQUAMAT Mortality and AQUAMAT In-hospital Mortality Subgroup Analyses

Subpopulation	SEAQUAMAT		AQUAMAT	
	Artesunate (N=730)	Quinine (N=731)	Artesunate (N=2712)	Quinine (N=2713)
Hyperparasitemia (>10%)				
Yes	26/121 (21%)	52/108 (48%)	56/584 (10%)	77/573 (13%)
No	70/609 (11%)	98/623 (16%)	140/1766 (8%)	167/1779 (9%)

Subpopulation	SEAQUAMAT		AQUAMAT	
	Artesunate (N=730)	Quinine (N=731)	Artesunate (N=2712)	Quinine (N=2713)
Missing			34/362 (9%)	53/361 (15%)
Pretreatment with any antimalaria treatment				
Yes	17/167 (10%)	34/142 (24%)	132/1325 (10%)	162/1330 (12%)
Ineffective			38/387 (10%)	38/371 (10%)
Effective			94/938 (10%)	124/959 (13%)
No	79/563 (14%)	116/589 (20%)	86/1281 (7%)	125/1270 (10%)
Missing			12/106 (11%)	10/113 (9%)
Acidosis				
Yes	70/308 (23%)	117/334 (35%)	149/1009 (15%)	189/975 (19%)
No	13/354 (4%)	13/314 (4%)	49/1275 (4%)	52/1314 (4%)
Missing	13/68 (19%)	20/83 (24%)	32/428 (7%)	56/424 (13%)
Severe anemia (hemoglobin<50 g/L)				
Yes	5/40 (13%)	9/54 (17%)	77/737 (10%)	93/692 (13%)
No	86/643 (14%)	124/621 (20%)	136/1696 (8%)	163/1734 (9%)
Missing	5/47 (11%)	17/56 (30%)	17/279 (6%)	41/287 (14%)
Shock				
Yes	11/78 (14%)	23/92 (25%)	57/323 (18%)	66/339 (19%)
No	85/652 (13%)	127/639 (20%)	173/2389 (7%)	231/2374 (10%)

Source: The publications and statistical reviewer's analyses

8.2.2 Integrated Assessment of Effectiveness

Although patient-level data were only available from SEAQUAMAT, the data were considered adequate to provide substantial evidence of effectiveness based on the persuasive finding of mortality benefit in this trial with a similarly robust mortality benefit reported in the AQUAMAT trial publication.

SEAQUAMAT demonstrated the efficacy of IV artesunate in the treatment of severe malaria in adults and pediatric patients. Significantly reduced mortality was observed in the artesunate group compared to the quinine group. In this trial, the observed in-hospital mortality was 96/730 (13.2%) in the artesunate group and 150/731 (20.5%) in the quinine group, with an OR of 0.59 (95% CI: 0.44, 0.79, p=0.0002). The in-hospital mortality was lower in the subgroup of subjects who received any prior antimalaria treatment than in the subgroup of subjects who did not. AQUAMAT provided supportive information for IV artesunate's efficacy in severe malaria in a younger patient population in a different region of the world; however, patient level data were not available for review.

A third trial, EDCTP/MMV07-01, demonstrated high proportions of early parasite reduction in subjects treated with artesunate IV. Slight differences in the treatment regimens in that trial, demonstrated the importance of the 12-hour dose; however, patient level data were also not available.

8.3 Statistical Issues

No major statistical issues were identified. However, there were a few minor statistical issues worthy of discussion. In SEAQUAMAT, according to the protocol, an interim analysis was to be performed after enrollment of 500 patients and after that at time points chosen by the DSMC. It is noted that the DSMC was advised that clinical practice would only change if there was compelling evidence of at least a 25% treatment effect for artesunate compared to quinine, although it is not clear a 25% treatment effect was on absolute difference in mortality rates, relative risk, or odds ratios. There were two interim analyses conducted in the trial. The first interim analysis was conducted in June 2004 with 659 patients included (129 deaths). The second interim analysis was conducted in February 2005 when there were 244 deaths for the 1294 patients included. The p-value at that point was 0.0007. The trial was recommended to stop at this interim analysis based on the strength and robustness of the efficacy findings and supporting safety results. The trial was stopped on May 11, 2005 with 1461 patients included. Despite not having plans in place to control the Type I error, it is noted that the study results would have been significant based on the commonly used and acceptable stopping rules (the O'Brien-Fleming or the Haybittle-Peto procedure) even if 3-5 interim analyses were considered.

Intravenous quinine requires a carefully rate-controlled infusion with a continuous or three times daily administration to avoid dangerous hypotension. In the AQUAMAT ITT analysis population, more subjects died before receiving the assigned treatment in the quinine group. More early deaths in the quinine group might be pointing to an impact of the delay in the quinine group and a potential weakness/vulnerability of that group. It should be clear that a direct comparison of artesunate to quinine might not be appropriate based on this study. It was a practical comparison of efficacy, given the constraint in drug administration, not a direct (theoretical) comparison of administering the drug in the same time period. The trial still demonstrated superior efficacy, but it might not have demonstrated such a large effect over a prompt treatment with quinine. In addition, lack of patient-level data was a limitation of this study.

In these two trials, as there was no specific follow-up procedure after discharge, mortality data after discharge may not have been consistently collected. Therefore, the interpretation of comparative overall mortality results between the two treatment groups should be made with caution.

Both adjusted and unadjusted analyses of in-hospital mortality by study site (hospital) produced a similar or the same results, indicating that the treatment effects were homogeneous and not greatly impacted by hospital.

8.4 Conclusions and Recommendations

The SEAQUAMAT study was considered by the statistical reviewer as an adequate and well-controlled trial that demonstrated a statistically significant improvement in the in-hospital

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

mortality in patients receiving IV artesunate compared to IV quinine for the treatment of severe malaria. The results were strong and consistent across multiple subgroups. AQUAMAT provided supportive evidence from a different region and in younger patients. The application meets the evidentiary standard of effectiveness and demonstrated efficacy of artesunate. Therefore, artesunate is recommended for approval for the proposed indication of treatment of patients with severe malaria.

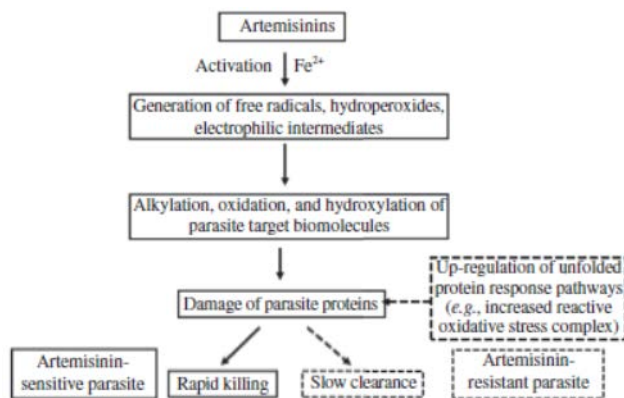
9 Clinical Microbiology Review

9.1 Nonclinical Microbiology

9.1.1 Mechanism of Action

The precise mechanism by which artesunate and DHA exhibit activity against *Plasmodium* species is not clearly understood. Studies suggest that the mechanism of action of artesunate and DHA, like other artemisinins, is likely multifactorial. Endoperoxide bridge is essential for the antiplasmodial activity (Figure 9-1). Upon activation by heme (liberated as a result of plasmodial protease-mediated degradation of host hemoglobin), artesunate and DHA, like other artemisinins, generate free radicals (e.g., superoxide anions, H_2O_2 and other intermediates), increase oxidative stress within the infected RBCs, cause alkylation of proteins and lipids as well as ultrastructural changes leading to decrease in parasite function, growth and survival. The mitochondria, endoplasmic reticulum, as well as digestive vacuole appear to be the sites of damage.

Figure 9-1: Diagrammatic representation of the mechanism of action and resistance to qinghaosu/artemisinin.



The activation by Fe^{2+} releases free radicals, hydroperoxides, and other intermediates, and the addition of these products to target biomolecules, (e.g., proteins, forming damaged proteins), leads to rapid parasite death (shown in solid line symbols). The resistant parasites have up-regulated unfolded protein response pathways including reactive oxidative stress complex, decreasing protein damage caused by artemisinin action and slowing parasite growth rate (shown in broken line symbols).

Source: ([Krungskrai and Krungskrai 2016](#))

The uptake of artemisinins varies with different developmental forms of the *P. falciparum* parasite; the ring forms accumulate lower amounts of DHA compared to trophozoites. At the early ring stage, activation appears to rely primarily on the parasite's heme biosynthesis pathway and at latter stages on hemoglobin digestion; it appears that most of the proteolysis of hemoglobin occurs during the trophozoite stage that the parasite endocytosed from the RBC into an acidic digestive vacuole.

The artesunate treatment regimen, proposed by the Applicant, spans the entire asexual life cycle of the *Plasmodium* parasite in the blood.

For details see Section 18.5.1.

9.1.2 *In vitro* Activity

Methods for measurement of *in vitro* sensitivity of *Plasmodium* species are not standardized and limited to testing in research laboratories. The most common *Plasmodium* species used for *in vitro* testing is *P. falciparum* and the testing is based on blood stage parasites.

9.1.2.1 *Exoerythrocytic forms of Plasmodium species*

Meister *et al.* ([Meister et al. 2011](#)) reported that artemisinin is not active against the liver stage parasites of *Plasmodium yoelii*. Artemisinin class of drugs are not active against the exoerythrocytic (liver) stage parasites of *Plasmodium* species ([Tilley et al. 2016](#)).

9.1.2.2 *Erythrocytic forms of Plasmodium species*

The studies supporting the activity of artesunate and DHA against the different erythrocytic stages of *P. falciparum* and other *Plasmodium* species are summarized in Section 18.5.2.

Overall, the studies suggest that artesunate and DHA are active *in vitro* against the blood stage parasites of *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*. The artesunate 50% inhibitory concentrations (IC₅₀) against the laboratory strains and clinical isolates were ≤ 2.14 ng/mL and ≤ 2.69 ng/mL, respectively; the DHA IC₅₀ against the laboratory strains and clinical isolates were ≤ 6.54 ng/mL and ≤ 3.98 ng/mL, respectively.

The activity may vary against the different developmental forms in the blood. The mature gametocytes appear to be the least sensitive.

9.1.3 *In vivo* Activity (Animal Studies)

The suppressive activity of artesunate and/or DHA was measured in rodent and nonhuman primate malaria models including severe malaria animal models. Overall, the studies support the activity of artesunate and DHA in severe malaria animal models that include rodents infected with the erythrocytic stage parasites of *P. berghei*, immunocompromised chimeric mice infected with *P. falciparum*, nonhuman primates infected with *P. coatneyi*, *P. cynomolgi*, *P. falciparum* or *P. knowlesi*. However, recrudescence occurs after discontinuation of treatment.

DHA was also effective in curing immunocompromised mice infected with a chloroquine and quinine resistant strain of *P. falciparum*; it is noted that DHA was effective in curing mice that had failed chloroquine treatment.

For details see Section 18.5.2.

9.1.4 Drug Resistance

The nonclinical and clinical studies supporting a potential for development of resistance to artesunate or DHA in the blood stage parasites of *Plasmodium* species are summarized in Section 1.1.1. Briefly, studies in the *P. falciparum* humanized murine immunocompromised model suggest that delay in parasite clearance time was not associated with increased IC₅₀ values; however, in stable resistant strains, there was an association with an increase in the IC₅₀ values as well as mutation in the *RAD5* but not in the *Kelch13* gene.

Published clinical studies suggest that resistance to artemisinins, including artesunate and DHA, in the *P. falciparum* parasites is manifested as recrudescence and/or delay in parasite clearance time. This is thought to be due to short half-life of the artemisinin drugs. Therefore, artemisinin drugs are administered in combination with long-acting drugs such as lumefantrine.

The mechanism of resistance is likely to be multifactorial and complex with more than one genotype/phenotype associated with artemisinin resistance. A delay in parasite clearance time and/or recrudescence has been shown to be associated with alterations in some genetic regions, such as *Kelch13* and *Pfmdr1* of *P. falciparum* isolates from some of the regions. A possibility of different mechanism of resistance associated with delayed parasite clearance and recurrence (recrudescence) of parasites after initial cure exists.

There is no standardized assay to evaluate the in vitro sensitivity of the isolates to artemisinins, including artesunate and DHA. Recent studies ([Witkowski et al. 2013](#); [Klonis et al. 2013](#)) suggest that a ring-stage survival assay may be useful for testing sensitivity to artemisinins; there was a good correlation between in vitro sensitivity and a delay in parasite clearance time. Thanh et al. ([Thanh et al. 2017](#)) reported that recrudescence was associated with a decrease in the ex vivo response to DHA as measured by the ring survival assay.

9.1.5 Cross-resistance

The nonclinical studies in vitro and in animal models of malaria suggest a potential for development of cross-resistance to quinine, halofantrine, and amodiaquine (for details see Section 1.1.2). However, the clinical relevance of these findings is not known. Cross-resistance with quinine, amodiaquine, and halofantrine may be due to changes in the *Pfmdr1* gene, which encodes the P-glycoprotein pump essential for parasite detoxification.

9.2 Clinical Microbiology

Parasitological assessments, for the two phase 3 trials, SEAQUAMAT and AQUAMAT, to support the efficacy of artesunate in patients with severe malaria as well as other supportive studies are summarized.

9.2.1 Parasitological Assessments

Parasitological assessments, in the clinical trials (SEAQUAMAT, AQUAMAT, EDCTP/MMV07-01, Study 1263ab and Study CDC-060) include rapid diagnostic tests and/or microscopic examination of blood smears.

9.2.1.1 Study SEAQUAMAT (Dondorp et al., 2005)

The study was conducted in Southeast Asia (Myanmar, Bangladesh, India, and Indonesia) in adults and children ≥ 2 years of age. The initial malaria diagnosis was performed using a rapid Paracheck test that was confirmed by microscopic examination of blood smears. The details of the parasitological methods used were not included. Following is summarized based on published information.

Rapid test: The initial malaria diagnosis was performed using a histidine rich protein 2 (HRP-2) immunochromatographic Paracheck test (Orchid Biosystems, India). The Paracheck test is not cleared by the FDA. Based on the information in the test package insert, the sensitivity and specificity of the Paracheck test, compared to microscopic examination of blood smears, were 98.2% and 99.3%, respectively. Some of the endogenous (e.g., bilirubin, creatinine, triglycerides, and uric acid at high physiological levels) and exogenous (e.g., antimalarial drugs, antibiotics, and anti-inflammatory drugs at high concentrations) substances may interfere with the test performance. The test should be used for the diagnosis of *P. falciparum* malaria with signs and symptoms consistent with malaria infection. Also, the test results should be confirmed by microscopy.

The following information for the Paracheck test is from the WHO compendium ([World Health Organization 2014](#)) that summarized the performance characteristics of several malaria rapid diagnostic tests:

- For the *P. vivax* samples with 200 parasites/ μL (a low parasite burden) and 2,000-5,000 parasites/ μL (a higher parasite burden), there were no false-positive *P. falciparum* rates.
- For clean negative samples, the total *Plasmodium* species false positive rate was between 0.9 and 1.3%.

No information on *Plasmodium* species other than *P. falciparum* or *P. vivax* was included in the WHO compendium. The Paracheck test appears to be specific for *P. falciparum* species.

Microscopic examination of blood smears: Slide reading was performed in reference laboratories in the Shoklo Malaria Research Unit in Mae Sot, Thailand, or in the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. The details for the preparation of blood smears, slide reading and quality control parameters implemented were not included in the study report or the publication.

Patients with severe malaria, enrolled in the clinical trial had at least one of the 9 criteria for severe malaria that include asexual parasitemia $>10\%$, hematocrit $<20\%$ + $>100,000$

parasites/ μL (2%) or visible jaundice and $>100,000$ parasites/ μL (2%) besides being positive by the Paracheck test.

Of the 1461 patients enrolled based on the Paracheck test, 5.4 % (n=79; 41 in the artesunate arm and 38 in the quinine arm) were smear negative. The *Plasmodium* species identified in the smear positive patients was *P. falciparum*. Hyperparasitemia ($\geq 10\%$), severe malaria, and cerebral malaria were reported in 15.7%, 71.9%, and 40.2% of the patients, respectively. Overall, artesunate was more effective than quinine in reducing mortality (for details see Section 8).

No information on post-treatment follow-up of patients for parasitemia was included.

9.2.1.2 Study AQUAMAT (Dondorp et al., 2010)

The study was conducted in African children, ≤ 15 years of age, with malaria. The initial malaria diagnosis was performed using a rapid Optimal test that was confirmed by microscopic examination of blood smears. The following is summarized based on published information.

Rapid test: The initial malaria diagnosis was based on a positive pLDH test (Optimal, Switzerland); the test package insert was not available for review. The test is not cleared by the FDA. The following information for the Optimal test is from the WHO compendium ([World Health Organization 2014](#)):

- The false-positive rates when *P. falciparum* was present at 200 parasites/ μL (a low parasite burden) or 2,000-5,000 parasites/ μL (a higher parasite burden) were 1.5% and 0.5%, respectively.
- For *P. vivax* samples with low or high parasitemia, the false positive *P. falciparum* rate was 0.0% and 20.3%, respectively.
- For clean negative samples, the total *Plasmodium species* false positive rate was 2.0%.

Overall, the results suggest that the test is not specific for *P. falciparum* and that other *Plasmodium* species may show positive findings. No information on *Plasmodium* species other than *P. falciparum* or *P. vivax* was included in the WHO compendium.

Microscopic examination of blood smears: Thick and thin blood smears were prepared for determining parasitemia and *Plasmodium* species at the site laboratories as well as the Bangkok reference laboratory. The details for the preparation of blood smears, slide reading and quality control parameters implemented were not included in the study report or the publication.

Of the 5425 patients enrolled, central laboratory assessments for parasite counts were not available for 109 patients; therefore, parasitemia results based on slide reading at the site laboratory were used. Slides were missing for 211 patients (112 in the artesunate arm and 99 in the quinine arm) and 42 patients (17 in the artesunate arm and 25 in the quinine arm) were blood smear negative. Of the 5214 patients with a positive rapid diagnostic test result, 99.2% were blood smear positive. Hyperparasitemia ($>10\%$) was reported in 1157 patients (21.3%;

584 in the artesunate arm and 573 in the quinine arm) and severe malaria in 4618 (88.6%; 2280 in the artesunate arm and 2338 in the quinine arm) patients; although the number of patients with cerebral malaria was not specified, coma and convulsions were reported in 1825 (33.6%) and 1690 (31.2%) patients enrolled. Overall, artesunate was more effective than quinine in reducing mortality (for details see Section 8).

No information on post-treatment follow-up of patients for parasitemia was included.

9.2.1.3 Study EDCTP/MMV07-01 (Kremsner et al., 2012)

The study was conducted in African children with *P. falciparum* severe malaria; patients were randomized to either 3-dose (0, 24, and 48 hours) or 5-dose (0, 12, 24, 48, and 72 hours) IV artesunate groups. Parasitological assessments performed include qualitative and quantitative examination of blood smears for enrolling patients with ≥ 5000 *P. falciparum* parasites/ μL and severe malaria as well as evaluating efficacy ($\geq 99\%$ parasite clearance).

Thick and thin blood smears for parasitological assessments were prepared at 6-hourly interval after treatment as well as prior to the next dose of treatment for at least 72 hours following the first dose of study drug or until 2 consecutive negative smears were recorded within the last 24-hour period. The Giemsa stained blood smears were examined, at the site laboratories, by 2 qualified microscopists who were blinded to each other's results and to the treatment status of the patient. Non-concordance among microscopy interpretations was referred to a 3rd expert microscopist for final slide determination; additionally, 5% of the concordant results between Microscopist 1 and 2 were re-examined by the third expert microscopist, however, these results were not recorded on the CRFs. Non-concordance was defined as disagreement about any of the following:

- Presence of asexual forms of *Plasmodia* on blood smear.
- Species of *Plasmodium* present on blood smear.
- Density of parasitemia within a factor of 2, if either microscopist determined parasitemia to be >100 asexual parasites/ μL . If both microscopists agreed to the *Plasmodium* species as well as the presence of parasitemia, with both counts <100 parasites/ μL , the results were considered concordant even if the reported parasitemia were not within 2-fold of each other.
- Parasitemia within an absolute range of 50,000 parasites/ μL . For example, a count by one microscopist of 200,000/ μL and a count by the 2nd Microscopist of 300,000 parasites/ μL was not considered concordant.

It is noted that the criteria for non-concordance/concordance between >100 and $<50,000$ parasites/ μL were not specified.

The parasitological and clinical follow-up examinations were performed on Days 7, 14, 21, and 28. As soon as the patient was able to receive oral medication and no signs and symptoms of severe malaria were present (~50 hours after the start of artesunate treatment), a single dose

of sulfadoxine/pyrimethamine (in Gabon) or artemether-lumefantrine (in Malawi) was to be administered to ensure parasitological cure. Recurrent infection within 28 days was treated with artemether-lumefantrine.

Parasitemia at baseline ranged between 869 and 1870624 parasites/ μ L (0.02-37.41%, respectively). Within 24 hours of treatment initiation, 99% reduction in parasitemia (PC₉₉) was achieved in 85% of the patients in the 5 dose group compared to 78% in the 3 dose groups; the median parasite clearance time for PC₉₉ ranged between 24 and 18 hours in both 5-dose and 3-dose group.

Parasite clearance (PC₁₀₀; based on two consecutive negative blood smears), was achieved in 84% of the patients in the 5 dose group and 80% in the 3-dose group; the median time to parasite clearance was 36 hours in both groups.

The median time to fever clearance was 12 hours in both groups.

Of the 16 patients with recurrence of parasitemia at Day 28, 7 were in the 5-dose group compared to 9 in the low dose group. It is noted that the recurrence rate is based on initial parental artesunate treatment followed by oral treatment with either a combination of sulfadoxine-pyrimethamine or artemether-lumefantrine.

For details see Section 8.

9.2.1.4 Study 1263ab

Study 1263ab was conducted in Thailand and Kenya in patients with acute, uncomplicated *P. falciparum* malaria with parasitemia of $\geq 5,000/\mu$ L. Artesunate was administered, IV, at doses of 1.2, 2.4, and 4.8 mg/kg for 3 days followed by mefloquine or Malarone. For determining parasitemia, two thick and thin malaria smears were prepared at different timepoint and Giemsa stained at the site laboratories by trained microscopists. Slides were assessed for the quality of slide preparation and staining prior to slide interpretation; poorly prepared slides were not used for assessment. Slides were examined by two qualified microscopists who were blinded to each other's results and to the treatment status of the patient. A total of 200 oil immersion fields were examined before a blood smear was considered negative. A non-concordance of the two microscopists in the results of the initial smear (positive/negative; identification of *Plasmodium* species) resulted in an exclusion of the subject.

Parasite densities were calculated based on a count of parasites per 200 WBCs in a thick film (if ≥ 10 parasites seen), 500 WBC in thick film (if 9 or less parasites seen at 200 high power fields) or per 5000 RBCs in thin film (if parasites $> 500/200$ WBCs). The final parasite density was calculated as the arithmetic mean of the density determined by the two microscopists. Additionally, the individual microscopist's evaluation of the parasite density had to be less than 2-fold of each other or the resultant parasite density was considered non-concordant and

referred to a third certified expert microscopist as a referee. The 3rd expert microscopist reviewed both smears and determined a final interpretation. Additionally, if either of the two primary microscopists calculated a parasite density of ≤ 100 parasites/ μL , the slides were automatically referred to the certified expert microscopist for the final density determination. Overall, the malaria slide interpretation was managed by a slide coordinator, who was not a microscopist, to assess concordance between the first two readers and to ensure that blinding was maintained.

The Applicant states that for quality assurance, 5% of the concordant results were re-examined by the referee expert microscopist; however, these readings and parasite density evaluation were not considered as the final result.

A majority of the patients were infected with *P. falciparum* and treated with either 1.2, 2.4, 4.8 mg/kg of IV artesunate for 3 days followed by mefloquine or Malarone. The primary objective was to compare parasite clearance time. Overall, 90% parasite clearance was achieved by 48 hours in all subjects and 100% clearance by 72 hours range 12-164 hours (median 32-40 hours) in patients treated with 2.4 mg/kg artesunate for 3 days followed by mefloquine or Malarone. Recrudescence was reported on Day 4, in one patient (Subject ID (b) (6) treated with 2.4 mg/kg artesunate for 3 days (bid on Day 0, followed by once daily for 2 days).

One of the exclusion criteria was exclusion of subjects with “mixed malaria infection on admission by malaria smear (i.e., any parasite morphologies not consistent with a *P. falciparum* mono-infection)”; however, two patients with mixed infection due to *P. falciparum* and *P. ovale* from Kenya were enrolled. One patient was treated with 1.2 mg/kg and another with 4.8 mg/kg dose of IV artesunate. In both patients, the *P. falciparum* parasitemia was reduced by ≥ 42 -fold on Day 2 and became aparasitemic by Day 3 of treatment; for *P. ovale*, no parasites were detected on Day 2 (Table 9-1).

Table 9-1: Study 1263ab - Follow-up of *P. falciparum* and *P. ovale* parasitemia in 2 patients with mixed infection

Patient ID	Dose mg/kg	Screening/Baseline		Day 2 (Visit 2)*		Day 3 (Visit 2)*	
		<i>P. falciparum</i>	<i>P. ovale</i>	<i>P. falciparum</i>	<i>P. ovale</i>	<i>P. falciparum</i>	<i>P. ovale</i>
(b) (6)	1.2	31 x 10 ⁶ / μL	0.9 x 10 ⁶ / μL	0.04 x 10 ⁶ / μL	Negative	Negative	Negative
	4.8	36 x 10 ⁶ / μL	0.9 x 10 ⁶ / μL	0.02 x 10 ⁶ / μL	Negative	Negative	Negative

*In patient IV artesunate treatment

9.2.1.5 Study CDC-060

The Study R-CDC-060 was a treatment protocol performed in the United States. Microscopic examination of blood smears was performed at the local hospital clinical laboratory for a majority of the patients; specimens from some of the patients were sent to reference

laboratories. For one patient (Subject (b) (6)) *P. falciparum* species was confirmed by a PCR method at the CDC. The details of the methods used were not provided.

Of the 102 patients, 87 patients were evaluable based on microscopic confirmation of *P. falciparum* and met the criteria of need for IV treatment; parasitemia was $\geq 5\%$ in 65 patients. *Plasmodium* species other than *P. falciparum* were identified in 5 patients (3 with *P. vivax* and 2 with *P. ovale*).

Deaths were reported in 5 (5.7%) patients with *P. falciparum* (baseline parasitemia ranged between 12% and 90%) and one patient with *P. vivax* (baseline parasitemia 0.7%). Two patients with *P. ovale* infection survived (Table 9-2). The Applicant states that patient with *P. vivax* who died was “essentially moribund at presentation”.

Table 9-2: Study CDC-060 - Survival in patients with *P. vivax* and *P. ovale* malaria

Patient ID	<i>Plasmodium</i> species (%parasitemia at baseline)	# doses of IV artesunate	Smear negative (Hours)	Survival status
(b) (6)	<i>P. vivax</i> (1.0)	4	Yes (72)	Survived
(b) (6)	<i>P. vivax</i> (0.7)	2	No (108)	Died
(b) (6)	<i>P. vivax</i> (1.0)	4**	No (84)**	Survived
(b) (6)	<i>P. ovale</i> (Not specified)	4	Yes (84 hours)	Survived
(b) (6)	<i>P. ovale</i> (47859)	2	Not specified	Survived

^aPatient (b) (6) was unable to take oral medication and had circulatory collapse, ARDS, acidosis, and DIC at baseline.
^{**}Two doses of artesunate administered on Days 4 and 5

Source: Applicant submission

The Applicant summarized all available CDC surveillance data for resistance markers, collected between 2012 – 2016 i.e., when CDC began molecular surveillance for malaria drug resistance markers from patients with severe and uncomplicated malaria (Table 9-3). Three patients from Africa were stated to have artemisinin resistance *kelch13* marker; 2 (1.5%) were in 2014 and 1 (0.7%) in 2015. It is noted that the methods used for identifying artemisinin resistance were different in 2014 and 2015; details of the methods used were not provided.

Table 9-3: Artemisinin resistance among *P falciparum* samples - CDC (2012-2016)

Year	Resistance markers tested	No. of specimens tested	Regions represented	No. specimens resistant (markers)
2012	Polymorphisms located on: chromosome 10 (<i>MAL10-688956</i>) chromosome 12 (<i>MAL13-1718319</i>)	56	52 – Africa 2 – C. America 2 - Unknown	0
2013		76	60 – Africa 1 – C. America 1 – S. America 14 - Unknown	0

Year	Resistance markers tested	No. of specimens tested	Regions represented	No. specimens resistant (markers)
2014	Polymorphisms located on: chromosome 10 (<i>MAL10-688956</i>) chromosome 12 (<i>MAL13-1718319</i>) and <i>K13</i> -propeller domain	137	128 – Africa 2 – Asia 7 - Unknown	2 (<i>K13</i> propeller)*
2015		149	78 – Africa 5 – C. America 66 - Unknown	1 (<i>K13</i> propeller)*
2016	<i>K13</i> -propeller domain	153	146 – Africa 2 – C. America 5 - Unknown	0 But 1 specimen was positive for the 578S <i>K13</i> mutation that is not associated with resistance

*Specimens were from travelers from Africa
K13= *Kelch13*

Source: Applicant submission

9.2.1.6 Published studies

Some of the published studies reported efficacy of artesunate in patients with severe malaria due to *Plasmodium* species other than *P. falciparum*; these were mostly case series and are summarized in Table 9-4. Overall, the studies suggest that artesunate should be effective in improving survival of patients with severe malaria due to the asexual blood stage parasites of *P. vivax* (n=58), *P. knowlesi* (n=39) and *P. ovale* (n=1) as well as mixed infections.

Table 9-4: Literature reports - Intravenous artesunate treatment of patients with severe malaria due to *Plasmodium* species other than *P. falciparum*.

Country (Reference)	<i>Plasmodium</i> species	Patient characteristics	Artesunate dose and duration	Survival n/N (%)*
Germany (African descent) (Bélard et al. 2019)	<i>P. vivax</i>	Pediatric patients with imported severe malaria (African descent)	2.4 mg/kg (Guilin Pharmaceutical product)	1/1
	Mixed <i>P. vivax</i> and <i>P. falciparum</i>			1/1
Italy (Eritrea immigrant) (Tekle et al. 2018)	<i>P. vivax</i>	A 35-week old pre-term infant with sudden onset of fever on Day 19 after birth; blood smears performed on Day 22 and parasitemia of 0.1% (congenital malaria); became a parasitemic after 3 days of treatment and discharged from NICU on Day 37. Mother had one episode of fever with chills in a Libyan refugee camp; at the time of the neonate's malaria diagnosis, the mother's thick and thin	2.4 mg/kg bid on Day 1 followed by once daily for 3 days and clindamycin every 8 h for 7 days.	1/1

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Country (Reference)	Plasmodium species	Patient characteristics	Artesunate dose and duration	Survival n/N (%)*
		blood smears were positive for <i>P. vivax</i> malaria.		
Bikaner, India (Nayak 2011)	<i>P. vivax</i>	Patients ≥ 14 years having asexual form of <i>Plasmodium</i> parasites in peripheral smear and positive rapid antigen test and satisfying WHO criteria for severe malaria	2.4 mg/kg IV; the first 2 doses at 12-hour interval followed by every 24 hours for 7 days	65/65 (100) Results by species not included.
	Mixed <i>P. vivax</i> and <i>P. falciparum</i>			
	<i>P. falciparum</i>			
India (Kute et al. 2012)	<i>P. vivax</i>	Adults, mean age 30; all had acute renal injury	Not specified (treatment with artesunate + doxycycline)	22/25 (88)
India (Gehlawat et al. 2013)	<i>P. vivax</i>	Pediatric; 12 with prostration; 8 with multiple seizures; 6 with severe anemia; 5 with jaundice. The 2 patients that died: • One presented with features of cerebral malaria, acute respiratory distress syndrome, hypoglycemia, metabolic acidosis, and catecholamine refractory shock. • Second presented with cerebral malaria, hepatitis, deranged coagulation profile, and acute kidney injury.	Not specified; 3-day treatment followed by oral treatment with artemether + lumefantrine	16/18 (88.9)
India (Karanth, Marupudi, and Gupta 2014)	<i>P. vivax</i>	47-year-old, from a malaria endemic region, presented with high-grade, intermittent fever, rigors, intracerebral bleed, right hemiparesis, aphasia and seizures following vivax malaria. Initially treated with hydroxychloroquine. Follow-up treatment with primaquine for 14 days	IV artesunate 2.4 mg/kg at 0, 12, 24 h followed by daily for 5 days	1/1
India (Prashanth et al. 2012)	<i>P. vivax</i>	Transfusion transmitted malaria: A neonate born at 30-weeks of gestation with respiratory distress, was given packed RBC transfusion; after 24h of transfusion, the baby became lethargic, had multiple episodes of apnea necessitating bag and mask ventilation. <i>P. vivax</i> schizonts and gametocytes observed on blood smears; parasitemia was 18874/μL. Also positive by the pLDH and HRP rapid tests. Infant's condition improved within 24h and was blood smear negative by Day	IV artesunate 2.4 mg/kg/day for 6 days + clindamycin	1/1

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Country (Reference)	<i>Plasmodium</i> species	Patient characteristics	Artesunate dose and duration	Survival n/N (%)*
		7.		
India (Kaushik et al. 2013)	<i>P. vivax</i>	Adults with acute renal failure. *Represent patients that failed artesunate+ doxycycline (doxy) treatment	2.4 mg/kg at 0, 12, and 24 hours and then once daily for 7 days and oral doxy (100 mg/bid). In the absence of parasitological or clinical response to parenteral artesunate, patients received quinine	Artesunate: 0/2 Artesunate + Doxy: 51/63 (81%) *Artesunate + quinine + doxy: 12/63 (19%)
India (Thapa, Patra, and Kundu 2007)	<i>P. vivax</i>	Pediatric (age 12) with cerebral malaria. Repeat blood smear after 2 days showed clearance of the parasite. Both were discharged in a clinically stable condition and advised primaquine for 14 days. Follow up after one month showed no residual neurological deficit	Not specified; the authors state “the recommended dose”	2/2
India (Sarkar and Bhattacharya 2008)	<i>P. vivax</i>	Adults with cerebral malaria: seizures and symptoms of diffuse meningoencephalitis. Patients were advised to take follow-up treatment with primaquine.	Recommended dose	3/3
Sri Lanka (Ranaweera et al. 2018)	<i>P. vivax</i>	A Sri Lankan gem miner, returned from Madagascar, parasitemia of 26,793/ μ L. Co-infected with HIV and tuberculosis. Blood smear negative within 24 h. Oral artemether-lumefantrine commenced on Day 3 and primaquine on Day 5.	IV artesunate 2.4 mg/kg	1/1
Sabah, Malaysia (Barber et al. 2013)	<i>P. vivax</i> **	Adults, median age 43; hypotension and jaundice were most common at baseline	Not specified; ≥ 1 dose	7/7
	<i>P. knowlesi</i> **	Adults, median age 55; hyperparasitemia and jaundice were common at baseline		38/38 (100)
Sabah, Malaysia (William et al. 2011)	<i>P. knowlesi</i> **	Adults age 44-76; 4 ARDS; 3 shock; 3 acidosis; 2 ARF	Not specified	5/6
	Mixed <i>P. knowlesi</i> ** + <i>P.</i>	Adult male, 48 years; hyperbilirubinemia, ARF, and acidosis	Not specified	1/1

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Country (Reference)	Plasmodium species	Patient characteristics	Artesunate dose and duration	Survival n/N (%)*
	<i>vivax</i>			
Netherlands & Belgium (acquired in Africa) (Kreeftmeijer-Vegter et al. 2012)	Mixed <i>P. malariae</i> + <i>P. falciparum</i>	68 patients with imported malaria (In all but two patients, malaria was acquired in Africa); 55 of the 68 patients were with severe malaria based on WHO criteria. #survival data not available; however, the authors state that 2/55 (3.6%) patients with severe malaria died 4 and 8 days after parasite clearance; the <i>Plasmodium</i> species for the patients that died was not specified.	63% of patients received IV artesunate: 2.4 mg/kg, 3 doses at 12 hour interval, then switched to oral therapy with Malarone	NA#/1
	<i>P. vivax</i>			NA#/2
	<i>P. ovale</i>			NA#/1
France (Born in Afghanistan) (Izri et al. 2019)	<i>P. vivax</i>	Patient born in Afghanistan and living in France since 2012. The patient became a parasitemic and a pyretic within 24 hours. Relapse occurred at Month 9.	Artesunate/dose not specified	1/1
Indonesia (Tatura et al. 2018)	<i>P. vivax</i>	47-day old infant with cholestatic jaundice and sepsis-like signs and symptoms and blood smear positive (800 parasites/ μ L); mother was asymptomatic and blood smear positive for <i>P. vivax</i> . Clinical outcome of the infant improved after 7 days of treatment.	Artesunate/2.4 mg/kg for 7 days	1/1
Rome, Italy (returning from Africa) (D'Abramo et al. 2018)	<i>P. ovale</i>	A 34 y old Caucasian, returning from Angola, Africa was admitted at the National Institute for Infectious Diseases Lazzaro Spallanzani in Rome. He had 0.1% parasitemia of <i>P. ovale</i> and failed oral chloroquine treatment which then progressed to ARDS. 1 day after switching to IVAS, parasitemia cleared and the patient's condition improved. Parasite clearance achieved after 24 h; oral primaquine was administered. Patient was discharged after 10 days. Patient was examined after 2 months and no sequelae, relapse, etc. were reported.	IV artesunate (2.4 mg/kg) at 0 and 12 h, followed by daily + oral doxycycline	1/1
*Represent number survived (n)/Total number of patients (N); percentage shown if the number of patients was ≥ 10. **<i>Plasmodium</i> species confirmed by PCR. ARDS: a acute respiratory distress syndrome; ARF: a acute renal failure				

Reviewer comments:

Overall, the studies suggest that artesunate maybe effective in improving in treating patients with severe malaria to different Plasmodium species.

Post-treatment follow-up of patients for parasitemia was not assessed in the two Phase 3 studies (SEAQUAMAT and AQUAMAT). However, post-treatment follow-up for parasitemia was conducted in patients with severe malaria in Study EDCTP/MMV07-01 that showed that 99% parasite clearance was achieved by 24 hours in 85% of the patients in the 5 dose group compared to 78% in the 3 dose groups; the median parasite clearance time for 99% reduction ranged between 24 and 18 hours in both 5-dose and 3-dose group. A study in patients with uncomplicated malaria (Study 1263ab) showed that 100% parasite clearance was achieved between 12-164 hours (median 32-40 hours) in patients treated with 2.4 mg/kg artesunate for 3 days followed by mefloquine or Malarone.

Additional information on parasite clearance time, based on published studies is summarized in Table 9-5. Overall, the studies support that the parasite clearance time after treatment with artesunate is shorter (48-72 hours) compared to other antimalarials such as quinine (5 to 6 days).

Table 9-5: Time to parasite clearance in patients with severe malaria

Country (Reference)	Study design	PCT ₁₀₀
Uganda (Byakika-Kibwika et al. 2012)	150 patients with severe malaria were treated with 2.4 mg/kg IV artesunate, repeated at 0 h, 12 h and every 24 h thereafter until switched to oral treatment. All patients showed rapid recovery from symptoms and a ability to take oral medication within 24 hours. PCT was based on serial blood smears performed 6-hourly after start of treatment until 6 h post first negative blood smear when parasite clearance was achieved.	Median 2 days; none of the patients had parasitemia on Day 3.
United Kingdom – African ethnicity; traveled to West Africa (Eder et al. 2012)	22 severe malaria patients were administered IV artesunate (2.4 mg/kg at 0 and 12 h, followed by every 24 h treatment until smears were negative for trophozoites; patients were then treated with Malarone. The results were compared with 48 patients treated with IV quinine (10 mg/kg, 8 h) until patient was able to take oral treatment with doxycycline. Time of examination of blood smears and criteria for parasite clearance to determine PCT were not specified. Median parasite clearance was significantly faster (62 h) in artesunate treated patients (mean±SD: 65±36 h; range: 27-200 h) compared to 80 h (mean±SD: 85±29; range: 41-145h) in the quinine group. Parasite clearance time ≥48 hours (%) was reported in 12 (55%) patients in the artesunate group and 45 (94%) in the quinine group.	Median 62 hours (2.7 days). Range: 27-200 h (Days 1.12-8.3)

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Country (Reference)	Study design	PCT ₁₀₀
Europe (Kurth et al. 2015)	Observational study: The TropNet severe malaria database was analyzed to compare PCT after treatment with artesunate or quinine in 100 patients with severe malaria. Median parasite clearance time was 72 h in the artesunate group and 96 h in the quinine group.	Median 72 h
Africa (Kremsner et al. 2016)	The PCT of 3-dose IV (4 mg/kg at 0, 24, and 48 h; n=291), 3-dose IM (4 mg/kg at 0, 24, and 48 h; n=291) and 5-dose IM (2.4 mg/kg at 0, 12, 24, 48, and 72 h; n=303) artesunate regimen was compared in 1047 children. Parasitemia was measured at 6 h interval and prior to each dose for at least 48 h or until 3 consecutive negative smears were reported. Blood smears were also examined on Days 7, 14, and 28. The PCT in the 3 groups was comparable (Median 42 h; 95% CI 36, 42).	Median 42
Cameroon (Maka et al. 2015)	Children (≤5 years) with severe malaria were treated with parenteral 2.4 mg/kg artesunate (n=29) at 0, 12, 24 followed by once daily or quinine (3 different dose and regimens; n=29-30/group). PCT was based on 2 consecutive negative blood smears. The mean PCT (±SD) was 19.2±11.3 h. for artesunate and was shorter than quinine. The overall PCT for the last patient with parasitemia was 48h for artesunate and between 66-72 h for the quinine groups.	48 h
Thailand (Newton et al. 2003)	Patients (Burmese, Karen, Thai and Hmong ethnic groups) with severe malaria were treated with either IV artesunate (n=59; 2.4 mg/kg, 1.2 mg/kg at 12 h followed by 1.2 mg/kg daily) or quinine (n=54; 20 mg/kg). Once the patient was able to swallow, oral therapy with artesunate combined with either mefloquine, tetracycline or doxycycline was administered. Blood smears were examined for parasite counts at 0, 1, 2, 4, 6, 8, 10, 12, 16, 20, and 24 h and then every 6 h until 6 h after parasite clearance. PCT in the quinine group was 76 h.	62.5 h
Thailand and Vietnam (Krudsood et al. 2000)	Effect of treatment with IV artesunate (2.4 mg/kg at 0 h, followed by 1.2 mg/kg at 12 h interval for 5 days; total dose 600 mg) was compared with IV artesunate followed by mefloquine, as well as 3 day course of artemether IM (total dose 480 mg/kg), followed by mefloquine, and IV quinine (200 mg/kg in divided doses) followed by tetracycline. PCT was based on the time from the start of a patient's treatment until the patient's first negative blood film, with the blood film then remaining negative for 24 hours. Mean PCT with artemisinin regimes was between 53 and 62 h and with quinine 92 h.	56 h
India (Nayak 2011)	Adult patients >14 years were treated with artesunate (2.4 mg/kg IV at 0 and 12 h followed by every 24 hours for 7 days; n=65) or quinine (n=35). Mean (SD) PCT was 1.62 (0.65) days in patients treated with artesunate compared to 3.46 (0.78) days in those treated with quinine.	Mean 1.62 days
Sudan	Patients were treated with IV artesunate (2.4 mg/kg at 0, 12, and 24 hours, then daily) or quinine (20 mg/kg loading dose followed by 10 mg/kg, tid). PCT	~ 24 h

Country (Reference)	Study design	PCT ₁₀₀
(Abdallah et al. 2014)	was defined as the interval between the start of treatment and the time of the first of two sequential negative thick blood smears. PCT was ≤24 h in 98% of the patients treated with artesunate compared to 96% in the quinine group	
PCT=parasite clearance time; h=hours; *PCT₁₀₀ information not shown		

*Recrudescence can occur after discontinuation of artesunate treatment. Patients with *P. vivax* or *P. ovale* infection should be treated with an anti-malarial that is active against the liver stage parasites of *Plasmodium* species.*

A postmarketing surveillance study should be conducted to evaluate the potential development of resistance to artesunate and DHA as obtained from ongoing resistance monitoring programs on antimalarials collected by the CDC as well as international consortia and organizations (e.g., World Health Organization).

9.2.2 Interpretive Criteria

The Applicant has not requested any interpretive criteria in the labeling. This is appropriate as the tests to measure in vitro sensitivity of parasites of the *Plasmodium* species are not standardized and their use is limited to research laboratories.

10 Review of Safety

10.1 Safety Review Approach

In this NDA, evidence for clinical safety of IV artesunate was based primarily on one randomized open label trial (SEAQUAMAT) and a retrospective analysis of data collected under an expanded access IND (CDC-060). Additional supportive evidence for safety was provided by two phase 1 studies in healthy volunteers (Studies 1128 and 1142), and two phase 2 studies in patients with uncomplicated malaria (Studies 1148 and 1263ab). Studies 1128, 1142, 1168 and 1263ab were sponsored and conducted by the U.S. Army and > 50% of CDC-060 was funded by the U.S. Army. The U.S. Army transferred rights for these studies to Amivas prior to the NDA submission. Study protocols, patient level datasets, annotated CRFs, and CSR were submitted for each of these studies. SEAQUAMAT was not conducted by the Applicant or the U.S. Army.

SEAQUAMAT was conducted in Southeast Asia and evaluated 730 patients aged 2 to 87 years who received IV artesunate, compared with 730 patients who received at least one dose of IV quinine ([Dondorp et al. 2005](#)). SEAQUAMAT included limited safety follow up as data were collected only during hospitalization, until death or discharge. Laboratory data were reported only for the admission timepoint. The primary endpoint was in-hospital mortality and secondary endpoints were neurological sequelae and recovery times (times to eat, speak sit and discharge), and development of severe complications. For adverse event analyses in SEAQUAMAT, the safety population in this section included all patients who received a least one dose of study medication.¹⁵

Study CDC-060 presented data retrospectively collected from the first 102 patients who received IV artesunate under IND 76725 for the treatment of severe malaria in the United States. Datasets collected information on mortality, adverse events, and laboratory data until death or discharge, with limited additional information reported in patient narratives.

The phase 1 studies 1128 and 1142 in healthy volunteers followed patients until Day 15 and routine laboratory evaluations including hematology panels (Hb, Hct, platelets, reticulocytes, and neutrophils), bilirubin, AST, blood urea nitrogen, and creatinine, as well as ECG datasets (machine read). The phase 2 studies in patients with uncomplicated malaria included laboratory evaluations at Day 7 if previously abnormal (WRAIR Study 1168) and at Days 7, 14 and 28 (WRAIR 1263ab).

Supportive information for safety was provided by AQUAMAT, a randomized open label trial of severe malaria in Africa, which studied 2715 pediatric patients less than 15 years old

¹⁵ In SEAQUAMAT, one patient (Subject (b) (6)) randomized to quinine did not receive study medication. Applicant's safety analyses used the ITT population.

administered IV artesunate compared with 2713 who received IV quinine ([Dondorp et al. 2010](#)). In this review, AQUAMAT data and analyses were derived solely from the publication as the patient level dataset was not provided in the NDA.¹⁶ The primary endpoint was in-hospital mortality, and secondary outcomes were similar to SEAQUAMAT and included the incidence of neurological sequelae and recovery times (i.e., times to eat, speak, sit, and discharge). One key difference with SEAQUAMAT was that after 11% of patients were enrolled in AQUAMAT, the study protocol was amended to assess patients who had not fully recovered at discharge at 28 days and at regular intervals to 12 months or until full recovery. Limited information on these assessments, however, were included in the publication.

Supportive safety information was also provided by Study EDCTP-MMV07-01, a study in pediatric patients 6 months to 10 years with severe malaria, comparing five-dose regimen of IV artesunate 2.4 mg/kg/dose at 0, 12, 24, 48, and 72 hours with a three-dose regimen of 4.0 mg/kg/dose once a day for three days, for the same total dose of 12 mg/kg. The application included the protocol, CRF, and CSR; datasets including PK data were not available.

Consultations from the QT-IRT team and Division of Pediatric and Maternal Health were summarized in Section 10.4.9 and Section 10.9.2, respectively.

The safety review focused primarily on the safety of IV artesunate at the proposed dose of 2.4 mg/kg/dose at 0, 12, 24 hours when administered to patients with severe malaria. While most patients in studies submitted to the NDA had malaria due to *P. falciparum*, review of safety included subjects with other malaria species (CDC-060). Adverse events of special interest such as hematologic, renal, and hepatic were analyzed across the clinical development program if the data were collected, see Table 10-1 below. In addition, see Section 6 for the clinical pharmacology reviewer’s assessment of Phase 1 studies.

Table 10-1: NDA 213036 Clinical Trials Reviewed for Safety Analysis

Study	Description	Data Available for Analysis
SEAQUAMAT	Phase 3 efficacy and safety study in adult and pediatric patients $\geq$ 2 years old with severe <i>P. falciparum</i> malaria	Patient level datasets, CRF, CSR,
CDC-060	Retrospective data abstraction of IND Expanded Access Study in adults and children (Travelers to US) with severe malaria	Patient level datasets, CRF, CSR
Study 1168	Phase 2 safety, efficacy, PK in patients 18 to 65 years old with uncomplicated malaria	Patient level datasets, CRF, CSR
Study 1263ab	Phase 2 dose-ranging efficacy and safety in subjects 5 to 65 years with uncomplicated	Patient level datasets, CRF, CSR

¹⁶ Safety population for AQUAMAT excluded patients who died before receiving study treatment, i.e., six patients randomized to artesunate and 22 randomized to quinine. (Dondorp 2010).

Study	Description	Data Available for Analysis
	malaria	
Study 1128	Phase 1 single dose, dose escalation in healthy volunteers	Patient level datasets, CRF, CSR
Study 1142	Phase 1 multiple dose, dose escalation in healthy volunteers	Patient level datasets, CRF, CSR
AQUAMAT	Phase 3 efficacy and safety in pediatric subjects < 15 years old with severe <i>P. falciparum</i> malaria	Publication only
EDCTP-MMV07-01	Phase 2 safety and efficacy in patients 6 months to 19 years with severe <i>P. falciparum</i> malaria.	Publication, CSR, CRF

Source: NDA213036 Clinical Overview

See Table 7-1: Clinical Trials Relevant to this NDA for summary of clinical studies reviewed for safety as well as efficacy.

In lieu of an Integrated Summary of Safety (ISS) and an Integrated Summary of Efficacy (ISE) the Applicant submitted a meta-analysis entitled “Meta-analysis of Intravenous and Intramuscular Artesunate in Complicated or Severe Malaria” conducted in 2013 by Sigma-Tau. The Amivas ISS/ISE meta-analysis focused primarily on the Cochrane meta-analysis which conducted its original search in November 2005 and an updated search in November 2010 identifying a total of eight randomized controlled trials¹⁷ ([Sinclair et al. 2012](#)). The ISS/ISE meta-analysis included an additional 25 publications through 2012, adding other controlled trials of IV artesunate versus quinine, retrospective cases of travelers, additional data on safety and efficacy in subgroups, uncontrolled or add-on trials, and studies with a control group other than quinine. SEAQUAMAT, AQUAMAT, and EDCTP-MMV07-01 were analyzed as part of the ISS/ISE meta-analysis but CDC-060, published in Twomey et al. 2015, was not included.

Note: This reviewer acknowledges the contributions of Scott Runyan, B.S., Senior Analyst, J Review Support team, to many of the tables and figures included in the Safety Review.

10.2 Review of Safety Database

10.2.1 Overall Exposure

In studies with subject-level data submitted to the application, 832 patients received at least one dose of IV artesunate for treatment of severe malaria. The application included safety data on an additional 130 patients who received IV artesunate for uncomplicated malaria, as well as

¹⁷ The Cochrane meta-analysis of IV artesunate for severe malaria included the following studies: ([Anh 1989](#)), ([Anh, Binh, and Kim 1995](#)), ([Hien et al. 1992](#)), ([Cao et al. 1997](#)), ([Newton et al. 2003](#)), ([Dondorp et al. 2005](#)), ([Dondorp et al. 2010](#)), and ([El tahir et al. 2010](#)).

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

50 healthy volunteers. Across the clinical development program including supportive studies, more than 3000 subjects were exposed to either IM¹⁸ or IV artesunate in single and multiple doses.

Approximately 3638 subjects received parenteral artesunate for severe malaria at 2.4 mg/kg/dose at 0, 12 and 24 hours before transitioning to oral regimen. These studies included SEAQUAMAT, AQUAMAT, CDC-060, and one arm of EDCTP-MMV07-01 which studied a 5-dose regimen of 2.4 mg/kg/dose.

The datasets and studies submitted in this NDA are summarized in the tables below. Clinical datasets are located at <\\CDSESUB1\evsprod\NDA213036\0001\m5>.

Table 10-2: Overall Exposure to IV Artesunate in Clinical Development Plan (Safety Population)

SAFETY DATABASE (Studies Included)	No. Receiving ≥1 dose IV or IM¹ Artesunate	No. Receiving ≥1 dose comparator (Quinine)²
Studies of IV artesunate with subject level data for severe malaria indication (SEAQUAMAT, CDC-060)	832	730
Studies of IV artesunate with subject level data (SEAQUAMAT, CDC-060, Phase 1-2 Studies, all dosages and regimens)	1012	730
Studies of parenteral artesunate for severe malaria at 2.4 mg/kg/dose at 0, 12, and 24 hours (SEAQUAMAT, CDC-060, AQUAMAT, EDCTP-MMV07-01 for 5 dose regimen)	3638	3421
Controlled, active comparator trials for severe malaria (SEAQUAMAT, AQUAMAT)	3436	3421
Studies for severe malaria indication (SEAQUAMAT, AQUAMAT, CDC-060, EDCTP-MMV07-01 all doses and regimens)	3732	3421
WRAIR formulation (Ph 1-2 Studies, CDC-060 and EDCTP-MMV07-01, all dosages and regimens)	476	0
Total: (all dosages and regimens, phases and indications) (SEAQUAMAT, AQUAMAT, CDC-060, EDCTP-MMV-07-01, Phase 1 and 2 Studies)	3912	3421

Source: Clinical Reviewer from Study CSRs (Module 5)

¹In AQUAMAT, 933 (34.4%) of artesunate recipients received IM artesunate. Dondorp 2010 noted that 6 patients randomized to artesunate and 22 patients randomized to quinine died before receiving antimalarial treatment; the safety population for artesunate included 2706 patients who received at least one dose of parenteral artesunate.

²Quinine: 20 mg/kg IV or IM loading dose, then 10 mg/kg three times per day.

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Note: Safety population for AQUAMAT drawn from study publication and for EDCTP-MMV07-01 from publication and CSR. (AQUAMAT safety population: artesunate N=2706 and quinine N=2691; EDCTP: N=194 all dosages and regimens)

The following two tables provides details regarding the dose regimens evaluated in each study.

Table 10-3: Exposure to IV Artesunate Across the Studies Submitted, Including Severe and Uncomplicated Malaria (Safety Population)

Indication	Study/ Population	IV Artesunate Proposed Dose	IV Artesunate Other Doses				Total IV Artesunate
		2.4 mg/kg at 0, 12, 24 hr then daily n=2682	2.4 mg/kg for 3 days N=55	1.2 mg/kg for 3 days N=25	4.8 mg/kg for 3 days N=119	8.0 mg/kg for 3 days N=12	
Severe malaria	SEAQUAMAT Adults & Children	730	0	0	0	0	730
	CDC-060 Adults & Children	102	0	0	0	0	102
	AQUAMAT ¹ Children	2706	0	0	0	0	2706
	EDCTP- MMV07-01 ² Children	100	0	0	94 ³	0	194
Uncompli- cated malaria	1168 Adults & children	0	30	0	0	0	30
	1263ab Adults	25	25	25	25	0	100

Source: Clinical Reviewer from Study CSRs (Module 5) and 2.7.4 Summary of Clinical Safety

¹Datasets not provided for AQUAMAT; analysis from data included in the publication (Dondorp 2010); AQUAMAT includes 933/2712 (34.4%) artesunate recipients who were randomized to IM Artesunate; 6 patients in the artesunate group died before receiving study drug.

²According to Kremsner 2012 and the EDCTP-MMV07-01 CSR, Safety Population was defined as all patients receiving at least one dose; the ITT population excluded 12 patients that had protocol violations (3), consent withdrawal (1), and a parasitemia (1).

³Patients in this group received IV artesunate 4.0 mg/kg for 3 days rather than 4.8 mg/kg

Table 10-4: Exposure to IV Artesunate in Healthy Volunteers (Safety Population)

	Study	IV Artesunate					Total IV Artesunate	Placebo
		0.5 mg/kg for 3 days N=6	1.0 mg/kg for 3 days N=6	2.0 mg/kg for 3 days N=12	4.0 mg/kg for 3 days N=12	8.0 mg/kg for 3 days N=12		N=16
Phase 1 healthy volunteers	1128	6	6	6	6	6	30	10
	1142	0	0	6	7	7	20	6

Source: Clinical Reviewer from Study CSRs (Module 5) and 2.7.4 Summary of Clinical Safety

10.2.2 Relevant characteristics of the safety population

The safety population for this application included all randomized subjects who received at least one dose of IV artesunate. Studies for which patient-level data were submitted included subjects 18 months to 85 years of age who received IV artesunate. In SEQUAMAT, among subjects who were treated with IV artesunate 161 (22.1%) were 2 - <18 years old and 17 (2.3%) were 65 years of age or older. CDC-060 included patients 18 months to 72 years of age: 12 (11.8%) were <18 years old and 3 (2.9%) were 65 years of age and older.

Regarding the supportive studies, EDCTP-MMV07-01 included subjects as young as 6 months and up to 10 years of age. The lower age range for AQUAMAT is uncertain as the datasets were not submitted in the application and the publication (Dondorp 2010) included only interquartile range for the enrolled pediatric patients. ([Dondorp et al. 2010](#))

Demographic characteristics are summarized in Section 8 (Statistical and Clinical Evaluation) for SEQUAMAT, AQUAMAT and EDCTP-MMV07-01, and in Section [18.6](#) for CDC-060 and Studies 1168, 1263ab, 1128 and 1142. See Section 12 for a discussion of the safety database in pediatric subjects.

In studies of severe malaria, SEQUAMAT enrolled patients in Myanmar, Bangladesh, India, and Indonesia. The highest enrolling sites for SEQUAMAT were in Myanmar (40%), Bangladesh (30.4%) and Indonesia (19.6%). CDC-060 enrolled patients in U.S. hospitals who acquired malaria while traveling outside the U.S. According to the study publication, AQUAMAT enrolled patients at 11 centers in 9 African countries including Mozambique, The Gambia, Ghana, Kenya, Tanzania, Nigeria, Uganda, Rwanda, and Democratic Republic of the Congo. Study sites in EDCTP-MMV07-01 were in Gabon and Malawi.

In the clinical trials SEQUAMAT, CDC-060, EDCTP-MMV07-01 and phase 1 and 2 studies, patients received IV artesunate; in AQUAMAT, 933 of 2712 (34.4%) subjects received artesunate intramuscularly.

For SEQUAMAT, datasets included selected clinical events and laboratory evaluations on admission as well as selected clinical events and adverse event (AE) data after admission and at discharge. CDC-060 datasets included AEs, labs and vital signs through death or discharge. For those subjects with longer hospital stays, data were generally collected through day 7, with data for a few patients with prolonged courses reported through days 8-9. Phase 2 study 1168 of uncomplicated malaria included AEs, safety labs and vital signs through day 3, or day 7 if abnormal, and Study 1263 included AEs, labs and vital signs through day 28. Phase 1 studies 1128 and 1142 of healthy volunteers (with placebo controls) followed patients for AEs, labs, ECG, and vital signs through day 15.

10.2.3 Adequacy of the safety database

The number of patients exposed to IV artesunate in the studies submitted to the NDA appeared adequate to perform a safety evaluation. The numbers of patients in the greater than 65 years or in other demographic categories were insufficient to conduct subgroup analyses. ([Dondorp et al. 2010](#))

In SEAQUAMAT, AEs, per se were not collected by the Wellcome Trust, the sponsor of the study. Clinical events and complications associated with severe malaria disease were captured along with a free text field on admission, after admission, and upon discharge. The Applicant coded legacy data obtained from the Wellcome Trust according to MedDRA preferred terms.

Overall, the safety assessment for this application was constrained by the limited safety data collected in key trial SEAQUAMAT and supportive trial AQUAMAT. Both studies focused primarily on efficacy, limiting safety observations to the clinical events associated with malaria, e.g., coma, anemia, etc., and the duration of in-patient hospitalization, until death or discharge. AQUAMAT included neurological follow up until day 28 but information on outcomes on day 28 was limited. CDC-060 included safety observations until death or discharge for most subjects but contained some prolonged follow up information for select patients, for example, pregnancy outcome. No patient narratives were available for SEAQUAMAT and AQUAMAT. Narratives for patient deaths and SAEs were available for CDC-060 and EDCTP-MMV07-01.

The lack of patient-level data for AQUAMAT prevented robust cross-study comparisons with SEAQUAMAT and prevented ascertainment of the lower age range of enrolled pediatric patients, as the AQUAMAT publication (Dondorp 2010) reported only the median and interquartile range. The AQUAMAT publication did not contain an analysis of adverse events by route of administration (IV versus IM).

While a clinical study report was provided for EDCTP-MMV07-01, the report lacked Appendix 12 which included the statistical analysis plan; patient data listings for discontinued patients, compliance data, adverse event listings, and individual laboratory measurements; and CRFs for deaths, SAEs, and withdrawals; the Applicant was unable to obtain this information from the study authors.

10.3 Adequacy of Applicant's Clinical Safety Assessments

Issues Regarding Data Integrity and Submission Quality

The Applicant submitted patient-level data, annotated CRFs and clinical study reports for SEAQUAMAT and CDC-060 (severe malaria), Phase 2 Studies in uncomplicated malaria 1168 and 1263ab, and Phase 1 studies 1128 and 1142. No issues were identified regarding data integrity and submission quality. A clinical study report was submitted for EDCTP-MMV07-01 but no patient-level datasets were included, limiting the analyses that could be performed. The

Applicant included statements of Good Clinical Practice for each trial.

Categorization of Adverse Events

AEs were coded using MedDRA Version 22.0 (SEAQUAMAT), Version 14.0 (CDC-060), Version 10.0 (Study 1263ab), Version 8.1 (Study 1168), version 12.0 (Study 1142), and Version 8.0 (Study 1128). SAEs were not recorded for SEAQUAMAT and AQUAMAT. In CDC-060, SAEs were not prospectively recorded but in the retrospective data abstraction, Grade 4 AEs were coded as SAEs. SAEs were recorded in EDCTP-MMV07-01. One SAE was recorded in Phase 2 Study 1168 with no SAEs reported in the remaining phase 1 and 2 studies.

Symptoms and sequelae of malarial disease were included as AEs in the phase 3 studies of severe malaria and phase 2 trials of uncomplicated malaria.

Routine Clinical Tests

Phase 1 studies (1128 and 1142), phase 2 trials (1168 and 1263 ab), EDCTP-MMV07-01 and CDC-060 included routine laboratory testing of blood chemistries, liver profiles, and hematology panels. Datasets for SEAQUAMAT did not include routine blood tests following admission; AQUAMAT publication included limited information on anemia, hypoglycemia and renal function (BUN).

Vital signs were reported for phase 1 and 2 studies as well as EDCTP-MMV07-0 and CDC-060. SEAQUAMAT datasets included only diastolic blood pressure and patient weight. Height was not recorded in Phase 2 or 3 studies.

ECGs were not performed in phase 2 or 3 trials but were performed in phase 1 studies 1128 and 1142. ECGs were evaluated by a cardiologist at the clinical site and were also digitized and analyzed by Cardiocore. In addition to the reports from Cardiocore, the Applicant submitted machine-read ECG data for these two clinical studies. A summary of the findings by the FDA QT-IRT can be found in Section 10.4.9.

10.4 Safety Results

Overview

The following table provides an overview of adverse events in the Artesunate for Injection clinical development program.

Table 10-5: Overview of Adverse Events and Deaths in IV Artesunate Recipients (All Dose Regimens) (Safety Population)

Adverse Events	SEAQUAMAT N=730	CDC-060 N=102	Phase 1 1128 N=30 1142 N=20	Phase 2 1168 N=30 1263ab N=100	EDCTP- MMV07-01 N=194	AQUAMAT N-2706
Submission	Dataset, CSR, CRF, publication	Dataset, CSR, CRF, publication	Dataset, CSR, CRF, publication	Dataset, CSR, CRF, publication (1168 only)	CSR, publication	publication
AEs - No. subjects (%)	296 (40.5%)	94 (92%)	26 (86.7% in 1128) 19 (95% in 1142)	30 (100% in 1168) 98 (98%) 1263ab	133 (68.6%)	NR
SAEs - No. of subjects (%)	NR	31 (30.4%) ¹ ; Total no. of SAEs = 56 ²	0	1/130 (0.8%) in 1168 0 in 1263ab	23 (11.9%)	NR
Deaths (%)	101 (14.7%)	7(6.9%)	0	0	2 (1.0 %)	230 (8.5%)
Withdrawals (%)	NR	2 ³	0 (1128) 2 (10% in 1142) ⁴	3 ⁵	5 (2.6%) ⁶	NR
Discontinuation of IV artesunate (%)	NR	2 ³	0 (1128) 2 (10% in 1142) ⁴	0	5 (2.6%)	NR

Source: NDA 213036, ADCE datasets (SEAQUAMAT), CSR (EDCTP-MMV07-01) and Adverse Event datasets (ADAE) (Phase 1 and 2 Studies), J Review 13.1

AEs: Adverse events; SAEs: Serious adverse events; NR= Not recorded

¹ SAEs not recorded for CDC-060; number represents Grade 4 AEs.

²From J Review analysis of CDC-060 Grade 4 AEs

³ See CDC-060 CSR Table 3 (Includes Subject (b) (6) – withdrawal of consent, and Subject (b) (6) -- investigator judgment; does not include subjects who died.)

⁴In Study 1142, two subjects withdrew from study but only one subject withdrew because of an AE; this was due to an upper respiratory tract infection felt to be unrelated to artesunate administration.

⁵ In Study 1263ab, 3 subjects were categorized as early withdrawal/lost to follow up.

⁶In EDCTP-MMV07-01, in subjects who had received at least one dose of IV artesunate, patient withdrawals included two subjects who died and three subjects who withdrew consent. See CSR, Table 6.1-2, p. 43.

10.4.1 Treatment Emergent Adverse Events and Adverse Reactions

Differences among the clinical trials in dose regimens (dose administered, timing, and length of therapy), indications (severe versus uncomplicated malaria), study populations, available supportive care, and adverse event data collection, made cross-study analysis of TEAEs difficult. Moreover, the Applicant generally did not distinguish between the clinical symptoms due to malaria and treatment emergent adverse events following artesunate therapy. Treatment emergent adverse events reported in each study are summarized below.

For SEAQUAMAT, common adverse events were coded by the Applicant. TEAE by preferred term with an incidence greater than 2% are found in the table below.

Table 10-6: SEAQUAMAT -- Treatment Emergent Adverse Event by PT Incidence >2% (Safety Population)

TEAEs	Artesunate (n=730)	Quinine (n=730)	Totals (n=1460)
Subjects with at least 1 AE after admission	296 (40.5%)	339 (46.4%)	635 (43.5%)
Acute renal failure requiring dialysis ¹	65 (8.9%)	53 (7.3%)	118 (8.1%)
Sepsis	61 (8.4%)	76 (10.4%)	137 (9.4%)
Coma	54 (7.4%)	68 (9.3%)	122 (8.4%)
Hemoglobinuria	49 (6.7%)	33 (4.5%)	82 (5.5%)
Seizure	31 (4.2%)	45 (6.2%)	76 (5.2%)
Shock	26 (3.6%)	36 (4.9%)	62 (4.2%)
Respiratory failure	26 (3.6%)	39 (5.3%)	65 (4.4%)
Anemia	20 (2.7%)	21 (2.9%)	41 (2.8%)
Jaundice	17 (2.3%)	14 (1.9%)	30 (2.1%)
Lower respiratory tract infection	16 (2.2%)	25 (3.4%)	41 (2.8%)

Source: Clinical Reviewer (J Review 13.1)

¹Includes terms dialysis, hemodialysis, and peritoneal dialysis

Reviewer comments: In SEAQUAMAT, AEs that occurred more frequently in recipients of artesunate compared to those receiving quinine included dialysis (8.9% vs 7.3%, respectively), hemoglobinuria (6.7% vs 4.4%, respectively), and jaundice (2.3% vs 1.9%, respectively). The FDA analysis of renal failure requiring dialysis included those patients with CRFs recording the pre-specified term of dialysis as well as free-text fields that included the terms hemodialysis, peritoneal dialysis, intraperitoneal dialysis (IPD) and peritoneal dialysis (PD).

For Study CDC-060, TEAE by preferred term with an incidence of greater than 2% are found in the table below.

Table 10-7: CDC-060 - TEAE by PT, Incidence > 2% (Safety Population)

TEAEs	No. of Subjects (%) n=102
Subjects with at least 1 TEAE > 2% -	94 (92.2%)
Anemia	66 (64.7%)
Transaminases increased	28 (27.5%)
Aspartate aminotransferase increased	22 (21.6%)
Thrombocytopenia	18 (17.6%)
Hyperbilirubinemia	14 (13.7%)
Leukocytosis	10 (9.8%)
Renal failure acute	10 (9.8%)
Acute respiratory distress syndrome	8 (7.8%)

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

TEAEs	No. of Subjects (%) n=102
Lymphopenia	7 (6.9%)
Neutropenia	5 (4.9%)
Acidosis	4 (3.9%)
Mental status changes	4 (3.9%)
Disseminated intravascular coagulation	3 (2.9%)
Blood creatinine increased	3 (2.9%)
Alanine aminotransferase increased	3 (2.9%)
Pneumonia	3 (2.9%)
Pulmonary edema	3 (2.9%)
Diarrhea	3 (2.9%)

Source: Clinical Reviewer (J Review 13.1)

Reviewer comment: Adverse events included anemia, thrombocytopenia and leukocytosis, ARDS, acidosis, and mental status changes that are associated with severe malaria.

In phase 2 studies of uncomplicated malaria (Studies 1168 and 1263ab), TEAEs occurring at $\geq 5\%$ are found in the tables below.

Table 10-8: Study 1168 - TEAE by Preferred Term $\geq 5\%$

TEAEs	Study 1168 Artesunate 2.4 mg/kg n=30
Subjects with at least 1 TEAE - No. subjects with data	30 (100.0%)
Neutropenia ¹	17 (56.7%)
Hypotension	16 (53.3%)
Anemia	13 (43.3%)
Pyrexia	10 (33.3%)
Headache	9 (30.0%)
Bradycardia	6 (20.0%)
Tachycardia	6 (20.0%)
Vomiting	5 (16.7%)
Eosinophilia	5 (16.7%)
Infusion site pain	3 (10.0%)
Chills	3 (10.0%)
Hyperglycemia	3 (10.0%)
Leukopenia	2 (6.7%)
Fatigue	2 (6.7%)
Viral infection	2 (6.7%)
Upper respiratory tract infection	2 (6.7%)
Hypoglycemia	2 (6.7%)
Anorexia	2 (6.7%)
Abdominal pain	2 (6.7%)

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

TEAEs	Study 1168 Artesunate 2.4 mg/kg n=30
Cough	2 (6.7%)
Tachypnea	2 (6.7%)

Source: Clinical Reviewer (J Review 13.1)

Definition of neutropenia: ANC <2.0 x 10⁶/L, with occurrence on day 3 or later, see discussion in Section 10.5.3.

Table 10-9: Phase 2 Study 1263ab - TEAE by SOC/PT >=5% (Safety Population)

TEAEs	Study 1263ab (Pooled Artesunate Groups) n=100
Subjects with at least 1 TEAE	98 (98.0%)
Neutropenia ¹	28 (28.0%)
Anemia	23 (23.0%)
Thrombocytopenia	21 (21.0%)
Leukopenia	20 (20.0%)
Dizziness	16 (16.0%)
Pyrexia	15 (15%)
Abdominal pain	15 (15%)
Headache	14 (14.0%)
Lymphocytosis	14 (14.0%)
Cough	11 (11.0%)
Leukocytosis	10 (10.0%)
Rhinorrhea	9 (9.0%)
Diarrhea	9 (9.0%)
Nausea	8 (8.0%)
Vomiting	8 (8.0%)
Rhinitis	8 (8.0%)
Lymphadenopathy	7 (7.0%)
Oral herpes	7 (7.0%)
Eosinophilia	5 (5.0%)
Thrombocytopenia	5 (5.0%)
Lymphopenia	5 (5.0%)

Source: Clinical Reviewer (J Review 13.1)

¹Definition of neutropenia: ANC <1.5 x 10⁶/L, see discussion in Section 10.5.3.

Table 10-10: Studies 1168 and 1263ab - Severe Adverse Events (Grade 4) in Patients with Uncomplicated Malaria

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

Source: NDA213036 Module 2, 2.1.1.2, Table 14

In studies of healthy volunteers (Studies 1128 and 1142), the most common AEs, occurring in $\geq$ 5% of artesunate subjects, included dysgeusia, dizziness, headache, nausea, vomiting, fatigue, myalgia, and anemia. Of these, only dysgeusia was dose-dependent and was only observed in patients receiving artesunate at 4.0 and 8.0 mg/kg. All the TEAEs were considered mild and resolved without treatment.

One subject in Study 1128 who received artesunate at 8.0 mg/kg experienced urticaria which was considered moderate in severity and resolved after receiving Benadryl.

TEAE $\geq$ 5% observed in studies of healthy volunteers are listed in the table below; TEAEs included in the table were observed in Study 1128 and/or Study 1142 in artesunate recipients.

Table 10-11: Study 1128 and 1142 (Healthy Volunteers) - TEAE by Preferred Term (Pooled Artesunate $\geq$ 5%) (Safety Population)

	Study 1128		Study 1142	
	Artesunate Pooled n=30	Placebo n=10	Artesunate Pooled n=20	Placebo n=6
Subjects with at least 1 TEAE*	26 (86.7%)	6 (60.0%)	19 (95%)	6 (100%)
Dysgeusia	10 (33.3%)	1 (10.0%)	16 (80.0%)	1 (16.7%)
Dizziness	10 (33.3%)	1 (10.0%)	3 (15.0%)	0 (0.0%)
Headache	5 (16.7%)	1 (10.0%)	6 (30.0%)	1 (16.7%)
Vomiting	3 (10.0%)	0	0	0
Nausea	3 (10.0%)	0	0	0
Fatigue	3 (10.0%)	1 (10.0%)	1 (5.0%)	0
Myalgia	3 (10.0%)	0	0	0
Anemia	3 (10.0%)	0	5 (25.0%)	3 (50.0%)
Paresthesia	2 (6.7%)	0	2 (10.0%)	0 (0.0%)
Erythema	2 (6.7%)	0	1 (5.0%)	0 (0.0%)
Ecchymosis	2 (6.7%)	0	0	1 (16.7%)
Hyperhidrosis	2 (6.7%)	0	0	0
Hot flush	2 (6.7%)	0	1 (5.0%)	0
Cough	2 (6.7%)	0	0	0
Somnolence	0	0	2 (10.0%)	0
Upper respiratory tract infection	0	0	2 (10.0%)	0

Source: Clinical Reviewer (J Review 13.1)

*TEAEs with incidence $\geq$ 5% in pooled artesunate group were included.

The tables below provide the TEAEs by dose regimen in Studies 1128 and 1142.

Table 10-12: Study 1128 - TEAE by Study Dose by PT, Incidence $\geq$ 5% (Safety Population)

	Artesunate 0.5 mg/kg n=6	Artesunate 1.0 mg/kg n=6	Artesunate 2.0 mg/kg n=6	Artesunate 4.0 mg/kg n=6	Artesunate 8.0 mg/kg n=6	Placebo n=10
Subjects with at least 1 TEAE	5 (83.3%)	4 (66.7%)	6 (100.0%)	5 (83.3%)	6 (100.0%)	6 (60.0%)
Dysgeusia	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (83.3%)	5 (83.3%)	1 (10.0%)
Dizziness	4 (66.7%)	0 (0.0%)	1 (16.7%)	2 (33.3%)	3 (50.0%)	1 (10.0%)
Headache	0 (0.0%)	1 (16.7%)	2 (33.3%)	1 (16.7%)	1 (16.7%)	1 (10.0%)
Vomiting	1 (16.7%)	0 (0.0%)	1 (16.7%)	0 (0.0%)	1 (16.7%)	0 (0.0%)
Nausea	1 (16.7%)	0 (0.0%)	1 (16.7%)	0 (0.0%)	1 (16.7%)	0 (0.0%)
Fatigue	0 (0.0%)	0 (0.0%)	1 (16.7%)	1 (16.7%)	1 (16.7%)	1 (10.0%)
Myalgia	0 (0.0%)	1 (16.7%)	1 (16.7%)	0 (0.0%)	1 (16.7%)	0 (0.0%)
Anemia	0 (0.0%)	1 (16.7%)	2 (33.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Paresthesia	1 (16.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (16.7%)	0 (0.0%)
Erythema	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (33.3%)	0 (0.0%)
Ecchymosis	0 (0.0%)	1 (16.7%)	1 (16.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

	Artesunate 0.5 mg/kg n=6	Artesunate 1.0 mg/kg n=6	Artesunate 2.0 mg/kg n=6	Artesunate 4.0 mg/kg n=6	Artesunate 8.0 mg/kg n=6	Placebo n=10
Hyperhidrosis	0 (0.0%)	0 (0.0%)	1 (16.7%)	0 (0.0%)	1 (16.7%)	0 (0.0%)
Hot flush	1 (16.7%)	1 (16.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Cough	0 (0.0%)	1 (16.7%)	1 (16.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Diarrhea	0 (0.0%)	0 (0.0%)	1 (16.7%)	0 (0.0%)	0 (0.0%)	1 (10.0%)
Hematuria	0 (0.0%)	1 (16.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (10.0%)

Source: Clinical Reviewer (J Review 13.1)

Table 10-13: Study 1142 - TEAE by Study Dose by PT, in Any Artesunate Group >=5%, by Dose Regimen (Safety Population)

	Artesunate 2.0 mg/kg n=6	Artesunate 4.0 mg/kg n=7	Artesunate 8.0 mg/kg n=7	Placebo n=6
Subjects with at least 1 TEAE -	6 (100.0%)	6 (85.7%)	7 (100.0%)	6 (100.0%)
Dysgeusia	4 (66.7%)	6 (85.7%)	6 (85.7%)	1 (16.7%)
Headache	3 (50.0%)	2 (28.6%)	1 (14.3%)	1 (16.7%)
Anemia	1 (16.7%)	4 (57.1%)	0 (0.0%)	3 (50.0%)
Dizziness	1 (16.7%)	1 (14.3%)	1 (14.3%)	0 (0.0%)
Paresthesia	1 (16.7%)	1 (14.3%)	0 (0.0%)	0 (0.0%)
Somnolence	0 (0.0%)	1 (14.3%)	1 (14.3%)	0 (0.0%)
Upper respiratory tract infection	0 (0.0%)	1 (14.3%)	1 (14.3%)	0 (0.0%)
Parosmia	1 (16.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Syncope vasovagal	0 (0.0%)	1 (14.3%)	0 (0.0%)	0 (0.0%)
Leukopenia	0 (0.0%)	0 (0.0%)	1 (14.3%)	0 (0.0%)
Neutropenia	0 (0.0%)	0 (0.0%)	1 (14.3%)	0 (0.0%)
Erythema	1 (16.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Dermatitis	0 (0.0%)	0 (0.0%)	1 (14.3%)	0 (0.0%)
Pruritus generalized	1 (16.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Venipuncture site inflammation	1 (16.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Fatigue	0 (0.0%)	1 (14.3%)	0 (0.0%)	0 (0.0%)
Tenderness	1 (16.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Rash pruritic	0 (0.0%)	0 (0.0%)	1 (14.3%)	0 (0.0%)
Decreased appetite	0 (0.0%)	0 (0.0%)	1 (14.3%)	0 (0.0%)
Anorexia	0 (0.0%)	1 (14.3%)	0 (0.0%)	0 (0.0%)
Joint stiffness	0 (0.0%)	0 (0.0%)	1 (14.3%)	0 (0.0%)
Arthralgia	0 (0.0%)	0 (0.0%)	1 (14.3%)	0 (0.0%)
Back pain	1 (16.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Hot flush	0 (0.0%)	1 (14.3%)	0 (0.0%)	0 (0.0%)
Flushing	0 (0.0%)	0 (0.0%)	1 (14.3%)	0 (0.0%)
Eye pain	1 (16.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Photophobia	0 (0.0%)	1 (14.3%)	0 (0.0%)	0 (0.0%)
Asthenopia	0 (0.0%)	1 (14.3%)	0 (0.0%)	0 (0.0%)
Alanine aminotransferase increased	0 (0.0%)	1 (14.3%)	0 (0.0%)	0 (0.0%)

	Artesunate 2.0 mg/kg n=6	Artesunate 4.0 mg/kg n=7	Artesunate 8.0 mg/kg n=7	Placebo n=6
Aspartate aminotransferase increased	0 (0.0%)	1 (14.3%)	0 (0.0%)	0 (0.0%)
Body temperature increased	0 (0.0%)	0 (0.0%)	1 (14.3%)	0 (0.0%)
Nasal congestion	0 (0.0%)	0 (0.0%)	1 (14.3%)	0 (0.0%)
Muscle strain	1 (16.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Source: Clinical Reviewer (J Review 13.1)

Reviewer comments: *Dysgeusia appears to be dose related in both studies of normal volunteers. Neutropenia ($ANC < 1.0 \times 10^6/L$) was observed in one patient in Study 1142 at the highest dose regimen of 8 mg/kg/dose for three days.*

10.4.2 Deaths

Note: A detailed analysis of mortality in SEAQUAMAT, AQUAMAT, and EDCTP-MMV07-01, can be found in Section 8.1, Review of Individual Trials Used to Support Efficacy.

Cross-study comparisons of mortality between studies are complicated by the differences in populations, study design, dose regimens, indications, available supportive care, and other factors. All deaths that occurred in the clinical development program were consistent with outcomes associated with the underlying clinical illness (severe malaria) and were generally associated with the severity of symptoms at presentation and known risk factors. In SEAQUAMAT, for example, higher mortality was observed in those who met the definition of severe malaria, had hyperparasitemia, cerebral malaria, and age ≥ 65 years. (See Table 10-14.)

There were no deaths in the Phase 1 Studies 1128 and 1142 or in the Phase 2 studies 1168 and 1263ab.

The table below summarizes the overall mortality in studies of severe malaria.

Table 10-14: Overall Mortality in Studies of Severe Malaria (Safety Population)

Study	Population	Deaths in Artesunate Recipients n/N (%)
SEAQUAMAT (Dondorp 2005) ¹	2 -87 years (SE Asia)	101/730 (14.7%)
SEAQUAMAT (FDA analysis) ²	2-87 years (SE Asia)	96/730 (13.2%)
AQUAMAT (Dondorp 2010)	< 15 years (Africa)	230/2712 (8.5%)
EDCTP-MMV07-01 ³ (Kremsner 2012 and CSR)	6 months – 10 years (Africa)	2/194 (1.0 %)
CDC-060 (Twomey 2015 and FDA analysis)	1-72 years (Travelers to US)	7/102 (6.9%)

Source: Clinical Reviewer from study CSRs, publications, and clinical/statistical analyses

¹Dondorp 2005 reported in-hospital mortality but some deaths occurred following discharge.

²FDA Statistical review limited mortality to deaths occurring before hospital discharge

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

³EDCTP-MMV07-01 - Mortality for pooled 5-dose and 3-dose IV artesunate regimens

In SEAQUAMAT a total of 96 of in-hospital deaths (13.2%) was recorded in patients receiving IV artesunate. Higher mortality rates were observed in subjects with hyperparasitemia, those meeting the protocol definition of severe malaria, cerebral malaria, and in subjects 65 years of age or older. Analyses of mortality in subgroups can be in found in the Section 8, **Error! Reference source not found..**

Of the seven patients who died in CDC-060, three deaths were associated with either brain edema or brainstem herniation, most likely due to severe malaria. Six of seven (85.7%) were infected with *P. falciparum* and 5 of 7 had hyperparasitemia at baseline (range 12 to 90%). One subject death was associated with *P. vivax* malaria. All subject deaths were associated with patients who presented with severe neurological symptoms including seizures and coma and elevated hepatic transaminases. Clinical characteristics of CDC-060 patients who died can be found in the table below.

Table 10-15: CDC-060 - Clinical Characteristics of Patients who Died (Safety Population)

Clinical Characteristic	No. of Subjects n=7
Unable to take oral medication	7 (100.0%)
Impaired consciousness	6 (85.7%)
Acidosis	6 (85.7%)
Circulatory collapse/shock	5 (71.4%)
Parasitemia > 5%	5 (71.4%)
Abnormal bleeding or DIC	3 (42.9%)
Pulmonary edema or ARDS	2 (28.6%)
Jaundice	2 (28.6%)
Acute renal failure	2 (28.6%)
Severe anemia with hemoglobin under 7 g/dL	1 (14.3%)
Seizures	1 (14.3%)

Source: Clinical Reviewer (J Review 13.1)

In EDCTP-MMV07-01, two patients (2/194 or 1%) died. Available details from submission are provided below:

- Subject (b) (6) (Group B, 3-dose regimen of IV artesunate) was a 4-year old female enrolled in Gabon who was admitted with vomiting, fever and fatigue for 2 days. On admission the hemoglobin was 4.2 g/dL and parasite count was 92,700 *P. falciparum*. On day 1 following admission the patient developed coma (Blantyre score 3/5) with respiratory distress, tachypnea, thrombocytopenia and hemoglobinuria and she was started on ampicillin, ceftriaxone, and gentamicin for UTI and sepsis. Following the third

dose of study medication, the parasite count was 8000 but the patient deteriorated further with tonic-clonic seizures and progression to deep coma. Despite clearance of *P. falciparum* in her blood, the patient expired on the second day of admission.

- Subject (b) (6) (Group B, 3-dose regimen of IV artesunate) was a 34-month old male in Gabon with a four-day history of fever and vomiting, and one day history of seizures and coma (Blantyre score 2/5 on admission). His hemoglobin on admission was 4.9 g/dL and he had a parasite count of 224,000 *P. falciparum*. Ten hours after the first dose of artesunate he experienced prolonged seizures associated with respiratory distress and a Blantyre coma score of 1/5. He received a second dose of IV artesunate and was transferred to the ICU. He died later that day.

Both subjects who died in EDCTP-MMV07-01 presented with symptoms consistent with severe malaria and hyperparasitemia. Deaths were likely associated with complications of malaria.

AQUAMAT reported an imbalance of deaths prior to initiating therapy, with 22 deaths in patients randomized to receive quinine and 6 deaths in patients randomized to receive artesunate. As the two groups appeared to be balanced with respect to baseline characteristics, the authors speculated the higher number of deaths in patients assigned to quinine was caused by a delay in administration due of parenterally-administered quinine by controlled infusion compared with bolus administration of IV artesunate. In SEAQUAMAT, only one patient (assigned to quinine) died before receiving study drug.

Reviewer comments: *Observed mortality was higher in SEAQUAMAT than in AQUAMAT. The authors of AQUAMAT propose several reasons for this finding. African children generally have some background immunity to malaria which facilitates recovery. In addition, severe malaria may be over diagnosed in African children, a population with a relatively high incidence of sepsis and with a high background incidence of P. falciparum parasitemia. The older population enrolled in SEAQUAMAT and differences in available medical care in the two studies may have played a role in the higher incidence of deaths in SEAQUAMAT. The lower mortality observed in EDCTP-MMV07-01 may be due to a more expansive case definition for severe malaria which required only that symptoms were severe enough to require hospitalization.*

10.4.3 Serious Adverse Events

Due to the significant clinical illness associated with severe malaria, morbidity associated with malarial disease was difficult to distinguish from adverse effects that may have been associated with treatment. For example, in SEAQUAMAT, subjects had a high incidence of malaria complications on admission, including convulsions (49% in artesunate recipients), coma (39%), shock (11%), and severe anemia (6%), among other manifestations. Before receiving study treatment, 23.5% of patients in CDC-060 had impaired consciousness, 23.5% had circulatory collapse or shock, and 34.3% had acidosis. Patients enrolled in AQUAMAT had a high baseline incidence of coma (32% in artesunate recipients), convulsions (30%), severe anemia (30%), and

shock (12%). In EDCTP-MMV07-01 among patients who received at least one dose of study medication, 70.1 % had prostration and 17.6% had severe anemia at baseline. SEAQUAMAT did not report SAEs in the datasets, CSR, or in the publication (Dondorp 2005). AQUAMAT did not report SAEs in the publication.

While the CDC-060 Case Report Form had specific fields for recording data on SAEs, these data were not provided in the datasets and were not reported in the publication. (Twomey 2015) In lieu of SAEs, Grade 4 (life-threatening) AEs were reported instead. The most common SAEs were anemia (12.7%), thrombocytopenia (5.9%), acute respiratory distress syndrome (5.9%), hyperbilirubinemia (3.9%), and acute renal failure (2.9%), see table below.

Table 10-16: CDC-060 - Serious Adverse Events (SAE), Safety Population*

SAE (Grade 4 SAE)	Intravenous Artesunate n=102
Subjects with at least 1 GRADE 4 SAE	31 (30.4%)
BLOOD AND LYMPHATIC SYSTEM DISORDERS	19 (18.6%)
Anemia	13 (12.7%)
Thrombocytopenia	6 (5.9%)
Disseminated intravascular coagulation	2 (2.0%)
Lymphopenia	1 (1.0%)
Leukocytosis	1 (1.0%)
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	9 (8.8%)
Acute respiratory distress syndrome	6 (5.9%)
Respiratory distress	2 (2.0%)
Pneumothorax	1 (1.0%)
Pulmonary hemorrhage	1 (1.0%)
Pneumonia aspiration	1 (1.0%)
HEPATOBIILIARY DISORDERS	6 (5.9%)
Hyperbilirubinemia	4 (3.9%)
Acute hepatic failure	1 (1.0%)
Cholecystitis	1 (1.0%)
RENAL AND URINARY DISORDERS	3 (2.9%)
Renal failure acute	3 (2.9%)
NERVOUS SYSTEM DISORDERS	3 (2.9%)
Brain edema	2 (2.0%)
Depressed level of consciousness	1 (1.0%)
CARDIAC DISORDERS	1 (1.0%)
Cardiac failure congestive	1 (1.0%)
INVESTIGATIONS	3 (2.9%)
Transaminases increased	2 (2.0%)
Pulse absent	1 (1.0%)
INFECTIONS AND INFESTATIONS	3 (2.9%)
Septic shock	1 (1.0%)

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

SAE (Grade 4 SAE)	Intravenous Artesunate n=102
Sepsis syndrome	1 (1.0%)
Fungemia	1 (1.0%)
PSYCHIATRIC DISORDERS	1 (1.0%)
Mental status changes	1 (1.0%)
METABOLISM AND NUTRITION DISORDERS	1 (1.0%)
Hyperkalemia	1 (1.0%)
VASCULAR DISORDERS	1 (1.0%)
Hypotension	1 (1.0%)

Source: Clinical Reviewer (J Review 13.1)

*SAEs were not recorded for this study. Applicant reported Grade 4 AEs (life-threatening AEs) in lieu of SAEs.

In CDC-060, the majority of Grade 4 AEs (49 of 56 or 88%) were assessed by the Clinical Benefits Endpoint Committee to be unlikely to be related to IV artesunate or unrelated to IV artesunate administration, with the exception of anemia or hyperbilirubinemia.

During the Phase 1 Studies 1128 and 1142, no SAEs were reported.

In Phase 2 studies, one SAE was reported in Study 1168, in a 54-year old female subject who developed Stevens-Johnson syndrome (verbatim term bullous erythema multiforme) and was hospitalized following onset of symptoms. Symptoms of pruritis first developed within 24 hours of the final dose of IV artesunate and within 1 hour and 40 minutes of receiving the second dose of follow-on therapy with Malarone. Given this timing, it is not possible to determine whether this adverse event was independently associated with IV artesunate administration. A more detailed description of this SAE can be found in Section 18.6.5.

Of the total 194 patients in the EDCTP-MMV07-01 Safety Population, 23 subjects (11.9%) experienced 31 SAE, with 10 (10%) children enrolled in the 5-dose group (Group A) and 13 (13.8%) receiving the 3-dose regimen (Group B). See table below for SAEs by SOC/PT and Section 18.6.4 for additional details.

Table 10-17: EDCTP-MMV07-01 Serious Adverse Events by SOC/PT (Safety Population)

SYSTEM ORGAN CLASS	PREFERRED TERM	A		B		All		Fisher P=
		Subjects N=10	Events N=13	Subjects N=13	Events N=18	Subjects N=23	Events N=31	
BLOOD AND LYMPHATIC SYSTEM DISORDERS	ANAEMIA	2 (20.0%)	2	3 (23.1%)	3	5 (21.7%)	5	1.000
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	DEATH	0 (0.0%)	0	1 (7.7%)	1	1 (4.3%)	1	1.000
INFECTIONS AND INFESTATIONS		7 (70.0%)	9	4 (30.8%)	4	11 (47.8%)	13	
	BACTERIAL INFECTION	1 (10.0%)	1	0 (0.0%)	0	1 (4.3%)	1	0.435
	BRONCHOPNEUMONIA	0 (0.0%)	0	1 (7.7%)	1	1 (4.3%)	1	1.000
	CELLULITIS	0 (0.0%)	0	1 (7.7%)	1	1 (4.3%)	1	1.000
	LUNG INFECTION	1 (10.0%)	1	0 (0.0%)	0	1 (4.3%)	1	0.435
	MALARIA	2 (20.0%)	4	0 (0.0%)	0	2 (8.7%)	4	0.178
	PLASMODIUM FALCIPARUM INFECTION	0 (0.0%)	0	1 (7.7%)	1	1 (4.3%)	1	1.000
	SALMONELLA SEPSIS	1 (10.0%)	1	0 (0.0%)	0	1 (4.3%)	1	0.435
	SEPSIS	1 (10.0%)	1	0 (0.0%)	0	1 (4.3%)	1	0.435
	STAPHYLOCOCCAL SEPSIS	1 (10.0%)	1	1 (7.7%)	1	2 (8.7%)	2	1.000
NERVOUS SYSTEM DISORDERS		0 (0.0%)	0	3 (23.1%)	7	3 (13.0%)	7	
	CONVULSION	0 (0.0%)	0	2 (15.4%)	2	2 (8.7%)	2	0.488
	GRAND MAL CONVULSION	0 (0.0%)	0	2 (15.4%)	5	2 (8.7%)	5	0.488
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	SKIN SWELLING	1 (10.0%)	1	0 (0.0%)	0	1 (4.3%)	1	0.435
SURGICAL AND MEDICAL PROCEDURES	HOSPITALISATION	0 (0.0%)	0	1 (7.7%)	1	1 (4.3%)	1	1.000
VASCULAR DISORDERS		1 (10.0%)	1	2 (15.4%)	2	3 (13.0%)	3	
	PHLEBITIS	1 (10.0%)	1	2 (15.4%)	2	3 (13.0%)	3	1.000

Source: EDCTP-MMV07-01 CSR Table 8.3-2

As was noted in CDC-060, in EDCTP-MMV-0701, anemia was the most commonly reported SAE. In EDCP, three of the 5 subjects with SAE of anemia had anemia diagnosed 7 to 14 days following admission and received transfusions:

- Patient 1045 had severe anemia 7 days after enrollment that required a blood transfusion.
- Patient 2019 was a 7-year old male who was transfused on Day 2 after the fourth dose of IV artesunate. On Day 12 he was transfused again for a drop in Hct.
- Subject 3074 was a 59-month female who returned on Day 14 with severe anemia (Hct 14%) and received a blood transfusion.

Reviewer Comments: Although CDC-060 and EDCTP-MMV07-01 were conducted before post-artemisinin delayed hemolysis was described, the most commonly reported SAE was anemia. Given the timing of these SAE cases relative to artesunate administration, they may represent post-artesunate hemolytic anemia, although confirmatory laboratory evaluations were not performed.

10.4.4 Dropouts and/or Discontinuations Due to Adverse Effects

Withdrawal of subjects from SEAQUAMAT and AQUAMAT was not recorded in documents provided in the application.

For EDCTP-MMV07-01, withdrawals in subjects who had received at least one dose of Artesunate for Injection included two subjects who died and three subjects who withdrew consent. According to the CSR, two patients were discontinued due to adverse events, one each from Groups A and B.

Additional details on subject disposition in EDCTP-MMV07-01 can be found in Section 8.1.3, Table 8-24.

In CDC-060, 2 subjects in the Safety Population did not complete Artesunate for Injection therapy for reasons other than death; one subject withdrew consent and the second subject was withdrawn at the judgement of the investigator.

Note: reports for EDCTP-MMV07-01 and CDC-060 do not specify whether discontinuation was due to adverse effects of the study medication.

Only one subject in the phase 1 and 2 studies of Artesunate for Injection withdrew because of an adverse event. This subject from Study 1142 withdrew because of an upper respiratory tract infection that was considered unrelated to IV artesunate administration.

10.4.5 Significant Adverse Events

An evaluation of reported AEs according to CDER's list of Designated Medical Events (DME) was performed to identify subjects who experienced one or more of the following serious medical conditions that may be related to the study drug: acute pancreatitis, acute respiratory failure, agranulocytosis, anaphylaxis or anaphylactoid reaction, aplastic anemia, blindness, bone marrow depression, deafness, disseminated intravascular coagulation, hemolytic anemia, liver failure, liver necrosis, liver transplant, pancytopenia, renal failure, seizure, Stevens-Johnson syndrome, *torsade de pointes*, toxic epidermal necrolysis, thrombotic thrombocytopenic purpura, and ventricular fibrillation.

The following tables provide the results of a search of SEAQUAMAT and CDC-060 for DMEs.

Table 10-18: SEAQUAMAT - Designated Medical Events (Safety Population)

Dictionary Derived Term	Artesunate N=730	Quinine N=730	Total N=1460
Acute renal failure requiring dialysis ¹	65 (8.9%)	53 (7.3%)	118 (8.1%)
Seizure	31 (4.2%)	45 (6.2%)	76 (5.2%)
Respiratory failure	26 (3.6%)	39 (5.3%)	65 (4.4%)
Aplastic anemia	1 (0.1%)	0	1 (0.1%)

Source: Clinical Reviewer (J Review 13.1)

¹Includes terms dialysis, hemodialysis, and peritoneal dialysis

Table 10-19: CDC-060 - Designated Medical Events

Dictionary Derived Term	Artesunate N=102
Renal failure, acute	10 (9.8%)
Disseminated intravascular coagulation	3 (2.9%)

Source: Clinical Reviewer (J Review 13.1)

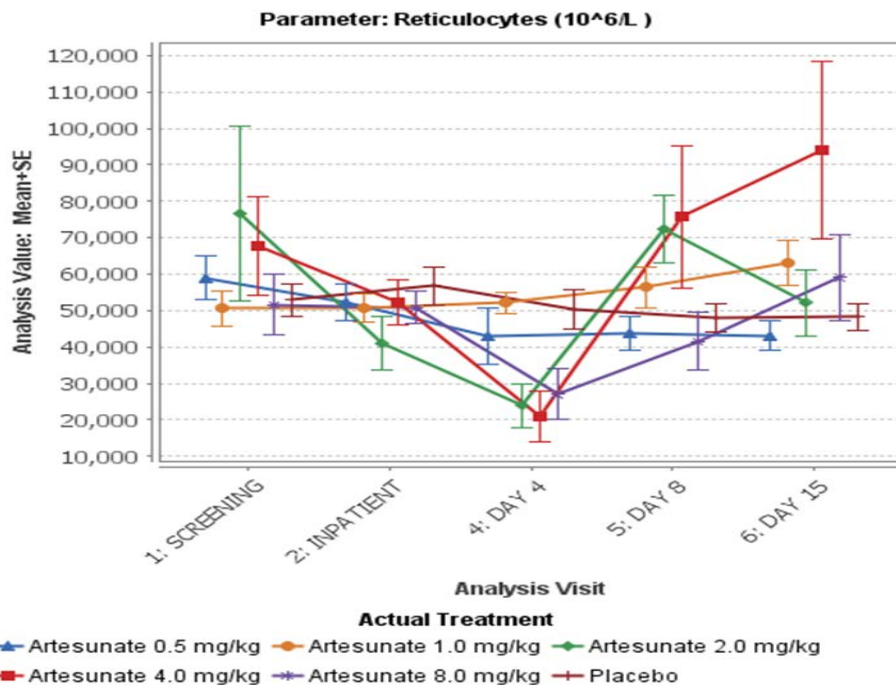
One case of Stevens-Johnson syndrome was reported in Study 1168, in a 54-year old female subject (# (b) (6)) who bullous erythema multiforme and was hospitalized. Symptoms of pruritis developed on Day 3, following completion of 3 days of IV artesunate at 2.4 mg/kg/day and within one hour 40 minutes of receiving the second dose of concomitant Malarone. As the prescribing information for Malarone notes that Stevens-Johnson syndrome has been reported as a post-marketing adverse event,¹⁹ it is not possible to determine an unequivocal association with IV artesunate alone. A more detailed description of this SAE can be found in Section 18.6.5.

10.4.6 Laboratory Findings

See Section 10.5 Submission-Specific Events for discussion of hematology abnormalities associated with IV artesunate administration including anemia, including post-artesunate delayed hemolysis, neutropenia, and decrease in reticulocytes; and hepatic effects including elevation of transaminases. See discussion of TEAEs, above.

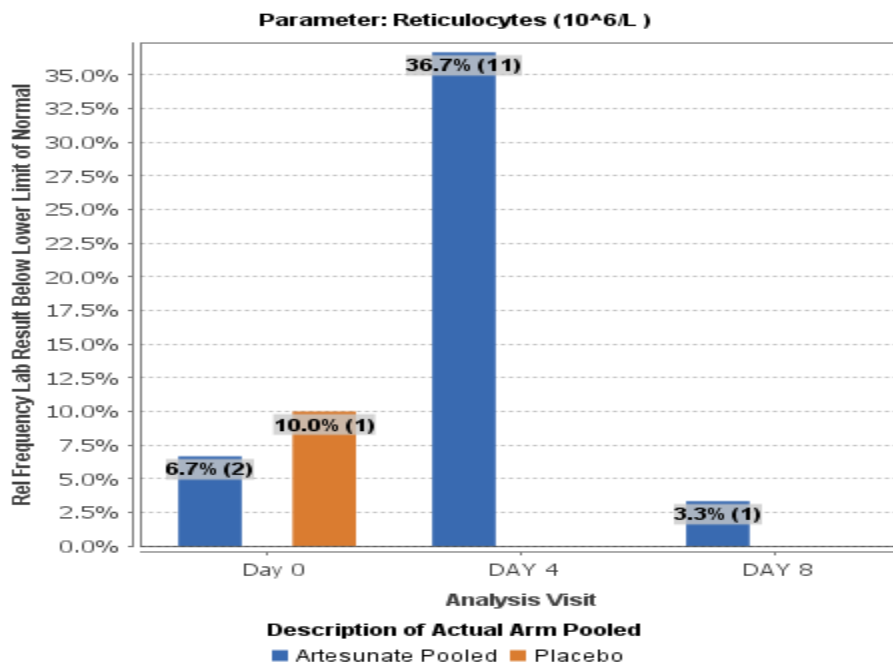
Studies of healthy volunteers (Study 1128 and 1142) showed a decrease in reticulocyte counts following administration of IV artesunate, returning to approximately baseline by days 7- 8, see figures below.

Figure 10-1- Study 1128 Reticulocytes following IV Artesunate by Study Dose



Source: Clinical Reviewer (J Review 13.1)

Figure 10-2- Study 1128 - Reticulocytes below Lower Limit of Normal (Pooled Artesunate)



Source: Clinical Reviewer (J Review 13.1)

A similar pattern with respect to reticulocytes was observed in Study 1142, see Section 18.6.5, Laboratory Findings at Figure 18-38. Phase 2 Study 1263ab in patients with uncomplicated also demonstrated a drop in reticulocytes on Day 3 in patients receiving the two higher dose regimens. This pattern was seen in both the absolute and corrected reticulocyte counts.

Reviewer comments: *In Study 1128, a decrease in reticulocyte count with a return to baseline by Day 8 was observed following IV artesunate administration. The appeared to be dose dependent. In the pooled artesunate groups, a decrease in reticulocyte count below the lower limit of normal was observed in 11 of 30 (36.7%).*

Phase 1 studies of healthy volunteers, transient elevations of ALT and AST in one subject receiving 4.0 mg/kg/dose. In Study CDC-060 transaminase elevations were observed in 27.5 % of subjects.

10.4.7 Vital Signs

There was no pattern of clinically significant changes in vital signs observed in the Phase 1-3 studies included in this application.

10.4.8 Electrocardiograms (ECGs)

Phase 1 Studies 1128 and 1142 included ECG monitoring and cardiac safety analyses.

In addition to the Cardiac Core Cardiac Safety Reports submitted in the CSRs for studies 1128 and 1142 (1128 CSR 16.1.12.10 and 1142 CSR 16.1.12.2, respectively), the Applicant submitted machine-read ECGs which were reviewed by the QT-IRT.

In phase 2 Study 1168 of uncomplicated malaria, 20% of patients receiving IV artesunate at 2.4 mg/kg/daily for 3 days experienced bradycardia and tachycardia each, but these findings were not observed in Study 1263ab at any dose or reported in SEAQUAMAT. In Study CDC-060, 6 patients reported a total of 7 cardiac-related adverse events; in only one subject was the AE considered possibly related to IV artesunate (subject # (b) (6) who experience Grade 2 cardiomyopathy beginning on study day 3). CDC-060 reported two patients with cardiac arrhythmias, one patient with a mild bradycardia and one with supraventricular tachycardia (SVT). The patient with SVT (# (b) (6) had a past medical history significant for significant cardiovascular disease, including congestive heart failure, mitral valve regurgitation, hypertension and coronary artery disease.

10.4.9 QT Interval

In SEAQUAMAT, one subject (# (b) (6) - (b) (6)) a 55-year old male assigned on artesunate arm experienced an AE of QT prolongation and bundle branch block at the time of admission.

The FDA QT-IRT reviewed data from Studies 1128 and 1142, the proposed product labeling and

the Applicant's review of clinical pharmacology and cardiac safety, see QT-IRT review dated 2/18/2020.

The data were evaluated using exposure-response analysis. The QT-IRT concluded that there was no significant QTc prolongation effect of artesunate.

The findings were supported by a time analysis using non-parametric method with the primary analysis based on exposure-response analysis and a categorical analysis. The Applicant used digitized data for their analyses while the QT-IRT used the dataset derived from single paper ECGs with automated ECG measurements. No significant outliers were found in the Applicant's and FDA's analyses for QTcF > 500 msec. The FDA analysis identified one subject in the artesunate 8.0 mg/kg group who experienced Δ QTcF greater than 60 msec, while the Applicant's analysis has not identified any subjects in either study who experienced Δ QTcF greater than 60 msec.

The QT-IRT noted that the clinical trials submitted with this application included a placebo control but did not utilize a positive control such as moxifloxacin for assay sensitivity. Per the QT-IRT, the lack of a positive control was mitigated by using ECG data performed using automated ECG measurements that would eliminate the potential for measurement bias. The QT-IRT recommended the following wording for the product labeling:

Cardiac Electrophysiology

At the maximum approved recommended dose, artesunate does not prolong the QT interval to any clinically relevant extent.

10.4.10 Immunogenicity

Not applicable.

10.5 Analysis of Submission-Specific Safety Issues

10.5.1 Neurological Events

Neurological complications of severe malaria due to *P. falciparum* include coma, seizures and altered mental status and may result in long-term sequelae, particularly after recovery from cerebral malaria. While first described in adults, residual neurological impairment has also been described in children with sequelae that included hemiplegia, cortical blindness, aphasia, and ataxia. ([Brewster, Kwiatkowski, and White 1990](#)).

Artemisinins have been associated with neurotoxicity in animals, with the manifestations dependent upon the particular drug, route of administration, half-life and duration of exposure. ([Efferth and Kaina 2010](#)) Artesunate may pose less risk for neurotoxicity given its rapid clearance with shorter central nervous system exposure. ([Medhi et al. 2009](#)) Human studies of

artemisinins evaluating neurological outcomes have shown equivocal results, with some clinical studies of artemether, for example, observing prolonged recovery times from coma in patients treated with artemether compared with quinine ([Hien et al. 1996](#); [Tran et al. 1996](#)). These findings were not confirmed by a meta-analysis that included over 1900 patients. ([Stepniewska 2001](#)) A Cochrane meta-analysis of eight randomized controlled trials (including SEAQUAMAT and AQUAMAT) compared artesunate (intravenous, intramuscular, or rectal) with quinine (intravenous or intramuscular) for the treatment of adults (n=1664) and children (n=5765) with severe malaria. This meta-analysis showed a reduced risk of death in both adults and children but found an increased incidence of neurological sequelae in children at the time of hospital discharge. The review characterized the majority of sequelae as transient with no significant difference observed on follow up at 28 days following discharge. ([Sinclair et al. 2012](#))

Under the SEAQUAMAT protocol, patients were evaluated on discharge with a check box for specified neurological adverse events including the following: cortical blindness, cranial nerve palsies, ataxia, loss of balance, extrapyramidal rigidity, hemiplegia/paresis, neuropsychiatric conditions (like psychosis), and included a separate check box for other significant neurological sequelae with a free text field to fill in details.²⁰ Patients were not followed further after discharge. The following table describes the neurological deficits observed at the time of discharge. Specific findings from the free text field for “other significant neurological sequelae” results are also provided.

Table 10-20: SEAQUAMAT – Number of Patients with Neurological Sequelae on Discharge (Safety Population)

Outcome	Artesunate (n=730)	Quinine (n=730)	All (n=1460)
SEAQUAMAT publication ¹	7 (1.0%)	3 (0.4%)	10 (0.7%)
Neurological sequelae on discharge ²	7 (1.0%)	3 (0.4%)	10 (0.7%)
Neurological sequelae on discharge based on reported neurological term ³	12 (1.6%) ⁴	7 (1.0%)	19
Ataxia	2 (0.3%)	0 (0%)	2 (0.1%)
Poor balance	4 (0.5%)	3 (0.4%)	7 (0.5%)
Paresis	2 (0.3%)	0 (0%)	2 (0.1%)
Neuropsychiatric conditions (like psychosis)	4 (0.5%)	4 (0.5%)	8 (0.5%)
Other significant neurological sequelae on discharge	4 (0.5%)	0 (0%)	4 (0.3%)
Tremor	1 (0.1%)	0 (0%)	1 (0.1%)
Monoparesis	1 (0.1%)	0 (0%)	1 (0.1%)
Asthenia	1 (0.1%)	0 (0%)	1 (0.1%)
Confusional state	1 (0.1%)	0 (0%)	1 (0.1%)

Source: Clinical Reviewer (J Review) and Statistical Reviewer, Event numbers by subject.

¹ Dondorp 2005, Table 3

² Based on investigator response “yes” to question significant neurological sequelae on discharge

³ CRF listed prespecified terms: cortical blindness, cranial nerve palsies, ataxia, loss of balance, extrapyramidal rigidity, hemiplegia/paresis, neuropsychiatric (like psychosis) and other (please specify). One additional patient

²⁰ Per the annotated CRF, neurological sequelae at discharge were captured in the ADCE dataset as CETERM and data were also included in the ADAE dataset as AETERM.

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

receiving artesunate, patient # (b) (6) - (b) (6) had the following free text recorded: “feels extremely weak. Unable to walk properly, unstable gait”, but was not classified as “other significant neurological sequelae” on discharge.

⁴ One subject had 4 events and one subject had 2 events.

Reviewer Comments: *When evaluating the prespecified neurological terms, 7 patients (1%) receiving artesunate compared with 3 patients (0.4%) receiving quinine experienced neurological sequelae. When data from the free text field were included, 12 (1.6%) artesunate patients and 7 (1%) quinine patients had a specific neurological sequela noted on discharge. Two subjects had multiple events.*

Review of neurological adverse events in AQUAMAT was limited to data reported in the publication. According to the publication, neurological status was initially assessed only at hospital discharge. The protocol was amended after 11% of patients had been enrolled to include neurological exam 28 days following discharge. Dondorp 2010 reported that 99 artesunate and 71 quinine recipients had neurological sequelae at discharge; denominators were not provided. According to the publication, 34 artesunate and 27 quinine recipients had neurological sequelae at day 28. Of these sequelae, 28 artesunate recipients compared with 20 quinine recipients had sequelae described as severe; these included severe motor impairment, cortical blindness, and severe speech or hearing impairment.

In patients who had cerebral malaria, the overall incidence of persistent neurological sequelae at 28 days was 3.4% (24 of 706) in artesunate recipients and 3.1% (23 of 737) in quinine recipients. The incidence of severe neurological sequelae was 2.3% in regardless of whether the patients received artesunate (17 of 706) or quinine (17 of 737). See table below for presentation of data from the publication.

Figure 10-3: AQUAMAT – Neuro Sequelae at discharge and 28 days in children with severe falciparum malaria (Per Protocol)

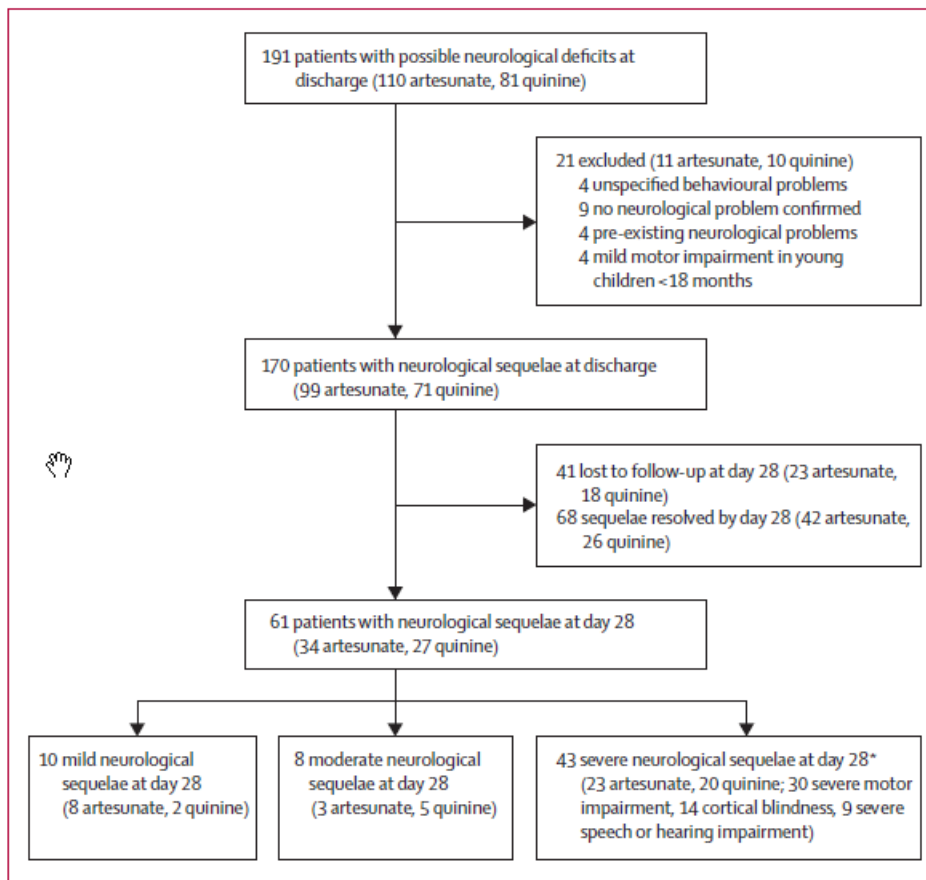


Figure 4: Neurological sequelae at discharge and after 28 days (range 3–8 weeks) in children with severe falciparum malaria

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

Source: Dondorp 2010

Reviewer Comments: At the time of hospital discharge, a higher incidence of neurological sequelae was observed in both SEAQUAMAT and AQUAMAT trials. In both trials artesunate recipients demonstrated lower in-hospital mortality and the higher incidence of residual neurological deficits in children has been postulated to be due to the greater survival of patients with more severe disease rather than direct toxicity from artesunate. The absence of patient level data from AQUAMAT precluded FDA's analysis of neurological outcomes from this trial.

The Applicant's ISS/ISE meta-analysis relied heavily on studies included in the Cochrane meta-analysis. Few of these trials included safety endpoints. Other than SEAQUAMAT and AQUAMAT, only one study reported on neurological sequelae but the sample size of 37 subject in each treatment was too small to demonstrate a significant difference. (Cao et al. 1997)

10.5.2 Delayed Hemolysis and Anemia

Following the recommendation by WHO in 2010 for use of IV artesunate as the first line treatment of severe malaria, investigators began reporting the occurrence of delayed anemia 1 to 3 weeks following artesunate therapy, first in travelers and subsequently in patients from endemic regions. ([Zoller et al. 2011](#)) ([Kreeftmeijer-Vegter et al. 2012](#)) ([Briggs and Arguin 2013](#)) ([Rolling et al. 2014](#)) While transient anemia following artesunate therapy has been ascribed to reversible bone marrow suppression ([Finaurini et al. 2012](#)) and intravascular hemolysis ([Dondorp et al. 2010](#)), the delayed hemolytic anemia observed following artesunate therapy (post-artemisinin delayed hemolysis or PADH) appears to be a separate condition with a different pathogenesis, as follows.

The rapid action of artesunate kills malaria parasites which are then removed by the spleen, resulting in “pitted” once-infected erythrocytes that remain in circulation. ([Arguin 2014](#)) By examining hematologic parameters in travelers who received IV artesunate for severe malaria, Jaureguiberry found a correlation between the pitted RBCs and the occurrence of PADH, with the once-infected RBCs being removed by the spleen 7-21 days after IV artesunate therapy. Jaureguiberry proposed a case definition of 10% drop in hemoglobin associated with haptoglobin <0.1 g/L and either an increase in LDH to >390 IU/L or 10% rise >7 days after treatment initiation with IV artesunate. ([Jaureguiberry et al. 2014](#))

While anemia was a frequently observed adverse event in studies submitted in this application, the occurrence of PADH could not be confirmed in this review because of the limited duration of follow up. Anemia following IV artesunate was observed in phase 1 studies of healthy volunteers (Studies 1128 and 1142), with 10% of subjects receiving single dose regimen experiencing anemia compared with 30% of subjects receiving multiple dose regimens. Anemia was also observed in phase 2 studies in uncomplicated malaria, with 13 of 30 (43%) of subjects in Study 1168 (2.4 mg/kg/dose) experiencing anemia and 23 of 100 (23%) of patients in Study 1263ab pooled IV artesunate regimens (1.2 mg/kg dose to 4.8 mg/kg/dose), without a dose response relationship. SEAQUAMAT recorded severe anemia after admission, defined as hematocrit <20, as occurring in 2.7% of patients receiving artesunate versus 2.9% of patients receiving quinine; however, no information on transfusions was provided in the datasets. In study CDC-060, 65.7% of subjects had anemia and transfusions were administered in 49 patients (48%), but because follow up was limited to the period of hospitalization, it is not clear whether any of these transfusions were due to PADH. The AQUAMAT publication reports that transfusions were given to 1487 of 2712 (55%) of patients randomized to artesunate and 1495 of 2713 (55%) of patients randomized to quinine; however, details on timing were not provided. EDCTP-MMV07-01 provided descriptions of transfusions in some serious adverse event narratives but complete data and timing of transfusions for all patients were not provided. Three subjects had SAE of anemia diagnosed 7 to 14 days following admission and received transfusions.

Two case reports have described an immune-mediated hemolysis following IV artesunate associated with a direct antiglobulin (Coomb's) test. ([Raffray et al. 2014](#)) ([Chamnanchanunt 2017](#)) These patients experienced a drop in hemoglobin 12-14 days following IV artesunate therapy, had a positive Coomb's test, and improved with oral steroid therapy. The patients with immune-mediated hemolysis appear to be subset of patients with delayed hemolysis after IV artesunate.

10.5.3 Neutropenia

Dose-dependent neutropenia has been described in clinical studies of patients treated with oral artesunate for *P. falciparum* malaria, with 5 of 26 patients (19%) treated with 6 mg/kg/day for 7 days experiencing neutropenia (defined in this study as absolute neutrophil count or ANC less than 1000 per mm³) and geometric mean ANCs in this high dose group remaining significantly lower than in patients receiving 2 mg/kg/day or 4 mg/kg/day on both day 6 and day 14. ([Bethell et al. 2010](#)) In a study of uncomplicated malaria, the risk of neutropenia 14 days after starting treatment with oral artesunate was higher in HIV-infected than in HIV-uninfected children and 16% of HIV-infected children experienced severe neutropenia (ANC <750 cells/mm³). ([Gasasira et al. 2008](#))

During the clinical development of Artesunate for Injection, different studies used different definitions of neutropenia as a TEAE, hampering an overall assessment of this adverse event. In phase 1 Study 1128 for example, no patients were identified as having neutropenia as a TEAE, but 2 subjects (one receiving artesunate at 4.0 mg/kg/dose and one receiving 8.0 mg/kg dose) demonstrated a drop in neutrophil count to < 1.5 x 10⁶/L (one placebo recipient experienced a similar drop.) In phase 1 Study 1142, one subject receiving Artesunate for Injection at 8.0 mg/kg/dose (20% of total artesunate recipients) was coded as having neutropenia as a TEAE with an ANC <1.0 x 10⁶/L but an additional 2 subjects receiving 8.0 mg/kg/dose experienced neutrophil counts <1.5 x 10⁶/L (as did one placebo recipient.)

During phase 2 studies of uncomplicated malaria, severe neutropenia was defined as neutrophil count <0.75 x 10⁶/L. Severe neutropenia occurred in 4 of 30 (13.3%) of patients in Study 1168 and in 9 of 100 (9%) patients in Study 1263ab, primarily in patients who received doses greater than 2.4 mg/kg/day, but there was no strict dose-response relationship. In Study 1263ab for example, 4 of 25 patients in the 1.2 mg/kg/dose group, 0 of 50 in the 2.4 mg/kg/dose group and 5 of 25 patients in the 4.8 mg/kg/dose experienced severe neutropenia. While 5% of patients experienced neutropenia in CDC-060, only one of 102 subjects was identified as having severe neutropenia. An analysis of SEAQUAMAT data did not find any TEAEs of neutropenia, and neutropenia was not reported as an AE in the AQUAMAT publication. A transient decrease in neutrophils was observed in Study EDCTP-MMV07-01 in both the 3-dose and 5-dose treatment groups.

In summary, despite the variable coding of neutropenia as a TEAE and inconsistent assignment of severity, certain trends were apparent upon analysis of neutrophil counts for different dose

regimens. A transient, reversible drop in neutrophil count was observed in the phase 1 - 2 studies with a tendency for a greater incidence and severity at higher dose regimens.

10.5.4 Hypersensitivity

Hypersensitivity reactions including anaphylaxis have been reported with artemisinin drugs, including Coartem (artemether and lumefantrine) ("[Coartem \(artemether/lumefantrine\) Tablets \[package insert\]](#)") and oral artesunate. ([Leonardi et al. 2001](#)) Anaphylaxis following IV artesunate has been described in the literature. ([Mohapatra et al. 2009](#)), ([Dube et al. 2012](#)) ([Neki 2015](#))

Within the phase 1 clinical development of Artesunate for Injection, one case of urticaria was reported in a healthy volunteer (Study 1142) who received single dose of artesunate at 8.0 mg/kg. The rash, which was considered moderate in severity, started on the day of dosing and resolved with 24 hours following receipt of diphenhydramine. In phase 2 studies in patients with uncomplicated malaria, there was one case of bullous erythema multiforme (preferred term Stevens-Johnson Syndrome) in a patient with uncomplicated malaria who also received Malarone (see Section 18.6.3 for more details).

10.5.5 Reduced Reticulocyte Count

Artesunate and other artemisinins have been associated with reversible bone marrow depression. ([Finaurini et al. 2012](#)) A Thai study in healthy volunteers demonstrated a decrease in hemoglobin 7 days after administration of DHA dosed orally at 300 mg daily for two days compared to oral artesunate therapy. ([Kongpatanakul et al. 2009](#)) This finding was accompanied by a reduction in reticulocytes with nadir on day 5 (DHA 47% reduction in reticulocyte count, artesunate 75% reduction), with the authors proposing transient bone marrow suppression as a direct effect of artemisinin therapy. A reversible reduction in reticulocyte counts in healthy volunteers was previously described in healthy volunteers at total doses of IV artesunate up to 16.88 mg/kg. ([Barradell and Fitton 1995](#)) A drop in reticulocyte counts by day 5 treatment has also been described in a clinical study of patients with severe malaria receiving IV artesunate relative to patients receiving quinine. ([Cao et al. 1997](#))

Phase 1 and 2 studies of IV artesunate submitted with this application showed a transient but consistent drop in reticulocyte count. Study 1128, a decrease in reticulocyte count with a return to baseline by Day 8 was observed which appeared to be dose dependent. In the pooled artesunate groups, a decrease in reticulocyte count below the lower limit of normal was observed in 11 of 30 (36.7%) subjects. A similar pattern with respect to reticulocytes was observed in Study 1142, see Section 18.6.5, Laboratory Findings. Phase 2 Study 1168 in patients with uncomplicated malaria also found a dose-related drop in reticulocyte count, reaching its nadir by day 4 and returning to baseline by day 7 for most patients. ([Li et al. 2014](#)) A similar dip in reticulocytes was seen in phase 2 Study 1263ab, reaching lowest levels on reticulocytes on

Day 3 in patients receiving the two higher dose regimens. This pattern was seen in both the absolute and corrected reticulocyte counts.

10.6 Clinical Outcome Assessment (COA) Analyses Informing Safety/Tolerability

Not applicable

10.7 Safety Analyses by Demographic Subgroups

Safety analyses by gender, age, race and ethnicity (CDC-060 and Studies 1128 and 1142), and by gender, age, country, and study site (SEAQUAMAT) were performed. Race and ethnicity were not collected for SEAQUAMAT.

The incidence of at least one TEAE was balanced by gender, age above and below age 65 years, age above and below 18 years, weight above and below 20 kg, and country, see table below.

Table 10-21: SEAQUAMAT – Incidence of TEAE by Demographic Subgroups¹ (Safety Population)

Subgroup	SEAQUAMAT Incidence of TEAE Per Demographic Subgroup			
	Artesunate		Quinine	
	N	n (%)	N	n (%)
Females	184	77 (41.8%)	202	96 (47.5%)
Males	546	219 (40.1%)	528	243 (46.0)
≥65y	17	10 (58.8)	21	13(61.9)
<65y	713	286 (40.1)	709	326 (46.0)
≥18 y	569	246 (43.2)	578	287(49.7)
<18 y	161	50 (31.1)	152	52 (34.2)
Pediatric patients ² ≥20 kg	109	37 (33.9%)	95	35 (36.8%)
Pediatric patients ² <20 kg	52	13 (25.0%)	57	17 (29.8%)
Country				
Myanmar	295	130 (44.4%)	281	141 (50.2%)
Bangladesh	222	75 (34.2%)	231	96 (41.6%)
Indonesia	143	62 (43.4%)	146	66 (45.2%)
India	70	39 (55.7%)	72	41 (56.9%)

Source: Clinical Reviewer (J Review 13.1)

¹ TEAE Incidence: ADAE dataset "After Admission"

² Pediatric patients less than 18 years of age

Reviewer comments: In SEAQUAMAT, TEAE incidence was consistently higher in patients receiving quinine across all subgroups. SEAQUAMAT enrolled 38 total subjects (2.6%) age 65

years or older; although adverse events were observed more frequently in this age group, the numbers were too small to determine its clinical significance. TEAEs were more commonly observed in those 18 years of age or older compared with those younger than 18 years. Pediatric patients younger than 18 years of age constituted 21.4% of total patients; in this population patients weighing less than 20 kg showed a trend towards decreased incidence of TEAE compared with patients weighing $\geq$ 20 kg for both artesunate and quinine arms. No other specific patterns were identified.

The incidence of TEAEs for patients in CDC-060 is found below.

Table 10-22: CDC-060 -- TEAE by Demographic Subgroups¹ (Safety Population)

Subgroup	CDC-060 No. of TEAEs	
	Artesunate	
	N	n (%)
Females	40	37 (92.5%)
Males	62	57 (91.9%)
$\geq$ 65y	3	3 (100%)
<65y	99	91 (91.9%)
$\geq$ 18 y	90	83 (92.2%)
<18 y	12	11 (91.7%)
Pediatric patients ² $\geq$ 20 kg	8	7 (87.5%)
Pediatric patients ² < 20 kg	4	4 (100%)

Source: Clinical Reviewer (J Review 13.1)

¹ TEAE Incidence: ADAE dataset "After Admission"

² Pediatric patients less than 18 years of age

Reviewer comments: In Study CDC-060, the incidence of TEAE in patients appeared similar across demographic subgroups, although numbers were too small to make meaningful comparisons.

For additional analyses of SEAQUAMAT and CDC-060 in pediatric patients, see Section 12.

10.8 Specific Safety Studies/Clinical Trials

No specific safety studies were performed for this application. For a detailed review of clinical studies evaluating Artesunate for Injection, See Appendix 18.6.

10.9 Additional Safety Explorations

10.9.1 Human Carcinogenicity or Tumor Development

Not applicable

10.9.2 Human Reproduction and Pregnancy

In malaria endemic regions, pregnant women have a higher prevalence of malaria and an increased risk of severe malaria; these risks extend through the early postpartum period. ([Diagne et al. 2000](#)) Malaria during pregnancy is associated with increased maternal morbidity and mortality and adverse fetal outcomes including spontaneous abortions, still births, preterm delivery, low birth weight, and congenital infection. ([World Health Organization 2019](#)) ([Fried and Duffy 2017](#))

CDC Guidelines for Treatment of Malaria in the US (December 2019) recommend IV artesunate for all patients with severe malaria, including pregnant women in all trimesters. ([Centers for Disease Control and Prevention 2019](#)) WHO Guidelines for the Treatment of Malaria ([World Health Organization 2015](#)) also recommend parenteral artesunate as the treatment of choice for severe malaria in all trimesters, noting that treatment must not be delayed. In the event that artesunate is unavailable, IM artemether is recommended; if this is unavailable, parenteral quinine should be started immediately until artesunate becomes available. Limited data have been published regarding the safety of artemisinins during breastfeeding.

Embryotoxicity and fetal malformations have been reported in published nonclinical studies of artemisinin drugs administered during pregnancy at or below the equivalent therapeutic doses in humans, with timing of effects seen early in gestation; see discussion in Section 5.5.4, Reproductive and Development Toxicology. The toxicity of artemisinins, which has been observed in several animal species, has varied depending on the artemisinin drug and the route of administration. Clinical studies evaluating the use of oral artesunate and other artemisinin drugs during pregnancy, including randomized controlled clinical trials, observational studies and cohort studies have not identified adverse maternal or fetal outcomes but limited clinical data are available evaluating the safety of IV artesunate during the first trimester. Oral artesunate and other drugs in the artemisinin class have had widespread clinical use over the past several decades, however, and have been administered to scores of pregnant women without reports of significant congenital malformations or other adverse fetal or maternal outcomes.

Two studies in this application, SEQUAMAT and CDC-060, included pregnant patients with severe malaria and were analyzed for available data. In both studies, patients were generally followed only until hospital discharge so limited data were available on outcomes.

The following table provides the number of pregnancies identified during SEQUAMAT, along with the estimated period of gestational and numbers of fetal and maternal deaths.

Table 10-23: SEAQUAMAT – Pregnancies, Maternal Deaths, and Still Births and Spontaneous Abortions (Safety Population)

	Artesunate (n=730)	Quinine (n=730)	Totals (n=1460)
Pregnant Flag	23 (3.2%)	26 (3.6%)	49 (3.4%)
Estimated Gestation Time Weeks			
Subjects with data	23	26	49
mean [SD]	24.35 [6.46]	27.38 [7.94]	25.96 [7.37]
median	0	0	0
range	8-36	8-40	8-40
Fetal Death Flag - Still birth or spontaneous abortion	5/23 (21.7%)	5/26 (19.2%)	10/49 (20.4%)
Maternal Deaths	2/23 (8.7%)	3/26 (11.5%)	5/49 (10.2%)

Source: Clinical Reviewer – J Review 13.1

Reviewer comments: In SEAQUAMAT a total of 49 women, 23 receiving artesunate and 26 receiving quinine, were flagged as being pregnant. Most patients were in the second or third trimester with a mean of 24 weeks for artesunate recipients. Only two subjects were ≤ 12 weeks gestation, one in artesunate and one in the quinine group. The incidence of fetal and maternal deaths was similar in the two treatment groups although numbers were too small to make a meaningful comparison.

The following table presents data on the occurrence of prespecified clinical events.

Table 10-24: SEAQUAMAT -- Clinical Events After Admission in Pregnant Patients (Safety Population)

Clinical Event or Treatment	Artesunate n=23	Quinine n=26	Totals n=49
Antibiotics	6 (26.1%)	8 (30.7%)	14 (28.6%)
Still Birth or Spontaneous Abortion	5 (21.3%)	5 (19.2%)	10 (20.4%)
Severe Anemia (Hct < 20%)	4 (17.4%)	3 (11.5%)	7 (14.2%)
Coma or Deterioration in GCS	4 (17.4%)	1 (3.8%)	5 (8.2%)
Other Complications	3 (11.5%)	3 (11.5%)	6 (12.2%)
Black Water Fever/ Hemoglobinuria	2 (8.7%)	1 (3.8%)	3 (6.1%)
Shock	1 (4.3%)	2 (7.7%)	3 (6.1%)
Suspected Sepsis	1 (4.3%)	4 (15.4%)	5 (8.2%)
ICU Admission	1 (4.5%)	0	1 (2.0%)
Hypoglycemia	1 (4.3%)	2 (7.7%)	3 (6.1%)
Dialysis	1 (4.3%)	0	1 (2.0%)
Respiratory Insufficiency or Mechanical Ventilation	1 (0.5%)	1 (3.8%)	2 (4.1%)
Severe Jaundice	1 (4.3%)	0	1 (2.0%)

Clinical Event or Treatment	Artesunate n=23	Quinine n=26	Totals n=49
Vasopressor Drugs Required	1 (4.3%)	0	1 (2.0%)
Convulsions	0	1 (3.8%)	1 (2.0%)

Source: Clinical Reviewer (J Review 13.1)

Reviewer comments: Observed clinical events after admission were generally balanced between pregnant patients receiving artesunate compared with those receiving quinine. While coma or deterioration in GCS occurred more commonly in artesunate recipients and suspected sepsis was observed more frequently in quinine recipients, numbers were too small for meaningful comparisons.

The following table provides additional details on maternal and fetal outcomes for all patients with pregnancy flag.

Table 10-25: SEAQUAMAT -- All Pregnancies: Maternal and Fetal Outcomes (Safety Population)

Study Drug	Subject Identifier	No. Doses Study Drug	Maternal Age	Estimated Gestation Time Weeks	Mother Outcome Description	Day of Maternal Death if Applicable	Fetal Death Flag
Artesunate	(b) (6)	4	33	32	No Death	-	N
Artesunate	(b) (6)	1	18	20	Death	1	N
Artesunate	(b) (6)	4	20	24	No Death	-	N
Artesunate	(b) (6)	6	30	24	No Death	-	N
Artesunate	(b) (6)	6	28	34	No Death	-	N
Artesunate	(b) (6)	8	23	18	No Death	-	N
Artesunate	(b) (6)	6	25	16	No Death	-	Y
Artesunate	(b) (6)	4	22	28	No Death	-	N
Artesunate	(b) (6)	2	21	32	No Death	-	N
Artesunate	(b) (6)	6	18	24	No Death	-	N
Artesunate	(b) (6)	4	22	20	No Death	-	N
Artesunate	(b) (6)	3	35	32	No Death	-	N
Artesunate	(b) (6)	3	30	20	No Death	-	N
Artesunate	(b) (6)	3	24	24	No Death	-	N
Artesunate	(b) (6)	3	28	22	No Death	-	Y
Artesunate	(b) (6)	3	25	24	No Death	-	N
Artesunate	(b) (6)	3	24	24	No Death	-	N
Artesunate	(b) (6)	1	34	22	Death	1	Y
Artesunate	(b) (6)	5	19	8	No Death	-	Y
Artesunate	(b) (6)	5	31	36	No Death	-	Y
Artesunate	(b) (6)	4	26	26	No Death	-	N
Artesunate	(b) (6)	3	35	20	No Death	-	N
Artesunate	(b) (6)	3	19	30	No Death	-	N
Quinine	(b) (6)	5	20	20	No Death	-	N

Study	(b) (6) No. Doses Study Drug	Maternal Age	Estimated Gestation Time Weeks	Mother Outcome Description	Day of Maternal Death if Applicable	Fetal Death Flag
Quinine	5	22	12	No Death	-	Y
Quinine	6	22	28	No Death	-	N
Quinine	16	22	21	No Death	-	Y
Quinine	5	18	18	Death	2	N
Quinine	3	35	32	Death	2	N
Quinine	7	20	32	No Death	-	N
Quinine	6	25	27	No Death	-	N
Quinine	5	30	36	No Death	-	N
Quinine	5	24	32	No Death	-	N
Quinine	6	20	24	No Death	-	N
Quinine	12	30	26	No Death		N
Quinine	10	32	30	No Death		N
Quinine	18	18	16	No Death		Y
Quinine	11	22	26	No Death		N
Quinine	18	28	36	No Death		N
Quinine	6	28	34	No Death		N
Quinine	3	26	32	No Death		N
Quinine	9	33	32	No Death		N
Quinine	6	24	38	No Death		N
Quinine	4	18	32	No Death		N
Quinine	7	35	22	No Death		Y
Quinine	7	22	8	Death	4	N
Quinine	10	21	40	No Death		N
Quinine	10	17	28	No Death		N
Quinine	12	23	30	No Death		Y

Source: Clinical Reviewer (J Review 13.1)

Color code: Maternal Deaths; Fetal Death Flag

¹Subject (b) (6) had the following recorded in "Other Complications" free text field: "spontaneous vaginal delivery, alive (b) (6)", no additional details provided.

²Subject (b) (6) had the following recorded in "Other Complications" free text field: "retained placenta and manual removal done, no additional details provided."

Reviewer Comment: This table includes maternal outcomes in all pregnant subjects and as well as fetal death flags. In SEQUAMAT, a total of 5 maternal deaths was observed, 2 in patients receiving artesunate and 3 in patients receiving quinine. Four out of 5 deaths occurred in the first two days after admission. Pregnant artesunate recipients received between 1 and 8 doses of study drug.

If all maternal deaths were flagged as fetal death, fetal deaths occurred in 6 of 23 (26.1%) of pregnant artesunate recipients, and 8 of 26 (30.8%) of pregnant quinine recipients. Pregnancy outcomes were not recorded in the datasets with the exception of one subject who delivered a liveborn infant via spontaneous vaginal delivery at 30 weeks gestation.

CDC-060 Pregnancy Outcomes

Three pregnant patients, all in the third trimester, were enrolled in the CDC IND protocol during the time period covered by CDC-060. Two patients delivered prior to receiving IV artesunate. The two remaining patients (35 and 36-37 weeks respectively) recovered from severe malaria without sequelae and delivered healthy infants by vaginal delivery within one month of initiating treatment.

Division of Pediatric and Maternal Health Review

The Division of Pediatric and Maternal Health conducted a review of available data to assist the review team in providing guidance in product labeling. DPMH concluded that although artesunate and artemisinin derivatives are teratogens in animals, available data in humans have not identified adverse maternal or fetal outcomes, including congenital malformations, miscarriage, and still birth. DPMH and the review team collaborated in drafting language in the prescribing information for Artesunate for Injection that conveyed the risks associated to both mother and fetus of delaying or withholding treatment for severe malaria; noted that extensive experience with oral artesunate and other artemisinin class drugs in pregnant women has not identified a risk of major birth defects, miscarriage, or adverse maternal or fetal outcomes; and described the observation in animals of embryo-lethality and fetal malformations when artesunate was administered early in gestation but noted that the clinical relevance was uncertain.

10.9.3 Overdose, Drug Abuse Potential, Withdrawal, and Rebound

No abuse or withdrawal potential has been described in the literature with IV artesunate.

The application reports one case of a fatal overdose from the literature in a 5 year old child following the administration of rectal artesunate at a dose of 200 mg BID or 88 mg/kg/day for 4 days. ([Campos 2008](#)) The patient became obtunded and developed seizures, jaundice and gastrointestinal hemorrhage on the fifth day after starting treatment. Prior to death on the 12th day after the start of artesunate therapy, the patient also experienced hypotension, hypoglycemia, pancytopenia and multiorgan system failure.

10.9.4 Dosing in Patients $\geq$ 100 kg

The proposed artesunate dosing is 2.4 mg/kg/dose regardless of the age or weight of the patient, with no maximum dose recommended. No data were found in the literature regarding safety of artesunate in patients > 100 kg. In SEAQUAMAT only one recipient of quinine had a weight greater than 100 kg. CDC-060 included 14 of 102 patients (13.7%) with weight greater than 100 kg. See table below for analysis of TEAE by weight $\leq$ 100 kg and >100 kg.

Table 10-26: CDC-060 TEAE > 5% by PT According to Weight 100 kg (Safety Population)

TEAE	<= 100kg n=88	> 100kg n=14	Totals n=102
Subjects with at least 1 TEAE	81 (92.0%)	13 (92.9%)	94 (92.2%)
Anemia	55 (62.5%)	11 (78.6%)	66 (64.7%)
Thrombocytopenia	17 (19.3%)	1 (7.1%)	18 (17.6%)
Leukocytosis	8 (9.1%)	2 (14.3%)	10 (9.8%)
Lymphopenia	6 (6.8%)	1 (7.1%)	7 (6.9%)
Transaminases increased	24 (27.3%)	4 (28.6%)	28 (27.5%)
Aspartate aminotransferase increased	20 (22.7%)	2 (14.3%)	22 (21.6%)
Acute respiratory distress syndrome	8 (9.1%)	0 (0.0%)	8 (7.8%)
Hyperbilirubinemia	13 (14.8%)	1 (7.1%)	14 (13.7%)
Renal failure acute	10 (11.4%)	0 (0.0%)	10 (9.8%)

Source: Clinical Reviewer (J Review 13.1)

Reviewer Comment: In CDC-060, TEAE observed in patients weighing more than 100 kg were generally comparable or occurred less frequently than TEAEs in patients weighing less than 100 kg, with the exception of anemia and leukocytosis. All subjects in Studies 1168 and 1263ab were less than 100 kg so it was not possible to assess safety of IV artesunate by weight > 100 kg in these studies.

10.10 Safety in the Postmarket Setting

Safety Concerns Identified Through Postmarket Experience

Parenteral artesunate has been studied extensively in multiple clinical trials in various malaria-endemic regions and has been recommended by WHO as first line therapy for severe malaria since 2010.

The Applicant submitted an integrated Summary of Safety (ISS) and an Integrated Summary of Efficacy (ISE) that consisted of a meta-analysis entitled “Meta-analysis of Intravenous and Intramuscular Artesunate in Complicated or Severe Malaria” conducted in 2013 by Sigma-Tau. The Amivas ISS/ISE meta-analysis focused primarily on the Cochrane meta-analysis which conducted its original search in November 2005 and an updated search in November 2010 identifying a total of eight randomized controlled trials²¹ ([Sinclair et al. 2012](#)). The ISS/ISE meta-analysis included an additional 25 publications through 2012, adding added other controlled trials of IV artesunate versus quinine, retrospective cases of travelers, additional data on safety and efficacy in subgroups, uncontrolled or add-on trials, and studies with a control group other than quinine. SEAQUAMAT, AQUAMAT, and EDCTP-MMV07-01 were analyzed as part of the

²¹ The Cochrane meta-analysis of IV artesunate for severe malaria included the following studies: ([Anh 1989](#)), ([Anh, Binh, and Kim 1995](#)), ([Hien et al. 1992](#)), ([Cao et al. 1997](#)), ([Newton et al. 2003](#)), ([Dondorp et al. 2005](#)), ([Dondorp et al. 2010](#)), and ([El-tahir et al. 2010](#)).

ISS/ISE meta-analysis but CDC-060, published in Twomey et al. 2015, was not included.

Adverse reactions identified in the ISS/ISE that were identified with higher frequency in recipients of artesunate compared with recipients of quinine were blackwater fever and neurological disorders, similar to FDA's findings in this application. Additional adverse reactions included in the WHO/Guilin artesunate SPC include elevated serum amylase and pancreatitis, tinnitus with associated hearing loss, and insomnia.

10.11 Integrated Assessment of Safety

The safety database included 832 adults and children with severe malaria treated with IV artesunate in a randomized active-controlled trial in South East Asia (SEAQUAMAT). Supportive evidence for safety was provided by a retrospective analysis of data from an uncontrolled open label study in the US (CDC-060), published studies of severe malaria in Africa consisting of 2712 pediatric patients (less than 15 years of age) receiving parenteral artesunate enrolled in a randomized active-controlled trial (AQUAMAT) and 194 children 6 months to 10 years of age in randomized controlled trial comparing two dose regimens of IV artesunate (EDCTP-MMV-07-01), and Phase 2 studies in patients with uncomplicated malaria. Both SEAQUAMAT and AQUAMAT compared IV artesunate to IV quinine (the previous standard of care) and demonstrated superior in-hospital mortality and a lower incidence of treatment-emergent adverse events overall in patients receiving IV artesunate.

IV artesunate was generally well tolerated. Most common adverse reactions in SEAQUAMAT in patients receiving IV artesunate included renal failure requiring dialysis, hemoglobinuria, and jaundice. In CDC-060 the most frequent adverse events were anemia, elevation of transaminases, thrombocytopenia, hyperbilirubinemia, and acute renal failure. In SEAQUAMAT, at the time of hospital discharge, the incidence of neurological sequelae in artesunate recipients was higher compared to patients receiving quinine. Similarly, in AQUAMAT, incidence of neurological findings at discharge and on subsequent follow up was higher in the artesunate arm as compared to quinine arm; residual neurological sequelae at day 28 included severe motor impairment, cortical blindness, and speech or hearing impairment.

Serious adverse events were not reported in SEAQUAMAT. In CDC-060, Grade 4 TEAEs were reported in lieu of SAEs; the most common were anemia, thrombocytopenia, acute respiratory distress syndrome, and hyperbilirubinemia. One subject in a phase 2 study of uncomplicated malaria experienced an SAE of Stevens-Johnson Syndrome which was associated with concomitant Malarone therapy.

Post-artesunate delayed hemolysis, characterized by a drop in hemoglobin of 10% occurring at least 7 days or more after start of artesunate therapy and associated with laboratory markers of hemolysis, has been described in the literature both in patients from malaria-endemic countries and in travelers from endemic regions. Anemia and hemoglobinuria were among the most common TEAEs reported in pivotal and supportive trials, and transfusions were reported

in some studies during the typical timeframe for PADH; these observations point to the possible occurrence of PADH but limited follow up of patients in studies submitted in this application precluded confirmation of this adverse reaction.

Severe hypersensitivity reactions to IV artesunate such as anaphylaxis have been reported in the literature but were not observed in studies submitted in this application. Within the phase 1 clinical development of IV artesunate, one case of urticaria moderate in severity was reported in a healthy volunteer (Study 1128) who received single 8.0 mg/kg dose artesunate. Transient neutropenia and decrease in reticulocyte count have been reported in published studies of IV artesunate. These hematologic findings were also observed in phase 1 studies in healthy volunteers and phase 2 studies of uncomplicated malaria submitted in this application.

Limitations of the safety assessments in this application included the restricted period of follow up (until hospital discharge) in SEAQUAMAT and AQUAMAT as well as the uncontrolled US trial CDC-060. In addition, SEAQUAMAT and AQUAMAT focused largely on the occurrence of clinical events associated with malaria, did not record adverse events' severity, and had limited data on longer-term outcome. Moreover, it was not possible to discern whether higher incidence for some adverse events (e.g. neurological impairment at discharge) reported with IV artesunate compared to quinine may have resulted from improved survival of patients who presented with more severe disease. While study CDC-060 provides information on clinical outcomes and adverse reactions in patients treated in the US, the lack of a comparator arm makes it difficult to ascribe causality to the adverse reactions observed. Most of the reported adverse events in this study may be attributable to severe malaria, further complicating interpretation of safety as it was not possible to determine which AEs would have occurred more commonly with Artesunate for Injection than with the comparator, if one was used.

Based on limited findings from phase 1 studies, no clinically significant changes in QT were observed in patients receiving IV artesunate.

Overall, IV artesunate administered at a dose of 2.4 mg/kg/dose at 0, 12, 24 hours and once daily until able to tolerate oral antimalarial therapy was shown to have an acceptable safety profile with the risks outweighed by improved survival in patients with severe malaria.

10.12 Conclusions and Recommendations

Evidence for the safety of IV artesunate in the treatment of severe malaria was derived from results of the randomized controlled trial SEAQUAMAT conducted in South East Asia and from a retrospective analysis of the data from the open-label uncontrolled study of IV artesunate use under an expanded access IND in the US (CDC-060). Published studies AQUAMAT, a randomized controlled trial, and EDCTP-MMV07-01, a controlled study comparing two dose regimens, that evaluated IV artesunate for severe malaria in African children, provided supportive safety information. Additional supportive evidence for safety was drawn from phase 1 studies in healthy volunteers and phase 2 studies of uncomplicated malaria. As summarized

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

in Section 8, IV artesunate was superior to quinine, the previous standard of care, for the primary endpoint of mortality and displayed an acceptable safety profile for the treatment of adults and children with severe malaria.

There were no safety signals in the application that would preclude approval.

11 Advisory Committee Meeting and Other External Consultations

No Advisory Committee meeting was held for this application as there were no issues that needed input from external experts.

12 Pediatrics

Due to the Orphan Drug Designation, this application is exempt from the requirement to conduct pediatric studies under the Pediatric Research Equity Act; this was communicated to the U.S. Army on March 28, 2019; ownership of Orphan Drug Designation for Artesunate for Injection was subsequently transferred to Amivas.

Young children are more likely than adults to suffer severe symptoms of malaria, e.g., seizures and concomitant invasive bacterial infections, and experience complications of malaria, e.g., neurological sequelae. ([World Health Organization 2015](#)) Children also bear a heavy burden of disease with 86% of malarial deaths globally occurring in children less than 5 years of age, underscoring the need for effective antimalarial therapies in this population. ([World Health Organization 2012](#))

Following the publication of SEAQUAMAT and AQUAMAT, the WHO updated its guidelines to recommend intravenous artesunate in preference to quinine for the treatment of severe malaria in children, recommending a dose of 2.4 mg/kg/dose on admission, at 12 hours and 24 hours, then once a day until able to tolerate oral therapy. ([World Health Organization 2010](#)) In 2015 WHO revised its pediatric dosage recommendations for children less than 20 kg to 3.0 mg/kg/dose based on its review of pharmacokinetic data which projected under exposure in younger children at the 2.4 mg/kg/dose regimen ([World Health Organization 2015](#)) ([Hendriksen et al. 2013](#))

The Applicant has proposed a dose regimen of Artesunate for Injection at 2.4 mg/kg/dose at 0, 12, 24, (b) (4) hours for adults and children; no lower age limit for the pediatric indication was proposed. Additional analyses were performed by the review team on the effectiveness and safety of intravenous artesunate, and Clinical Pharmacology/Pharmacometrics review team evaluated the dose regimen and lower age range for the pediatric indication, see discussion below.

This application included pediatric data from five studies: SEAQUAMAT, AQUAMAT, EDCTP-MMV-0701 and CDC-060 for the treatment of severe malaria and Study 1263ab for uncomplicated malaria. Characteristics of the pediatric database are presented in the table below.

Table 12-1: NDA 213036 - Size of Pediatric Database < 18 years old for Artesunate Recipients (Safety Population)

Study	Indication	Pediatric Safety Population Any Dose Regimen/Total number of artesunate recipients (%)	Pediatric Safety Population Dose 2.4 mg/kg/day at 0, 12, 24 hrs	Lower Limit of Age Range of Enrolled Patients
SEAQUAMAT ¹	Severe malaria	161/730 (22.0%)	161	2 years
CDC-060 ²	Severe malaria	12/102 (11.8%)	12	18 months
AQUAMAT ³	Severe malaria	2712 (100%)	2712	Not specified in publication ²
EDCTP-MMV07-01 ⁴	Severe malaria	194 (100%)	100	6 months
Study 1263ab ⁵	Uncomplicated malaria	59/100 (59%)	14	5 years
Total		3138	3009	-

¹ SEAQUAMAT and AQUAMAT did not specify the minimum number of doses of IV artesunate.

² CDC-060 evaluated Artesunate for Injection at 2.4 mg/kg/dose at 0, 12, 24 and 48 hours. 12 (11.8%) of patients were less than 18 years of age. Two patients were less than 2 years of age; one subject was 18 months and one was 23 months of age.

³ Dondorp 2012 reported Median (IQR) of 2.9 (1.7-4.3); Applicant was unable to provide additional details.

⁴ Subjects in EDCTP-MMV07-01 who received 2.4 mg/kg/dose received 5 doses

⁵ Of a total 59 patients < 18 years of age enrolled in Study 1263ab, 14 subjects received 2.4 mg/kg/dose at 0, 12, 24 and 48 hours (minimum of 4 doses) and an additional 14 pediatric patients received IV artesunate 2.4 mg/kg/dose once daily for 3 days.

In SEAQUAMAT, 22.0% of IV artesunate recipients were 2 to <18 years of age. CDC-060 included 12 of 102 or (11.8%) patients <18 years old. Regarding the supportive studies, EDCTP-MMV07-01 included subjects as young as 6 months and up to 10 years of age. The lower age range for AQUAMAT is uncertain as the datasets were not submitted in the application and the publication (Dondorp 2010) included only interquartile range for the enrolled pediatric patients. ([Dondorp et al. 2010](#))

Reviewer comment: In response to an IR, the Applicant was unable to obtain additional details from the study Sponsor on the lower age range of pediatric subjects in AQUAMAT. Although the lower age limit for pediatric patients enrolled was not specified, patients less than 6 months of age were likely included given the epidemiology of severe malaria in the geographic regions included in the study.

Selected additional analyses were conducted for SEAQUAMAT and CDC-060, see below.

12.1 SEAQUAMAT

See table in Section 8.1.1 for mortality analyses in subgroups, including pediatric patients and patients by weight.

In SEAQUAMAT 109 of 1461 (7.5%) patients enrolled weighed less than 20 kg. Due to the small numbers it is not possible to determine whether mortality was affected by weight < or ≥ 20 kg.

The following table presents the TEAE by preferred term for pediatric patients < 18 years old. The most common adverse events observed in artesunate recipients are renal failure, hemoglobinuria, seizure, meningitis, sepsis, coma, and anemia.

Table 12-2: SEAQUAMAT - TEAE (Preferred Term) for Patients < 18 years old, Incidence ≥ 5% in Artesunate Group (Safety Population)

TEAEs	Artesunate n=161	Quinine n=152	Totals n=313
Hemoglobinuria	11 (6.8%)	5 (3.3%)	16 (5.1%)
Seizure	11 (6.8%)	8 (5.3%)	19 (6.1%)
Meningitis	8 (5.0%)	1 (0.7%)	9 (2.9%)
Sepsis	8 (5.0%)	16 (10.5%)	24 (7.7%)

Source: Clinical Reviewer (J Review 13.1)

Given the WHO recommendation for 3.0 mg/kg/dose in pediatric patients who weigh less than 20 kg, the following tables evaluated whether there is any observable difference in mortality and AEs based on whether the pediatric subjects weighed less than 20 kg. In SEAQUAMAT and CDC-060, all subjects received 2.4 mg/kg/dose.

Table 12-3: SEAQUAMAT TEAE (SOC/PT) ≥ 1% in Pediatric¹ Subjects by Weight 20 kg (Safety Population)

TEAE (SOC/PT)	Artesunate n=161		Quinine n=152		Totals n=313
	Pediatric < 20 kg n=52	Pediatric ≥ 20 kg n=109	Pediatric < 20 kg n=57	Pediatric ≥ 20 kg n=95	
Seizure	3 (5.8%)	8 (7.3%)	1 (1.8%)	7 (7.4%)	19 (6.1%)
Hemoglobinuria	0 (0.0%)	11 (10.1%)	0 (0.0%)	5 (5.3%)	16 (5.1%)
Sepsis	3 (15.8%)	5 (4.6%)	6 (10.5%)	10 (10.5%)	24 (7.7%)
Meningitis	3 (15.8%)	5 (4.6%)	1 (1.8%)	0 (0.0%)	9 (2.9%)
Coma	2 (3.8%)	5 (4.6%)	1 (1.8%)	7 (7.4%)	15 (4.8%)
Renal failure requiring dialysis	1 (1.9%)	4 (3.7%)	0 (0.0%)	5 (5.3%)	10 (3.2%)

TEAE (SOC/PT)	Artesunate n=161		Quinine n=152		Totals n=313
	Pediatric < 20 kg n=52	Pediatric >= 20 kg n=109	Pediatric < 20 kg n=57	Pediatric >= 20 kg n=95	
Anemia	1 (1.9%)	5 (4.6%)	3 (5.3%)	5 (5.3%)	14 (4.5%)
Bacterial infection	1 (1.9%)	4 (3.7%)	0 (0.0%)	1 (1.0%)	6 (1.9%)
Respiratory failure	1 (1.9%)	3 (2.7%)	0 (0.0%)	3 (3.2%)	7 (2.2%)
Lower respiratory tract infection	0 (0.0%)	3 (2.7%)	2 (3.5%)	4 (4.2%)	9 (2.9%)
Shock	0 (0.0%)	3 (2.7%)	2 (3.5%)	1 (1.0%)	6 (1.9%)
Hemorrhage	1 (0.6%)	2 (1.8%)	1 (1.8%)	1 (1.0%)	5 (1.6%)
Diarrhea	0 (0.0%)	2 (1.8%)	0 (0.0%)	0 (0.0%)	2 (0.6%)
Jaundice	0 (0.0%)	1 (0.9%)	0 (0.0%)	3 (3.2%)	4 (1.3%)
Hypoglycemia	0 (0.0%)	0 (0.0%)	2 (3.5%)	0 (0.0%)	2 (0.6%)

Source: Clinical Reviewer (J Review 13.1)

¹ Subjects < 18 years of age

Reviewer comments: Numbers in children are too small to make a conclusion regarding TEAEs by weight less than or greater or equal to 20 kg in artesunate recipients compared with quinine recipients.

12.2 CDC-060

There were no pediatric deaths in CDC-060.

Table 12-4: CDC-060 - TEAE in Pediatric Patients < 18 years old (Safety Population)

TEAEs	Totals n=12
Subjects with at least 1 TEAE	11 (91.7%)
Anemia	6 (50.0%)
Aspartate aminotransferase increased	4 (33.3%)
Thrombocytopenia	3 (25.0%)
Neutropenia	1 (8.3%)
Lymphocytosis	1 (8.3%)
Cardiac murmur functional	1 (8.3%)
Transaminases increased	1 (8.3%)
White blood cell count decreased	1 (8.3%)
Abdominal distension	1 (8.3%)
Vomiting	1 (8.3%)
Retinal exudates	1 (8.3%)
Sepsis	1 (8.3%)

TEAEs	Totals n=12
Hypotension	1 (8.3%)
Acidosis	1 (8.3%)
Hyperbilirubinemia	1 (8.3%)
Ecchymosis	1 (8.3%)

Source: Clinical Reviewer (J Review 13.1)

Reviewer comments: In CDC-060, TEAEs in pediatric subjects were similar to those observed in adult patients.

Table 12-5: CDC-060 Pediatric* Enrollment by Weight < or ≥ 20 kg (Safety Population)

Weight Pediatric Subjects	Artesunate (N=12 of 102 Total Subjects)
< 20 kg	4/12 (33.3%)
≥ 20 kg	8/12 (66.7%)

Source: Clinical Reviewer

*Subjects < 18 years old

Table 12-6: CDC-060 - Pediatric Time to Discharge by Weight < 20 kg or ≥ 20 kg (Safety Population)*

	Pediatric <20kg n=4	Pediatric ≥20kg n=8	Totals n=12
Time to Discharge (Days)			
subjects with data	4	8	12
mean [SD]	4.50 [1.29]	5.00 [2.27]	4.83 [1.95]
median	4.50	4.50	4
range	3-6	3-9	3-9

Source: Clinical Reviewer (J Review 13.1)

*There were no pediatric subject deaths in CDC-060

Reviewer comment: In CDC-060, mean time to discharge was 0.5 days less in pediatric patients < 20 kg compared to patients ≥ 20 kg, but numbers were too small to evaluate significant differences.

1.1. Dose Regimen and Lower Age Range for Pediatric Indication

To address the absence of data in patients less than 6 months of age, the Applicant reviewed the literature for additional studies that evaluated the use of artesunate in the treatment of severe malaria in infants. Only two studies were found that enrolled children less than 6 months of age that also provided data on safety and/or efficacy. ([Ferrari et al. 2015](#)) ([Burri et al.](#)

[2014](#)) Both studies enrolled patients as young as 2 months of age but the number of patients less than 6 months was not available from either publication.

The Applicant identified a total of 10 case reports of infants less than 6 months of age treated with parenteral artesunate in 8 publications, see table below.

Table 12-7: Case Reports of Infants Less Than 6 Months of Age with Severe Malaria Treated with Artesunate

Reference	Pediatric Patient	IV Artesunate	Parasite Clearance Data	Outcome	Location
Poespoprodjo-2010	Female, age 1 day, weight 2.35 kg, hypothermic, respiratory distress, unable to feed Initial <i>Pf</i> parasitemia 7,575/ μ L; rose to 26,700/ μ L before treatment	3.4 mg/kg at 24, 36, and 48 h	Smear negative at 48 hours after start of IV artesunate	Clinical improvement within 24 hours after start of IV artesunate. Discharge on Day 11	Papua, Indonesia
Odinaka-2015	Male, age 25 days, weight 3.8 kg, febrile severe anemia <i>Pf</i> parasitemia	2.4 mg/kg at 0, 12, 24, and 48 h	--	Discharge after 5 days	Nigeria
Onyiriuka-2016	Female, age 26 days, hypoglycemic, in respiratory distress 3+ <i>Pf</i> parasitemia	2.4 mg/kg at 0, 12, 24, and 48 h	Decrease to 1+ parasitemia in 24 hours.	Malarial symptoms subsided after 48 hours of treatment	Benin
Tekle-2018	Male pre-term infant (35 weeks), with abrupt onset of fever at 18 days after birth <i>Pv</i> parasitemia 0.1%	2.4 mg/kg given twice on Day 1, and then once daily for 3 days	Parasitemia cleared after 3 days of treatment	Discharged from the NICU on Day 37 in good condition.	Eritrea
Prashanth-2012	Female, pre-term at 30 weeks gestation, acquired malaria via transfusion in NICU at age 18 days. Developed pallor and episodes of apnea. Initial <i>Pv</i> parasitemia 18,874/ μ L	2.4 mg/kg daily x 7 days	Day 2 – <i>Pv</i> 10.2/ μ L; Day 3 – <i>Pv</i> 0.8/ μ L; Day 7- No parasites	Clinical improvement within 24 hours after start of IV artesunate. Normal feeding at 48 hours. At 6 months follow-up, normal neurologic,	India

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Reference	Pediatric Patient	IV Artesunate	Parasite Clearance Data	Outcome	Location
				ophthalmic, and hearing assessments.	
Tatura-2018	Male, age 47 days, with high fever, cholestatic jaundice, severe anemia. Initial <i>Pv</i> parasitemia 800/ μ L	2.4 mg/kg daily x 7 days	Three negative smears prior to discharge (Day 7)	After 7 days of treatment, all symptoms had improved	Indonesia
Bukeyenzeza-2017	Preterm infant, gestational age 29 weeks, weight 1.098 kg, smear positive for <i>Pf</i>	2.4 mg/kg daily for 7 days	--	Full recovery	Rwanda
Bukeyenzeza-2017	Infant, age 8 days, smear positive for <i>Pf</i>	2.4 mg/kg daily for 7 days		Baby responded well	Rwanda
Bukeyenzeza-2017	Preterm infant, gestational age 31 weeks. Extremely low birth weight of 700g	2.4 mg/kg daily for 7 days (empiric treatment)	--	Baby responded well	Rwanda
Belard-2019	Infant age 0.4 years, weight 7.5 kg <i>Pf</i> parasitemia 18%	2.0 mg/kg "per injection"	Slide negative for <i>Pf</i> on Day 4	Cured with no residual problems (last follow-up at Day 22)	Germany (history of travel to Nigeria)

Source: Adapted from NDA 213036, SN 0032, Table 3

Reviewer comments: *In a review of the literature, case reports published between 2010 and 2019 identified 10 infants less than 6 months of age who received IV artesunate for severe malaria. Age range was newborn (preterm infant 31 weeks gestational age, 700 g) to 0.4 years of age; 7 infants had malaria due to P. falciparum, 3 infants were infected with P. vivax. All 10 patients were considered cured; in patients where parasite clearance was described, clearance occurred within 1 to 3 days. While safety was not the primary focus of these reports, no safety issues with IV artesunate were identified.*

The Applicant also reviewed published reports of infants treated under the CDC IV artesunate expanded access protocol from 2011-2016 (time period subsequent to that covered in Study CDC-060), see table below.

Table 12-8 - Information from Published CDC Malaria Surveillance Summaries - Infants with Severe Malaria treated under CDC-060 from 2011-2016

Reference	No. of Patients Total/Age <3 yrs, n (%)	Patients of Any Age Treated with IV Artesunate n (%)	No. cases Congenital Malaria	Relevant Clinical Information for Each Congenital Case	Treatment	Outcome	Safety
Cullen-2013	275 total 9 (3.3%)	51 (18.5%)	2	Male, age 1 day, with <0.01% <i>Pf</i> parasitemia	Unk	Survived	NR
				Female, age 3 months, with 0.2% <i>Pv</i> parasitemia	AP	Survived	NR
Cullen-2014	231 total 6 (2.6%)	26 (11.3%)	NR	--	--	--	--
Cullen-2016	270 total 13 (4.8%)	43 (15.9%)	2	Male, age 3 weeks, with smear positive for <i>Pv</i>	CQ	survived	No clinical complications
				Male, age 1 day, with 0.1% <i>Pf</i> parasitemia	AP	Survived	No clinical complications
Mace-2017	293 total NR	42 (14.3%)	1	Female, age 13 days, with 5.4% <i>Pf</i> parasitemia and RSV infection	IVQG and CL	survived	NR
Mace-2018	259 total NR	54 (20.8%)	1	Infant, age 3 weeks, with 2.2% <i>Pv</i> parasitemia, thrombocytopenia, anemia, and splenomegaly	IVQG and CL	survived	NR
Mace-2019	306 total NR	138 (45.1%)	2	Male, age 1 day, smear positive for <i>Pf</i> , hypoglycemic, poor feeding	QN and CL	survived	NR
				Female, age about 1 month, with 1.6% <i>Pv</i> parasitemia	Unk	survived	NR

Source: Adapted from NDA 21036, SN 0032, Table 1

Abbreviations: AP = Atovaquone/Proguanil; CQ=Chloroquine; CL=Clindamycin; IV=intravenous; NR = Not Reported; *Pf* = *P. falciparum*; *Pv* = *P. vivax*; QN = Quinine; QG = Quinidine Gluconate; RSV = Respiratory syncytial virus; Unk = Unknown

Reviewer comments: *According to MMWR Surveillance Summaries, up to 4.8% of children with severe malaria in the US were less than 5 years of age; however, the specific age groups and number of children receiving IV artesunate were not reported. Of the Surveillance Summaries with information on infants < 6 months, none of the reports cited IV artesunate as the drug administered although some were missing this information and patients may have received IV artesunate.*

To evaluate the proposed dose regimen in pediatric subjects < and $\geq$ 20 kg, the Clinical Pharmacology/Pharmacometrics review team employed PK modeling and simulation with data from published studies (for details see Pharmacometrics review in Section 18.4). PK modeling and simulation found that pediatric patients younger than 6 months of age had comparable or higher predicted PK steady-state AUC of DHA when compared with older children or adults at the recommended 2.4 mg/kg dose regimen of Artesunate for Injection. The Clinical Pharmacology/Pharmacometrics review team recommended no dose adjustment for pediatric patients regardless of age or bodyweight. In addition, review of the literature for use of intravenous artesunate for severe malaria in pediatric patients less than 6 months of age identified no significant safety concerns.

Conclusion

The safety and efficacy of Artesunate for Injection for treatment of severe malaria in children is supported by evidence from adequate and well-controlled studies in pediatric patients 6 months of age or older; support of efficacy and safety in patients less than 6 months was provided by PK modeling and simulation and published studies. The Applicant has agreed to a postmarketing commitment to evaluate safety, tolerability, and PK of IV artesunate in pediatric patients with severe malaria from 0 to 12 months of age receiving 2.4 mg/kg Artesunate for Injection, with the majority of pediatric patients to be 6 months of age and younger.

13 Labeling Recommendations

13.1 Prescription Drug Labeling

Prescribing information

Table 13-1: Summary of Significant Labeling Changes

Summary of Significant Labeling Changes (High level changes and not)		
Section	Proposed Labeling	Approved Labeling
Indications and Usage	(b) (4)	Artesunate for Injection is an antimalarial indicated for the initial treatment of severe malaria in adult and pediatric patients. Treatment of severe malaria with artesunate should always be followed by a complete treatment course of an appropriate oral antimalarial regimen. <i>Deleted</i> (b) (4) <u>Limitations of Use</u> Artesunate for Injection does not treat the hypnozoite liver stage forms of <i>Plasmodium</i> and will therefore not prevent relapses of malaria due to <i>Plasmodium vivax</i> or <i>Plasmodium ovale</i>. Concomitant therapy with an antimalarial agent such as an 8-aminoquinoline drug is necessary for the treatment of severe malaria due to <i>P. vivax</i> or <i>P. ovale</i>.
Dosage and Administration	(b) (4)	The recommended dosage of Artesunate for Injection is 2.4 mg/kg administered intravenously at 0 hours, 12 hours, and 24 hours, and thereafter, administered once daily until the patient is able to tolerate oral antimalarial therapy. Administer diluted Artesunate for Injection intravenously as a slow

Summary of Significant Labeling Changes (High level changes and not)		
Section	Proposed Labeling	Approved Labeling
Use in Specific Populations	8.1 Pregnancy	<i>Standard PLLR language was added on background risk of major birth defects, fetal loss, or other adverse outcomes.</i>
	Human data	<i>Additional data added regarding first trimester use of intravenous artesunate in humans.</i>
	Animal data	<i>Added findings from animal reproductive studies conducted using clinically relevant route.</i>
	8.2 Lactation Risk Summary	<i>Edits made to reflect the finding of DHA in human milk and the lack of data on the effects of artesunate on the breastfed infant or milk production.</i>
(b) (4)		
	8.4 Pediatric Use	<i>Regarding pediatric patients less than 6 months of age, text added to reflect that a PK extrapolation approach using modeling and simulation indicated comparable or higher than predicted PK steady-state AUC of DHA between this age group and older children or adults at the recommended 2.4 mg/kg dose regimen.</i>
	8.5 Geriatric Use	<i>Revised to reflect that clinical studies did not include sufficient numbers of patients 65 years and older to determine whether they responded differently than younger subjects.</i>
	8.6 Renal Impairment 8.7 Hepatic Impairment	<i>Edited to note that in patients with renal or hepatic impairment, no specific PK studies have been carried out and no specific dose adjustments are needed.</i>

Summary of Significant Labeling Changes (High level changes and not		
Section	Proposed Labeling	Approved Labeling
Overdosage	(b) (4)	<i>Sentence removed</i> (b) (4)
Nonclinical Toxicology		<i>Added additional information from the published literature regarding the genotoxic potential of artesunate.</i> <i>Added findings on spermatogenic assessment and histopathology from 28-day toxicity studies in rats and dogs</i>
Clinical Studies	(b) (4)	<i>Removed</i> (b) (4) <i>Revised primary efficacy endpoint for SEAQUAMAT to reflect in-hospital mortality rather than</i> (b) (4)
Patient Counseling Information		<i>Added text to advise patients on the need for regular blood tests during the 4-week period following completion of artesunate to monitor for post-treatment delayed hemolysis.</i> <i>Added text to inform patients to monitor for signs and symptoms of hypersensitivity reactions and anaphylaxis.</i> <i>Additional text added to advise pregnant patients of the potential for drug-related embryofetal toxicity based on animal studies.</i>

14 Risk Evaluation and Mitigation Strategies (REMS)

No REMS was deemed necessary for this application.

15 Postmarketing Requirements and Commitments

Postmarketing Requirements (PMR)

Clinical Microbiology:

Conduct a 5-year surveillance study to evaluate the potential development of resistance to artesunate and DHA as obtained from ongoing resistance monitoring programs on antimalarials.

Draft protocol submission:	May 2021
Final protocol submission:	November 2021
Interim study report:	November 2022
Interim study report:	November 2023
Interim study report:	November 2024
Interim study report:	November 2025
Interim study report	November 2026
Study completion:	November 2026
Final study report submission:	May 2027

Clinical

Conduct a single-arm descriptive international study collecting data in women exposed to IV artesunate during pregnancy to assess risk of pregnancy and maternal complications and adverse effects on the fetus, neonate, and infant. Infant outcomes will be assessed through at least the first year of life. The study will collect information for a minimum of 7 years.

Draft protocol submission:	November 2020
Final protocol submission:	April 2021
Interim study report:	April 2022
Interim study report:	April 2023
Interim study report:	April 2024
Interim study report:	April 2025
Interim study report:	April 2026
Interim study report:	April 2027
Study completion:	May 2028
Final study report submission:	November 2028

Pharmacology/Toxicology

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Conduct a female fertility study in rats that tests the adverse effects of intravenous artesunate when administered prior to mating and continued through mating and implantation in accordance with ICH S5(A) and 21 CFR 58 Good Laboratory Practice for Nonclinical Laboratory Studies.

Draft protocol submission:	July 2020
Final protocol submission:	October 2020
Study completion:	July 2021
Final study report submission:	October 2021

Conduct an In Vivo Micronucleus Assay that evaluates intravenous artesunate at doses demonstrated to achieve exposure at the target tissue site(s) and in accordance with the OECD Guideline for the Testing of Chemicals #474 and 21 CFR Part 58 Good Laboratory Practice for Nonclinical Laboratory Studies.

Final protocol submission:	June 2020
Study completion:	June 2020
Final study report submission:	August 2020

Postmarketing commitment (PMC):

Clinical

Conduct a descriptive 5-year study on the use of Artesunate for Injection in the U.S.

Draft protocol submission:	November 2020
Final protocol submission:	April 2021
Interim study report:	April 2022
Interim study report:	April 2023
Interim study report:	April 2024
Interim study report:	April 2025
Study completion:	April 2026
Final study report submission:	October 2026

Clinical Pharmacology

Conduct a multiple-dose safety, tolerability, pharmacokinetics (PK) study in pediatric patients with severe malaria from 0 to 12 months of age receiving 2.4 mg/kg Artesunate for Injection. The majority of pediatric patients in this age group is to be 6 months of age and younger.

Draft protocol submission:	November 2020
Final protocol submission:	November 2021
Interim study report:	November 2022

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Interim study report:	November 2023
Interim study report:	November 2024
Interim study report:	November 2025
Study completion:	November 2026
Final study report submission:	May 2027

16 Division Director Comments

I agree with the review team's assessment and recommendations.

17 Office Director Comments

I agree with the review team's assessment and recommendations.

18 Appendices

18.1 Financial Disclosure

Studies CDC-060,1128, 1142, 1263ab and 1168 were conducted by the U.S. Army which transferred rights for these studies to Amivas. Form FDA 3454 was signed by the Applicant certifying that based on information from the sponsor or participating clinical investigators, the listed clinical investigators did not participate in any financial arrangement, had no proprietary interest in the product or significant equity interest, and had not received significant payments of other sorts. The Applicant stated all investigators were Active Duty members of the US Army or U.S. Government employees and none of these investigators had reportable financial disclosures.

The Applicant was not the Sponsor of SEAQUAMAT, AQUAMAT or EDCTP-MMV07-01. The Applicant noted that these studies were sponsored by either the Wellcome Trust (SEAQUAMAT and AQUAMAT) or Medicines for Malaria Venture (EDCTP-MMV07-01) and provided a list of trial investigators for each study. Form FDA 3454 was submitted certifying that as the Applicant who submitted studies sponsored by a firm other than the Applicant, the listed clinical investigators did not participate in any financial arrangement with the sponsor whereby the value of compensation for conducting the study could be affected by the outcome of the study, have no proprietary or significant equity interest, and had not received significant payments of other sorts.

Covered Clinical Study (Name and/or Number): SEAQUAMAT

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: <u>46</u>		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): <u>0</u>		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): <u>0¹</u>		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)): NA		
Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: _____		
Significant payments of other sorts: _____		
Proprietary interest in the product tested held by investigator: _____		
Significant equity interest held by investigator in S		
Sponsor of covered study: _____		

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☒	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☒	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3) <u>0</u>		
Is an attachment provided with the reason:	Yes ☐ NA	No ☐ (Request explanation from Applicant)

¹ According to the Applicant, no investigators had disclosable financial interest or arrangements. Three investigators disclosed potential conflicts of interests without financial interests in the study publication

(b) (6)

(b) (6)

Covered Clinical Study (Name and/or Number): AQUAMAT

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: <u>94</u>		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): <u>0</u>		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): <u>0¹</u>		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)): NA		
Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: _____		
Significant payments of other sorts: _____		
Proprietary interest in the product tested held by investigator: _____		
Significant equity interest held by investigator in S		
Sponsor of covered study: _____		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☒	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☒	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3) <u>0</u>		
Is an attachment provided with the	Yes ☐	No ☐ (Request explanation from

NDA Multi-disciplinary Review and Evaluation NDA 213036
 Artesunate for Injection

reason:	NA	Applicant)

¹According to the Applicant, no investigators had disclosable financial interest or arrangements. (b) (6)

(b) (6)

Covered Clinical Study (Name and/or Number): EDCTP-MMV07-01

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: <u>4</u>		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): <u>0</u>		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): <u>0</u>		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)): NA Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: _____ Significant payments of other sorts: _____ Proprietary interest in the product tested held by investigator: _____ Significant equity interest held by investigator in S _____ Sponsor of covered study: _____		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☒	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☒	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3) <u>0</u>		
Is an attachment provided with the reason:	Yes ☐ NA	No ☐ (Request explanation from Applicant)

18.2 Nonclinical Pharmacology/Toxicology

Safety Pharmacology

Behavioral and neural toxicity of the artemisinin antimalarial, arteether, but not artesunate and artelinate, in rats. *Pharmacology Biochemistry and Behavior*. 2000;67(1):37-44. Adult male rats (Sprague-Dawley) were administered equivalent molar concentrations of arteether (25 mg/kg in sesame oil; n=6), artesunate (31 mg/kg in sodium carbonate; n=6), and artelinate (36 mg/kg in saline; n=6) via IM injection daily for 7 days, with performance on an auditory discrimination task and neurohistology conducted. Control animals received IM injection of sodium carbonate (n=6).

No observable effects on any performance measures of the auditory discrimination task were observed following artesunate or artelinate treatment compared with vehicle control. In contrast, significant performance deficits (percent correct and stimulus response) were observed in rats receiving arteether. These behavioral effects were greater during the latter portion of the treatment regimen and during post-administration testing sessions. No apparent histopathological changes were observed in brainstem nuclei of rats treated with artesunate or artelinate compared with control. Brainstem nuclei of rats treated with arteether showed evidence of chromatolysis, pyknosis, and gliosis.

General Toxicity

Dose Range Finding Study of Injectable Artelinate and Artesunate in Rats/SR01-1

Key findings:

- The maximum tolerated dose (MTD) for single IV administration of artesunate in rats was between 200 and 400 mg/kg; the MTD for multiple IV doses (up to 7 days) was observed to be ≥ 75 mg/kg.
- Artesunate-related hematologic changes following 7 days of repeated IV dosing included increased MCV, reticulocyte % and count, neutrophil and monocyte values and decreased RBC, HGB, and HCT values. There were no macroscopic or microscopic lesions observed in hindbrain from animals at any dose tested.

Conducting laboratory and location:

(b) (4)

GLP compliance: No

Methods

Dose and frequency of dosing:	Phase 1: single dose of 0, 50, 100, 200, or 400 mg/kg Phase 2: 0, 9.4, 18.8, 37.5, or 75 mg/kg daily, for 7 days Phase 3: 0 or 150 mg/kg daily, for 7 days
Route of administration:	Intravenous
Formulation/Vehicle:	5% Sodium bicarbonate or 0.9% sterile saline
Species/Strain:	Rat/Crl:CD(Sprague-Dawley) IGS BR

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Number/Sex/Group: Phase 1: 2/sex/group
Phase 2: 3/sex/group
Phase 3: 3/sex/group

Age: 6 to 8 weeks

Satellite groups/unique design: Positive control, IM injection of 25 mg/kg arteether formulated in sesame oil, daily for 7 days (3/sex).

Deviation from study protocol affecting interpretation of results: None.

Observations and Results:

Parameters	Major findings
Mortality	Phase 1 (HD): All animals were found dead on the day after dosing Phase 3 (HD): 1 animal (female) was found dead on Day 6
Clinical Signs	Phase 1 (HD): 3 of 4 animals displayed transient ataxia within 30 minutes after dosing. Phase 2 (HD): females displayed diarrhea, hunched posture and wet skin/fur on 2 or more days between Days 3 and 8.
Body Weights	Up to 10% decreased bodyweight gain and/or weight loss $\geq$ 75 mg/kg (F) and at 150 mg/kg (M)
Hematology	HD (150 mg/kg; F only): increased MCV (108%), reticulocyte % and count (2 to 4-fold), neutrophil and monocyte values and decreased RBC (79%), HGB, and HCT values.
Clinical Chemistry	HD (150 mg/kg; F only): decreased albumin (76%) and A/G values (49%).
Gross Pathology	None
Histopathology Hindbrain only	None for AS. AE: 1 out of 3 females displayed degenerative lesions in trapezoid nucleus.

LD: low dose; MD: mid dose; HD: high dose.

Reviewer comment: The study also included an arm in each phase in which rats were dosed with artelinate in lysine. Only findings from artesunate-treated animals and the positive control (arteether) are discussed. The study was not conducted in compliance with GLP regulations, but notes that all study procedures, data recording, and reporting were performed in a manner consistent with the standard of GLPs.

Reproductive Toxicology

Fertility

Histological studies of the toxicity of artesunate on the testes in Wistar rats (Biology and Medicine. 2010. Vol 2(2):49-56)

Key Study Findings: Oral administration of artesunate for 7 days in rats caused mild sloughing of germ cells from the seminiferous tubule basement membrane, with a dose-related reduction in the population of germ cells.

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Conducting laboratory and location

(b) (4)

GLP compliance:

Unknown

Methods

Dose and frequency of dosing:

2, 4, and 8 mg/kg

Route of administration:

Oral (gavage)

Formulation/Vehicle:

Distilled water

Species/Strain:

Rat/Wistar

Number/Sex/Group:

5 males/group

Study design:

Males were treated with either 4 mg/kg/day for 3 days followed by 2 mg/kg/day for 4 days, or 8 mg/kg/day for 3 days followed by 4 mg/kg/day for 4 days.

Deviation from study protocol

Unknown

affecting interpretation of results:

Reviewer comment: Limited findings were presented in the publication, with only 3 H&E images of testes from control and artesunate-treated animals.

Modulatory Effects of Ascorbic Acid on Spermatotoxicity of Artesunate in Rats (Nigerian Medical Practitioner 53 (2008) 62-65)

Key Study Findings

- Artesunate significantly reduced sperm count, percent sperm motility and serum testosterone levels in rats following a 7-day repeated oral administration. The artesunate-induced effects were ameliorated with concomitant treatment with ascorbic acid. There were no noted changes in testicular histology other than an apparent reduction in gonial cell layers of the seminiferous epithelium.

Conducting laboratory and location

(b) (4)

GLP compliance:

Unknown

Methods

Dose and frequency of dosing:

2 mg/kg/day for 7 days

Route of administration:

Oral (gavage)

Formulation/Vehicle:

Water

Species/Strain:

Rat/Sprague-Dawley

Number/Sex/Group:

5 males/group

Satellite groups:

None

Study design: Animals were given 2 mg/kg artesunate daily for 7 days. A second group received 8 mg/kg vitamin C for 7 days in addition to artesunate. At the end of dosing, testicular histology, epididymal sperm parameters and serum testosterone levels were evaluated.

Deviation from study protocol affecting interpretation of results: Unknown

Reviewer comment: Only group means $\pm$ SD of hormone and evaluated sperm parameters across treatment groups were provided with statistical analysis. No histopathologic data was shown to support the noted findings.

Embryo-Fetal Development

Histopathological Investigation of the Embryonic Effect of SB-714702 (Artesunate) in Cynomolgus Monkeys/Study No. CD2006/01492/00 ((b) (4) No. SBL097-004)

This study was conducted to investigate the effects of artesunate on surviving embryos from dams in the 30 mg/kg treatment group in Study CD2006/00794/00. Histopathological examinations were done on the three live embryos collected from dams in the 30 mg/kg group on Days 26, 32, and 36 of gestation and compared against an untreated Day 30 gestation embryo obtained from a historical collection.

GLP compliance: The EFD study in cynomolgus monkeys from which the embryos were removed (Study No. CD2006/00744/00) was conducted under GLP conditions. The histopathological examination of the embryos was not performed in compliance with GLP regulations.

Key Study Findings

- An overall relative reduction in erythroblasts was observed in embryos from artesunate-treated dams compared with the control embryo.
- Embryos from artesunate-treated dams had atrial chamber enlargement with thin atrial and ventricular walls compared to the Day 30 gestation embryo from the historical collection; the embryo collected on Day 36 gestation displayed an enlarged ventricular space and evidence of pyknotic nuclei in hepatocytes.

Reviewer comment: The presence of cardiovascular malformations in surviving embryos of the high dose group are consistent with those reported in other species (i.e., rat and rabbit) following oral artesunate exposure. The reduction in erythroblasts in embryos from artesunate-treated dams should be interpreted with caution given the lack of gestation day-matched control embryos allowing for a direct comparison.

Artesunate: In Vitro Effects on Embryonic and Fetal Blood Cells of Cynomolgus Monkeys/Study No. CD2006/00795/00 ((b) (4) Study No. I05188)

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Key Study Findings

- An approximate 6 to 14% nucleated RBCs was observed in untreated fetal blood collected on Day 50 of gestation, whereas no nucleated RBCs were noted in untreated fetal blood collected on Day 100 of gestation.
- Fetal blood cells were more sensitive than maternal blood cells to the cytotoxic effects of artesunate and DHA on Day 50 of gestation compared with Day 100 of gestation, with up to 25% greater fetal blood cell death observed following exposure to 50 mcM artesunate and 20 mcM DHA.

Conducting laboratory and location: (b) (4)
GLP compliance: No

Methods

Species/Strain: Monkey/Cynomolgus
Study design: Fetal (cord) and maternal (peripheral venous) blood samples collected on Day 50 (n=3) or 100 (n=4) of gestation were cultured in vitro with up to 500 mcM artesunate or DHA for 19 hr and examined for cell viability and lysis. DMSO (0.5%) served as the vehicle control.
Deviation from study protocol affecting interpretation of results: No.

A Study for the Investigation of Developmental Toxicity of SB-714702 (Artesunate), in Two Different Treatment Periods, in Cynomolgus Monkeys/Study No. CD2007/00245/00 ((b) (4) Study No. SBL097-010)

Key Study Findings

- Administration of 12 mg/kg/day oral artesunate to cynomolgus monkeys between Days 27 to 33 or between Days 29 to 31 of gestation did not induce any increases in embryofetal death or fetal malformations compared with control-treated animals.

Conducting laboratory and location: (b) (4)
GLP compliance: Yes

Methods

Dose and frequency of dosing: 12 mg/kg/day
Route of administration: Oral (naso-gastric intubation)

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Formulation/Vehicle: 1% w/v methyl cellulose
 Species/Strain: Monkey/ Cynomolgus
 Number/Sex/Group: 15 pregnant females/group
 Satellite groups: None
 Study design: Animals were administered 12 mg/kg day artesunate in two different treatment periods: Days 29 to 31 of gestation and Days 27 to 33 of gestation). Fetal viability was determined by ultrasonography on day prior to dosing and on Days 30, 35, 40, 45, 51, 60, 70, 80, and 90 of gestation. Cesarean sectioning was performed on Day 100 of gestation and fetuses were removed and examined.

Deviation from study protocol affecting interpretation of results: No

Observations and Results

Parameters	Major findings
Mortality	None
Clinical Signs	None
Body Weights	None
Hematologic findings	Treatment-related trend toward lower reticulocyte counts on the day after final dosing, though 2 animals treated between Day 27 and 33 gestation displayed high reticulocyte counts.
Necropsy findings Cesarean Section Data	GD 29 to 31: Embryonic death and fetal death occurred in 1 dam on Days 35 and 60 gestation, respectively GD 27 to 33: Embryonic death occurred in 1 dam on Day 35 The observed embryonic and/or fetal deaths were considered to be spontaneous as their incidence was within the range of control background data.
Necropsy findings Offspring	None

Reviewer comment: Based on the lack of artesunate-induced embryofetal toxicity when administered between days 27-33 of gestation with the same dose causing embryonic death between days 30 and 55 of gestation when administration began on day 20 of gestation (Study No. CD2006/00794/00), it can be inferred that the critical window of embryotoxicity following oral administration in cynomolgus monkey falls between gestation day 20 and 27.

SB-714702 (Artesunate): Oral Critical Window Embryo-Fetal Development Study in Rats/Study No. CD2004/00133/00 ((b) (4) Study No. G03312)

Key Study Findings

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

- The critical window for artesunate-induced embryoletality in rats following oral administration of 10 mg/kg was determined to be between Days 9 and 12 post-coitum (pc). A higher dose of 30 mg/kg administered orally later during gestation (Days 14-17 pc) induced nearly total implantation loss.
- Cardiac and skeletal malformations in developing fetuses occurred when artesunate was orally administered between Days 9 and 10 pc. The malformations included ventricular septal and subclavian artery defects, curved/misshapen scapulae and long bones, and brachyury.

Conducting laboratory and location:



GLP compliance:

Methods

Dose and frequency of dosing: 10 and 30 mg/kg/day
 Route of administration: Oral (gavage)
 Formulation/Vehicle: 1% methyl cellulose
 Species/Strain: Rat/CD™(Sprague-Dawley)IGSBR
 Number/Sex/Group: 10-20 pregnant females/group
 Satellite groups: None
 Study design: Animals were administered 10 mg/kg/day artesunate on Days 9-10, 11, 12, 13, or 14-17 pc or 30 mg/kg/day on Days 14-17 pc. Control animals were treated from Days 9-17 pc. C-sections were performed on Day 21 pc and uterine contents examined.

Deviation from study protocol affecting interpretation of results: No. Mating dates were determined to be incorrect for some females, causing some animals in the 10 mg/kg group to be dosed on Days 8-9, 9, 10-11, and 14 pc and some animals in the 30 mg/kg group to be dosed on Days 13-16 pc. Impact was considered to be minimal given the objective of the study (i.e., determine critical windows for developmental toxicity of artesunate) was met.

Observations and Results

Parameters	Major findings
Mortality	None
Clinical Signs	None
Body Weights	None

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Necropsy findings Cesarean Section Data	LD: Total litter resorption was observed in 2 of 21 litters after dosing on Day 11 pc, 1 of 19 litters after dosing on Day 12 pc, and 1 partially resorbed litter in each group after dosing on Days 9-10 (of 13 litters) or Day 10 pc (of 18 litters). No increased resorptions were observed in animals dosed on 8-9, 9, 13, or 14-17 pc. HD: Total litter resorption in 90% of litters with remaining litters displaying significant increase in postimplantation loss (up to 86% of implants resorbed).
Necropsy findings Offspring	LD: cardiovascular defects (ventricular septal defects and retroesophageal right subclavian arteries in 6 fetuses from 2 litters) and skeletal malformations (curved, shortened and/or misshapen scapula, radius, femur, humerus, or tibia, unossified or incomplete ossification of pelvic bones in 6-7 fetuses from 3 litters) were observed following treatment from Days 9-10 pc. Short tails were also observed in fetuses with other treatment-related effects. Variations in liver shape (absence of deep fissure and/or ligamentum teres hepatic) occurred in 8 fetuses from 1 litter with other artesunate-related findings dosed on Day 10 pc. No treatment-related abnormalities were observed after dosing on Days 8-9, 9, 13 or 14-17 pc. HD: Observed skeletal changes in the small number of surviving fetuses following administration between days 13 and 16 pc were similar to those observed in LD treatment group

SB-714702 (Artesunate): Oral Embryo-Fetal Development Study During Critical Development Windows in Rats/Study No. CD2004/00353/00 ((b) (4) Study No. G04015)

Key Study Findings

- A critical window for artesunate-induced embryoletality and developmental toxicity was determined to be days 10 to 14 pc. A single treatment with 17 mg/kg artesunate on Days 10 or 14 pc induced significant postimplantation loss (up to 60%). In surviving fetuses, treatment-related cardiovascular, liver, and skeletal malformations were noted, with greater incidence occurring following treatment on Day 10 pc.
- No increases in artesunate-related embryoletality or fetal abnormalities were noted following a single dose of 17 mg/kg on Day 9 pc or 30 mg/kg on Day 16 pc.

Conducting laboratory and location:

(b) (4)

GLP compliance:

Yes

Methods

Dose and frequency of dosing: 17 and 30 mg/kg, single dose
Route of administration: Oral (gavage)
Formulation/Vehicle: 1% methyl cellulose
Species/Strain: Rat/CD™(SD)IGSBR
Number/Sex/Group: 15 pregnant females/group

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Satellite groups: None
 Study design: Animals were administered a single dose of 17 mg/kg artesunate on Days 9, 10, or 14 pc or 30 mg/kg on Day 16 pc. Control animals were treated on Days 9 to 17 pc. C-sections were performed on Day 21 pc and uterine contents examined.
 Deviation from study protocol affecting interpretation of results: No.

Observations and Results

Parameters	Major findings
Mortality	None
Clinical Signs	None
Body Weights	None
Necropsy findings Cesarean Section Data	LD: Significant increases in embryo lethality occurred following a single dose administered on Day 10 or 14 pc (61% and 40%, respectively). Eight litters were 100% resorbed following administration on Day 10 pc, with 2 additional litters displaying 5 or more resorptions. Following administration on Day 14 pc, 2 litters were 100% resorbed with 4 additional litters having 4 or more resorptions. A 13-14% decrease in fetal weight was noted following treatment on Day 10 or 14 pc (13-14% and 5-6%, respectively). No increased embryo lethality was observed after single dose on Day 10 pc. HD: No increased embryo lethality was observed following administration on Day 16 pc.
Necropsy findings Offspring	LD: Treatment-related fetal abnormalities occurred following administration on Days 10 or 14 pc, but not Day 9 pc. Following treatment on Day 10 pc, fetal abnormalities included brachyury (10 fetuses in 3 litters), ventricular septal defects (9 of 71 fetuses from 3 litters), retroesophageal right subclavian vessels (4 fetuses from 2 litters), and skeletal defects and abnormalities such as curved scapula and femurs, short femur, knobby ribs and variation and shape and incomplete ossification of sternbrae (4 fetuses from 2 litters). Several fetuses also displayed liver variations (absence of deep fissure of median lobe and associated absent ligamentum teres hepatis). Following treatment on Day 14 pc, fewer fetal malformations were observed compared with Day 10 pc (i.e., 3 fetuses from 3 litters displaying VSD with no great vessel or liver abnormalities noted). Fetuses from this group did display more skeletal variations (i.e., increased incidence of knobby ribs) and unique skeletal variations including incomplete ossification of cervical vertebral arches (15 of 121 fetuses from 6 litter) and incompletely ossified skull bones. HD: No treatment-related fetal abnormalities were observed following administration on Day 16 pc.

Artemisinins: Oral Embryo-Fetal Development Comparison Study in Rats/Study No. CD2004/00606/01 ((b) (4) Study No. G04117)

Key Study Findings

- Artesunate, DHA, artemether and arteether induced significant embryoletality (55 to 75% postimplantation loss) in rats following oral administration of a single dose on Day 10 pc. An approximate 20% reduction in mean fetal weight was observed in all artemisinin-treated groups compared with control. No maternal toxicity was noted.
- Similar treatment-related fetal malformations were noted following exposure to all artemisinins. These included cardiovascular malformations (ventricular septal defects and interrupted aortic arch), skeletal defects (curved scapula, curved, misshapen or short humerus/femur, duplicate sternbrae), and a distinct liver variation (absence of the deep fissure of the median lobe).

Conducting laboratory and location:

(b) (4)

GLP compliance:

Yes

Methods

Dose and frequency of dosing:

Route of administration:

Oral (gavage)

Formulation/Vehicle:

1% methyl cellulose

Species/Strain:

Rat/CD™ (Sprague-Dawley)IGSBR

Number/Sex/Group:

25 pregnant females/group (24/group for control)

Satellite groups:

None

Study design:

Animals were administered a single dose of 15 mg/kg artesunate, 11.1 mg/kg DHA, 19.4 mg/kg artemether or 20.3 mg/kg arteether on Day 10 pc. C-sections were performed on Day 21 pc and uterine contents examined.

Deviation from study protocol affecting interpretation of results:

No.

Observations and Results

Groups	0 (Control)	Artesunate	DHA	Artemether	Arteether
No. Females/Group	24	25	25	25	25
No. Pregnant	23	25	25	25	25
Died or euthanized moribund	1	0	0	0	0
No. Pregnant Evaluated	22	25	25	25	25

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Groups	0 (Control)	Artesunate	DHA	Artemether	Arteether
No. with Total resorption of Litter	0	12	7	11	15
Body Weight Gain, g					
Day 11 to 11 pc	5	7	6	6	7
Day 11 to 21 pc	107	58 (-42%)	74 (-31%)	61 (-43%)	57 (-47%)
Mean No. Corpora Lutea	12.7	13.4	14.2*	13.8*	13.2
Mean No. Implantations	12.2	12.6	13.5*	13.0	12.1
Mean % Preimplantation Loss	3.7	4.9	4.7	5.0	7.3
Mean No. Total Postimplantation Loss	0.7	8.8*	7.6*	8.9*	8.7*
Mean (%) Total Postimplantation Loss	5.7	69.7	55.4	68.5	73.6
Litters: No. Litters Evaluated	22	13	18	14	10
Mean No. Live Fetuses	11.5	7.3*	8.1*	7.1*	8.3
Fetal Malformations (% Litter Incidence)					
Bent Tail	0	23	33	43*	20
Brachyury	0	38	67	71*	60
Kinked Tail	0	31	28	43*	30
Ventricular Septal Defect	0	62*	67*	79*	70*
Liver, Lobe Variation in Shape	4.5	31	39*	64*	40
Ulna, curved	0	23	22	36*	10
Rib, Knobby	14	62	72*	71	70
Scapula, Curved	0	38	44	50*	50
Sternebrae, Incompletely Ossified	4.5	62	39	50*	30
Sternebrae, Variation in Shape	14	38	44*	11	40

* p<0.05

18.3 OCP Appendices (Technical documents supporting OCP recommendations)

Study 1128:

This study was a Phase 1a, randomized, double-blind, placebo-controlled, in-patient, ascending single IV dose study to determine the safety, tolerability, and pharmacokinetics of Intravenous Artesunate (IV AS) in healthy male and non-pregnant, non-lactating female subjects aged 18 to 55 years old. Five cohorts of 8 volunteers per cohort were studied. Within each cohort, 6 subjects received a single dose of IV AS infused over a period of 2 minutes at 0.5, 1.0, 2.0, 4.0, and 8.0 mg/kg, and 2 subjects in each cohort received a similar infusion of placebo (mannitol in phosphate buffer).

PK results and conclusions: The PK parameter values of artesunate and dihydroartemisin (DHA) following single IV Artesunate doses of 0.5 mg/kg to 8 mg/kg are summarized in Table 18-1 and Table 18-2. The mean plasma concentration profiles are shown in Figure 18-1 and the dose-proportionality relationship for C_{max} and AUC is shown in Figure 18-2.

Table 18-1. Compartmental Analyses of AS by Intravenous Infusion Modeling after Single IV Administrations of 0.5, 1, 2, 4 and 8 mg/kg by Short-term Infusion (2 minutes) in healthy volunteers

PK parameters	Mean (% CV)				
	0.5 mg/kg (N = 1) ^b	1 mg/kg (N = 5)	2 mg/kg (N = 6)	4 mg/kg (N = 6)	8 mg/kg (N = 6)
C _{max} after infusion (ng/mL)	4.8	6.1 (28)	19.4 (19)	36.1 (24)	83.3 (73)
T _{max} (min)	2	2	2	2	2
AUC _{inf} (ng·h/mL)	386	593 (37)	1595 (16)	3038 (25)	6994 (60)
t _{1/2} elimination (h)	0.1	0.1 (22)	0.2 (89)	0.2 (7)	0.2 (29)
CL (mL/min/kg)	21.6	30.6 (38)	21.4 (17)	23.5 (31)	26.2 (57)
V _d (mL/min/kg)	92	187 (46)	106 (35)	109 (26)	165 (75)

Table 18-2. Pharmacokinetic Parameters of DHA Following Single Doses of IV Artesunate after Single IV Administrations of 0.5, 1, 2, 4 and 8 mg/kg by Short-term Infusion (2 minutes) in healthy volunteers

PK parameters	Mean (%CV)				
	0.5 mg/kg (n = 6) ^a	1 mg/kg (n = 6)	2 mg/kg (n = 6)	4 mg/kg (n = 6)	8 mg/kg (n = 6)
NCA modeling					
C _{max} (ng/mL)	428 (15)	802 (22)	1286 (20)	3,148 (30)	4744 (36)
T _{max} (min)	0.16 (66)	0.25 (56)	0.16 (63)	0.12 (0)	0.40 (65)
AUC _{0-6h} (ng·h/mL)	379 (19)	1008 (29)	1801 (17)	4645 (24)	10057 (28)
AUC _{inf} (ng·h/mL)	385 (18)	1082 (28)	1850 (17)	4886 (24)	10410 (26)
t _{1/2} elimination (h)	0.96 (26)	1.54 (41)	1.15 (23)	1.37 (9)	2.14 (38)
CL (mL/min/kg)	22.2 (17)	16.4 (26)	18.5 (19)	14.4 (27)	13.6 (28)
V _d (mL/kg)	1734 (25)	2201 (49)	1860 (37)	1701 (27)	2403 (34)

Figure 18-1. Mean plasma concentration-time profiles of AS (top) and DHA (bottom), an active metabolite of AS measured by LC-MS/MS after a single intravenous dosage with a 2-minute short-term infusion of AS at 0.5, 1, 2, 4, and 8 mg/kg in healthy volunteers (*N* = 6 for each dose cohort).

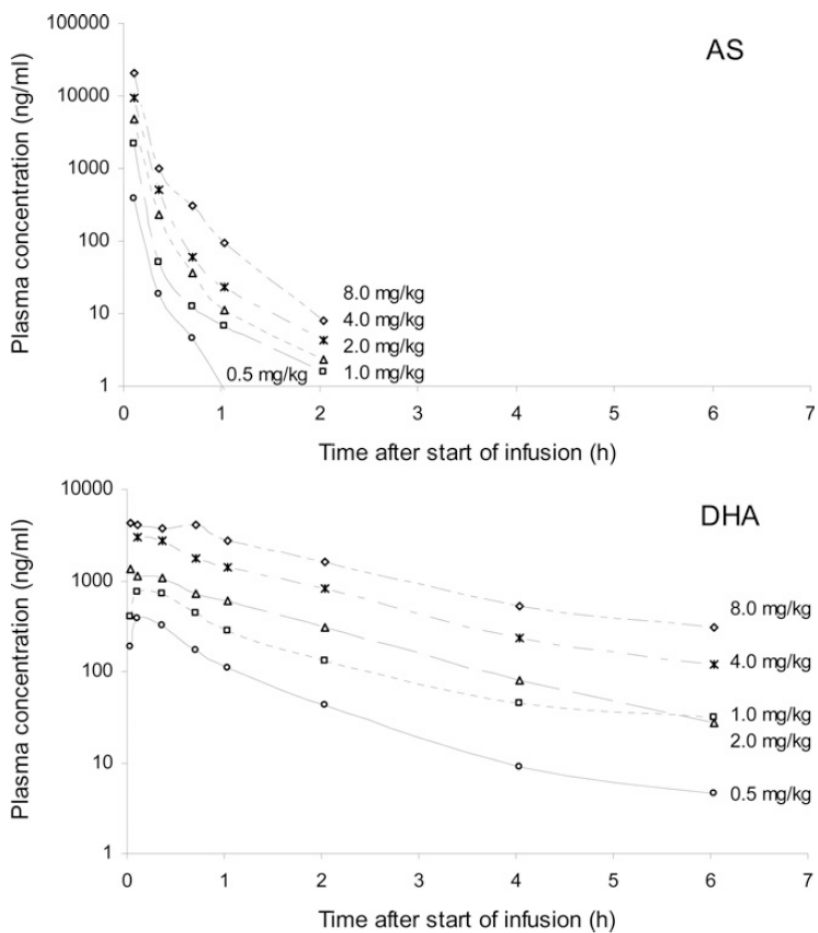
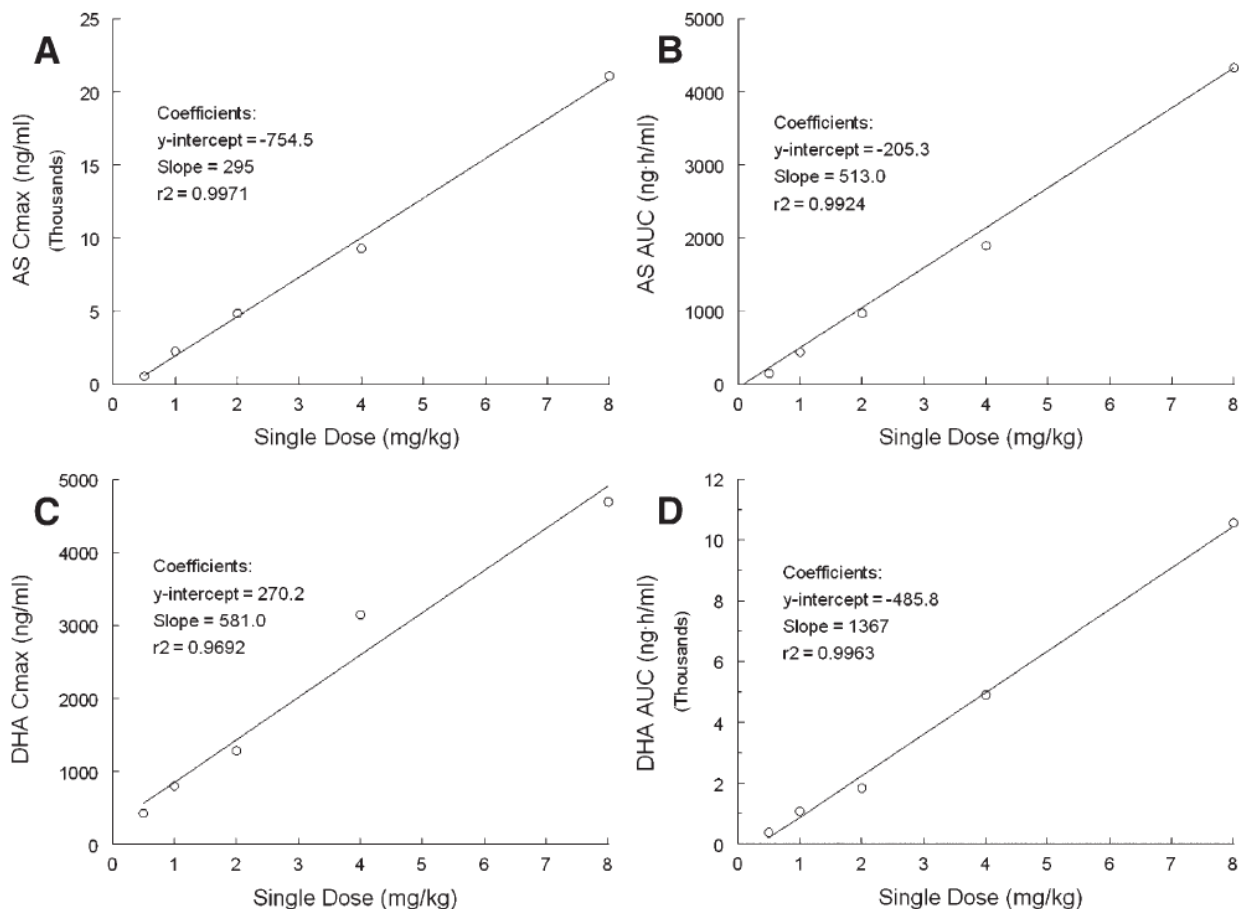


Figure 18-2. Dose proportional increases of AS and DHA Cmax and AUC Single Doses of IV Artesunate after Single IV Administrations of 0.5, 1, 2, 4 and 8 mg/kg by Short-term Infusion (2 minutes) in healthy volunteers



Artesunate displayed approximately dose proportional increases in respective AUC and Cmax estimates when administered IV to healthy volunteers over the dose range of 2.0 mg/kg – 8.0 mg/kg. At doses of 0.5 mg/kg and 1.0 mg/kg insufficient plasma concentration-time data was available to draw firm conclusions for doses below 2.0 mg/kg. Artesunate half-lives ranged from 5 to 37 min.

DHA appeared rapidly (quantifiable within 5 minutes of the end of the infusion), achieved a maximum concentration within about 20 minutes and disappeared with a half-life of about 1-2 hours. Across the range of 1.0 mg/kg - 8.0 mg/kg, the DHA AUC and Cmax estimates showed approximate dose proportional increases. The clearance of DHA was approximately 50 L/hr, and the volume of distribution was approximately 100 L. Thus, in comparison with the parent compound, DHA has a smaller clearance and a larger volume of distribution, which together yield a half-life that is longer than that of artesunate.

Study 1142: Multiple Ascending Dose Study

Study 1142 was Phase 1a, randomized, double-blind, placebo-controlled trial using multiple ascending doses of intravenous artesunate to determine its safety, tolerability and PK in healthy male and female subjects. Six subjects in each dose group received AS over about 2 min as a short-term IV infusion. Blood samples for the measurements of AS and DHA levels in plasma was approximately 10 min before dosing (0 h), and at 5, 20, 40 min, 1, 2, 4, 6, and 8 h on Days 1, 2, and 3 post-dose. During the multiple-dose period, samples were collected immediately before morning dose on days 2 and 3 for additional trough values.

PK parameters for artesunate and DHA are summarized in Table 18-3 and Table 18-4, respectively.

Table 18-3. Mean (CV%) PK parameters for AS by Compartmental Modeling: IV AS

Administered at Doses of 2 mg/kg, 4 mg/kg, and 8 mg/kg daily for 3 Days

Date of dose	PK Parameters	Mean (%CV)		
		2 mg/kg (n = 6)	4 mg/kg (n = 6)	8 mg/kg (n = 6)
Day 1	Cmax (ng /mL)	28411 (59)	40574 (68)	63677 (55)
	Tmax (min)	2	2	2
	AUCinf (ng·h/mL)	2051 (66)	4640 (69)	6022 (48)
	t½ elimination (h)	0.16 (52)	0.21 (46)	0.20 (40)
	CL_obs (mL/min/kg)	21.19 (33)	19.39 (48)	27.87 (51)
	Vss_obs (mL/kg)	103.60 (48)	104.34 (80)	187.99 (89)
Day 2	Cmax (ng/mL)	17920 (57)	34387 (50)	74588 (45)
	Tmax (min)	2	2	2
	AUCinf (ng·h/mL)	1410 (49)	2628 (30)	6669 (47)
	t½ elimination (h)	0.19 (51)	0.23 (67)	0.19 (19)
	CL_obs (mL/min/ kg)	29.50 (45)	27.26 (28)	26.66 (64)
	Vss_obs (mL/kg)	185.24 (57)	139.76 (51)	129.10 (68)
Day 3	Cmax (ng/mL)	16487 (42)	51164 (61)	67613 (83)
	Tmax (min)	2	2	2
	AUCinf (ng·h/ mL)	1270 (26)	3518 (51)	5916 (61)
	t½ elimination (h)	0.15 (40)	0.20 (46)	0.20 (60)
	CL_obs (mL/min/kg)	27.87 (26)	23.02 (35)	28.91 (44)
	Vss_obs (mL/kg)	122.00 (42)	122.17 (49)	164.58 (55)
	Accumulation Index (AUCD3/D1)	0.81 (56)	0.91 (45)	1.01 (31)

Table 18-4. Mean (CV%) PK parameters of DHA by Noncompartmental Analysis Following Multiple Administrations of IV AS (2 Min Infusion) at Doses of 2, 4, and 8 mg/kg Daily for 3 days in Healthy Volunteers

Date of dose	PK Parameters	Mean (%CV)		
		2 mg/kg (n = 6)	4 mg/kg (n = 6)	8 mg/kg (n = 6)
Day 1	C _{max} (ng/mL)	1735 (31)	3015 (29)	6056 (40)
	T _{max} (hr)	0.29 (45)	0.25 (55)	0.25 (59)
	AUC _{inf} (ng·h/mL)	2121 (18)	4391 (22)	9697 (37)
	t _½ elimination (h)	1.19 (16)	1.74 (59)	1.42 (21)
	CL/F (mL/min/kg)	16.18 (19)	15.92 (26)	15.51 (37)
	V _{ss} /F (mL/kg)	1634 (14)	2377 (62)	1870 (39)
Day 2	C _{max} (ng/mL)	1710 (41)	2923 (35)	5943 (35)
	T _{max} (hr)	0.20 (63)	0.25 (55)	0.22 (69)
	AUC _{inf} (ng·h/mL)	2012 (27)	3740 (28)	8732 (35)
	t _½ elimination (h)	1.59 (55)	1.42 (22)	1.91 (60)
	CL/F (mL/min/kg)	17.55 (24)	19.37 (35)	16.96 (35)
	V _{ss} /F (mL/kg)	2224 (31)	2249 (21)	2525 (35)
Day 3	C _{max} (ng/mL)	2358 (26)	3018 (25)	5762 (59)
	T _{max} (hr)	0.16 (63)	0.29 (45)	0.29 (45)
	AUC _{inf} (ng·h/mL)	2385 (24)	3960 (19)	9205 (33)
	t _½ elimination (h)	1.31 (16)	1.24 (20)	1.34 (12)
	CL/F (mL/min/kg)	14.63 (22)	17.49 (23)	15.60 (27)
	V _{ss} /F (mL/kg)	1691 (33)	1895 (34)	1776 (25)
	Accumulation Index (AUC _{D3} /D1)	1.14 (21)	0.93 (24)	0.98 (15)

Reviewer Conclusion: After IV injection of AS, parent drug rapidly declined and was converted to DHA with overall mean elimination half-lives ranging 0.1 – 0.23 h for AS and 1.23 – 1.63 h for DHA. The C_{max} and AUC values of AS and DHA increased proportionally to the AS dose. AS and DHA CL, V_{ss}, and T_½ all remained relatively constant across all doses and with respect to days of dosing. Thus, the PK of AS and DHA are linear. Plasma accumulation of AS was not apparent, while accumulation of DHA was minimal; this is to be expected with the observed short half-lives of each moiety. The peak concentration (C_{max}) of AS was much higher than that of DHA with a range of 3.08 – 3.78.

Study 1168: A Phase II, Open Label, Study of the Safety, Tolerability, Efficacy and Pharmacokinetics of Intravenous Artesunate in Adults with Uncomplicated Malaria.

Study 1168 was an unblinded, non-randomized, phase II clinical study to evaluate the pharmacokinetics, safety, tolerability, and efficacy (parasite clearance) of intravenous artesunate for the treatment of uncomplicated *Plasmodium falciparum* malaria in adults in Kenya. This study involved the treatment of 30 adult subjects, aged 18 to 65 years with uncomplicated falciparum malaria, with intravenous artesunate (2.4 mg/kg) once a day for 3 days as an IV injection given over 2 minutes. Following completion of the intravenous artesunate treatment, all subjects received Malarone follow-on therapy (4 tablets once daily for 3 days) to ensure parasitologic cure. Pharmacokinetic samples were obtained at 9 approximate time points (0, 5 min, 10 min, 20 min, 40 min, 1 h, 2 h, 4 h, and 6 h) on Day 0. Pharmacokinetic results for artesunate and DHA are summarized in below.

Table 18-5. Pharmacokinetic Parameters for artesunate following administration of 2.4 mg/kg once a day for 3 days.

Parameters (Units)	AUCinf (ng·h/ml)	Cmax (ng/ml)	t1/2 (hr)	CL (ml/min/kg)	Vss (ml/kg)
Mean	1877.6	28557.8	0.17	1727.8	138.7
SD	1189.6	28530.7	0.08	982.6	123.8
% CV	63.36	99.91	47.4	56.9	89.2
Minimum	477.	3361.5	0.11	373.0	41.1
Maximum	3521.4	55873.4	0.43	5029.9	670.4

Table 18-6. Pharmacokinetic Parameters for DHA following administration of 2.4 mg/kg once a day for 3 days.

Parameters (Units)	AUC0-last (ng·h/ml)	AUCinf. (ng·h/ml)	Cmax (ng/ml)	Tmax (hr)	t1/2 beta (hr)	CL (ml/min/kg)	Vss (ml/kg)
Mean	3443.2	3587.4	2789.6	0.20	1.3	723.8	1240.3
SD	933.2	1024.7	931.9	0.08	0.4	205.6	355.5
% CV	27.1	28.6	33.4	38.8	34.0	28.4	28.7
Minimum	2012.5	2207.9	1493.3	0.08	0.6	442.5	500.4
Maximum	5038.9	5424.0	5568.9	0.38	2.2	1185.6	1934.6

The results show that DHA was detected in all volunteers' plasma until 6 hours, while the parent drug was undetectable at time-points as early as 1-2 hours after the single short-term infusion. Although the plasma concentration of DHA was higher than AS with the ratio of AUC_{DHA/AS} of 1.89, the peak concentration of AS was much higher than that of DHA with a ratio of Cmax_{AS/DHA} of 10.35. It is known that DHA has a longer half-life of 1.26 hours, which is 7.9 folds longer than AS (T_{1/2}~ 0.16 hours).

In Vitro Studies

Melendez, 2010: In Vitro Biotransformation and Drug Interaction Studies of Artesunate and its Active Metabolite Dihydroartemisinin Using Plasma and Liver Preparations Followed by LCMS and Fluorometric Analyses

Study #	Melendez, 2010
Objective	<i>In vitro</i> study designed to report a simple, fast, and reliable LCMS method for the simultaneous quantification of low nanomolar concentrations of AS and DHA from biological fluids and its application to profiling the compounds in plasma, liver microsomes from several species, and cryopreserved human hepatocytes.
Cell System Used	Cryopreserved human hepatocytes
Investigational drug concentration	Metabolic stability of AS at 100 µM. Incubations containing 10 and 25 µM final AS concentrations yielded 4.2 and 10.5 µM DHA, respectively.
Are the methods appropriate	Yes

Results and Conclusions: A simple LC-MS assay for the simultaneous determination of AS and DHA in plasma and from *in vitro* preparations was developed. The assay has acceptable specificity, sensitivity, accuracy and precision. Incubation of AS at 37°C in the presence of plasma or physiological buffer (phosphate, K-H) resulted in at least 25% spontaneous conversion to DHA after one hour. Hepatic microsomal metabolism of AS yielded an estimated half-life of 20 minutes and resulted in nearly complete (greater than 95%) turnover of AS into DHA after one hour. Hepatocytes incubation resulted in approximately 50% biotransformation after two hours; presumably due to membrane barriers and less CYP 450 activity compared to microsomal preparations. Small amounts of putative DHA-glucuronide and acetyl CoA were also detected but not confirmed using authentic standards. This data supports the notion that administered AS not transformed in plasma, will undergo first pass liver metabolism into a more stable compound, DHA, which is subsequently conjugated for elimination. Drug interactions studies identified CYP 450 1A2 and 2C19 as isoenzymes moderately affected by DHA (Table 1) and suggested the need for *in vivo* studies and/or monitoring of patients when combination of AS with other therapies is needed. The C_{max} of DHA following administration of 2.4 mg/kg IV Artesunate in malaria patients is approximately 3 µg/mL. The IC₅₀ values derived for CYP1A2 (32.3 µM) and 2C19 (75.4 µM) in this *in vitro* study correspond to approximately 9 µg/mL and 21 µg/mL. Thus, DHA is unlikely to affect the substrates of CYP1A2 and 2C19.

Table 1. IC50 (µM) values calculated for Artesunic acid and Dihydroartemisinin. Data represent the mean of 3 different assays done by duplicate ± standard deviation.

Isoenzyme	AS	DHA
1A2	> 100 µM	32.3 ± 2.94
2C9	> 100 µM	> 100 µM
2C19	> 100 µM	75.4 ± 5.57
2D6	> 100 µM	> 100 µM
3A4	> 100 µM	> 100 µM

Role of P Glycoprotein in Absorption of Novel Antimalarial Drugs	
Study #	Crowe et al, 2006
Objective	Role of P Glycoprotein(P-gP) in Absorption of Novel Antimalarial Drugs
Cell System Used	Monolayer of a CLEFF9 subclone of human Caco-2 cells with high P-gp-mediated efflux
Investigational drug concentration	DHA - 1 μ M Naphthoquine - 5 μ M, Pyronaridine - 5 μ M, and 30 μ M, and 10 μ M in presence of reference inhibitor for each transporter
Reference Inhibitor (concentration)	P-gP: PSC-833 and GF120918 (4 μ M)
Are controls and control concentrations appropriate?	Yes
Methods: Drug transport was studied with an in vitro gastrointestinal model by using a monolayer of a CLEFF9 subclone of human Caco-2 cells with high P-gp-mediated efflux and previously reported experimental protocols. Briefly, cells were seeded onto 0.6-cm² polycarbonate filters in 24-well plates and grown in high-glucose Dulbecco's modified Eagle's medium for 3 weeks. Following incubation in buffered Hanks balanced salt solution (HBSS) with or without specific efflux inhibitors (PSC-833 and GF120918) for 30 min at 37°C, transepithelial electrical resistance was measured and assay medium/inhibitors were placed in the receiver chambers. Antimalarial drugs were added to the donor chamber of each well. The apical and basolateral chambers received 0.3 and 0.6 ml medium, respectively. Transepithelial electrical resistance measurements were repeated at the conclusion of the studies to ensure continued monolayer integrity Results and Conclusions: As shown in the table below, the ratio of apical/basolateral transport is <1 for DHA, suggesting that there is no net efflux. Thus, it is unlikely that P-gp has a significant role in the gastrointestinal absorption of DHA. Given the lack of an effect of PgP on the transport of DHA, no dosage adjustment of IV Artesunate is needed upon coadministration of P-gP inhibitors or inducers.	

Table 18-7. Transport rates for 5 and 20 μ M DHA through Caco-2 subclone (CLEFF9) monolayers in both apical-to-basolateral (A $\rightarrow$ B) and basolateral-to-apical directions (B $\rightarrow$ A)

pH in apical/basolateral chamber	Concentration (μ M) of efflux modifier	Transport Rate (cm/s) (10^6)		Fold Difference
		A $\rightarrow$ B	B $\rightarrow$ A	
7.4/7.4	1	32.0 $\pm$ 2.6	21.9 $\pm$ 1.7	0.7 (uptake)
7.4/7.4	1 (4 μ M PSC-833)	30.8 $\pm$ 1.5	21.1 $\pm$ 2.4	0.7 (uptake)
7.4/7.4	20	38.6 $\pm$ 5.0	29.9 $\pm$ 0.6	0.8 (no net flux)

PSC-833 is the PgP inhibitor

Pharmacokinetics of mefloquine alone or in combination with artesunate

Bulletin of the World Health Organization, 1994, 72 (1): 83-87

J. Karbwang, K. Na Bangchang, A. Thanavibul, D.J. Back, D. Bunnag, & T. Harinasuta

A randomized comparative trial of the pharmacokinetics and pharmacodynamics of oral doses of mefloquine and of mefloquine in combination with oral artesunate was carried out on 20 Thai male patients with acute, uncomplicated falciparum malaria. The patients were randomized to receive either mefloquine alone (8 patients; 1250 mg of mefloquine-initial dose, 750 mg; followed 6 hours later by 500 mg), or in combination with oral artesunate (12 patients-initial dose, 200 mg of artesunate; followed by 750 mg and 500 mg of mefloquine 6 hours and 12 hours later, respectively).

Treatments. The patients were randomized into two groups (A and B) to receive two drug regimens as follows:

- Group A (8 patients): an initial oral dose of 750 mg of mefloquine (Lariam[®], Hoffmann-La Roche; 250 mg per tablet) followed 6 hours later by 500 mg.
- Group B: an initial oral dose of 200 mg of artesunate followed by mefloquine (750 mg and 500 mg, 6 hours and 12 hours after the artesunate, respectively).

Blood for mefloquine concentrations. Blood samples were collected for determination of mefloquine concentrations at 0, 2, 4, 6, 8, 10, 12, 16, 20, 24, 30, 36, and 42 hours after the initial dose of mefloquine was administered, and then on days 2, 3, 4, 5, 7, 14, 21, 28, 35, and 42.

Pharmacokinetic analysis: The whole blood mefloquine concentrations were determined using HPLC and the concentration-time profiles were analyzed using an iterative, nonlinear curve-fitting program (PCNONLIN, Metzler & Weiner, 1984). A multiple dosing approach was used to fit a two-compartment model to the unweighted data.

Results:

Concomitant administration of oral artesunate with mefloquine resulted in a lowered C_{max} and AUC of mefloquine (Table 18-8). The apparent oral clearance of mefloquine increased by 25% following concomitant administration of oral artesunate.

Table 18-8: Mefloquine Pharmacokinetic parameters (Mean ± SD) in healthy volunteers receiving mefloquine alone or mefloquine +artesunate

Pharmacokinetic parameter	Mefloquine alone	Mefloquine + artesunate
C _{max} (µg/ml)	2.2 ± 0.5	1.6 ± 0.4
T _{max} (hours)	20.3 ± 5.2	15.0 ± 3.0
AUC (µg-day/mL)	17.2 ± 6.4	12.8
T _{1/2} (days)	11.9 ± 2.7	11.0 ± 7.0
CL/F (mL/min)	1.1 ± 0.5	2.9 ± 2.6

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Pharmacokinetic parameter	Mefloquine alone	Mefloquine + artesunate
Vd/F (l/kg)	25.0 ± 6.0	31.8 ± 5.1

Reviewer's Comments: The results of this publication indicate that the mefloquine AUC and C_{max} are reduced by about 37% when mefloquine and oral artesunate are administered together compared to when mefloquine is administered alone. The authors have hypothesized that since mefloquine is highly protein-bound, the lower concentrations that resulted when mefloquine was taken 6 hours after artesunate could arise because of competition for protein binding sites between mefloquine and artesunate or its metabolite(s), resulting in higher free mefloquine concentrations and therefore, a wider mefloquine concentration distribution. Free mefloquine can be cleared faster. This explanation is supported by the larger volume of distribution and faster clearance in the patients who received the combined regimen relative to those patients who received mefloquine alone. Alternatively, the decrease in mefloquine concentration could have resulted from the reduction in its oral bioavailability, induced by artesunate.

Overall, despite the exposure difference (~37% lower C_{max} and AUC) of mefloquine when administered with oral artesunate versus when given alone, given that the favorable results of the fever clearance time and parasite clearance time, no dosage adjustment is deemed necessary. It is to be noted that the authors evaluated the effect of oral artesunate on the PK of mefloquine. However, oral artesunate is not approved in the US.

Assessment of the Effect of Mefloquine on Artesunate Pharmacokinetics in Healthy Male Volunteers

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 2007, p. 1099–1101

Timothy M. E. Davis, Michelle England, Anne-Marie Dunlop, Madhu Page-Sharp, Nathalie Cambon, Thomas G. Keller, Ja'nos L. Heidecker, and Kenneth F. Ilett

The effect of mefloquine on artesunate pharmacokinetics was assessed using crossover design in 20 volunteers given artesunate for 3 days, and after a washout period of 21 days, by combination therapy of artesunate and mefloquine for 3 days. Each subject received 200 mg oral artesunate (Mepha Ltd., Switzerland) after an overnight fast on three consecutive mornings (period 1). After a washout phase of 21 days, this schedule was repeated, but mefloquine (Mepha) at 250 mg daily was given at the same time as artesunate (period 2). On days 1 and 3 of each period, blood samples were drawn for drug assay under a predetermined schedule from immediately before (0h) to 8 h post-dose. Additional samples were taken for mefloquine assay on the mornings of days 2, 4, 5, and 6 during and after period 2. Drug assays were by a validated HPLC-MS method.

Results:

Artesunate was measurable transiently and in low concentrations relative to DHA (Figure 18-3). For DHA, the Day 3 C_{max} and AUC were similar when artesunate was administered alone (period 1) versus when given with mefloquine (period 2) (Table 18-9). The Day 1 C_{max} of DHA following coadministration with mefloquine appeared to result in a 32% decrease compared to when artesunate was given alone.

Figure 18-3. Mean plasma dihydroartemisinin and artesunate concentrations during days 1 and 3 of periods 1 and 2 in healthy male volunteers.

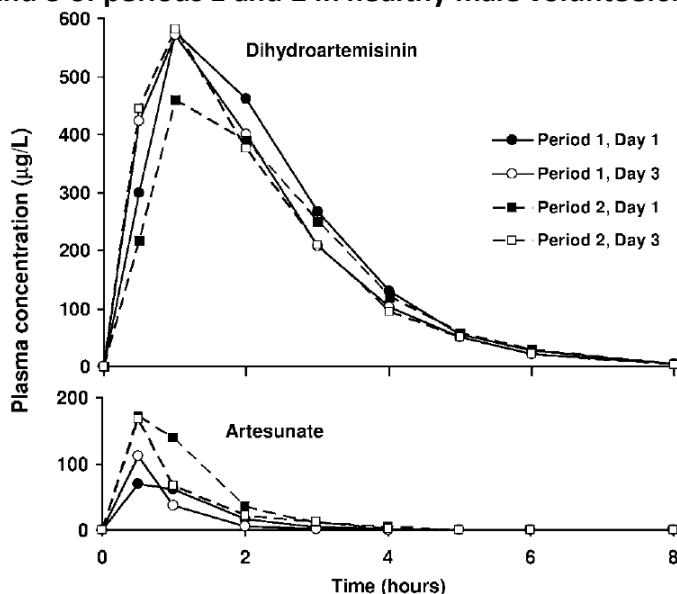


Table 18-9: Pharmacokinetic parameters for artesunate and dihydroartemisinin on days 1 and 3 of periods 1 (artesunate alone) and period 2 (artesunate given with mefloquine)

Drug and parameter ^a	Value ^b				P value ^c
	Period 1		Period 2		
	Day 1	Day 3	Day 1	Day 3	
Artesunate					
C_{max} (µg/liter)	135 (58–316)	113 (44–290)	91 (44–189)	109 (39–304)	0.42
T_{max} (h)	0.6 (0.4–0.9)	0.6 (0.4–1.1)	0.5 (0.3–0.7)	0.6 (0.3–1.0)	0.19
Dihydroartemisinin					
C_{max} (µg/liter)	675 (522–873)	643 (450–917)	508 (345–748)	591 (357–978)	0.11
T_{max} (h)	1.0 (0.6–1.8)	0.8 (0.4–1.5)	1.3 (0.7–2.3)	0.9 (0.5–1.6)	0.06
AUC _{0-∞} (µg · h/liter)	1,443 (1,082–1,924)	1,365 (1,038–1,795)	1,217 (850–1,742)	1,307 (903–1,892)	0.12
$t_{1/2\beta}$ (h)	1.14 (0.98–1.31)	1.14 (0.98–1.30)	1.02 (0.90–1.94)	1.09 (0.92–1.26)	0.93
V_z/F (liters)	174 (143–205)	184 (148–221)	201 (160–243)	186 (150–222)	0.75
CL/F (liters/h)	106 (94–119)	112 (99–126)	128 (111–146)	120 (102–138)	0.07

^a $t_{1/2\beta}$, elimination half-life; V_z/F and CL/F, volume of distribution and clearance, respectively, relative to bioavailability.

^b Values are geometric means (standard deviation ranges) for C_{max} , T_{max} , and AUC_{0-∞} and means (95% confidence intervals) for $t_{1/2\beta}$, V_z/F , and CL/F.

^c The P values are derived from general linear modeling for repeated measures.

Reviewer’s Comments: The results of this publication indicated that mefloquine does not alter the pharmacokinetics of artesunate when the two drugs are coadministered. In contrast to the case for artemisinin from the literature on autoinduction by artemisinin drugs, the authors reported that no significant time-dependent changes in artesunate-DHA pharmacokinetics were observed.

It is to be noted that the authors evaluated the effect of oral artesunate on the PK of mefloquine. However, oral artesunate is not approved in the US.

Title: Absence of an interaction between artesunate and atovaquone ± proguanil

Eur J Clin Pharmacol (1999) 55: 469±474

M. van Vugt, M. D. Edstein, S. Proux, K. Lay M. Ooh, S. Looareesuwan, N. J. White, F. Nosten

The authors investigated whether artesunate would alter the pharmacokinetics and tolerability of atovaquone and proguanil in an open, randomized, two-period crossover study in healthy volunteers. The study was carried out in 12 healthy adult volunteers at Shoklo, a camp for displaced people on the western border of Thailand. The study was an open, randomized, two-period crossover with a washout period of at least 90 days between drug administrations. After giving written informed consent, the volunteers were allocated randomly to receive either 1000 mg atovaquone plus 400 mg proguanil, or 1000 mg atovaquone plus 400 mg proguanil plus 250 mg oral artesunate. The fixed combination atovaquone plus proguanil (Malarone) was dispensed as pink, film-coated tablets, each containing 250 mg atovaquone and 100 mg proguanil (b) (4). Each tablet of artesunate contained 50 mg artesunate and was manufactured by Guilin Pharma in China. All three doses were administered orally with 200 ml of milk-ovaltine (8% fat) after 30 min of having a standard meal (boiled rice plus chicken curry), with a high fat content. The volunteers were also randomized to receive either the combination tablet or the same doses of atovaquone and proguanil separately. Each volunteer, therefore, received the combination on one occasion and the individual drugs on another.

Blood sampling: Blood samples (5 ml) were obtained before the first dose (day 1) and on days 3-10 following the third dose. At the beginning of day 3, sampling was done through an indwelling Teflon cannula inserted in a forearm vein and kept patent with heparinized saline for 24 h and thereafter by venipuncture. Blood was drawn before the third dose and at the following times: 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 32, 48, 72, 96, 120, 144 and 168 h. Over 10 days, the maximum volume of blood drawn for drug analysis was 90 ml. Blood samples were centrifuged (2000 g, 10 min), and plasma was stored at -20 °C

Drug analysis

Plasma concentrations of atovaquone, proguanil and cycloguanil (the active metabolite of proguanil) were measured by means of a validated HPLC method.

Results:

Although marked inter-individual variability was observed in the kinetics of atovaquone, there were no significant differences in the pharmacokinetic parameters of atovaquone whether it was administered as a separate drug or in the combined Malarone formulation. There was also no significant difference between these parameters with or without artesunate coadministration. The mean concentration-time profiles comparing atovaquone levels with and without artesunate coadministration is shown in Figure 18-4. The profiles were essentially superimposable, confirming the absence of any effect of co-administered artesunate on the

disposition of atovaquone. The pharmacokinetic parameters of atovaquone derived from the plasma concentration-time data are shown in Table 18-10.

Figure 18-4. Plasma concentration-time curves (mean $\pm$ SEM) of atovaquone in 12 healthy adults following the third daily dose of either 1000 mg atovaquone plus 400 mg proguanil (open circles) or 1000 mg atovaquone plus 400 mg proguanil plus 250 mg artesunate (closed circles)

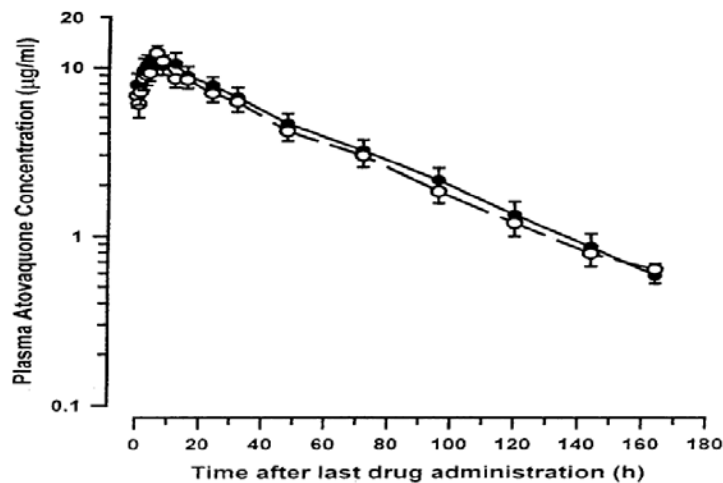


Table 18-10: Mean (SD) pharmacokinetics of atovaquone following the administration of the third dose of either atovaquone plus proguanil or atovaquone plus proguanil plus artesunate in 12 healthy adult volunteers

Parameter	Atovaquone plus proguanil	Atovaquone plus proguanil plus artesunate
C_{max} ($\mu\text{g/ml}$)	13.02 $\pm$ 8.28	13.27 $\pm$ 6.14
C_{min} (before last dose) ($\mu\text{g/ml}$)	6.75 $\pm$ 3.44	7.66 $\pm$ 4.49
C_{max} (corrected) ^b ($\mu\text{g/ml}$)	6.09 $\pm$ 6.17	5.58 $\pm$ 2.39
t_{max} (h)	5.7 $\pm$ 4.0	5.5 $\pm$ 4.4
$t_{1/2}$ (h)	42.2 $\pm$ 22.0	38.5 $\pm$ 15.6
$AUC_{48 \rightarrow \infty}$ ($\mu\text{g/ml} \cdot \text{h}$)	612 $\pm$ 224	663 $\pm$ 280
$AUC_{48 \rightarrow \infty}^b$ ($\mu\text{g/ml} \cdot \text{h}$)	265 $\pm$ 120	293 $\pm$ 163
CL/F^c (ml/h/kg)	90 $\pm$ 47	93 $\pm$ 61
Vd/F^c (l/kg)	4.9 $\pm$ 3.0	4.7 $\pm$ 3.3

^a Data from single drugs (Mepron and Paludrine) and the fixed atovaquone-proguanil combination (Malarone) are combined

^b Corrected for carry-over from previous doses

^c F is the fraction of the oral drug that reaches the systemic circulation

Plasma concentration time curves for proguanil following the last dose of the two regimens are shown in Figure 18-5. As for atovaquone, plasma concentrations of proguanil were similar, with no statistical differences in the kinetic parameters following separate or combined tablet administration or the two-drug versus three-drug combinations (Table 18-11).

Figure 18-5. Plasma concentration time curves of proguanil in 12 healthy adults following the third daily dose of either 1000 mg atovaquone plus 400 mg proguanil (open circles) or 1000 mg atovaquone plus 400 mg proguanil plus 250 mg artesunate (closed circles). Cycloguanil concentrations in seven volunteers after atovaquone plus proguanil (open squares) and atovaquone plus proguanil plus artesunate (closed squares)

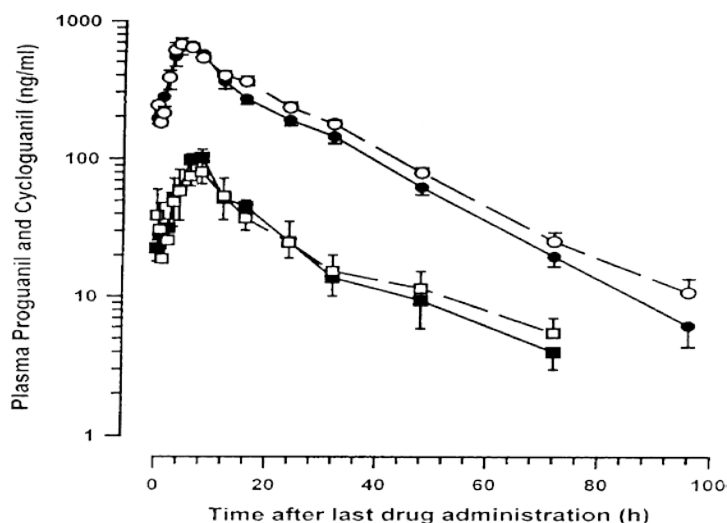


Table 18-11. Mean PK parameters of proguanil and cycloguanil following the administration of the third dose of either atovaquone plus proguanil or atovaquone plus proguanil plus artesunate in 12 healthy volunteers. Data from single drugs (Mepron and Paludrine) and the fixed atovaquone-proguanil combination (Malarone) are combined

Parameter	Atovaquone	Atovaquone plus proguanil
Proguanil		
Proguanil C_{max} (ng/ml)	742 ± 220	751 ± 242
C_{min} (before last dose) (ng/ml)	240 ± 63	193 ± 59
C_{max} (corrected) ^a (ng/ml)	501 ± 185	558 ± 256
t_{max} (h)	4.4 ± 1.2	5.2 ± 1.9
$t_{1/2}$ (h)	14.4 ± 2.7	14.3 ± 2.6
$AUC_{48 \rightarrow \infty}$ (ng/ml · h)	15433 ± 4316	13504 ± 3270
$AUC_{48 \rightarrow \infty}^a$ (ng/ml · h)	10425 ± 3290	9428 ± 2811
CL/F (ml/h/kg)	710 ± 250	764 ± 203
Vd/F (l/kg)	14.5 ± 4.8	15.8 ± 5.5
Cycloguanil		
C_{max} (ng/ml)	60 ± 76	67 ± 72
C_{min} (before last dose) (ng/ml)	21 ± 25	16 ± 9
C_{max} (corrected) ^a (ng/ml)	51 ± 67	39 ± 52
t_{max} (h)	6.4 ± 2.3	6.4 ± 3.1
$t_{1/2}$ (h) ^b	17.7 ± 5.9	15.6 ± 3.9
$AUC_{48 \rightarrow \infty}$ (ng/ml · h) ^b	1748 ± 1639	1810 ± 1308

^a Corrected for carry-over from previous doses

^b $t_{1/2}$ and $AUC_{0 \rightarrow \infty}$ for cycloguanil based on seven paired observations

Reviewer's Conclusions/Comments: Although this study shows that *orally* administered artesunate does not alter the pharmacokinetics of atovaquone plus proguanil (Mepron) in healthy volunteers, it is not known if the same results would be observed for IV artesunate since the absolute bioavailability of PO artesunate is not known.

Pharmacokinetics and tolerability of artesunate and amodiaquine alone and in combination in healthy volunteers

Eur J Clin Pharmacol (2008) 64:683–690

Catherine Orrell, Francesca Little, Peter Smith, Peter Folb, Walter Taylor, Piero Olliaro, Karen I. Barnes

In a randomized, three-phase, cross-over study, amodiaquine (10 mg/kg) and artesunate (4 mg/kg) were given as single oral doses to 15 healthy volunteers. Artesunate was given to all volunteers on day 0. On day 7 they received either amodiaquine or amodiaquine plus artesunate and the alternative regimen on day 28. The pharmacokinetics of artesunate and amodiaquine and their respective main active metabolites, dihydroartemisinin and desethylamodiaquine, were compared following monotherapy and combination therapy.

Blood sampling: Volunteers in phase one had blood samples collected before taking artesunate and at 0.25, 0.50, 0.75, 1.0, 1.5, 2, 3, 4, 6, 8 and 12 h after artesunate administration. In phases two and three (i.e. volunteers receiving artesunate plus amodiaquine or amodiaquine alone) venous blood samples were collected pre-dose and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48 and 72 h, and days 4, 5, 7, 10, 14 and 20 post-dose. Each sample was centrifuged within 5 min of collection and the plasma transferred to separate plastic cryotubes and frozen at -70°C until analyzed for artesunate.

Drug analysis

Artesunate and DHA were analyzed using a validated HPLC method. Amodiaquine and desethylamodiaquine were analyzed by a validated LC mass spectrometry.

Results:

Effect of amodiaquine on artesunate pharmacokinetic parameters

Figure 18-6a shows the mean plasma concentration time curves of artesunate and dihydroartemisinin following administration of artesunate alone and artesunate plus amodiaquine. Table 18-12 provides the mean PK parameter estimates for artesunate (AS) and dihydroartemisinin (DHA) when AS was given alone and with amodiaquine combination therapy (ACT).

Figure 18-6. Profiles of mean drug concentrations over time following monotherapy (*mono*) and combination (*combo*) therapy for a) artesunate (AS) and dihydroartemisinin (DHA) and b) amodiaquine (AQ) and desethylamodiaquine (DEAQ)

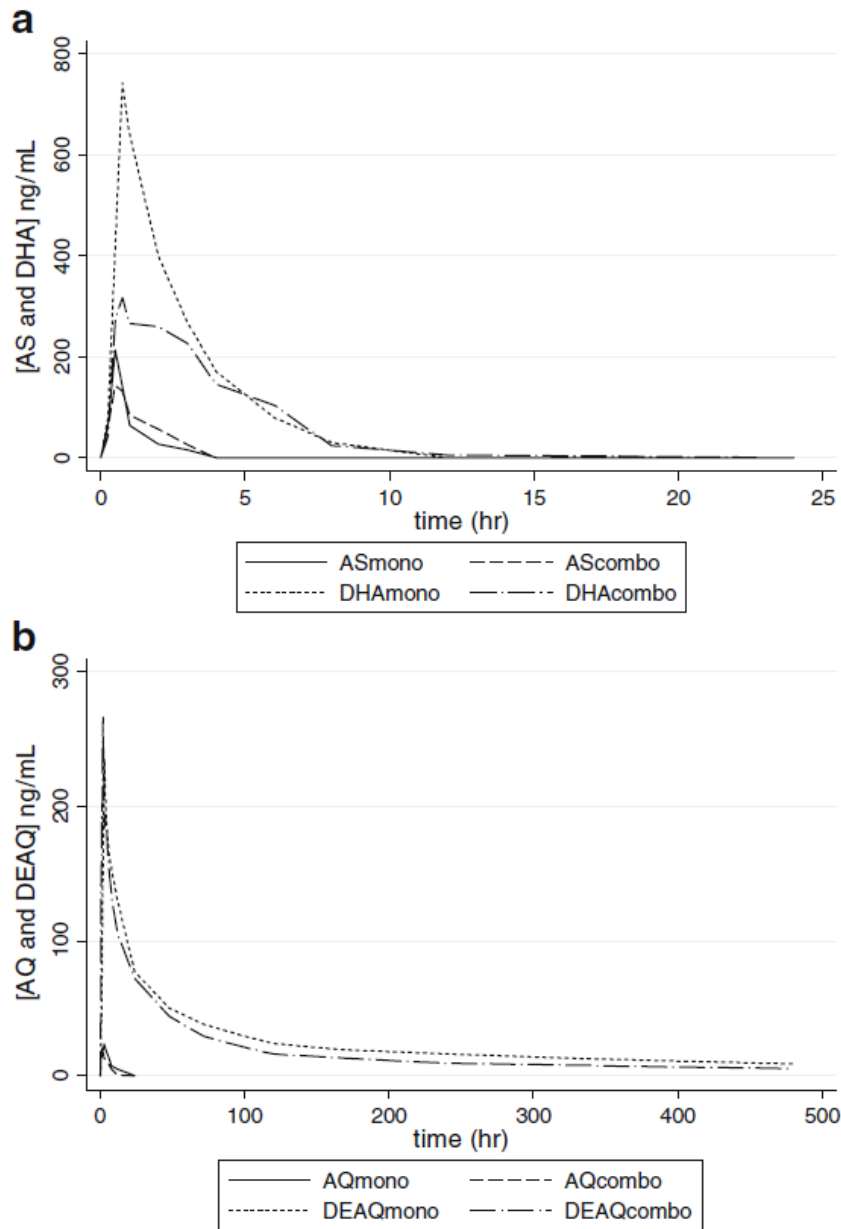


Table 18-12: Pharmacokinetic parameters for artesunate and dihydroartemisinin when artesunate was administered alone and with amodiaquine (ACT)

	AUC (ng·h/ml)	C _{max} (ng/ml)	T _{max} (h)	T _{1/2} (h)	V _d /f (l)	Cl/f (l/min)
Artesunate						
AS alone (mean±SD)	206.4±135.5	231.8±155.0	0.62±0.28	NA	NA	NA
ACT (mean±SD)	183.3±146.5	141.6±117.5	0.86±0.67	NA	NA	NA
ACT-to-monotherapy ratio (%) (mean, 95% CI)	36 (8–173)	25 (6–105)	125 (76–202)	NA	NA	NA
Significance (ANOVA)	0.18	0.057	0.33			
Dihydroartemisinin						
AS alone (mean±SD)	2,044.4±564.2	844.5±309.4	1.10±0.95	1.46±0.48	4.89±1.67	2.46±0.86
ACT (mean±SD)	1,410.5±543.6	446.2±239.5	2.08±1.72	2.20±0.85	9.68±4.16	3.08±0.82
ACT-to-monotherapy ratio (%) (mean, 95% CI)	67 (51–88)	51 (33–78)	165 (82–334)	157 (115–213)	192 (133–275)	122 (96–156)
Significance (ANOVA)	0.008	0.005	0.15	0.008	0.003	0.097

Treatment effects generated from the ANOVA were exponentiated to express within-subject comparisons between monotherapy and combination therapy as a ratio, adjusted for period effects

AUC Area under the plasma concentration time curve, C_{max} maximum concentration, T_{max} time to C_{max}, T_{1/2} elimination half-life, V_d volume of distribution, *f* oral bioavailability or fraction of the drug absorbed, Cl clearance, AS artesunate, SD standard deviation, CI confidence interval, NA not available (too few detectable concentrations for calculation of T_{1/2}, Cl, V_d)

The AUC, C_{max} and time to C_{max} (T_{max}) of artesunate (AS) were similar following monotherapy and combination therapy with amodiaquine (ACT; Table 1). Following the combination therapy, dihydroartemisinin (DHA) AUC was lower [ratio 67% (95% CI 51–88%); *P*=0.008], C_{max} was lower [ratio 51% (95% CI 33–78%); *P*=0.005], T_{1/2} was longer [ratio 157% (95% CI 115–213%); *P*=0.008] and V_d was larger [ratio 192% (95% CI 133–275%); *P*=0.003], when compared with AS monotherapy.

Effect of artesunate on amodiaquine pharmacokinetic parameters

Figure 18-6b (above) shows the mean plasma concentrations of desethylamodiaquine (DEAQ) over time following administration of amodiaquine (AQ) alone and in combination with AS. Table 2 provides the mean PK parameter estimates for AQ and DEAQ following administration of AQ alone and with AS. The volunteer ^{(b) (6)} who had the highest observed AUC for desethylamodiaquine following combination therapy [29,504 ng·h/ml compared with the mean of 8,437 (95% CI 5,744–11,131) ng·h/ml] was excluded from the pharmacokinetic analyses of desethylamodiaquine, as this was an extreme outlier and influential observation. The impact of adding artesunate to amodiaquine on the pharmacokinetic parameters of amodiaquine and desethylamodiaquine is reported for the remaining 11 volunteers.

Table 18-13. Pharmacokinetic parameters for desethylamodiaquine (DEAQ) when amodiaquine (AQ) was administered alone and with artesunate (ACT)

	AUC (ng·h/ml)	C _{max} (ng/ml)	T _{max} (h)	[Day 7] (ng/ml)	T _{1/2} (h)	V _d /f (l)	Cl/f (l/min)
Amodiaquine							
AQ alone (mean±SD)	162.4±101.4	29.2±10.9	2.32±1.16	NA	5.3±4.1	361.0±128.3	69±59
ACT (mean±SD)	108.5±56.0	22.7±9.0	2.18±1.61	NA	3.9±1.2	467.7±180.5	86±26
ACT-to-monotherapy (%) (mean, 95% CI)	77 (47–127)	78 (58–103)	92 (57–147)	NA	74 (29–189)	128 (93–175)	172 (84–357)
Significance (ANOVA)	0.27	0.07	0.68		0.44	0.11	0.11
Desethylamodiaquine							
AQ alone (mean±SD)	12,041± 3,480	268.7±70.8	3.68±1.85	19.4±7.3	240.8±146.9	234.1±97.5	768±252
ACT (mean±SD)	8,437±4,009	301.4±166.1	2.18±1.03	13.3±7.3	136.9±83.8	210.8±92.9	1,330±735
ACT-to-monotherapy ratio (%) (mean, 95% CI)	65 (46–90)	103 (73–147)	60 (45–80)	56 (30–104)	53 (25–111)	88 (51–149)	164 (112– 243)
Significance (ANOVA)	0.015	0.82	0.003	0.064	0.08	0.58	0.016

Treatment effects generated from the ANOVA have been exponentiated to express within-subject comparisons between monotherapy and combination therapy as a ratio, adjusted for period and sequence effects
AUC Area under the plasma concentration time curve, C_{max} maximum concentration, T_{max} time to C_{max}, [Day 7] day 7 concentration, T_{1/2} elimination half-life, V_d apparent volume of distribution, f oral bioavailability or fraction of the drug absorbed, Cl clearance, AQ amodiaquine, SD standard deviation, CI confidence interval, NA not available (as too few patients had quantifiable amodiaquine concentrations)

The pharmacokinetic parameters of amodiaquine were similar when administered as monotherapy or in combination with artesunate (ACT; Table 18-13). The desethylamodiaquine AUC is lower [ratio 65% (95% CI 46–90%); P=0.015], T_{max} shorter [ratio 60% (95% CI 45–80%); P=0.003] and clearance more rapid [ratio 164% (95% CI 112–243%); P= 0.016] when amodiaquine was administered in combination with artesunate rather than as monotherapy. There was a trend towards a lower day 7 concentration of desethylamodiaquine [ratio 56% (95% CI 30–104%); P= 0.064] when amodiaquine was administered in combination.

Author’s Conclusions: To conclude, the total exposure to the main active metabolites of both artesunate and amodiaquine was significantly reduced when administered in combination to healthy African volunteers; this might be important clinically. However, because cure rates with this combination are generally higher than with amodiaquine monotherapy, artesunate and amodiaquine could remain in the armamentarium of drugs used to combat falciparum malaria, provided efficacy continues to be monitored and adequate safety precautions can be taken.

Reviewer’s Comments:

- This study shows that oral artesunate does not alter the pharmacokinetics of amodiaquine in healthy volunteers
- The coadministration of amodiaquine reduced the C_{max} of DHA by 51% and AUC by 67% when given as oral artesunate.
- Coadministration of oral artesunate reduced C_{max} and AUC of amodiaquine by approximately 75% and reduced the AUC of desethylamodiaquine (DEAQ) by 65% but did not alter the C_{max} of DEAQ.
- It is to be noted that the authors evaluated the effect of oral artesunate on the PK of amodiaquine. Oral artesunate is not approved in the US. Overall, no dosage adjustments for the administration of IV Artesunate with amodiaquine are necessary, given that this study evaluated oral artesunate and also given that IV Artesunate, if approved, will not be given with amodiaquine.

Pharmacokinetics of Artesunate Alone and in Combination with Sulfadoxine/Pyrimethamine in Healthy Sudanese Volunteers

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Kamal M. Matar, Abdelmoneim I. Awad, and Sakina B. Elamin

The authors conducted a study to assess the potential impact of sulfadoxine/pyrimethamine (SP) on the pharmacokinetics of orally administered artesunate (AS) and its active metabolite, dihydroartemisinin (DHA), in healthy adults. A single-dose, randomized, open-label, crossover study design with a washout period of three weeks was performed with 16 volunteers. After a 12-hour overnight fasting, the first group received a single AS oral dose of 200 mg and the second group received a single oral dose of AS (200 mg) in combination with Fansidar (Roche, Basel, Switzerland) (3 tablets: 500 mg of sulfadoxine/25 mg of pyrimethamine per tablet). The first and second groups received the opposite treatment after a washout period of three weeks.

Blood sampling:

Blood (5 mL) samples were withdrawn into heparinized tubes before dosing and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4, 4.5, 5, 6, 9, and 12 hours after drug administration. Plasma samples were immediately separated and kept frozen at -80°C .

Drug analysis

A validated liquid chromatography-mass spectrometry analytical method for determination of artesunate, and DHA was developed.

Results: The pharmacokinetic parameters of AS and DHA for both treatments are shown in Table 18-14 and Table 18-15, respectively.

Table 18-14. Pharmacokinetic parameters of artesunate (AS) after a single oral dose of AS (200 mg) alone and in combination with a single dose of 3 sulfadoxine/pyrimethamine (SP) tablets (sulfadoxine 500 mg +pyrimethamine 25 mg/tablet), Sudan*

Parameter	AS alone, median (IQR) n = 16	AS + SP, median (IQR) n = 16	P
$t_{1/2}$ (h)	0.36, (0.24–1.18)	0.34 (0.23–0.72)	0.61
$T_{\max}$ (h)	1.0 (0.5–1.5)	1.5 (1.1–2.0)	0.02†
$C_{\max}$ (ng/mL)	65.5 (48.6–143.2)	78.0 (32.5–177.6)	0.68
$AUC_{0-\infty}$ (ng.h/mL)	94.5 (63.7–163.7)	89.7 (54.7–149.0)	0.84
Vd/F (L/kg)	24.6 (9.4–40.9)	24.2 (8.8–44.8)	0.64
CL/F (L/h/kg)	33.8 (21.6–46.5)	32.1 (19.2–54.0)	0.76

*IQR = interquartile range; $t_{1/2}$ = elimination half-life; $T_{\max}$ = time needed to attain maximum concentration; $C_{\max}$ = maximum plasma concentration; $AUC_{0-\infty}$ = areas under the plasma concentration-time curves; Vd/F = volume of distribution; CL/F = oral clearance.

†Statistically significant ($P < 0.05$) by Wilcoxon matched-pair signed-rank test.

Table 18-15. Pharmacokinetic parameters of dihydroartemisinin (DHA) after a single oral dose of artesunate (AS) (200 mg) alone and in combination with a single dose of 3 sulfadoxine/pyrimethamine (SP) tablets (sulfadoxine 500 mg + pyrimethamine 25 mg/Tablet), Sudan*

Parameter	DHA after administration of AS, median (IQR) n = 16	DHA after administration of AS + SP, median (IQR) n = 16
t _{1/2} (h)	1.18 (0.74–2.04)	1.55 (0.84–2.0)
T _{max} (h)	1.5 (1.0–1.5)	2.0 (1.6–3.8)
C _{max} (ng/mL)	900.1 (365.7–1,116.5)	951.5 (257.0–1,671.2)
AUC _{0–∞} (ng.h/mL)	1,364.5 (1,047.2–1,802.9)	1,738.4 (923.0–2,066.2)
Vd/F (L/kg)	4.9 (2.5–7.3)	4.8 (2.5–7.0)
CL/F (L/h/kg)	2.2 (1.7–3.3)	2.1 (1.4–2.9)

*IQR = interquartile range; t_{1/2} = elimination half-life; T_{max} = time needed to attain maximum concentration; C_{max} = maximum plasma concentration; AUC = area under the concentration-time curves; Vd/F = volume of distribution; CL/F = oral clearance.

As seen above, the PK parameters of AS and DHA were similar during AS monotherapy or when given in combination with SP. It does not appear that SP affects the PK of AS.

Reviewer’s Comments:

It is to be noted that the authors evaluated the effect of SP on the PK of oral artesunate. Oral artesunate is not approved in the US. Also, in this study the authors evaluated the effect of single doses of SP and oral artesunate. However, based on the results from this study, no dosage adjustment for the administration of IV Artesunate with SP would be needed if the two drug products are co-administered.

Artemisinin antimalarials moderately affect cytochrome P450 enzyme activity in healthy subjects

Fundamental & Clinical Pharmacology 21 (2007) 307–316

Sara Asimus, Doaa Elsherbiny, Trinh N. Hai, Britt Jansson, Nguyen V. Huong, Max G. Petzold, Ulrika S.H. Simonsson, Michael Ashton

The aim of this study was to investigate which principal human cytochrome P450 (CYP450) enzymes are affected by artemisinin antimalarial drugs and to what degree the artemisinin derivatives differ with respect to their respective induction and inhibition capacity. Seventy-five healthy adults were randomized to receive therapeutic oral doses of artemisinin, dihydroartemisinin, arteether, artemether or artesunate for 5 days (days 1–5). A six-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.

Study design

The volunteers were randomized to treatment with one of five different artemisinin study drugs; artemisinin (2×250 mg capsules; Mediplantex, Hanoi, Vietnam), dihydroartemisinin (60 mg tablets; Mediplantex), artemether (Artenam[®], 2×50 mg tablets; Arenco Pharmaceutica nv, Geel, Belgium), arteether (Artecef[®], 2 ×1 mL ampoules of the intramuscular preparation containing 50 mg arteether in sesame oil; Artecef BV, Maarsen, The Netherlands) or artesunate (Plasmotrim[®], 2×50 mg tablets; Mepha Ltd, Aesch, Switzerland). Repeated oral doses were administered in the morning for 5 days (day 1–5). The cocktail of probe drugs, caffeine (Koffein Recip[®], 100 mg tablets, Stock-holm, Sweden), coumarin (5 mg capsules, Laboratory of the National Corporation of Swedish Pharmacies, Goteborg, Sweden), midazolam (Dormicum[®], 7.5 mg tablets; Roche a/s, Hvidovre, Denmark), mephenytoin (Epilan Gerot[®], 100 mg tablets; Gerot Pharmazeutika, Vienna, Austria), metoprolol (Seloken[®], 100 mg tablets; AstraZeneca Sverige, Molndal, Sweden) and chlorzoxazone (Paraflex[®], 250 mg tablets, AstraZeneca Sverige) were given orally 1 week before (day -6) administration of the artemisinin drugs. On day 1 and day 5, the administration of the probe drug cocktail was repeated, 1 h after intake of the artemisinin drugs. After a washout period of 5 days (day 10), the probe drug cocktail was given again. Study drug and the cocktail were each taken with 200 mL water.

Blood sampling:

Probe compounds were measured in samples obtained pre-dose and at 4 h after intake of the cocktail drugs on days -6, 1, 5 and 10. Ten milliliters of blood was collected, and all plasma samples were immediately frozen and kept at -70°C.

Drug analysis: Plasma concentrations of caffeine, paraxanthine, chlorzoxazone, 6-OH-chlorzoxazone, 7-OH-coumarin, metoprolol, a-OH-metoprolol, midazolam and 1-OH-midazolam were measured in b-glucuronidase-treated samples by use of a validated LC/MS/MS method.

Data analysis: The plasma concentration ratio of paraxanthine to caffeine at 4 h was used to assess CYP1A2 activity. The total amount of 7-OH-coumarin excreted in 0- to 8-h urine was used as an index of CYP2A6 activity. The ability to hydroxylate S-mephenytoin (CYP2C19) was estimated by the S-4'-OH-mephenytoin to S-mephenytoin plasma ratio at 4 h post-dose. The 4-h- α -OH-metoprolol to metoprolol ratio and 6-OH-chlorzoxazone to chlorzoxazone ratio were used to evaluate the activities of CYP2D6 and CYP2E1, respectively. The concentration of 1-OH-midazolam divided by that of midazolam in the 4-h plasma sample was used to indicate CYP3A activity. A repeated ANOVA model with Gaussian random effects was applied to the log-transformed (natural base) data. Results are presented as quotients (mean, 98.75% CI) based on anti-logarithms of the contrasts for the different occasions. Quotients larger, or smaller, than unity may be associated with enzyme induction or inhibition, respectively.

Results: The effects of the artemisinin antimalarials on the major CYP450 enzymes are summarized in Table 18-16.

Table 18-16. Phenotyping metrics in the five different treatment groups [artemisinin (ART), dihydroartemisinin (DHA), arteether (ARE), artemether (ARM) and artesunate (AS)]. The presented quotients (mean, 98.75% CI) are based on anti-logarithms of the contrast for the different occasions.

Enzyme	Phenotyping metric	Quotients of metric for different occasions ^b	ART	DHA	ARE	ARM	AS
CYP1A2	Paraxanthine/caffeine 4-h concentration ratio	day 1/day -6	0.27 (0.18–0.39) ^a	0.73 (0.59–0.90) ^a	0.70 (0.55–0.89) ^a	0.83 (0.69–1.02)	0.87 (0.69–1.09)
		day 5/day -6	0.59 (0.41–0.85) ^a	0.85 (0.69–1.06)	0.70 (0.55–0.89) ^a	0.81 (0.67–0.98) ^a	1.00 (0.80–1.26)
		day 5/day 1	2.22 (1.54–3.21) ^a	1.17 (0.95–1.45)	1.00 (0.78–1.27)	0.97 (0.80–1.18)	1.16 (0.92–1.45)
		day 10/day -6	1.26 (0.88–1.81)	0.94 (0.76–1.16)	0.84 (0.66–1.06)	1.06 (0.87–1.30)	1.10 (0.88–1.38)
CYP2A6	7-OH-coumarin excreted in 0- to 8-h urine	day 1/day -6	0.74 (0.40–1.40)	1.17 (0.73–1.88)	0.81 (0.38–1.71)	1.01 (0.63–1.62)	0.73 (0.38–1.44)
		day 5/day -6	0.87 (0.48–1.60)	1.34 (0.84–2.14)	0.95 (0.45–2.02)	0.91 (0.57–1.45)	0.60 (0.30–1.17)
		day 5/day 1	1.17 (0.62–2.23)	1.15 (0.71–1.85)	1.18 (0.56–2.51)	0.90 (0.56–1.44)	0.81 (0.41–1.61)
		day 10/day -6	0.96 (0.53–1.74)	1.38 (0.87–2.19)	1.17 (0.55–2.47)	1.22 (0.77–1.94)	0.86 (0.44–1.68)
CYP2C19	S-4'-OH-mephenytoin/S-mephenytoin 4-h concentration ratio	day 1/day -6	0.95 (0.83–1.09)	0.97 (0.78–1.21)	0.93 (0.80–1.08)	0.95 (0.79–1.14)	0.91 (0.73–1.14)
		day 5/day -6	1.69 (1.47–1.94) ^a	1.16 (0.93–1.44)	1.33 (1.15–1.55) ^a	1.20 (1.00–1.44)	1.12 (0.89–1.40)
		day 5/day 1	1.77 (1.54–2.04) ^a	1.19 (0.96–1.49)	1.44 (1.24–1.67) ^a	1.26 (1.05–1.52) ^a	1.22 (0.98–1.53)
		day 10/day -6	1.65 (1.44–1.88) ^a	1.13 (0.91–1.41)	1.26 (1.08–1.46) ^a	1.14 (0.94–1.38)	1.18 (0.94–1.49)
CYP2D6	α -OH-metoprolol/metoprolol 4-h concentration ratio	day 1/day -6	0.82 (0.70–0.96) ^a	0.83 (0.71–0.96) ^a	0.89 (0.75–1.05)	0.90 (0.76–1.05)	0.90 (0.79–1.04)
		day 5/day -6	1.10 (0.94–1.29)	0.95 (0.81–1.10)	1.02 (0.86–1.21)	0.97 (0.82–1.13)	1.02 (0.89–1.18)
		day 5/day 1	1.34 (1.14–1.58) ^a	1.14 (0.99–1.33)	1.15 (0.97–1.37)	1.08 (0.92–1.27)	1.13 (0.99–1.30)
		day 10/day -6	1.15 (0.98–1.34)	0.93 (0.80–1.08)	0.98 (0.83–1.17)	0.92 (0.78–1.09)	1.07 (0.93–1.24)
CYP2E1	6-OH-chlorzoxazone/chlorzoxazone 4-h concentration ratio	day 1/day -6	0.68 (0.54–0.86) ^a	0.93 (0.66–1.31)	1.13 (0.84–1.51)	1.06 (0.85–1.33)	0.96 (0.73–1.26)
		day 5/day -6	0.74 (0.58–0.94) ^a	1.00 (0.70–1.41)	0.99 (0.74–1.32)	1.08 (0.86–1.35)	1.09 (0.83–1.43)
		day 5/day 1	1.08 (0.85–1.38)	1.07 (0.76–1.52)	0.88 (0.66–1.17)	1.02 (0.81–1.28)	1.13 (0.86–1.48)
		day 10/day -6	0.90 (0.71–1.14)	0.83 (0.59–1.17)	1.05 (0.78–1.42)	1.07 (0.85–1.35)	1.03 (0.79–1.36)
CYP3A	1-OH-midazolam/midazolam 4-h concentration ratio	day 1/day -6	1.60 (1.26–2.02) ^a	1.11 (0.94–1.30)	0.97 (0.79–1.20)	1.22 (0.90–1.65)	1.17 (0.94–1.47)
		day 5/day -6	2.66 (2.10–3.36) ^a	1.25 (1.06–1.47) ^a	1.16 (0.94–1.43)	1.54 (1.14–2.09) ^a	1.25 (1.00–1.56)
		day 5/day 1	1.67 (1.31–2.12) ^a	1.13 (0.96–1.33)	1.19 (0.97–1.47)	1.27 (0.93–1.72)	1.06 (0.85–1.33)
		day 10/day -6	1.25 (0.99–1.58)	1.16 (0.98–1.36)	1.12 (0.90–1.38)	1.15 (0.84–1.57)	1.26 (1.01–1.57) ^a

^aP < 0.0125 (α adjusted for multiple testing).

^bQuotients >1 indicate increased enzyme activity, quotients <1 indicate decreased enzyme activity.

The reviewer focused on the effects of AS and DHA, since these moieties are most relevant for this NDA for IV AS. As seen in Table 1, AS inhibits the metabolism of the CYP2A6 substrate, coumarin, and induce the metabolism of the CYP3A substrate, midazolam. The potential for AS to affect the other CYP enzymes was not apparent, as evidenced by the day5/day-6 ratio that centered mostly approximately 1 for the other CYP enzyme drug substrates. DHA appeared to slightly induce the CY2A6 and CYP3A enzyme and inhibit CYP1A2 slightly.

Reviewer's Comments:

- This result of this study should be interpreted with caution because the drug-enzyme effect was evaluated by only one PK sample collected at 4 hours post-dose. The authors should have measured multiple concentrations and calculated the AUC of the probe drug in the presence and absence of artesunate to determine the ratio to evaluate the effect of the drugs on various enzymes.
- It is to be noted that the authors evaluated the effect of oral artesunate on various CYP enzyme substrates. Oral artesunate is not approved in the US. Based on the results of this DDI cocktail study, there appeared to be some evidence that oral AS inhibited the metabolism of the CYP2A6 substrate, coumarin, and induced the metabolism of the CYP3A substrate, midazolam. DHA also showed some evidence to inhibit the metabolism of the CYP1A2 substrate, caffeine and induce the metabolism of the CYP2A6 substrate, coumarin and the CYP3A substrate, midazolam. These levels of respective inhibition and/or induction by AS and DHA is deemed by the reviewer to be no clinical concern. Thus, no dosage adjustments for drugs metabolized by CYP1A2, 2D6, 2C19, 2A6, 2E1 or 3A enzymes when co-administered with IV Artesunate are needed.

Drug-Drug Interaction Analysis of Pyronaridine/Artesunate and Ritonavir in Healthy Volunteers

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Carrie A. Morris, Luis Lopez-Lazaro, Donald Jung, Janthima Methaneethorn, Stephan Duparc, Isabelle Borghini-Fuhrer, Rolf Pokorny, Chang-Sik Shin, and Lawrence Fleckenstein

A multiple dose, parallel group study was conducted to assess for a drug-drug interaction between the pyronaridine/artesunate (PA) combination antimalarial and ritonavir. Thirty-four healthy adults were randomized (1:1) to receive PA for 3 days or PA with ritonavir (100 mg twice daily for 17 days, PA administered on Days 8–10). After enrollment, subjects were randomly assigned in a 1:1 ratio to Arm A or Arm B.

- Subjects in Arm A were administered ritonavir soft gelatin capsules (Norvir; Lot no.: 6008303; Abbott Laboratories, Abbott Park, IL) 100 mg once on Day 1, followed by ritonavir 100 mg every 12 hours on Days 2–17; these subjects were additionally administered three or four PA tablets, depending on weight, once daily on Days 8, 9, and 10. Subjects weighing < 65 kg were administered three PA tablets (180 mg pyronaridine tetrakisphosphate: 60 mg artesunate per tablet) per day; subjects weighing 65 kg or more were administered four tablets per day.
- Subjects in Arm B were administered three or four PA tablets, depending on weight, on Days 1, 2, and 3.

Blood sampling:

In Arm A, plasma samples for analysis of ritonavir concentrations were collected on Days 1 and 10 at pre-dose and 1, 2, 3, 4, 6, 8, 9, 12, 18, and 24 hours post-dose. Plasma samples for artesunate/DHA analysis were collected on Day 10 at pre-dose, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, and 12 hours post-dose. Blood samples for pyronaridine analysis were collected on Day 10 at pre-dose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours after the last dose and at 2, 3, 5, 12, 19, 26, 33, and 40 days after the last dose. In addition, trough concentrations for all analytes were assessed on study Day 8 (pre-dose for pyronaridine and artesunate/DHA) and Day 9 before the morning dosing.

In Arm B, plasma samples for artesunate/DHA analysis were collected on Day 3 at pre-dose, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, and 12 hours post-dose. Blood concentrations for pyronaridine were collected on Day 3 at pre-dose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours post-dose, and at 2, 3, 5, 12, 19, 26, 33, and 40 days post-dose. Trough concentrations of pyronaridine, artesunate, and DHA were assessed on study Days 1 and 2 before morning dosing.

Drug analysis

A validated liquid chromatography-mass spectrometry analytical method for determination of artesunate, DHA, and ritonavir was developed.

Results:

The PK parameters of pyronaridine+artesunate when given with and without ritonavir are given in Table 18-17. The AS and DHA pharmacokinetic parameters in the two study arms are presented in Table 18-18. The 90% CI for the ratios of geometric means of the PK parameters for pyronaridine, AS and DHA are shown in Table 18-19.

Table 18-17. Pharmacokinetic parameters of pyronaridine in subjects administered pyronaridine/artesunate (PA) + ritonavir (Arm A) or administered PA only. C_{max} and area under the curve (AUC) values are normalized to 9.72 mg/kg†

	Arm A (PA + ritonavir) [N = 17]	Arm B (PA only) [N = 16]
Half-life (days)	14.2 (50.3%)‡	13.1 (20.5%)
T _{max} (days)	0.078 (65.5%)	0.059 (25.7%)
C _{max} (ng/mL)	462.0 (28.5%)	377.2 (42.6%)
C _{trough} (ng/mL)	119.2 (47.5%)	114.1 (36.8%)
AUC _{0-t} (day*ng/mL)	955 (38.0%)§	1059 (34.7%)
AUC _{0-inf} (day*ng/mL)	1212 (24.3%)‡	1248 (34.4%)
AUC _{0-tau} (day*ng/mL)	222 (36.5%)	210 (36.8%)

† All values given as geometric mean (geometric %CV).

‡ N = 15.

§ N = 16.

Table 18-18. Pharmacokinetic parameters of artesunate and dihydroartemisinin (DHA) in subjects administered pyronaridine/artesunate (PA) + ritonavir (Arm A) or administered PA only*

	Artesunate		DHA	
	Arm A (PA + ritonavir) [N = 17]	Arm B (PA only) [N = 16]	Arm A (PA + ritonavir) [N = 17]	Arm B (PA only) [N = 16]
Half-life (hours)	0.410 (28.0%)†	0.433 (39.5%)‡	2.04 (52.6%)¶	2.18 (42.4%)§
T _{max} (hours)	1.05 (61.4%)	0.75 (54.2%)	1.77 (40.5%)	1.39 (29.1%)
C _{max} (ng/mL)	95.1 (108%)	95.5 (61.9%)	539.5 (63.9%)	734.3 (37.6%)
AUC _{0-t} (hours*ng/mL)	120 (86%)	105 (55.5%)	1151 (61.2%)	1790 (35.1%)
AUC _{0-inf} (hours*ng/mL)	157 (70.5%)†	124 (49.3%)‡	1164 (63.9%)¶	1887 (34.4%)§
AUC _{0-tau} (hours*ng/mL)	157 (70.5%)†	124 (49.1%)‡	1162 (63.8%)¶	1884 (34.2%)§

*C_{max} and area under the curve (AUC) values are normalized to an artesunate dose of 3.25 mg/kg. All values given as geometric mean (geometric %CV).

† N = 9.

‡ N = 13.

¶ N = 16.

§ N = 14.

Table 18-19: Geometric mean ratios (90% confidence intervals [CI]) for the pharmacokinetic parameters of pyronaridine, artesunate, and DHA after administration with ritonavir (Arm A) or without ritonavir (Arm B).

	Ratio: Arm A/Arm B (90% CI)		
	Pyronaridine [N = 33]	Artesunate [N = 33]	DHA [N = 33]
C _{max}	1.2255 (0.9983, 1.5045)	0.9954 (0.6408, 1.5462)	0.7348 (0.5500, 0.9815)
AUC _{0-t}	0.9020 (0.7277, 1.1181)*	1.1470 (0.7833, 1.6797)	0.6432 (0.4875, 0.8486)
AUC _{0-inf}	0.9717 (0.8132, 1.1610)†	1.2707 (0.8481, 1.9038)‡	0.6169 (0.4562, 0.8343)¶
AUC _{0-tau}	1.0590 (0.8589, 1.3058)	1.2691 (0.8476, 1.9001)‡	0.6170 (0.4565, 0.8338)¶

*N = 32.

†N = 31.

‡N = 21.

¶N = 30.

AUC = area under the curve.

As seen in Table 3, the point estimates for the ratios of geometric means indicate that on average, ritonavir coadministration was associated with decreases in DHA C_{max}, AUC_{0-t}, and AUC_{0-inf} of ~27%, 36%, and 38%, respectively. The authors concluded that ritonavir coadministration decreases exposure to DHA. This finding is of clinical relevance because currently available ACTs contain artesunate or artemether, which are metabolized to DHA, or DHA itself. The reduced exposure to DHA associated with ritonavir coadministration poses an increased risk of suboptimal antimalarial efficacy; the clinical effects of reduced exposure may be augmented by the immunocompromised state of the patient population receiving ritonavir therapy. Pharmacokinetic parameters for ritonavir are shown in Table 18-20.

Table 18-20. Pharmacokinetic parameters of ritonavir from Day 1 and Day 10*. Day 1 represents Ritonavir alone and Day 10 represents Ritonavir coadministered with PA.

	Day 1 [N = 17]	Day 10 [N = 17]
Half-life (hours)	6.46 (27.3%)	3.96 (25.1%)
T _{max} (hours)	3.46 (51.6%)	2.27 (39.5%)
C _{max} (ng/mL)	492 (81.1%)	2751 (52.4%)
C _{12hr} (ng/mL)	147.7 (59.3%)	416 (57.7%)
AUC _{0-12hr} (hours*ng/mL)	3072 (73.8%)	14825 (51.8%)
AUC _{0-inf} (hours*ng/mL)	4633 (62.4%)	
AUC _{0-24hr} (hours*ng/mL)	4127 (65.2%)	

*C_{max} and area under the curve (AUC) values are adjusted for 1.4 mg/kg q12hr dose. All values given as geometric mean (geometric %CV).

Coadministration of PA resulted in substantially increased exposure to ritonavir, suggesting that either total body clearance decreased and/or the extent of ritonavir absorption increased. From *in vitro* evidence, pyronaridine is an inhibitor of CYP2D6, which is involved in the metabolism of ritonavir, and of P-gp, which is involved in the efflux transport of ritonavir. Either CYP2D6 or P-gp inhibition could produce the observed increase in exposure. If the increase in exposure stemmed primarily from CYP2D6 inhibition, some increase in ritonavir half-life on Day 10, as

compared with Day 1, would be anticipated. However, half-life decreased, suggesting that the increased ritonavir exposure may have stemmed primarily from pyronaridine inhibition of P-gp mediated ritonavir efflux. It should be noted that the design of this study does not allow for effects of PA on ritonavir pharmacokinetics to be differentiated from any intrinsic time-dependency of ritonavir pharmacokinetics.

Reviewer's Comments:

- This study shows that coadministration of ritonavir modestly decreased the systemic exposure (C_{max} and AUC) of DHA and slightly increased the systemic to artesunate (AS). However, it is to be noted that the authors evaluated oral artesunate in this study, which is not approved in the US. This effect of reduced DHA exposure oral artesunate following coadministration with ritonavir could be due to induction effect of ritonavir on DHA metabolism via CYP3A, 1A2, 2C9, 2C19 and 2B6 (US PI for ritonavir).
- The combination of pyronardine + artesunate (PA) substantially increased the PK exposure (C_{max} and AUC) of ritonavir. The reviewer agrees with the authors' interpretation of the mechanism for this interaction due to pyronaridine inhibition of P-gp.
- Based on published literature, ritonavir decreased the C_{max} and AUC_(0-inf) of DHA following co-administration with oral artesunate by approximately 27% and 38%, respectively, in healthy adult subjects. It is expected that co-administration of ritonavir with IV artesunate will also decrease the C_{max} and AUC of DHA. Therefore, ritonavir should not be co-administered with IV artesunate.

Nevirapine-Based Antiretroviral Therapy Impacts Artesunate and Dihydroartemisinin Disposition in HIV-Infected Nigerian Adults

AIDS Research and Treatment Volume 2012

Fatai A. Fehintola, Kimberly K. Scarsi, Qing Ma, Sunil Parikh, Gene D. Morse, Babafemi Taiwo, Ibrahim Tope Akinola, Isaac F. Adewole, Niklas Lindegardh, Aphiradee Phakderaj, Oladosu Ojengbede, Robert L. Murphy, Olusegun O. Akinyinka, and Francesca T. Aweeka

The authors conducted a parallel-group comparison to explore the pharmacokinetic interactions between nevirapine (NVP) and oral artesunate taken in combination with amodiaquine in asymptomatic 21 HIV-infected Nigerian adults by evaluating the disposition kinetics of artesunate and DHA in the presence and absence of steady-state NVP.

Artesunate/amodiaquine 200/600 mg was given daily for three days.

Patients were recruited into two groups: (1) NVP group and (2) control group.

- 10 Patients in the NVP group were on the same anti-retroviral therapy (ART), consisting of lamivudine (3TC) 150 mg, zidovudine (AZT) 300 mg, and NVP 200 mg taken twice a day for a minimum period of 8 weeks prior to study enrolment
- 11 Patients in the control group were not yet receiving ART.

In both groups of patients, artesunate-amodiaquine 200/600 mg was given daily for three days.

Measurement of drug concentrations occurred between 0 and 96 hours after the final dose.

Pharmacokinetic parameters were determined using noncompartmental analysis.

Blood sampling:

Samples for the determination of artesunate plasma concentrations were collected according to the following schedule: predose (0h) on the third day, and 0.5 h, 1 h, 1.5 h, 2h, 3h, 4h, 6h, 8h, 10h, 12h, 24h, 48h, 72h, and 96h after the 3rd and last dose of artesunate-amodiaquine.

Drug analysis

A validated liquid chromatography-mass spectrometry analytical method for determination of artesunate, and DHA was developed.

Results: Pharmacokinetic parameters of artesunate and DHA are presented in Table 18-21.

Table 18-21. PK parameters of artesunate and dihydroartemisinin following administration of oral artesunate with and without nevirapine

	Nevirapine Group, n = 10 Mean (SD)	Control Group, n = 11 Mean (SD)
Artesunate		
C _{max} (µg/ml)	108 (42)	71 (57)
AUC ₀₋₉₆ (µg * L/h)	105 (31)	69 (26)
T _{max} (h)	1.0 (0.5–2.0)	1.0 (0.5–1.5)*
Cl/F (L/h)	1950 (543)	2995 (1180)

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

	Nevirapine Group, n = 10 Mean (SD)	Control Group, n = 11 Mean (SD)
Vd/F (L)	1162 (856)	4525 (3535)
T1/2 (h)	0.4 (0.3)	1.1 (0.9)
DHA		
Cmax (µg/ml)	298 (107)	507 (429)
AUC ₀₋₉₆ (µg * L/h)	603 (218)	883 (607)
Tmax (h)	1.0 (0.5-3.0)	1.5 (0.5-6.0)
Cl/F (L/h)	1130 (425)	980 (616)
Vd/F (L)	2405 (1077)	4338 (2518)
T1/2 (h)	1.6 (0.8)	3.2 (1.4)

Tmax is presented as median

The authors reported an increase in artesunate AUC₀₋₉₆ in patients receiving NVP compared to those not on ART (105 versus 69 µg-L/hr; respectively; p-value =0.02) and no statistically significant difference in the DHA AUC₀₋₉₆ between the two groups (603 vs 883 µg-L/hr, p value =0.19). There was no statistically significant difference in the Cmax between the two groups of patients for both artesunate and DHA (p-value = 0.15). Notably the variability is very high for Cmax and AUC for both AS and DHA in the control group of patients.

Reviewer's Comments:

- It is to be noted that the authors evaluated the effect of NVP on the PK of oral artesunate. Oral artesunate is not approved in the US. NVP reduced mean DHA AUC by 68% and mean DHA Cmax by 59%. This reduction is likely to result in the loss of efficacy of artesunate. Based on this reduction of systemic exposure observed in this study, IV Artesunate should not to be given with NVP. The package insert for IV Artesunate should include language recommending that NVP should not be coadministered with IV Artesunate.

Pharmacokinetics and Pharmacodynamics of Intravenous Artesunate During Severe Malaria Treatment in Uganda

Open Access Journal: <http://www.malariajournal.com/content/11/1/132>

Pauline Byakika-Kibwika, Mohammed Lamorde, Jonathan Mayito, Lillian Nabukeera, Harriet Mayanja-Kizza, Elly Katabira, Warunee Hanpithakpong, Celestino Obua, Nadine Pakker, Niklas Lindegardh, Joel Tarning, Peter J de Vries and Concepta Merry

The aim of this study was to assess the pharmacokinetics and pharmacodynamics of IV artesunate in Ugandan adults with severe malaria. Fourteen adults with severe malaria, admitted to Mulago National Referral Hospital, Kampala, Uganda were enrolled in this trial. All participants received intravenous artesunate at a dose of 2.4 mg/kg at time 0, 2.4 mg/kg at 12 hours and 2.4 mg/kg/daily until they could tolerate oral therapy.

Blood sampling:

Blood for artesunate (AS) and dihydroartemisinin (DHA) assays was drawn after the initial dose of artesunate at 0 (pre-dosing), 5, 10, 15, 30, 45 minutes, 1, 1.5, 2, 3, 4, 5, 6, 8- and 12-hours post-dosing.

Drug analysis

A validated liquid chromatography-mass spectrometry analytical method for determination of artesunate, and DHA was developed.

Results:

Pharmacokinetic parameters of AS and DHA are presented in Table 18-22. Following IV AS administration, the C_{max} for AS and DHA were observed rapidly post-dose administration indicating rapid conversion of AS to DHA. Artesunate was cleared from circulation rapidly while DHA had a slightly longer elimination T_{1/2}. Although the peak concentration of AS was higher than that of DHA, the AUC of DHA was more than four times that of AS. The pharmacokinetic parameters observed in the present study are similar to findings from previous studies in malaria patients.

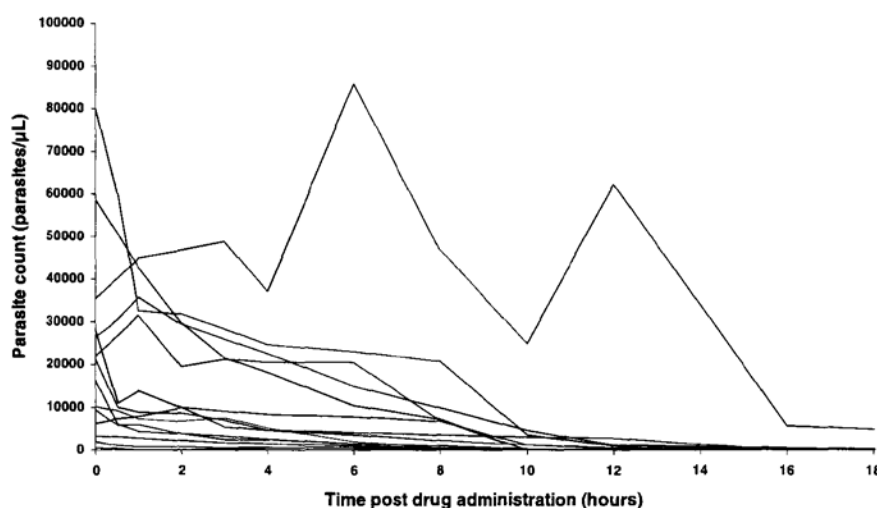
Table 18-22. Pharmacokinetic parameters of artesunate and dihydroartemisinin following IV administration of 2.4 mg/kg artesunate once a day for 3 days in 14 patients with severe malaria

Parameter	Artesunate	Dihydroartemisinin
	Median (range)	Median (range)
C_{max} (ng/ml)	3260 (1020-164000)	3140 (1670-9530)
AUC_{0-last} (ng·h/ml)	727 (290-111256)	3492 (2183-6338)
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)

Clinical response

All study participants tolerated IV AS very well and reported very rapid recovery from symptoms and ability to take oral medication after 24 hours. No immediate adverse events were recorded. The median (range) parasite clearance time was 17 (8 – 24) hours. Individual plots of parasite density against time post start of treatment are shown in Figure 18-7. Parasite clearance time was comparable to that from a previous study and shorter than a median of 66 hours from other studies.

Figure 18-7. Individual plots of parasite density against time post start of treatment



Reviewer's Comments:

- The PK parameters of both AS and DHA in patients with severe malaria reported in this study are comparable to those reported in other literature articles.
- The authors indicated that there was no correlation between AS or DHA exposure with parasite clearance times (Spearman's rho correlation coefficient -0.12 and -0.18 respectively). Further details of this analysis were not presented in this paper.

18.4 Pharmacometrics review of the proposed artesunate dosing in pediatric patients

Introduction

The Applicant relied on published literatures of population pharmacokinetics (PK) of parenteral artesunate (intravenous or intramuscular injections) to support the proposed artesunate dose modification in patients weighing <20 kg. Literature cited by the Applicant (and other published population PK analyses for artesunate/dihydro-artemisinin) were reviewed to assess their suitability for recommending the weight-based dosing. Table 18-23 summarizes population PK studies that were independently reviewed. A brief review of the studies follows.

Table 18-23. Population pharmacokinetic studies of artesunate that were reviewed by the pharmacometrics reviewer.

Author	Population	Subject characteristics Median (Range)	Doses	PK sampling designs	Sample handling	Bioassay method
Hendriksen et al., 2013	Children <= 14yrs (n = 70)	Age (yrs): 2.5 (0.6 - 11) Weight (Kg): 10.8 (9 – 13.5)	I.V: 2.4 mg/Kg at 0, 12, 24, 48, 72, then ALu	Five samples at baseline, 0-1hr, 1-4hr, 4-12hr, and 12-24hr.	- Collect in pre-chilled fluoride oxalate tubes - Immediate centrifuge at 4°C - Storage at -80°C, - Transport on dry ice	LC-MS (Hanpithakpong W. et al., 2008)
Zaloumis, 2014: POPPK of IV AS	Pooled PK data from 6 studies: Children (n = 195) and adults (n = 71)	Children Age (yrs): 3.5 (0.5 – 9.3) Weight (Kg): 13 (6-29) Adults Age (yrs): 24 (16 - 65) Weight (Kg): 50 (33 - 75)	I.V: 2.4 mg/Kg 4 mg/Kg I.R: 10 mg/Kg 20 mg/Kg I.M: 2.4 mg/Kg	Intensive sampling; typically, at 0, 5, 10, 15, and 30 minutes and at 1, 2, 4, 6, 8, and 12 hours post-dose. Other times were at 1, 3, and 6 minutes post dose	- Collect in pre-chilled heparinized tubes OR fluoride oxalate tubes - Immediate centrifuge - Storage at -20, OR -70, OR -80°C - Transport on dry ice	LC-MS (Teja-Isavadham et al., 2010, Maude et al., 2009) HPLC-ECD (Navaratnam V. et al., 1997) HPLC – UVD (Batty K, et al 1998)

Population Pharmacokinetics of Intramuscular artesunate in African children with severe malaria (Hendriksen ICE et al., 2013)

The published article by Hendriksen ICE et al., (2013)¹ describes population PK of artesunate and its metabolite dihydro-artemisinin in 70 Tanzanian children of ages 6 months to 11 years. All the children had severe malaria and received a 2.4mg/kg intramuscular injection of artesunate at 0, 12, and 24 hours. PK sampling was sparse as shown in Table 18-23. Population PK modeling using sparsely collected samples may limit identification of covariates for model parameter as information provided by the data may not be enough to characterize between subject variability (BSV) of the parameters. For the case of artesunate and dihydro-artemisinin, the 5 sparsely collected samples may be enough to characterize BSV as the 2 compounds have short half-life and follows a 1-compartment disposition kinetics.

Blood samples were adequately handled to prevent ex-vivo hydrolysis and degradation of artesunate. Artesunate is an unstable compound which, at room temperatures, undergoes hydrolysis to dihydro-artemisinin ex-vivo, and if hemolysis occurs, degrades upon contact with exposed heme. Proper sample collection tubes, immediate separation of plasma and cold storage, ensures that correct plasma exposures of artesunate and dihydro-artemisinin were assayed. The bioanalytical assay method was adequately developed and validated as reported by Hanpithakpong W. et al., (2008)².

Population pharmacokinetic modeling followed appropriate pharmaco-statistical principles. One and 2-compartment disposition models for artesunate and dihydro-artemisinin were tested, and one compartment model adequately described the data. Principles of allometry were considered as weight was included as a covariate on clearance and volume parameters. Despite a large percent of data falling below the limit of quantitation (BLQ), omission of this data did not cause model misspecification. The final model adequately predicted the probability of BLQ data. Stepwise covariate model building was used with forward inclusion of covariates at the significance level of $\alpha = 0.05$ and backward elimination at the significance level of $\alpha = 0.001$. Model building was guided by graphical analysis of goodness of fit, visual predictive check, and physiological plausibility of covariates and precision of parameter estimates (Parameter uncertainties). In addition to body-weight, hemoglobin was the only other identified covariate; Dihydro-artemisinin clearance increased by 10% per unit (g/dL) decrease of hemoglobin. Assessment of clearance maturation did not identify age as a covariate for clearance. Based on visual predictive check, the final model described the data well and was suitable for prediction of exposures at different doses of artesunate.

The population PK model was used to simulate artesunate and dihydro-artemisinin exposure in children with different body-weights from 6-25 kg. The simulations indicated that at 2.4 mg/kg dosing, children with body weights between 6 – 10 kg had a mean reduction of 20.4% in dihydro-artemisinin exposure compared to children with body-weight between 21-25 kg. This implies that patients with a body weight between 6-10 kg require a 20% higher dose to match total drug exposure over time to heavier children weighing 21-25 kg. Hendriksen ICE et al., proposed weight-band based dosing as indicated in Table 18-24.

Table 18-24. Proposed body weight–adjusted dosing regimen for IM artesunate.

Weight (kg)	Dose i.m. (mg)	Prepared solution (ml) ^a	Dose i.m. (mg/kg)
6–7	20	2	2.86–3.33
8–9	25	2.5 ^b	2.78–3.13
10–11	30	3 ^b	2.73–3.00
12–13	35	3.5 ^b	2.69–2.92
14–16	40	2	2.50–2.86
17–20	50	2.5	2.50–2.94
21–25	60	3	2.40–2.86

Source: Hendriksen et al., (2013), Clin Pharm & ther, vol 93, issue 5, page 447.

Population Pharmacokinetics of Intravenous artesunate (Zaloumis SG et al., 2014)

The published article by Zaloumis SG et al., (2014)³ describes the population pharmacokinetics of dihydro-artemisinin in adults and children with severe malaria after intravenous administration of artesunate. Patient data (adult and children) were obtained from 6 different studies with diverse study designs and dosing schemes^{4–9}. The PK data were pooled from the different studies for pharmacokinetic analysis. Patients received either intramuscular (I.M), intra-rectal (I.R), or intravenous (I.V) artesunate depending on study specific designs and dosing schemes. Only PK data following I.V artesunate were used for population PK modeling; this included I.V artesunate data collected in second periods for cross-over trials.

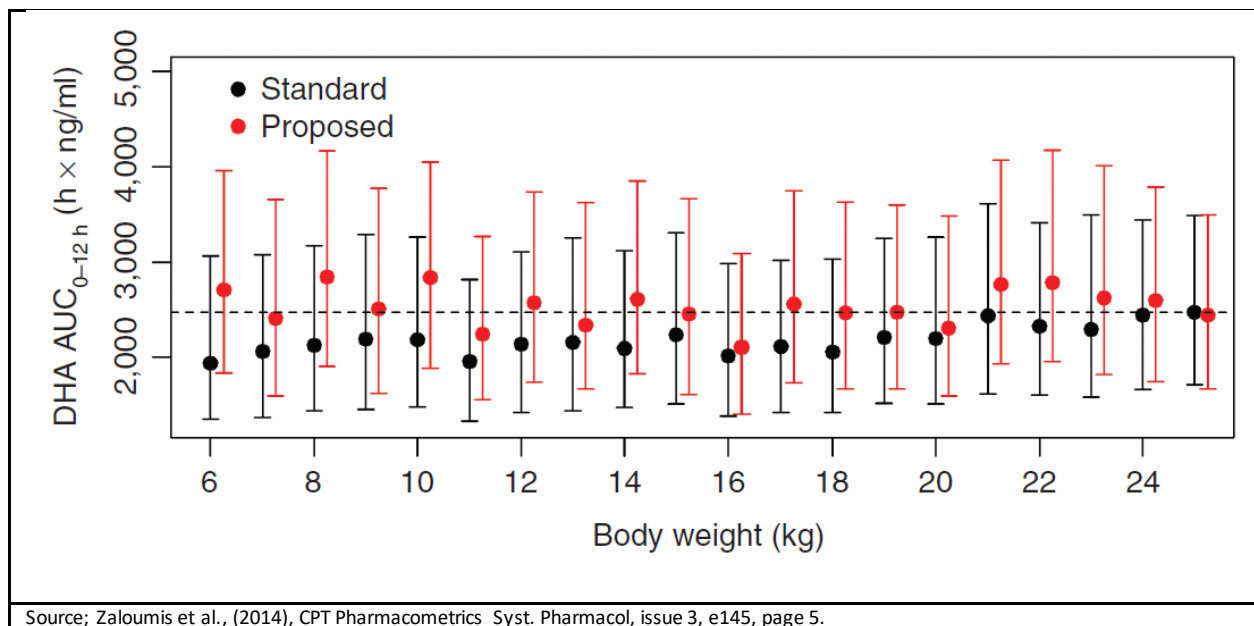
In all studies blood samples for PK analysis were intensively sampled at times that were specific for each study. Methods for sample handling and bioassay were different among the studies as indicated in table 1. In general, samples were appropriately collected and handled to prevent ex-vivo degradation of artesunate in all the studies.

Appropriate pharmaco-statistical steps were followed in developing the population PK model. Due to limited artesunate concentration data, only dihydro-artemisinin concentrations were analyzed. The model assumed bolus administration of dihydro-artemisinin instead of modeling conversion of artesunate to dihydro-artemisinin; this was a reasonable strategy since maximum concentration of dihydro-artemisinin was observed within minutes of I.V artesunate. The structural model consisted of one-compartment disposition kinetics, allometric scaling volume of distribution, and a sub-model for allometrically scaled age-dependent maturation of enzymatic clearance. The sub-model for maturation of enzymatic clearance is supported by similar practice in literature where by a similar sub-model has been used to describe maturation of UGT mediated morphine clearance. The sub-model was also supported by a significant decrease in the objective function ($\Delta\text{OFV} = 9.3$). Ignoring BLQ samples did not cause model misspecification, and BLQ samples could be predicted by the final model.

In a separate sensitivity analysis, PK data collected in period 2 of cross-over trials were excluded. This resulted in identifying baseline hemoglobin and body temperature as covariates for clearance. Dihydro-artemisinin clearance increased by 4% per unit (g/dL) decrease of hemoglobin and increased by 7% per unit increase in body temperature.

The final population PK model was used to simulate dihydro-artemisinin exposure after I.V 2.4 mg/Kg to each body weight from 6-25 kg (1-kg increments). A virtual pediatric population for simulation was created by sampling age from weight-dependent uniform distributions. It was found that children 6 – 10 kg had 13.7% less exposure compared with children 21-25 Kg. Zaloumis SG et al., further simulated exposure using adjusted dosing regimens proposed by Hendriksen ICE et al. The I.M dosing regimens proposed by Hendriksen ICE et al., were found to be applicable to I.V artesunate as they resulted into similar exposures in all weight bands as shown in Figure 18-8.

Figure 18-8. 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. (red).



Source; Zaloumis et al., (2014), CPT Pharmacometrics Syst. Pharmacol, issue 3, e145, page 5.
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

Reviewer's Analysis

In both studies, data collection, handling, bioassay and pharmacokinetic modeling were appropriately performed. However, the method used by Zaloumis et al. to create the virtual population is not appropriate as it eliminates correlation between age and body weight within the weight bands. For this reason, the reviewer performed simulations investigate of the effect of weight and age on dihydro-artemisinin exposure.

Based on the proposed weight-based dosing of artesunate, the reviewer performed an independent simulation of dihydro-artemisinin exposure in children 6-25 kg using the final population PK model developed in a sensitivity analysis by Zaloumis SG et al. This model was selected as it describes dihydro-artemisinin PK in severe malaria patients before convalescence. A virtual pediatric population was created from the CDC growth charts (https://www.cdc.gov/growthcharts/percentile_data_files.htm). The charts for boys and girls (0-36 months and 0-204 months) were used to create 10,000 individuals through bootstrap resampling with replacement. Weights and heights of the individuals were obtained as follows: For each individual in the bootstrapped dataset, the 3rd and 97th percentiles (of weight or height) were transformed to normality (Box-cox transformation). Then a value within the two values was randomly sampled and back transformed to obtain a weight or height for individuals. The resulting virtual population had 3rd and 97th percentiles of weight-by-age (or height-by-age) that were similar to the CDC growth charts. Hemoglobin values were sampled from a normal distribution with mean and standard deviation of 8.5 g/dL and 2.4g/dL respectively in children with severe malaria (These parameters were reported by Kremsner et al., (2016).

Figure 2 shows the distribution of dihydro-artemisinin exposure by weight-based dosing (3 mg/kg for children 2 – 20 kg and 2.4 mg/kg for children 21 – 25 kg). The figure show that the proposed dosing provides approximately 20% - 70% over-exposure in children 6-10 kg compared to children with 20-25 kg body weight. Children less than 6 kg had up to twice over-exposure compared to children 21-25 Kg. The simulated over-exposure is a result of age-dependent enzyme maturation effect in the simulation model. Inclusion of maturation effect in the model imply that neonates and infants have immature UGT enzymes (UGT1A9 and UGT2B7) and therefore lower clearance per body weight compared to older children. It is not clear at which age the UGT1A9 enzyme expression/activity in infants and children reaches adult level. Some literature suggest that maximal adult abundance is already reached between 4 months and 1.5 years after birth for UGT1A9 and UGT2B7¹⁰⁻¹³. Other literature suggests that protein abundance of UGT1A9 and UGT2B7 reaches half-maximal by 8.2 and 2.6 years respectively¹⁴. The CDC growth chart indicates that pediatric subjects weighting 6 kg to 10 kg are in the age range of 3 month to 1 year. The minimum age in the pediatric dataset analyzed by Zaloumis was 6 months. Due to uncertainty in the age at which adult UGT1A9 activity is attained, it is unclear whether the age-dependent enzyme maturation model in Zaloumis's analysis is justified. Nevertheless, considering the potential for over-exposure with longer than 6-month enzyme maturation time, the reviewer performed simulations to quantify over-exposure predicted by the age-dependent maturation model. Although the model was developed to

describe growth and maturation of renal function²², it was used in this analysis to describe growth and maturation of total clearance of DHA. This choice is based on observation of similarity between time course of GFR-maturation and time course of clearance-maturation of drugs metabolized by UGT enzymes²³. Furthermore, in population PK analyses by Hendriksen et al., and Zaloumis et al., the maturation model consistently showed improvement in model fit (although not statistically significant) over the allometric model. In addition to the empirical age dependent maturation model, the reviewer also assessed a physiological based DHA hepatic clearance model. The model was derived from literature data on invitro DHA metabolism, age dependent expression of UGT 1A9 relative to adult expression, liver microsomal protein quantity, hepatic blood flow, and plasma albumin binding. However, the model was not useful for extrapolation of exposure in neonates as it was unable to predict DHA clearance in adults: The model derived hepatic clearance in adults ($V_{max} = 177$ pmol/min/mg protein, $K_m = 90$ μ M) was about 10 times lower than the estimate by Zaloumis et al (0.073L/h/Kg vs 0.866L/h/Kg).

Taking age-dependent maturation effect into account, we performed simulation of dihydro-artemisinin exposure based on studied artesunate dose of 2.4 mg/kg for all children 2 – 25 kg. As shown in figure 3, based on this simulation, children 6-10 kg are 5% - 40% over exposed compared to children 21 – 25 kg while children less than 6 kg (neonates and infants less than 3-month-old) were up to 90% over-exposed. We also simulated dihydro-artemisinin exposure using a model without maturation effect. The results are shown in figure 4. As indicated in this figure, patients weighing 2-10 Kg have on average 25% less exposure compared to patients weighing 21-25Kg.

Artesunate dosing recommendation in infants depends on whether age-dependent maturation of UGT enzyme is considered. Hendriksen et al. ignored age-dependent maturation effect, and as a result recommended increasing artesunate dose in children 6 – 20 kg. However, our simulation indicates that, if age-dependent maturation is considered, artesunate dose of 2.4 mg/kg results into comparable dihydroartemisinin exposure in children 10 - 25 kg while children 2 – 9 kg are over-exposed.

²² Hayton, W. L. Maturation and growth of renal function: Dosing renally cleared drugs in children. *AAPS PharmSci* **2**, 22–28 (2000)

²³ Anderson, B. J. & Holford, N. H. G. Tips and traps analyzing pediatric PK data: Tips and traps. *Pediatr. Anesth.* **21**, 222–237 (2011)

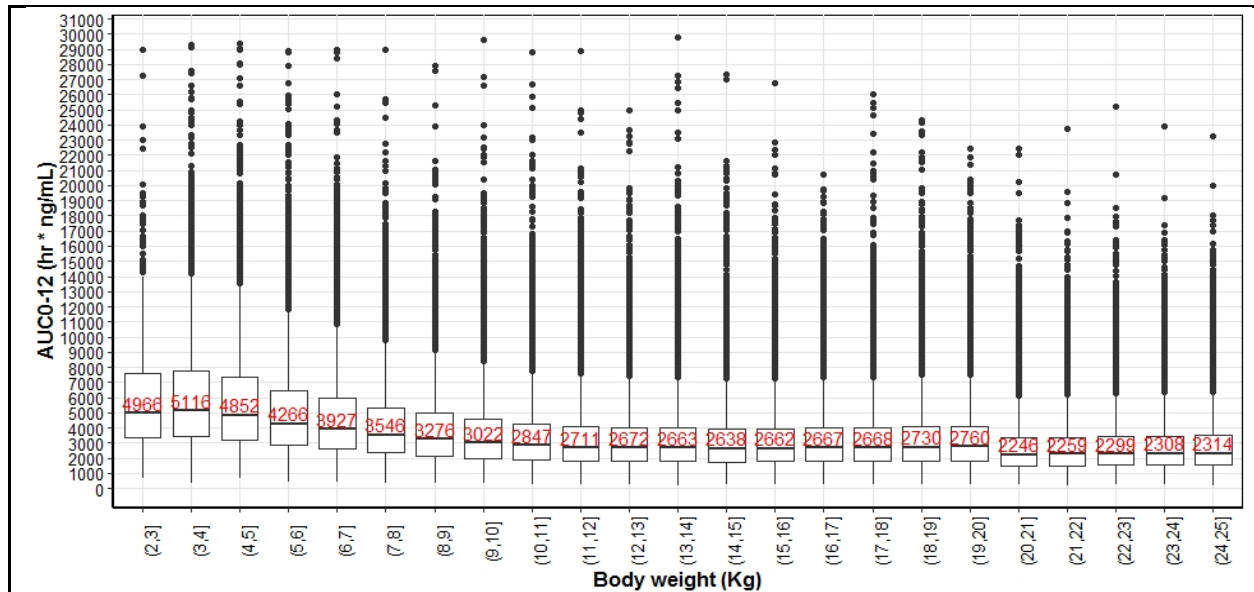


Figure 18-9. Simulated DHA exposure after first AS dose (3 mg/kg in children 2-20 kg and 2.4 mg/kg in children 20-25 kg).

Each box shows the 25th and 75th percentile of the DHA exposure measure. The bar i inside the box represents the median at its associated value, whiskers represent 1.5 times the interquartile range, and closed circles represent outliers. The minimum number of virtual subjects ranged between 1,000 to 15,000 to maintain covariate proportionality.

Source: Pharmacometrics reviewer’s independent simulation.

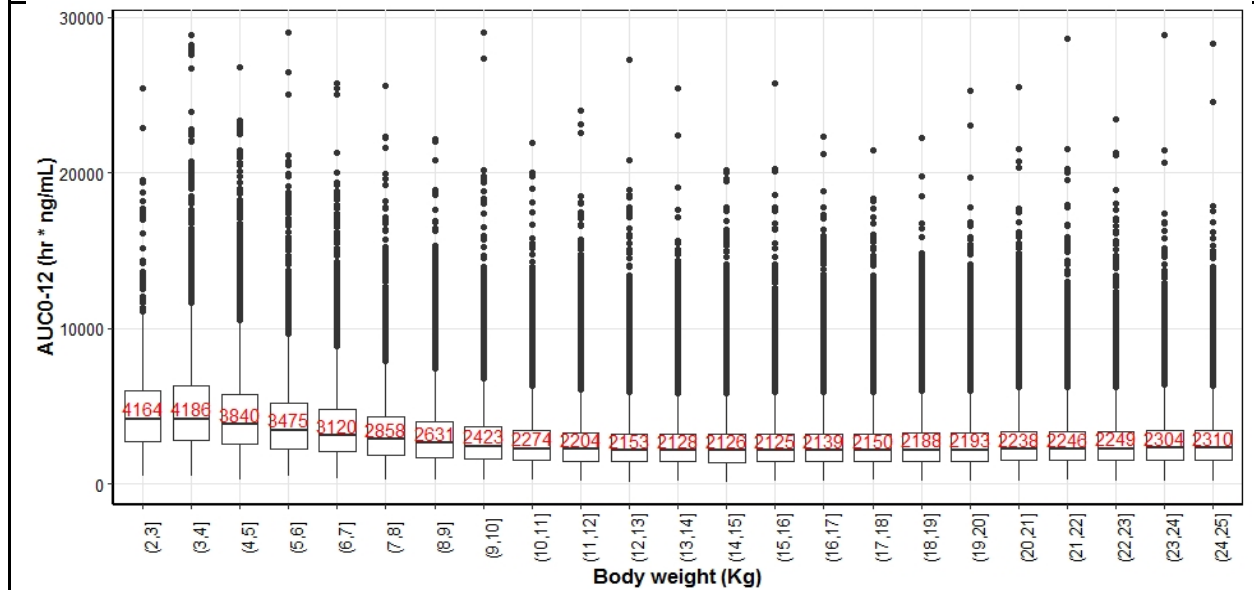


Figure 18-10. Simulated DHA exposure after first AS dose of 2.4 mg/kg regardless of body weight.

Each box shows the 25th and 75th percentile of the DHA exposure measure. The bar i inside the box represents the median at its associated value, whiskers represent 1.5 times the interquartile range, and closed circles represent outliers.

Source: Pharmacometrics reviewer’s independent simulation.

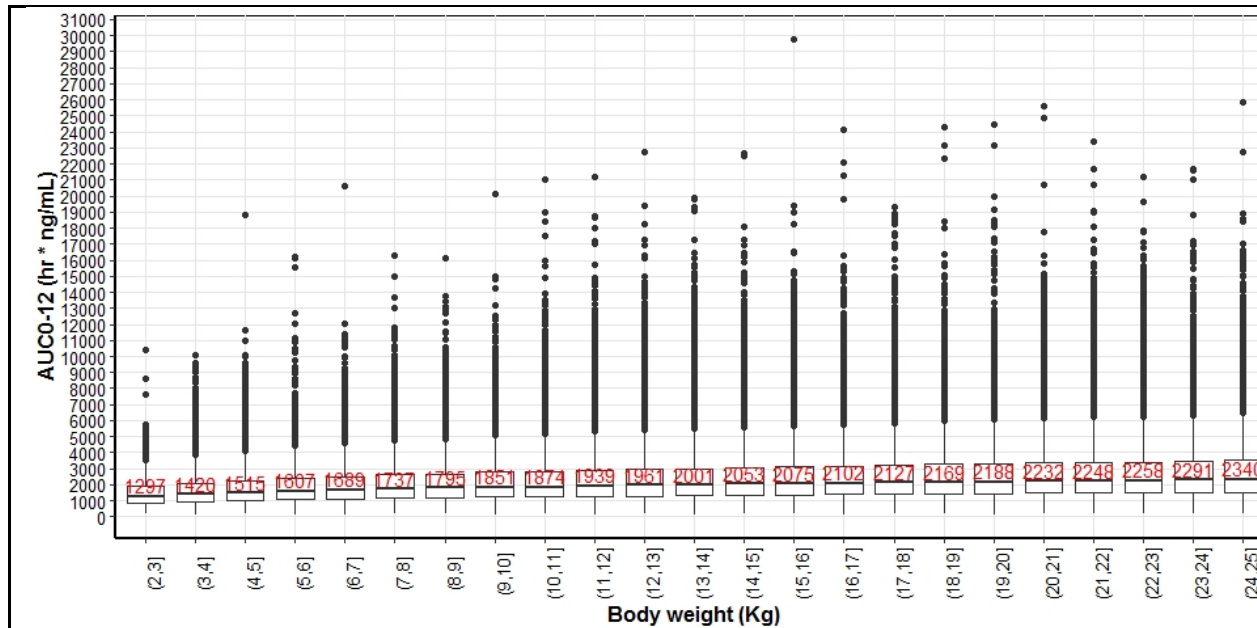


Figure 18-11. DHA exposure simulated using a model without maturation effect. The exposure is after an IV AS dose of 2.4 mg/kg regardless of body weight.

Each box shows the 25th and 75th percentile of the DHA exposure measure. The bar i inside the box represents the median at its associated value, whiskers represent 1.5 times the interquartile range, and closed circles represent outliers.

Source: *Pharmacometrics reviewer’s independent simulation.*

Conclusion: The estimated total systemic exposure (AUC) of DHA in virtual patients is sensitive to the method used to create the virtual patient population. After accounting for the correlation between age and body weight within the weight bands, simulations considering the age-dependent maturation effect do not support increasing the AS dose from 2.4 mg/kg to 3 mg/kg in children 2 kg to 20 kg based on a DHA population PK meta-analysis.

18.5 Clinical Microbiology Appendix

The nonclinical studies supporting mechanism of action, activity in vitro and in malaria animal models, drug resistance and cross-resistance are summarized.

18.5.1 Mechanism of Action

The precise mechanism by which artesunate, DHA and other artemisinins exhibit activity against *Plasmodium* species is not clearly understood. Studies using various derivatives of artemisinin, suggest a role of endoperoxide bridge in exhibiting anti-plasmodial activity. There appears to be an effect on the generation of superoxide anions, lipid peroxidation and protein alkylation. There is variability in uptake of the drug by the different development forms of the parasite within the red blood cells (RBCs). Some of the studies supporting the mechanism of action of artesunate, DHA, as well as artemisinin, including potential parasite targets, are summarized.

18.5.1.1 Role of the endoperoxide bridge structure and activation by heme

Brossi *et al.* ([Brossi et al. 1988](#)) reported that artemisinin, DHA and arteether (with endoperoxide bridge) are more active (≥ 142 -fold) than their deoxy derivatives (without the endoperoxide bridge) against the D6 strain of *P. falciparum*. Similar observations were reported in other studies. For example, Avery *et al.* ([Avery et al. 1993](#)) reported that the relative activity of the analogs of artemisinin was 1000- and $>10,000$ -fold higher compared to their deoxy derivatives against the D6 and W2 strains of *P. falciparum*; Mehra and Bhasin ([Mehra and Bhasin 1993](#)) reported that DHA was >500 -fold more active than deoxy-DHA against the FCD-3 strain of *P. falciparum*. Overall, the studies show that the endoperoxide bridge structure is important for the activity of artemisinins, including DHA.

The endoperoxide bridge is activated by heme resulting in the generation of free radicals, protein alkylation and cell death ([Meshnick et al. 1993](#)), ([Olliaro et al. 2001](#)). The activation of artemisinin and its derivatives appears to be due to binding with heme released from hemoglobin in the food vacuole; the binding affinity correlated with the anti-plasmodial activity in vitro ([Paitayatat et al. 1997](#)).

Wang *et al.* ([Wang et al. 2015](#)) reported that activation of artemisinins occurred inside the parasites and not in the uninfected RBCs; the killing effect was up to 100-fold higher at the later stages (trophozoite and schizonts) than the early ring stages. At the early ring stage, activation relied primarily on the parasite's heme biosynthesis pathway and at latter stages on hemoglobin digestion; the biosynthesis of heme was lower than that released by hemoglobin digestion.

18.5.1.2 Effect on oxidative stress and mitochondrial electron transport system

- **Oxidative stress**

The enzymes important in the oxidation of reactive oxygen radicals, which are by-products of metabolism and can damage nucleic acids, proteins and lipids include glutathione peroxidase (GSH-PX) and glutathione reductase (GR) in *Plasmodium* parasites as well as the host-derived superoxide dismutase (SOD) and catalase (Cat) enzymes.

The oxidative stress within the RBCs infected with the K1 strain of *P. falciparum* is increased compared to the uninfected RBCs ([Ittarat et al. 2003](#)). Pan *et al.* ([Pan, Lin, and Zhang 1989](#)) reported that in the presence of artesunate (concentration not specified), the activity of the antioxidant enzymes (SOD, Cat, GR, and GSH-PX) was similar in uninfected and *P. falciparum* (Hainan strain) infected RBCs. However, in another experiment, the exposure of RBCs to artesunate (961 ng/mL) resulted in ~2-fold increase in free radicals and H₂O₂ production in infected RBCs compared to uninfected RBCs. The activation of artemisinin by Fe²⁺/heme appears to be essential to release free radicals and other intermediates. Similar observations were reported by Ittarat *et al.* ([Ittarat et al. 2003](#)); significantly lower activity of GSH, Cat and GSH-Px enzymes was reported in parasitized RBCs, compared to uninfected RBCs, at DHA concentrations of 0.5 and 1.0 ng/mL.

Krungkrai and Yuthavong ([Krungkrai and Yuthavong 1987](#)) reported that with an increase in O₂ from 3% to 30% in cultures of the K1 strain of *P. falciparum*, the artesunate 50% inhibitory concentration (IC₅₀) values decreased from 0.11 ng/mL to 0.02 ng/mL. Synergistic activity in vitro was observed when artesunate was tested in the presence of the oxidant drugs e.g., miconazole or doxorubicin. However, the reducing agents [e.g., dithiothreitol (DTT)] as well as antioxidants (e.g., α -tocopherol, sodium ascorbate, reduced glutathione, and catalase) that scavenge free radicals decreased the activity of artemisinin. Overall, the study suggests that artesunate and artemisinin act through generation of oxidative stress.

Similar observations were reported by another group of investigators ([Meshnick et al. 1989](#)) using FCR3 strain of *P. falciparum* and different antioxidants (sodium ascorbate and reduced glutathione). The antimalarial activity was determined by incorporation of ³H-hypoxanthine. The erythrocytic forms of the parasites were cultured in the presence or absence of antioxidants and various concentrations of artemisinin. The results show that ascorbic acid increased the artemisinin IC₅₀ values suggesting that the artemisinin activity in the presence of antioxidants, is reduced.

It is known that genetically variant RBCs from patients with α -thalassemia [α -thal-1/ α -thal-2] exhibit an increase in the activity of antioxidant enzymes as well as impaired growth of *P. falciparum*. Yuthavong *et al.* ([Yuthavong et al. 1989](#)) reported decreased in vitro activity of artesunate and chloroquine when measured using genetically variant RBCs compared to RBCs from healthy volunteers infected with the synchronized cultures (containing schizonts) of the K1 strain of *P. falciparum*. However, the activity of pyrimethamine was not altered by the

source of RBCs. The study shows that an increase in the activity of antioxidant enzymes within the RBCs, which would lead to a decrease in oxygen radicals, can decrease the activity of artesunate.

Senok *et al.* ([Senok et al. 1999](#)) reported 2- to 3-fold increase in artemether IC₅₀ values when in vitro sensitivity testing was conducted using thalassemic RBCs compared to normal RBCs infected with synchronous cultures (0.5% parasitemia, 0.4% hematocrit and predominantly schizont stage) of the FC27 (CQ sensitive) strain of *P. falciparum*; drugs were added to cultures at 36 hours when the parasites were in the young trophozoite stage and the activity measured after 12 hours by microscopic examination of smears. For the K1 (CQ-resistant) strain, however, changes in IC₅₀ were ≤2-fold and not statistically significant. In the presence of vitamin E (antioxidant), the activity of artemether was decreased against both the FC27 and K1 strains; however, in the presence of riboflavin (pro-oxidant), significant increase in artemether activity was reported in both thalassemic and normal parasitized RBCs.

- **Mitochondrial electron transport system**

Krungkrai *et al.* ([Krungkrai et al. 1999](#)) reported that artemisinin, primaquine, and mammalian mitochondrial inhibitors (that target complexes I-IV of the respiratory system) inhibit oxygen consumption by both asexual and sexual stages of the *P. falciparum* parasites.

Wang *et al.* ([Wang et al. 2010](#)) reported that artemisinin induced rapid mitochondrial depolarization and increased production of reactive oxygen species by blood stage parasites of the *Plasmodium berghei* (rodent species); such an effect was observed in vitro at a concentration of 100 nM within 30 minutes as well as in vivo within 2 hours of drug administration. Addition of iron chelator (desferrioxamine) to *P. falciparum* parasites in culture, reduced mitochondrial electron transport chain activity, artemisinin-induced production of reactive oxygen species, and the sensitivity of the parasites to artemisinin.

18.5.1.3 Effect on oxidation of lipids

Pan *et al.* ([Pan, Lin, and Zhang 1989](#)) reported that exposure of parasitized RBCs to artesunate led to oxidation of lipids; this was based on the amount of lipid peroxidation product, i.e., malonyldialdehyde (MDA), in both uninfected and infected RBCs. The increase in the MDA content after exposure to artesunate was greater in infected RBCs compared to the uninfected cells. Similar observations were reported by Meshnick *et al.* ([Meshnick et al. 1989](#)) based on measurement of thiobarbituric acid-reacting substances (TBARS; products of lipid peroxidation); it is noted that the measurements of TBARS include malonyldialdehyde.

Meshnick *et al.* ([Meshnick et al. 1989](#)) reported that the formation of lipid hydroperoxides was inhibited when the uninfected RBCs were incubated with artesunate in the presence of maltol (an antioxidant), thereby suggesting that an increase in lipid peroxidation may be due to generation of free radicals. However, similar testing was not conducted using infected RBCs.

18.5.1.4 Effect on protein alkylation and proteasome

- **Protein alkylation**

Asawamahasakda *et al.* ([Asawamahasakda et al. 1994](#)) reported binding of radiolabeled DHA and other artemisinin derivatives [arteether or Ro 42-1611 (arteffene)] to different subcellular fractions of the ring and trophozoite forms of FCR3 (chloroquine-resistant) and D6 (chloroquine-sensitive) *P. falciparum* strains. Parasites were exposed to radiolabeled drugs for 6 hours and radioactivity as well as heme activity measured in host cell free parasites in different fractions by SDS-PAGE. The results show higher arteether uptake compared to DHA and Ro 42-1611; binding to parasite proteins occurred by all 3 drugs. Heme activity was observed in one of the radiolabeled subcellular fractions, representing food vacuoles of the parasite. The drug-labeled proteins appear to be associated with parasite membranes and not with hemozoin of both trophozoite and ring stages of both strains of *P. falciparum*. No proteins were labeled in the uninfected RBCs. The deoxy derivative of ³H-arteether did not alkylate any proteins.

Yang *et al.* ([Yang, Asawamahasakda, and Meshnick 1993](#)) reported that ³H-DHA and artemisinin alkylate the human serum proteins. DHA alkylated the thiol and amino moieties of the human serum protein; thiol (such as iodoacetamide or N-ethylmaleimide) and amine (succinic anhydride or N-ethylmaleimide) blocking agents reduced the binding of ³H-DHA to human serum by 15-58% under the experimental conditions tested. Protein alkylation by DHA appears to be iron dependent. Binding of artemisinin to human serum was increased in the presence of iron and hemin.

Bhisutthibhan *et al.* ([Bhisutthibhan et al. 1998](#)) reported that one of the proteins that binds to and is alkylated by ³H-DHA is a 25 kDa protein, translationally controlled tumor protein (TCTP) homologue; studies were conducted using the FCR3 and 3D7 strains of *P. falciparum*. The intensity of the 25 kDa band was stronger in trophozoites compared to the ring forms as measured by western blot using polyclonal rabbit antibodies against the recombinant TCTP. Several bands were observed for both the ring and trophozoite forms suggesting cross-reactivity of the TCTP antibodies with proteins other than 25 kDa in size.

Artemisinin binds to sites which are important for TCTP function ([Eichhorn et al. 2013](#)). The authors speculate that artemisinin disturbs TCTP's cytokine-like function in humans leading to the drug's anti-inflammatory activity. Wang *et al.* ([Wang et al. 2015](#)) reported that of the 124 targets identified for artemisinins, using an alkyne-tagged artemisinin analogue coupled with biotin as a probe, one of the targets was TCTP. The TCTP protein, found in eukaryotes, including protozoa and mammalian cells, is a calcium binding protein involved in a variety of cellular activities such as apoptosis and histamine release. The role of TCTP, found in the cytoplasm and food vacuole of *P. falciparum*, is unclear. Additionally, the relationship of TCTP alkylation to parasite death is not known.

Ismail *et al.* ([Ismail et al. 2016](#)) reported alkylation of several protein targets in situ, of the 3D7 strain of *P. falciparum*, that are important for glycolysis, hemoglobin degradation, antioxidant

defense, and biosynthesis of DNA, protein, and lipids. These biological pathways are important for parasite function and survival.

- **Proteasome**

DHA induced retardation of growth of erythrocytic parasites of *P. falciparum* was associated with an accumulation of ubiquitinated proteins suggesting cellular stress response (Dogovski *et al.* ([Dogovski et al. 2015](#))). Bridgford *et al.* ([Bridgford et al. 2018](#)) reported that DHA caused protein damage and inhibited proteasome function in the trophozoite and ring stages of the 3D7 strain of *P. falciparum*, leading to accumulation of unfolded/damaged and polyubiquitinated proteins, decreasing protein synthesis, activation of endoplasmic reticulum stress response, and parasite killing. Blocking protein synthesis with a translation inhibitor or inhibiting the ubiquitin-activating enzyme, E1, reduced the level of damaged polyubiquitinated proteins, and antagonized DHA activity.

18.5.1.5 Effect on phosphatidyl-inositol-3-kinase

Mbengue *et al.* ([Mbengue et al. 2015](#)) reported that DHA can inhibit phosphatidyl-inositol-3-kinase (*Pf*PI3K) of *P. falciparum*. *Pf*PI3K is exported from the endoplasmic reticulum to RBCs and important for generating phosphatidyl-inositol 3-phosphate (PI3P) as well as endocytosis of hemoglobin from the host and trafficking of hemoglobin within the parasite. Furthermore, increased (~2-fold) PI3P levels were observed in resistant strains compared to sensitive strains of *P. falciparum*.

18.5.1.6 Effect on sarcoplasmic reticulum calcium adenosine triphosphatase

Eckstein-Ludwig ([Eckstein-Ludwig et al. 2003](#)) *et al.* reported that artemisinin inhibits the SERCA orthologue (*Pf*ATP6) of *P. falciparum*; however, desoxyartemisinin, quinine, or chloroquine were ineffective. Chelation of iron by desferrioxamine abrogated the antiplasmodial activity of artemisinin and reduced inhibition of *Pf*ATP6. The inhibitory activity of artemisinin against *Pf*ATP6 appears to occur in the cytosol compartment; imaging of parasites with BODIPY-thapsigargin (used for intracellular localization that promotes Ca²⁺ release by inhibiting the endoplasmic reticulum Ca²⁺-ATPase) labeled the cytosolic compartment and was competed by artemisinin.

Wang *et al.* ([Wang et al. 2015](#)) identified 124 protein targets for artemisinins using an alkyne-tagged artemisinin analogue coupled with biotin as a probe; one of the targets was SERCA/*Pf*ATP6.

18.5.1.7 Uptake of dihydroartemisinin by ring and trophozoite forms

Gu *et al.* ([Gu, Warhurst, and Peters 1984](#)) reported 100- to 180-fold higher uptake of ³H-DHA (12 nM i.e., 3.41 ng/mL) in vitro by RBCs infected with the Wellcome/Liverpool/West African strain of *P. falciparum* compared to the uninfected RBCs. DHA uptake appears to depend on the type of developmental forms present in culture. For example, in cultures containing predominantly the ring form, lower amounts of DHA accumulated within 30 minutes compared

to the cultures consisting mainly of the trophozoites. The uptake of ^3H -DHA by parasitized RBCs was slower initially (for about 2 hours) in cultures containing predominantly the ring forms compared to the late stage developmental forms; however, after 2 hours of incubation, the uptake of ^3H -DHA was similar and maintained at steady level up to ~20 hours in cultures containing either predominantly early or late developmental forms. It is noted, that these experiments were conducted in medium containing high glucose and low protein concentrations. The relevance of such finding to accumulation of DHA *in vivo* is unclear.

18.5.1.8 Effect on ultrastructure

Ellis *et al.* ([Ellis et al. 1985](#)) reported ultrastructural changes in *P. falciparum* after exposure to ^3H -DHA *in vitro* and in mice infected with *P. berghei*. For *in vitro* assessments, DHA (10^{-5} M i.e. 28.44 ng/mL) was added to *P. falciparum* infected RBC cultures with 11% parasitemia and incubated for 1 hour. The cells were fixed and the sections examined by electron microscopy. ^3H -DHA was localized in the parasite limiting 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, blood was collected at different time intervals to evaluate ultrastructural changes of the parasites. The changes observed include an increase in free and bound ribosomes as well as swollen endoplasmic reticulum and mitochondria within 30 minutes of treatment. By 8 to 12 hours, the parasites' organelles were increasingly disorganized; perinuclear vacuolation without and aggregation of nuclear material within the nucleus was observed. After 12 hours, progressive disintegration of the parasite into membrane whorls and vacuoles occurred. By 24 hours, almost all the parasites had disappeared. The authors state that isolated organisms observed appeared either to have been unaffected by or to have recovered from drug treatment.

Maeno ([Maeno et al. 1993b](#)) *et al.* (1993b) reported alteration in morphology of the blood stage parasites of a Brazilian clone (ItG2) of *P. falciparum* parasites incubated with ^3H -DHA by electron microscopic/ autoradiography. Briefly, cultures were concentrated for schizonts and suspended to 5% parasitemia, 7% hematocrit; ^3H -DHA was added and cultures incubated for 1 to 4 hours and processed for electron microscopy and autoradiography. The results showed localization of silver grains (representing radiolabel) of DHA in approximately 70% of the food vacuoles (mainly in malaria pigment) and 40% in the mitochondria. No radiolabel was observed in other parts of the parasite or uninfected RBCs. Similar observations were made using radiolabeled artemisinin. Overall, the study suggests that the ultrastructural changes occur sooner (2 to 4 hours) than the reduction of parasite counts in culture.

Maeno *et al.* ([Maeno et al. 1993a](#)) reported similar observations in splenectomized and non-splenectomized nonhuman primates infected with *Plasmodium coatneyi* and treated with artesunate (for details see Section 18.5.2).

Presence of ring infected erythrocyte surface antigen (RESA) cells, without intracellular *Plasmodium* parasites, was reported in the blood of patients with uncomplicated or severe malaria treated with artesunate ([Angus et al. 1997](#)); Chotivanich *et al.* 2000 ([Chotivanich et al. 2000](#)). The clearance of artesunate exposed ring stage parasites appears to be due to pitting in the spleen, leading to removal of the intraerythrocytic parasite ([Chotivanich et al. 2000](#)); ([Buffet et al. 2006](#)). The RESA cells probably represent the once infected cells ([Woodrow and White 2017](#)).

18.5.2 In vitro Activity

Methods for measurement of *in vitro* sensitivity of *Plasmodium* species are not standardized and limited to testing in research laboratories. The most common *Plasmodium* species used for in vitro testing is *P. falciparum* and the testing is based on blood stage parasites.

Meister ([Meister et al. 2011](#)) *et al.* reported that artemisinin is not active against the liver stage parasites of *Plasmodium yoelii*. Artemisinin class of drugs are not active against the exoerythrocytic (liver) stage parasites of *Plasmodium* species ([Tilley et al. 2016](#)).

Some of the studies supporting the activity of artesunate and DHA against different stages of erythrocytic parasites of *P. falciparum* and other *Plasmodium* species are summarized.

18.5.2.1 Activity against the blood stage parasites

- ***Plasmodium falciparum***

Several studies have reported the in vitro activity of artesunate and DHA against the blood stage parasites of several **laboratory strains** ([Krungkrai and Yuthavong 1987](#)); ([Brossi et al. 1988](#)), ([Yuthavong et al. 1989](#)), ([Alin, Björkman, and Ashton 1990](#)); ([Shmuklarsky et al. 1993](#)); ([Skinner et al. 1996](#)); ([Fivelman et al. 1999](#)); ([Brockman et al. 2000](#)); ([Moreno et al. 2001](#)); ([Ramharter et al. 2003](#)); ([Fivelman, Adagu, and Warhurst 2004](#)); ([Teja-Isavadharm et al. 2004](#)); ([Li et al. 2007](#)); ([Vivas et al. 2008](#)); ([Lim et al. 2010](#)); ([Lelièvre et al. 2012](#)); ([Duffy and Avery 2013](#)); ([Zhang et al. 2019](#)); Study Report WRAIR 0432-16-1 ([Study Report WRAIR 0432-16-1 2009](#)) and **clinical isolates** ([Mehra and Bhasin 1993](#)); ([Bustos, Gay, and Diquet 1994](#)); ([Skinner et al. 1996](#)); ([Yang et al. 1997](#)); ([Wongsrichanalai et al. 1997](#)); ([Ringwald, Bickii, and Basco 1999](#)); ([Le 1998](#)); ([Wongsrichanalai et al. 1999](#)); ([Brockman et al. 2000](#)); ([Ramharter et al. 2003](#)); ([Lim et al. 2010](#)); ([Kaewpongsri et al. 2011](#)); ([Fall et al. 2015](#)); ([Chaorattanakawee et al. 2017](#)); ([Zhang et al. 2019](#)) of *P. falciparum*; however, the method used for testing varied. Some of the variations in the tests include the concentrations of parasitized and uninfected RBCs used for culture, source of RBCs, duration of incubation, and the method used to determine inhibition of parasite growth for example incorporation of ³H-hypoxanthine, flow cytometry, or microscopic examination of the parasites (for details see Table 18-25 and Table 18-26).

The artesunate IC₅₀ values against the laboratory strains and clinical isolates were ≤2.14 ng/mL and ≤2.69 ng/mL, respectively; the DHA IC₅₀ values against the laboratory strains and clinical

isolates were ≤ 6.54 ng/mL and ≤ 3.98 ng/mL, respectively (for details see Table 18-25 and Table 18-26).

Some of the clinical isolates tested were from regions known to be resistant to different antimalarial drugs; for example, isolates from India were resistant to chloroquine, Philippines to chloroquine and amodiaquine, Vietnam to chloroquine, amodiaquine, mefloquine and sulfadoxine/ pyrimethamine, China-Myanmar (Burma) and China-Lao PDR borders resistant to chloroquine, Thailand region stated to be resistant to mefloquine, and Cameroon and other parts of Africa to chloroquine. The activity of both artesunate and DHA appears to be similar against the sensitive and resistant strains of *P. falciparum*. Additionally, the activity of both artesunate and DHA was similar against primary as well as recrudescence isolates, from patients in Thailand, previously treated with mefloquine + artesunate.

Studies show that in the presence of antioxidants (which would lead to a decrease in oxygen radicals) as well as in the genetically variant RBCs (such as in patients with α -thalassemia), the in vitro activity of artesunate is decreased (for details see Section 18.5.1).

Table 18-25: In vitro activity of artesunate and DHA against the asexual stage parasites (black font) and/or gametocytes (green font) of the laboratory strains of *P. falciparum*

Experimental design (Reference)	Strain (Clone)	IC ₅₀ nM (ng/mL*)	
		Artesunate	DHA
Synchronized cultures (rings 89%; trophozoites 11%) in type O RBCs containing 0.3-0.5 % parasitemia, were incubated for 96 h; the medium with and without drugs was changed every 24 h. The number of parasites per 10,000 RBCs were counted on Giemsa stained smears and IC ₅₀ and IC ₉₀ values determined. The IC ₉₀ value was 0.5 ng/mL. Increase in oxygen tension from 3% to 17% to 30%, was associated with an increase in the activity of the drug. (Krungkrai and Yuthavong, 1987)	K1 ^a	1.1 (0.4)	ND
PRBCs (0.5 - 0.7%) were cultured at 37°C for 24 h; ³ H-hypoxanthine added, and the cultures incubated for an additional 18 to 20 h. (Brossi <i>et al.</i> , 1988)	D6 ^b	2.18 (0.83)	1.83 (0.52)
	W2 ^c	1.66 (0.64)	1.79 (0.51)
Synchronized cultures of PRBCs containing schizonts (~98%) were subcultured in genetically variant or normal RBCs at an initial parasitemia of 0.2-0.3% and incubated for 96 h. The culture medium was replaced with fresh medium containing drug every 24 h. The number of PRBCs was determined by counting 10,000 RBCs. The multiplication ratio, i.e., the ratio of % parasitemia at 96 h compared with that at 0 h, in the absence of drugs was slightly but significantly higher for normal RBCs (32.4±9.6) than for genetically variant RBCs [HbH (24.6±9.2, P < 0.02), HbH/CS (21.1±6.4, P < 0.02), homozygous HbCS (26.0±8.8, P < 0.02), and heterozygous HbCS (20.4±1.5, P < 0.02)]. The artesunate and CQ IC ₅₀ values were 5 to 10-fold higher using genetically variant RBCs compared to normal RBCs. However, the pyrimethamine IC ₅₀ value was not	K1 ^a	0.82 (0.32)	ND

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Experimental design (Reference)	Strain (Clone)	IC ₅₀ nM (ng/mL*)	
		Artesunate	DHA
altered by the source of RBCs. (Yuthavong <i>et al.</i> , 1989)			
The PRBCs (0.2%) of 3 CQ sensitive and one CQ resistant (D7) strains were incubated for 48 h and activity measured by microscopic examination of Giemsa stained smears. (Alin <i>et al.</i> , 1990)	F32	ND	4.7 (1.34)
	7G8 ^d		7.5 (2.13)
	Honduras ^e		7.0 (1.99)
	D7 ^f		23.0 (6.54)
Activity was measured by a semiautomated microdilution method in cultures with 0.5% parasitemia and 1% hematocrit; ³ H-hypoxanthine was added after 24 h incubation and the cultures incubated for additional 18-20 h. (Shmuklarsky <i>et al.</i> , 1993)	W2 ^c	0.88 (0.34)	ND
	D6 ^b	0.88 (0.34)	
	Vietnam Smith/RE ^g	1.02 (0.39)	
Synchronous cultures (2% parasitemia; 1% hematocrit) were incubated with ³ H hypoxanthine for 48 h. (Skinner <i>et al.</i> , 1996)	D10 ^h	0.3-0.5 (0.12-0.19)	1-3 (0.28-0.85)
	3D7 ⁱ	0.3 (0.12)	1-3 (0.28-0.85)
Testing conducted by the method of Desjardin <i>et al.</i> (AAC, 1979, 16: 710-718); probably by hypoxanthine incorporation. (Fivelman <i>et al.</i> , 1999)	D10 ^h	5.57 (2.14)	ND
	RSA11 ^m	3.44 (1.28)	
Synchronous cultures with 90% of the parasites at the ring stage were cultured with and without drug for 24 h; ³ H-hypoxanthine was added, and cultures incubated for additional 18 h. (Brockman <i>et al.</i> , 2000)	K1 ^a (1996)	2.15 (5.6)	1.02 (3.6)
	K1 ^a (1998)	1.77 (4.6)	0.88 (3.1)
Parasites were cultured with human O group RBCs, in the presence or absence of DHA for 72 h and activity measured by a commercial HRP2 ELISA. (Ramharter <i>et al.</i> , 2003)	3D7 ⁱ	ND	0.57 (0.16)
	7G8 ^d	ND	0.58 (0.16)
	Dd2 ^j	ND	0.78 (0.22)
	S007 ^k	ND	0.63 (0.18)
Synchronized cultures (2% hematocrit and 0.005% parasitemia) were incubated for 48 h, plates frozen and parasite growth measured by colorimetric DELI method based on detection of lactate dehydrogenase enzyme. (Moreno <i>et al.</i> , 2001)	NF54 ^l (Africa)	ND	4.00 (1.14)
	T24 (Thailand)	ND	3.00 (0.85)
Parasite cultures in Group A RBCs were incubated for 24 h and ³ H-hypoxanthine added; cultures were incubated for additional 24 h. (Fivelman <i>et al.</i> , 2004)	NGATV01	ND	3.83 (1.09)
	T996	ND	3.46 (0.98)
	D10 ^h	ND	3.76 (1.07)
	3D7 ⁱ	ND	3.55 (1.01)
	FC27	ND	2.24 (0.64)
	K1 ^a	ND	1.67 (0.47)
	RSA11 ^m	ND	1.98 (0.56)
	7G8 ^d	ND	2.30 (0.65)
B303 ^p	ND	1.16 (0.33)	
Cultures (0.5% parasitemia with 80% young rings at a 1.7% hematocrit) were incubated for 24 h, ³ H-hypoxanthine added and incubated for additional 18-20 h. (Teja-Isavadharm <i>et al.</i> , 2004)	W2 ^a	ND	(1.27) 0.36
The PRBCs were incubated for 48 h and the sensitivity of the	W2 ^a	FCM: 5.20	FCM: 1.09

NDA Multi-disciplinary Review and Evaluation NDA 213036
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Experimental design (Reference)	Strain (Clone)	IC ₅₀ nM (ng/mL*)	
		Artesunate	DHA
parasites determined by flow cytometry as well as ³ H-hypoxanthine incorporation (³ H added at 24 h). (Li <i>et al.</i> , 2007)		(2.02)	(0.31)
		³ H: 0.65 (0.25)	³ H: 1.20 (0.34)
	D6 ^b	FCM: 0.78 (0.30)	FCM: 0.91 (0.26)
		³ H: 0.91 (0.35)	³ H: 1.02 (0.29)
Cultures with 65–75% ring stage parasites were incubated for 48 h and ³ H-hypoxanthine added at 24 h. (Vivas <i>et al.</i> , 2008)	K1 ^a	4.14 (1.59)	2.35 (0.67)
	FCR3	5.82 (2.23)	1.64 (0.47)
	FCB	7.40 (2.84)	2.63 (0.75)
	3D7 ⁱ	9.39 (3.61)	2.22 (0.63)
	VS1	9.93 (3.82)	1.54 (0.44)
	TM90C2a	9.97 (3.83)	2.81 (0.80)
Testing performed by the 48 h ³ H-hypoxanthine incorporation method. (Lim <i>et al.</i> , 2010)	3D7 ⁱ	0.9 (0.35) Range: 0.7-1.07 (0.27-0.41)	ND
Parasitized cultures incubated for 48 h and growth examined on Giemsa stained smears of asexual and mature gametocytes (IV-V stages). 3D7HTGFP strain constitutively expresses green fluorescent protein (GFP). (Lelièvre <i>et al.</i> , 2012)	3D7A	37.00 (14.22)	2.80 (0.80)
	3D7HT-GFP	54.00 (20.76)	13.00 (3.70)
	Mature gametocyte	10830 (4163)	3560 (1012)
Synchronized cultures (ring stage, early trophozoites) were incubated for 72 h and growth of asexual and/or early (I-III stages), mature gametocytes (IV-V stages) measured by imaging after staining with 4',6-diamidino-2-phenylindole (DAPI). (Duffy <i>et al.</i> , 2013)	3D7 ⁱ	6.70 (2.58)	2.08 (0.59)
	NF54-pfs16-GFPa early gametocyte	3.8 (1.46)	18.3 (5.20)
	NF54-pfs16-GFPa late gametocyte	2.53 (0.97)	2.17 (0.62)
Ring stage synchronized cultures were incubated for 72 h and activity measured after staining with SYBR green I. (Zhang <i>et al.</i> , 2019)	3D7 ⁱ	5.00 (1.92)	1.00 (0.28)
Cultures with 0.3% parasitemia (ring stage 75%; trophozoites 20% and schizonts 5%) were incubated for 72 h; two lots [WR256283-5 (Starks Associates, Inc.) and WR256283-11 (Knoll AG)] of were tested by the Malaria SYBR Green I based fluorescence (MSF) assay. Weekly testing of the 4 strains, was conducted over a period of 6 years (2009-2015). (Study Report WRAIR 0432-16-1)	D6 ^b	1.14 (0.44)	ND
	C235 ⁿ (TM91-C235)	1.17 (0.45)	
	W2 ^c	0.55 (0.21)	
	C2B ^o (TM90-C2B)	0.78 (0.30)	
^a K1: Resistant to CQ, pyrimethamine and sulfadoxine. ^b D6: Sierra Leone clone - sensitive to CQ, pyrimethamine and sulfadoxine but resistant to mefloquine ^c W-2: Indochina clone is resistant to the antimalarial drugs CQ, pyrimethamine sulfadoxine and mefloquine ^d 7G8: CQ and pyrimethamine resistant ^e Honduras: CQ sensitive, pyrimethamine-resistant.			

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Experimental design (Reference)	Strain (Clone)	IC ₅₀ nM (ng/mL*)	
		Artesunate	DHA
^f D7: Chloroquine resistant ^g Vietnam Smith/RE clones: are resistant to CQ, quinine, sulfadoxine, and pyrimethamine but sensitive to mefloquine. ^h D10: CQ sensitive ⁱ 3D7: CQ sensitive ^j Dd2: Indochina, CQ, pyrimethamine and mefloquine resistant ^k S007: originally isolated from a Gabonese child presenting with complicated malaria and proved to be highly CQ resistant but sensitive to quinine and mefloquine. ^l NF54: CQ sensitive ^m RSA11: CQ resistant ⁿ C235: From RII mefloquine failure (Study done at the Bangkok Hospital for Tropical Diseases 1991), also resistant to Chloroquine, Quinine, DHFR/DHPS inhibitors (Thai/1991) ^o C2B: From a subject after an R1-Type recrudescence during a Phase 2 Atovaquone study. It is a hydroxynaphthoquinone-resistant isolate ^p B303: stated to be resistant; however, the drug not specified HB3: Honduras, CQ-sensitive ND=Not done; CQ=chloroquine; h=hours; PRBCs=parasitized RBCs; DELI method=double-site enzyme-linked lactate dehydrogenase immunodetection assay *Results against the asexual stage parasites are presented in black font and those against the gametocytes in green font.			

Table 18-26: In vitro activity of artesunate against the clinical/field isolates (asexual stage parasites) of *P. falciparum*

Experimental design (Reference)	Country (n)	IC ₅₀ nM (ng/mL)	
		Artesunate	DHA
The activity of DHA (labeled with DADF) was determined against a clinical isolate (FCD-3) by microscopic examination of Giemsa stained smears after 48 h of incubation with DHA. DHA, at 10 ng/mL, completely inhibited the development of asexual parasites; the IC ₉₀ value was 8.4 ng/mL. (Mehra and Bhasin, 1993)	India CQ resistant (1)	ND	7.02 (2.7)
Blood samples, collected from patients with at least 0.1% parasitemia, were processed for culture by the microdilution assay for 48 h and activity measured by incorporation of ³ H-hypoxanthine. (Bustos <i>et al.</i> , 1994)	Philippines CQ and amodiaquine resistant (24)	0.9 (0.35)	ND
Synchronous cultures (2% parasitemia; 1% hematocrit) were incubated with ³ H hypoxanthine for 48 h. (Skinner <i>et al.</i> , 1996)	CR14 (Vietnam)	0.2 (0.08)	0.8-1.0 (0.23-0.28)
	CR19 (Vietnam)	0.2 (0.08)	3.0 (0.85)
	CR25 (Vietnam)	0.3 (0.12)	1.0 (0.28)
	QA1 (China)	0.4-0.6 (0.15-0.23)	2.0 (0.57)
Microtechniques recommended by Rieckmann was used and activity based on maturation rate of rings to the schizont stage	China Myanmar (Burma) border (n=53)	6.0 (2.31)	13-14 (3.70-3.98) (n=15)

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Experimental design (Reference) (Yang <i>et al.</i> , 1997)	Country (n)	IC ₅₀ nM (ng/mL)	
		Artesunate	DHA
	China-Lao PDR Border Areas	5.0 – 7.0 (1.92-2.69) (n=42)	4.0 – 5.0 (1.14-1.42) (n=42)
Blood samples were collected during a malaria survey in 1995 from 10 patients with parasite density of 1000 to 30,000/μL; one patient was symptomatic. Specimens were cryopreserved, thawed within 2 months and asynchronous cultures adapted for 2 to 6 weeks. Testing was performed by a modified ³ H-hypoxanthine microtest method. (Wongsrichanalai <i>et al.</i> , 1997)	Vietnam: CQ resistant area (10)	3.22 (1.24) Range: 1.87-4.84 (0.72-1.86)	3.24 (0.92) Range: 1.97-4.61 (0.56-1.31)
Clinical isolates collected between 1997-1998, prior to treatment, were assessed by incorporation of ³ H-hypoxanthine added at 18 h after incubation and cultures incubated for additional 24 h. *CQ resistance was based on an IC₅₀ of >100 nM. (Ringwald <i>et al.</i> , 1999)	Yaounde, Cameroon CQ sensitive* (n=33)	ND	1.25 (0.36)
	Yaounde, Cameroon CQ resistant* (n=35)	ND	0.98 (0.28)
Isolates obtained from non-immune travelers returning from Africa to France in 1993-1996 were tested; with the exception of 26 patients returning from Comoros and Madagascar, the patients were infected in Africa, mainly in Central and West African. Most of them had not taken a correct chemoprophylactic regime. Infected RBCs were diluted with uninfected RBCs in medium containing 10% human serum, if the parasitemia exceeded 0.5% to obtain an initial parasitemia of 0.1-0.5%. Testing performed by a simplified <i>in vitro</i> test (hematocrit 1.5%) and a semi-microtest (the infected erythrocytes diluted with fresh uninfected erythrocytes at a parasite density 0.2-0.5% and hematocrit 0.5%). The activity was measured by incorporation of ³ H-hypoxanthine at 42 h. It is unclear if the results are based on the simplified or semi-microtest method. *CQ resistance based on DHA IC₅₀ ≥100 nM. (Le Bras, 1998)	Africa CQ sensitive (79)	ND	1.51* (0.43)
	Africa CQ resistant* (69)		0.89* (0.25)
Study conducted between 1991-1997. Blood samples from patients with parasitemia of >5000/μL were cryopreserved and processed as summarized above for the study by the same group (Wongsrichanalai <i>et al.</i> , 1997; for isolates from Vietnam). (Wongsrichanalai <i>et al.</i> , 1999)	Thailand (near the borders with Myanmar and Cambodia) MQ resistant	2.43-3.39 (0.93-1.30) (n=86)	1.86-2.55 (0.53-0.73) (n=35)
	Southern Thailand MQ sensitive (n=10)	1.33 (0.51)	1.4 (0.40)
Isolates collected between March 1995 and July 1999. Patients presenting with falciparum malaria within 42 days	Thailand Primary isolate	0.62 (1.62) (n=178)	0.35 (1.22) (n=84)

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Experimental design (Reference)	Country (n)	IC ₅₀ nM (ng/mL)	
		Artesunate	DHA
of antimalarial treatment (mefloquine+artesunate) were considered to be recrudescence. Synchronous cultures with 90% of the parasites at the ring stage were cultured with and without drug for 24 h; ³ H-hypoxanthine was added, and cultures incubated for an additional 18 h. (Brockman <i>et al.</i> , 2000)	Thailand Recrudescence isolates	0.90 (2.35) (n=72)	0.41 (1.43) (n=44)
Parasites were cultured with human O group RBCs, in the presence or absence of DHA for 72 h, and activity measured by a commercial HRP2 ELISA. (Ramharter <i>et al.</i> , 2003)	Gabon CQ and antifolate resistance (n=29)	ND	0.41 (1.43)
The isolates, obtained from nine sentinel sites during the years 2001 to 2007, were tested by the 48 h ³ H-hypoxanthine incorporation method. Both artesunate and CQ IC ₅₀ values were increased in the presence of leukocytes compared to those in the absence of leukocytes.	Southeast Asia Western Cambodia Artemisinin resistance* (n=495)	1.2 (0.46) Range: 0.13- 11.1 (0.05- 4.27)	ND
*P. falciparum resistance to artemisinin given as part of artemisinin-based combination therapy, was first reported from Western Cambodia in 2008 and spread across the Greater Mekong subregion. It is noted that resistance is based on a prolongation of parasitemia clearance i.e., delayed parasite clearance time, based on blood smears. (Lim <i>et al.</i> , 2010)	Southeast Asia Eastern Cambodia (n=325)	0.6 (0.23) 0.05-8.0 (0.02- 3.08)	ND
Blood samples with >90% parasites at the early ring stage (<6 to 8 h post-invasion) were incubated in the presence and absence of leukocytes, by the modified WHO recommended schizont maturation test. Both hemozoin/parasites and infected RBCs were phagocytosed by leukocytes (polymorphonuclear cells, monocytes, and lymphocytes) in the presence and absence of drug. Prior to culture, however, there was minimal (0-1.3%) detection of parasites or hemozoin in the leukocytes. (Kaewpongsri <i>et al.</i> , 2011)	MaeSod, Thailand (n=8)	1.39-1.88 (0.40-0.53)	ND
Synchronized cultures of isolates collected from patients, between 2013 and 2014 [approximately 7 years after the Senegalese National Malaria Control Programme recommended artemisinin-based combination therapy (in 2006)], were incubated for 72 h with and without drug and activity measured by the HRP2 ELISA. About 50% of the isolates were resistant to CQ and mefloquine. (Fall <i>et al.</i> , 2015)	Dakar, Senegal (n=18)	1.35 (0.52)	0.72 (0.20)

Experimental design (Reference)	Country (n)	IC ₅₀ nM (ng/mL)	
		Artesunate	DHA
Isolates collected between 2013–2015 were tested by the microscopy-based schizont maturation test (SMT) and a <i>Plasmodium</i> pan-species lactate dehydrogenase (pLDH) ELISA. <i>P. vivax</i> multidrug resistance gene 1 (<i>pvmdr1</i>) mutations, and copy number were analyzed in a subset of isolates. Samples with poor growth rates, defined as <40% schizonts in drug-free wells or a pLDH-OD ratio <1.7 between no-drug control wells and maximum tested drug concentrations, were excluded from data analysis for SMT or pLDH-ELISA, respectively. (Chaorattanakawee <i>et al.</i>, 2017)	Oddar Meanchey, Northern Cambodia (n=53)	pLDH: 5.7 (2.22) HRP2: 6.0 (2.31)	pLDH: 6.3 (1.79) HRP2: 7.4 (2.10)
Ring stage synchronized cultures of isolates collected between 2007-2016 were incubated for 72 h and activity measured after staining with SYBR green I. (Zhang <i>et al.</i>, 2019)	China-Myanmar border (n=133)	11.20 (4.31)	4.20 (1.19)
ND=Not done; CQ=chloroquine; h=hours; SMT=schizont maturation test			

- ***Plasmodium vivax***

Few studies (Kaewpongsri *et al.*, 2011; Lu *et al.*, 2011; Fernández *et al.* 2014; Chaorattanakawee *et al.*, 2017) reported the activity of artesunate and/or DHA, by the schizont maturation or *Plasmodium* lactate dehydrogenase (pLDH) tests, against the clinical isolates of *P. vivax*. The artesunate and DHA IC₅₀ values were ≤1.2 ng/mL and ≤1.02 ng/mL, respectively (for details see Table 18-27).

- ***Plasmodium malariae***

Siswanto *et al.* (Siswanto *et al.*, 2011) reported the activity of artesunate against trophozoite (n=17) and ring (n=7) stages of clinical isolates of *P. malariae* from Indonesia. The activity was measured by the schizont maturation test. The artesunate IC₅₀ values were ≤1.1 ng/mL (for details see Table 18-27).

- ***Plasmodium ovale***

Siswanto *et al.* (2011) reported the activity of artesunate against trophozoite (n=1) and ring (n=4) stages of clinical isolate(s) of *P. ovale* from Indonesia. The activity was measured by the schizont maturation test. The artesunate IC₅₀ value was ≤0.5 ng/mL (for details see Table 18-27).

- ***Plasmodium knowlesi***

Fatih *et al.* (Fatih *et al.*, 2013) reported the activity of artesunate and DHA against 1 laboratory strain and 9 clinical isolates of *P. knowlesi* from Malaysia. The activity was measured by pLDH assay. The artesunate and DHA IC₅₀ values were ≤0.77 and 0.45 ng/mL, respectively (for details see Table 18-27).

Table 18-27: In vitro activity of artesunate/DHA against the laboratory strains/clinical isolates (asexual stage parasites) of *P. vivax*, *P. knowlesi*, *P. malariae* and *P. ovale*

Experimental design (Reference)	Strain (Clone)	IC ₅₀ μM (μg/mL*)	
		Artesunate	DHA
<i>P. vivax</i> – clinical isolates			
Blood samples with >90% parasites at the early ring stage (~6 to 8 h post-invasion) were incubated in the presence and absence of leukocytes, by the modified WHO recommended SMT. Both hemozoin/parasites and infected RBCs were phagocytosed by leukocytes (polymorphonuclear cells, monocytes, and lymphocytes) in the presence and absence of drug. Prior to culture, however, there was minimal (0-1.3%) detection of parasites or hemozoin in the leukocytes. (Kaewongsri <i>et al.</i> , 2011)	MaeSod, Thailand (n=8)	0.88-1.42 (0.25-0.40)	ND
During 2005–2006 malaria season, the blood samples from patients, who had not received any anti-malarial treatment for the past 3 weeks, were tested by the SMT after removal of the leukocytes. Culture time varied between 17 and 24 h. (Lu <i>et al.</i> , 2011)	Central China (n=40)	0.34 (0.13)	ND
Isolates from patients, with a parasitemia level of >2,000 parasites/μL of blood and no anti-malarial treatment in the previous month, were tested by the SMT; the PRBCs were incubated, with and without different concentrations of the drug, until 40% of the initial parasitic stages of each isolate matured into schizonts in the drug-free control wells. Smears were examined by microscopy and inhibition percentage of schizont maturation in a treatment group was calculated by relating the number of schizonts present in the controls (>40%) with the number of schizonts in the treatment groups and IC ₅₀ s determined. Culture time was <48 h. The IC ₅₀ s of CQ and AS were higher in the isolates from mature trophozoites (CQ, 39.3 nM versus 17 nM; AS, 1.4 nM versus 0.3 nM), and 10% of the isolates showed lower sensitivity to one of the antimalarial drugs, 13.3% to two antimalarial drugs, and 3.3% to more than three antimalarial drugs. The authors state that despite the extensive use of CQ in Colombia, <i>P. vivax</i> continues to be susceptible to antimalarials. (Fernández <i>et al.</i> , 2014)	Urabá, Colombia (n=30)	0.6 (0.23)	ND

Experimental design (Reference)	Strain (Clone)	IC ₅₀ μM (μg/mL*)	
		Artesunate	DHA
Isolates collected between 2013–2015 were tested by a <i>Plasmodium</i> pan-species lactate dehydrogenase (pLDH) ELISA. <i>P. vivax</i> multidrug resistance gene 1 (<i>pvmdr1</i>) mutations, and copy number were analyzed in a subset of isolates. Samples with poor growth rates, defined as pLDH-OD ratio < 1.7 between no-drug control wells and maximum tested drug concentrations, were excluded from data analysis for pLDH-ELISA. (Chaorattanakawee <i>et al.</i> , 2017)	Oddar Meanchey, Northern Cambodia (n=76)	3.2 (1.23)	3.6 (1.02)
<i>P. knowlesi</i>– laboratory strain and clinical isolates			
Synchronized cultures were tested by the pLDH and/or SMT (Mark III) assays of the H strain (adapted from a primate infection) as well as the clinical isolates. (Faith <i>et al.</i> , 2013)	H strain	pLDH: 2.0 (0.77) SMT: 1.2 (0.34)	pLDH: 0.79 (0.22)
	Sarikei and Sibul, Malaysia Clinical isolates (n=9)	pLDH: 0.90 (0.35)	pLDH: 1.60 (0.45)
<i>P. malariae</i> trophozoites and ring stages			
Isolates from patients with symptomatic malaria, were tested by the modified SMT. The activity was measured in cultures containing either a majority of trophozoite or ring stage parasites. (Siswantoro <i>et al.</i> , 2011)	Majority trophozoites Indonesia (n=17)	2.84 (1.09)	ND
	Majority rings Indonesia (n=7)	1.77 (0.68)	ND
<i>P. ovale</i> trophozoites and ring stages			
Isolates from patients with symptomatic malaria, were tested by the modified SMT. The activity was measured in cultures containing either a majority of trophozoite or ring stage parasites. (Siswantoro <i>et al.</i> , 2011)	Majority trophozoites Indonesia (n=1)	1.28 (0.49)	ND
	Majority rings Indonesia (n=4)	1.16 (0.45)	ND
ND=Not done; h=hours; SMT= schizont maturation test; PRBCs=parasitized RBCs			

18.5.2.2 Stage specificity

The activity of artesunate, DHA and other artemisinins may vary against different developmental forms of the asexual forms and gametocytes of the erythrocytic parasites; *P. berghei* or *P. falciparum* parasites were used for these studies.

- **Asexual stage parasites of *Plasmodium berghei* and *Plasmodium falciparum***
Janse *et al.* (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 was 3.7-fold and 6.3-fold higher than artesunate or artemisinin, respectively.

Skinner *et al.* (1996) reported the activity of DHA and other antimalarial drugs (artemisinin, artemether, or quinine) varied against different stages of the asexual parasites of 4 clinical isolates and 2 laboratory strains of *P. falciparum*. Briefly, synchronized cultures (2% parasitemia with >90% rings; 1% hematocrit) were incubated for different time points (0 hour for ring stage; trophozoites 20-24 hours; schizonts 36 hours) with concentrations equivalent to 90% inhibitory concentration (IC₉₀) values of the drugs. The percent inhibition of parasite growth was measured by incorporation of ³H-hypoxanthine added to the cultures at the same time as the drug and cultures incubated for 0-10 hours. Data for only one isolate were shown; however, the authors state that the activity against all isolates was similar. The results show a rapid inhibition of growth (>80%) of all the 3 stages within 2 – 4 hours of exposure to DHA. Artemisinin inhibited >90% rings and schizonts within 4 to 6 hours and 60% trophozoites within 10 hours; artemether was the least active compared to artemisinin and DHA. Quinine was more active against the schizonts than the ring and trophozoite forms of *P. falciparum*.

- **Gametocytes (transmission blocking activity)**

Mehra and Bhasin (1993) reported the activity of DHA against the gametocytes of the FCD-3 isolate of *P. falciparum*. Briefly, gametocytes enriched cultures were prepared by incubating cultures containing 1% parasitemia for 8 days in flow vessels; the number of asexual and sexual stage parasites were determined and gametocytes classified into stages I to V. Gametocyte enriched cultures were incubated with DHA for 48 hours (fresh drug containing medium added at 24 hours) and the drug free medium added at 48 and 72 hours. Blood smears were prepared at 48 and 96 hours and scored for various stages of gametocytes. Complete killing of gametocytes was reported at DHA and artemisinin concentrations of 100 ng/mL at 96 hours; gametocyte inhibition at 48 hours was lower than at 96 hours. The DHA IC₅₀ and IC₉₀ values, based on a 96-hour reading, were 6.6 and 19 ng/mL, respectively; the artemisinin IC₅₀ and IC₉₀ values were 13 and 22 ng/mL, respectively. Younger stages of gametocytes (I and II), compared to late stages (III – V) were more sensitive to the drugs.

Chotivanich *et al.* ([Chotivanich et al. 2006](#)) reported that artesunate was effective in reducing the infectivity of mosquitoes fed on mature gametocytes of a AMB 47 Thai isolate incubated with the drug for 24 hours prior to mosquito feeding; the artesunate IC₅₀ and IC₉₀ values were 0.1 nM (0.04 ng/mL) and 0.4 nM (0.15 ng/mL), respectively.

Adjalley *et al.* ([Adjalley et al. 2011](#)) measured the activity of DHA against the gametocytes of a recombinant strain of *P. falciparum*; the DHA concentrations tested were 0.5-, 1-, and 5-times the IC₅₀ value (24 nM i.e., 6.82 ng/mL) against the asexual parasites. The results show that DHA at ≥0.5-times the IC₅₀ value, against the asexual blood stage parasites, was effective in inhibiting growth of nearly 100% recombinant *P. falciparum* gametocytes at early stages (stage I-II). However, at 5-times the IC₅₀ value, near complete inhibition of parasite growth was reported at gametocyte maturation stages I-III; 60% and 35% inhibition at stage IV and V gametocytes,

respectively, at the 5x concentration. A reduction in infectivity of the mosquitoes (*Anopheles stephensi*) was reported; a reduction in oocyte numbers in infected mosquitoes, by 25% was reported at the 1X, and 70% at the 5X DHA IC₅₀ concentrations.

Lelièvre *et al.* (2012) reported that artesunate and DHA were >200-fold less active (IC₅₀s: 4163 ng/mL and 1012 ng/mL, respectively) against the mature gametocytes, compared to that against asexual forms of 3D7A strain of *P. falciparum* (Table 18-25),

Peatey *et al.* ([Peatey et al. 2012](#)) reported that artesunate inhibited the viability of the late stage gametocytes of a chimeric strain of *P. falciparum*, based on the production of ATP; the IC₅₀ was 884.17 ng/mL.

Duffy *et al.* (2013) reported that artesunate and DHA IC₅₀ values against the early stage (I-III) gametocytes of the transgenic NF54 Pfs 16-GFP strain, by confocal fluorescence microscopy, were 1.46 ng/mL and 5.20 ng/mL, respectively; whereas the artesunate and DHA IC₅₀ values against the late stage (IV-V) gametocytes were 0.97 and 0.62 ng/mL, respectively.

18.5.3 In vivo Activity (Animal Studies)

The suppressive activity of artesunate and/or DHA was measured in rodent and nonhuman primate malaria models including severe malaria animal models.

18.5.3.1 Rodents infected with *Plasmodium berghei*

The suppressive activity of artesunate and/or DHA was reported in Swiss mice (Janse *et al.*, 1994) and Sprague-Dawley rats [([Yang et al. 1982](#)); Janse *et al.*, 1994; ([Li et al. 2003](#)); ([Xie et al. 2005](#)); ([Ohr et al. 2014](#)); ([Study Report WRAIR-8C 2003](#); [Study Report WRAIR-8F 2003](#); [Study Report WRAIR-8G 2003](#))], infected with either synchronous or asynchronous parasites of the ANKA strain of *P. berghei*. The experimental design varied in different studies; in general, the animals were infected, either intravenously or intraperitoneally, with the parasitized RBCs and treatment with artesunate or DHA initiated after animals became parasitemic. Animals were followed for survival and/or parasitemia. Both artesunate and DHA were effective in improving survival and decreasing parasitemia. However, recrudescence was reported after treatment was discontinued (for details see Table 18-28).

One study (Yang *et al.*, 1982) reported that activity of artesunate was similar against chloroquine-sensitive and chloroquine-resistant strains.

Table 18-28: Suppressive activity of artesunate and DHA in mice and rats infected with the parasitized erythrocytes of *Plasmodium berghei*

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

<i>Plasmodium</i> species/ strain (Reference)	Study summary
<i>P. berghei</i> /CQ resistant and sensitive (strain not specified) Mice (Strain not specified) (Yang <i>et al.</i> , 1982)	Mice were inoculated with 1.5×10^7 PRBCs, IP, and artesunate administered IV or IM once daily for 3 days 24 h PI. The SD_{50} and SD_{90} doses were similar against the CQ sensitive (0.3 and 1.14 mg/kg) and resistant (0.5 and 1.14 mg/kg) strains.
<i>P. berghei</i> / ANKA strain Swiss mice (Janse <i>et al.</i> , 1994)	The activity of artesunate and DHA was measured in Swiss mice infected intraperitoneally with 10^5 PRBCs of the ANKA strain (Clone 8417) of <i>P. berghei</i> . Artemisinin was used as a comparator. Mice were treated, for 3 days, with artesunate or DHA (dissolved in Miglyol 812, a propylene glycol diester of saturated vegetable fatty) by the IM or SC routes once the parasitemia was between 1 and 3%. Animals were followed, up to Day 60 PI, for parasitemia at frequent intervals by thin blood films and flow cytometry. The results show that parasitemia in the vehicle group of mice increased to 40 to 50% by 12 days; 5 of the 10 mice died by Day 6 due to cerebral complications. High doses (≥ 40 mg/kg) of DHA, artesunate, and artemisinin were effective in curing mice up to Day 60 PI. At a lower dose of 10 mg/kg, parasitemia decreased, but increased rapidly in mice treated with artesunate or artemisinin; however, DHA was effective in curing 7 of the 15 mice up to Day 60. All mice treated with 0.1-10 mg/kg of artemisinin or artesunate were not cured. At a lower dose of 1 mg/kg of DHA or 1.4 mg/kg of artesunate, a significant delay in the development of parasitemia was reported in the first 4 days after drug administration; however, none of the mice were cured.
<i>P. berghei</i> / ANKA strain Sprague-Dawley rats (Li <i>et al.</i> , 2003)	Artesunate , at an IV dose of 191.2 μ M/kg (73.4 mg/kg), was effective in clearing parasitemia with a PCT of 2.7 ± 0.5 days (2 consecutive negative smears, one day apart) in rats (7 week-old) infected with 2×10^7 PRBCs; treatment was administered daily for 3 days on Days 6, 7, and 8 PI and rats were followed to 21 days PI. Parasitemia increased gradually and peaked to ~30–40% in control group rats by Day 10-12; however, parasitemia gradually decreased by Day 15 and was cleared by Day 18-20. Lower doses (47.8 and 95.6 μ M/kg i.e., 18.4 and 36.8 mg/kg) of artesunate were less effective and reduced parasitemia in 67% of the rats. Recrudescence was reported at all the doses within 5 to 8 days of initial parasite clearance by Day 11,
<i>P. berghei</i> / ANKA strain Sprague-Dawley rats (Study Reports WRAIR-8C and 8F)	Experimental design was similar to that summarized above (Li <i>et al.</i> , 2003). No mortality was observed in rats treated with 36.7 mg/kg artesunate once daily for 3 days. Parasitemia was cleared in 38.9% of rats. The PCT was 52.8 h in 50% of the rats. All animals showed recrudescence at Day 6. PK parameters in infected rats were measured. • DHA peak plasma concentration of 9717 ± 858 ng/mL, at 0.17 ± 0.04 h, were 5-fold higher than artesunate concentration. The ratio of DHA to artesunate was 5.39 of total dose. • After 1 or 2 h of IV administration, the concentration of DHA in plasma remained higher than artesunate. • The elimination half-life of artesunate was 0.36 h and DHA 0.74 h. • All 3 infected rats treated with 3 doses of 120 mg/kg artesunate had minimal thymic lymphocyte depletion; also, moderate diffused cortical depletion with marked to severe lymphocytolysis in the cortex was reported. In the non-infected rats, no lymphocyte depletion was reported however, cortical thymic depletion was observed.

<i>Plasmodium</i> species/ strain (Reference)	Study summary
<i>P. berghei</i> / ANKA strain Sprague-Dawley rats (Study Report WRAIR-8G)	Experimental designs similar to that summarized above (Li <i>et al</i> , 2003) except that rats with >5% parasitemia were treated with different doses of artesunate and the suppressive dose that reduced parasitemia by 50% and 100% (SD ₅₀ and SD ₁₀₀ , respectively) determined. The mortality in untreated rats was 16.3% whereas all the rats treated up to a dose of 240 mg/kg survived; however, 4 out of the 5 rats died at a higher dose of 480 mg/kg. Parasitemia was cleared at doses of ≥60 mg/kg. The SD ₅₀ and SD ₁₀₀ values were 16.3 mg/kg and 60 mg/kg, respectively. Recrudescence was reported in all animals with initial parasite clearance even at the dose of 240 mg/kg. Based on the endpoints of the MTD, suppression and clearance, the therapeutic indices were calculated as 14.7 (240 mg/16.3 mg: 14.7) based on the SD ₅₀ data and 4 (240 mg/60 mg =4) for the SD ₁₀₀ data.
<i>P. berghei</i> / ANKA strain Sprague-Dawley rats (Xie <i>et al.</i> , 2005)	Experimental designs similar to that summarized above (Li <i>et al</i> , 2003) except that different doses of artesunate were tested and the suppressive doses that reduced parasitemia by 50% and 100% (SD ₅₀ and SD ₁₀₀ , respectively) were determined. The mortality in untreated rats was 16.7% whereas all the rats treated up to a dose of 240 mg/kg survived; however, 4 of 5 rats died at dosage of 480 mg/kg. Parasitemia was cleared at doses of ≥60 mg/kg, and clearance time ranged between 48 and 59 h. The SD ₅₀ and SD ₁₀₀ values were 7.4 mg/kg and 60 mg/kg, respectively. Recrudescence was reported in all animals with initial clearance even at the MTD of 240 mg/kg.
<i>P. berghei</i> / ANKA strain Sprague-Dawley rats (Ohrt <i>et al</i> , 2014)	Rats were inoculated with <i>P. berghei</i> -infected rat blood and different doses of IV artesunate administered for 3 days, when parasitemia of >4% was achieved. PCT was between 48-59 h in all rats treated with artesunate at doses of ≥60 mg/kg. The authors state that <i>P. berghei</i> is a self-curing parasite in rats: parasitemia spontaneously diminishes beginning approximately Day 12 after inoculation. In the control group of animals, parasitemia increased to approximately 10% on Day 8, reached a peak (10 and 35%) on Day 11, and then declined (self-cleared) to undetectable levels over the next seven to 11 days in the surviving animals; a 16.7% mortality from malaria was observed.
h=hours; PI=post-infection; CQ=chloroquine; SC=subcutaneous; IM=intramuscular; PCT=parasite clearance time; PRBCs=parasitized RBCs; MTD=maximum tolerated dose; SD ₅₀ and SD ₉₀ =50% and 90% suppressive dose	

18.5.3.2 Nonhuman primates infected with *Plasmodium coatneyi* and *P. cynomolgi*

The suppressive activity of artesunate was measured in splenectomized and non-splenectomized rhesus monkeys infected with *P. coatneyi* [(Maeno *et al.*, 1993a); ([Study Report WRAIR-8P 2004](#); [Study Report WRAIR-8Q 2001](#))] and *P. cynomolgi* (Ohrt *et al*, 2014). Treatment with artesunate was initiated after animals became parasitemic. The results show that artesunate was effective in rapidly reducing parasitemia; however, recrudescence occurred.

Maeno *et al.* (1993a) reported that in monkeys infected with *P. coatneyi*, artesunate treatment altered the morphology of the parasites. Within 4 hours of treatment, enlargement of the food vacuoles and cellular degeneration with ribosomal clumping of the trophozoites was observed. By 24 hours, the sequestration of the parasitized RBC in the cerebral microvessels was reduced to <0.1% in both splenectomized and non-splenectomized animals; the sequestration of

parasitized RBCs in cerebral microvessels was also reported. By electron microscopy, multiple electron-dense knobs protruding from the membranes of parasitized RBCs were observed in untreated animals; the knobs cytoadhered to the endothelial cells in cerebral microvessels and occasional endothelial cells exhibited swelling. In artesunate treated animals, very few parasitized RBCs in the cerebral microvessels and no electron-dense knobs were observed; however, some swollen endothelial cells were observed in the brain of treated animals. The authors state that cerebral edema could not be conclusively demonstrated, and cerebral hemorrhages were not seen either before or after treatment.

The Applicant conducted a study (Study Reports WRAIR-8P and 8Q) in splenectomized monkeys infected with $\sim 5 \times 10^6$ parasitized RBCs; spleen was removed 4 weeks prior to challenge. Untreated animals developed parasitemia within 1-2 days of inoculation that increased to 10^6 parasites/ μL (17-28% parasitemia) by Days 7-8. Early symptoms included onset of lethargy, anorexia and bloody urine; the infection was uniformly fatal if left untreated. Treatment with IV artesunate at doses of 4, 6, and 8 mg/kg for 3 days reduced mortality; such an effect was dose-dependent. Mortality was lower in artesunate treated animals (10% at doses between 4-8 mg/kg; overall 25%) compared to that in quinine (33%) treated animals. The most effective dose was 8 mg/kg and parasites were cleared in all the 3 monkeys treated with the 8 mg/kg dose of artesunate; at lower doses of 4 and 6 mg/kg, 66 and 50% reduction in parasitemia was observed. The parasite clearance time was shorter (~ 2.4 days) in artesunate treated animals compared to quinine (5.6 days). However, recrudescence occurred in all animals.

Similar observations were made in non-splenectomized monkeys with uncomplicated malaria.

For details see **Error! Reference source not found.**

Error! Reference source not found.: Suppressive activity of artesunate in nonhuman primates infected with the parasitized erythrocytes of *Plasmodium coatneyi* or *Plasmodium cynomolgi*

<i>Plasmodium</i> species (Reference)	Study summary
<i>Plasmodium coatneyi</i>	
<i>P. coatneyi</i>	Rhesus monkeys (n=13) in Groups 1-3, were infected IV with whole blood containing 2.2×10^8 PRBCs (13% ring forms and 87% schizonts); animals in Group 4 were infected with 1.2×10^8 PRBCs (77% ring forms and 23% schizonts). Three additional monkeys were uninfected and used for immunohistochemical staining of tissues.
Rhesus monkeys (Maeno <i>et al.</i> , 1993a)	Group 1: n=3 (splenectomized 2 days PI, treated 7 days PI with 3 doses: artesunate loading dose- 3.3, followed by 2 doses of 1.7 mg/kg, 8 and 24 h later). Group 2: n=3 (no splenectomy, dosings same as Group 1). Group 3: n=2 (no splenectomy, treated with a single dose of 3.3 mg/kg on Day 9 PI). Group 4: n=5 (infected with lower concentration of the inoculum: untreated).
	Prior to treatment, parasitemia ranged between 13.3-19.5%. Parasitemia rapidly decreased in all treated animals: In Groups 1 and 2 animals, parasitemia decreased to 2.2% and 0.1%, respectively; 50% reduction in parasitemia was between 3 and 5 h. In Group 3, parasitemia reduced to 10.6% at 3 h post-treatment with a single dose. Blood was collected for evaluating parasitemia for up to 24 h post-treatment.

<i>Plasmodium</i> species (Reference)	Study summary
	Groups 1-3 animals were sacrificed within an hour of the last dose i.e., Days 8 or 9 Group 4 untreated animals sacrificed on Days 7-8. Tissues and blood samples were processed for light and electron microscopy. The results show that in Group 1 and 2 animals, artesunate was effective in altering the morphology of the parasites, within 4 h of treatment, that include enlargement of the food vacuoles and cellular degeneration with ribosomal clumping of the trophozoites; by 24 h, the percentage of PRBC sequestration in the cerebral microvessels was reduced to <0.1%. In group 3 animals, the rate of PRBC sequestration also was reduced to 2.0% within 3 h after administration of a single dose of artesunate. The sequestration of PRBC in cerebral microvessels was reported in Groups 1-3 animals. The authors state that cerebral edema could not be conclusively demonstrated, and cerebral hemorrhages were not seen either before or after treatment; however, electron microscopy showed that untreated animals in Group 4, had multiple electron-dense knobs protruding from the membranes of RBCs; the knobs cytoadhered to the endothelial cells in cerebral microvessels and occasional endothelial cells exhibited swelling. In artesunate treated animals (Groups 1-3), very few PRBCs in the cerebral microvessels and no electron-dense knobs were observed; however, some swollen endothelial cells were observed in the brain of animals in Groups 1 and 2. Immunohistochemical staining of tissues was performed for IgG, C3, CD36, thrombospondin (TSP), ICAM-1, and endothelial-leukocyte adhesion molecule-1 (ELAM-1). In the untreated animals Group 4), IgG, C3, CD36, TSP, and ICAM-1 were observed in the cerebral microvessels from all five and ELAM-1 was found in 4 of 5 monkeys. In artesunate treated animals (Group 1), ELAM-1 was not detected in all three monkeys. However, IgG, C3, CD36, TSP and ICAM-1 were observed in cerebral microvessels from all eight monkeys in Groups 1-3. The brains from three uninfected monkeys were negative for IgG, C3, CD36, TSP, ICAM-1, and ELAM-1.
<i>P. coatneyi</i> Rhesus monkeys Severe malaria model (<i>Macaca mulatta</i>) (Study Report WRAIR-8P and 8Q)	Four year old splenectomized monkeys (spleen removed 4 weeks prior to challenge) were infected with PRBCs (monkeys were infected with 1 mL of infected blood from a donor monkey with parasitemia of >50,000/μL; about 5 x 10⁶ RBC with 1% parasitemia); animals developed parasitemia within 1-2 days of inoculation that exceeded to 10⁶ parasites/μL (17-28% parasitemia) on Day 7/Day 8 and were symptomatic. Early symptoms included onset of lethargy, anorexia and bloody urine; the infection was uniformly fatal if left untreated. Moderate (<50% normal food intake) to moderately severe (<15% reduction of food intake) anorexia preceded other symptoms by 12 h. PCT₉₀ and PCT₁₀₀ were the primary endpoints. IV artesunate, at doses of 4, 6, and 8 mg/kg administered for 3 days (when parasitemia reached ~25%), reduced mortality; such an effect was dose-dependent and parasites were cleared in all the 3 monkeys treated with the 8 mg/kg dose of artesunate; at lower doses of 4 and 6 mg/kg, 66 and 50% reduction, respectively, in parasitemia was observed. PCT was shorter (~58 hours) in animals treated with artesunate for 3 days compared to quinine (5.6 days). However, recrudescence occurred after 3 days in all animals. Mortality was lower in artesunate treated animals (10% at doses between 4-8 mg/kg; overall 25%) compared to that in quinine (33%) treated animals. The most effective dose was 8 mg/kg. Similar observations were made in non-splenectomized monkeys with uncomplicated malaria that were treated with artesunate when parasitemia reached 3%.
<i>Plasmodium cynomolgi</i>	
<i>P. cynomolgi</i> <i>bastianellii</i>	Splenectomized monkeys were inoculated with 10⁶ PRBCs; <i>P. cynomolgi</i> is fatal to splenectomized rhesus monkeys, if untreated. Artesunate at doses of 1 and 8 mg/kg/day, IV, for 3 days was effective in clearing

<i>Plasmodium</i> species (Reference)	Study summary
Rhesus monkeys (Ohrt <i>et al.</i> , 2014)	parasitemia in between Days 1-3 post-treatment; however, recrudescence occurred between Days 7 – 11 days post-treatment in the monkeys treated with 1 mg/kg dose. At the higher dose of 8 mg/kg, parasitemia was cleared by Day 3, in 3 of the 4 monkeys and 1 animal died on Day 1 of treatment. Recrudescence was observed, on Days 11 and 12, in 2 monkeys and 1 animal was cured.

PCT=parasite clearance time; PI=post-infection; h=hours; PRBC=parasitized RBC

18.5.3.3 Immunocompromised mice and monkeys infected with *Plasmodium falciparum*

- **Immunocompromised mice**

The activity of DHA was measured in immunocompromised [bg/bgxid/xid nu/nu (BXN)] mice infected with the parasitized RBCs (synchronized cultures) of the T24 strain of *P. falciparum* resistant to chloroquine and quinine (Moreno *et al.*, 2001). The grafted mice had 85 to 99% human RBCs (HRBCs) in their peripheral blood and parasitemia was between 0.1 % and 10%. Consistent and sustained parasite growth was observed that persisted as long as the HRBCs were regularly transfused and the immunomodulation maintained. Two-day oral treatment with chloroquine (mouse equivalents of the therapeutic regimens employed for human beings were used) was initiated when parasitemia was stable for at least 6 days and when ring forms were the predominant stage. Mice were followed for parasitemia on Giemsa stained thin blood smears and the parasite clearance time, parasite reduction rate after 48 hours of treatment, and effects upon different stages and parasite morphology assessed. Chloroquine was not effective in decreasing parasitemia; few if any, changes in the morphology of parasites or in the stage pattern were reported. Although a low percentage of pyknotic forms could be detected (11 to 16%), but the parasites still grew and developed normally over the following days. Mice were then treated orally with 50 mg/kg/day **DHA**, for 2 days. The results show that DHA was effective in decreasing parasitemia to below 0.005% within 24 hours; at 24 hours after initiation of treatment, pyknotic parasites (72%) and altered trophozoites (16%) were already predominant; however, small proportions of apparently healthy rings, trophozoites (1%), and schizonts (3%) were observed. Mice were cured within 2 days. At 48 hours, all of the rare forms still visible were pyknotic (for details see Table 18-29).

- **Aotus monkeys**

Ohrt *et al.* (2014) reported that artesunate dose of 8 mg/kg/day for 3 days administered on Day 5 post-infection, was effective in clearing parasitemia within 3-4 days after treatment initiation in Aotus monkeys infected with PRBCs of *P. falciparum*. Recrudescence was observed 3-9 days after initial parasite clearance (for details see Table 18-29).

18.5.3.4 Rhesus monkeys infected with *Plasmodium knowlesi*

Yang *et al.* (1982) reported that in rhesus monkeys infected with PRBCs of *P. knowlesi*, IV artesunate (at 6, 8, and 12 mg/kg once daily for 3 days), administered when parasitemia

reached 22-30%, was effective clearing parasitemia within 16-20 hours (for details see Table 18-29).

Table 18-29: Suppressive activity of artesunate in mice and nonhuman primates infected with the parasitized erythrocytes of *Plasmodium falciparum* or *Plasmodium knowlesi*

<i>Plasmodium</i> species/ strain (Reference)	Study summary
<i>P. falciparum</i> – mice and Aotus monkeys	
<i>P. falciparum</i> / T 24 strain resistant to chloroquine and quinine Immuno-compromised BXN (bg/bg xid/xid nu/nu) mice (Moreno <i>et al.</i> , 2001)	BXN mice were infected IP with synchronized cultures of PRBCs (1 mL of 0.3% to 0.6% [parasitemia; 50% hematocrit] of the multi drug-resistant T24 (Thailand) strain. After infection, 1 mL of washed huRBC at 50% hematocrit was injected IP together with NIMP-R14 (MoAb to control neutrophils) every 3 to 4 days; dichloromethylene diphosphonate (to control macrophages) was administered every 4 to 5 days. The grafted mice had 85 to 99% huRBC in their peripheral blood and parasitemia was between 0.1 % and 10%. Consistent and sustained parasite growth was observed that persisted as long as huRBC were regularly transfused and the immunomodulation protocol continued. Two-day oral treatment with chloroquine (mouse equivalents of the therapeutic regimens employed for human beings were used) was initiated when parasitemia was stable for at least 6 days and when ring forms were the predominant stage. Mice were followed for parasitemia on Giemsa stained blood smears and the PCT, parasite reduction rate after 48 h of treatment, and effects upon each stage and parasite morphology were assessed. Chloroquine was not effective in decreasing parasitemia of the resistant T24 strain; few if any, changes in the morphology of parasites or in the stage pattern were reported. A low percentage of pyknotic forms could be detected (11 to 16%), but the strain still grew and developed normally over the following days. Mice were then treated orally with 50 mg/kg/day DHA , for 2 days. The results show that DHA was effective in decreasing parasitemia to below 0.005% within 24 h in both mice and fully disappeared within 2 days. At 24 h after initiation of the treatment, pyknotic parasites (72%) and altered trophozoites (16%) were already predominant; however, small proportions of apparently healthy rings, trophozoites (1%), and schizonts (3%) were still observed. At 48 h, all of the rare forms, still visible, were pyknotic.
<i>P. falciparum</i> / FVO strain <i>Aotus</i> monkeys (Ohrt <i>et al.</i> , 2014)	Monkeys were inoculated with 5×10^6 PRBCs of FVO strain of <i>P. falciparum</i> . FVO strain is generally fatal in <i>Aotus</i> monkeys. Parasitemia in all animals increased from approximately 2,000 parasites/mm ³ on Day 4 to at least 50,000 parasites/mm ³ on Day 5 PI. By Day 8, parasitemia in 3 control monkeys was between 600,000-800,000 parasites/mm ³ and rescue therapy was administered. Artesunate at 8 mg/kg/day dose was administered for 3 days on Day 5 post-infection. Clearance of parasitemia was observed in all 3 animals, 3-4 days after treatment initiation. Recrudescence was observed 3-9 days after initial parasite clearance.
<i>P. knowlesi</i> - rhesus monkeys	
<i>P. knowlesi</i> / strain not specified Rhesus monkeys (Yang <i>et al.</i> , 1982)	Rhesus monkeys were infected with PRBC and artesunate administered IV, when parasitemia reached 22-30%, at 6, 8, and 12 mg/kg once daily for 3 days. Blood smears were examined every 4 h. Artesunate cleared parasitemia within 16-20 h after first IV injection.
IP=intraperitoneal; PCT=parasite clearance time; PI=post-infection; h=hours; PRBCs=parasitized RBCs; huRBCs=human RBCs	

18.5.4 Drug combination

Few studies reported an interaction between artemisinins and antiretroviral drugs *in vitro*. For example, He *et al.* ([He et al. 2010](#)) reported antagonistic activity between artemisinins (artesunate, DHA, and artemether) and antiretroviral protease inhibitors (ritonavir, saquinavir, and indinavir), against the 3D7 (chloroquine sensitive) and Dd2 (multi-drug resistant) *P. falciparum* strains. However, another study ([Mishra et al. 2010](#)) reported synergistic activity between artemisinin and indinavir or nelfinavir against 3D7 and RKL303 (chloroquine resistant) *P. falciparum* strains. The reasons for differences in activity between the two studies are unclear.

1.1.1. Drug Resistance

The nonclinical and clinical studies supporting a potential for development of resistance to artesunate, DHA, and other artemisinin derivatives are summarized.

18.5.4.1 Nonclinical studies

- ***In vitro***
 - **Laboratory strains and clinical isolates of *P. falciparum***

Jiang ([Jiang 1992](#)) reported the development of resistance by the FCR3 strain of *P. falciparum* to artesunate; the artesunate IC₅₀ values increased by about 3-fold after 13 days of exposure to the drug. However, such an effect was reversible after culture in drug-free medium.

Lim *et al.* (2010) reported lower *in vitro* sensitivity (i.e., higher IC₅₀ values) of *P. falciparum* isolates from Western Cambodia (multi-drug resistant malaria region), to artesunate and other antimalarials drugs, compared to the isolates from Eastern Cambodia; there was a positive correlation between artesunate and mefloquine, chloroquine, as well as quinine based on the IC₅₀ values. The IC₅₀s for isolates from participants who failed artesunate + mefloquine therapy, i.e., recrudescence isolates (n=37), were 2-fold higher than those from patients (n=558) who were cured ($P < 0.001$). Some of the isolates (n=851) were genotyped for single nucleotide polymorphisms (SNPs) in four genes (*pfdhfr*, *pfcr*, *pfmdr1*, and *pfatpase6*). The distributions of the SNPs differed between Eastern and Western Cambodia, suggesting different genetic backgrounds of the parasite populations. Although, there was no statistically significant correlation between increased *pfmdr1* copy numbers and increased artesunate IC₅₀s, *pfmdr1* codon Y184F appeared to be associated with an increased artesunate IC₅₀s (n=113), but only for Eastern Cambodia. For chloroquine, however, there was a significant association between the IC₅₀ values and *pfmdr1* codon Y184F (n = 114) or *pfdhfr* codon I64L (n = 162). The clinical significance of these findings is not known.

Witkowski *et al.* ([Witkowski et al. 2010](#)) reported a selection of artemisinin tolerant line (F32-ART), after long-term artemisinin pressure *in vitro*, of the parasitized RBCs of the F32-Tanzania strain of *P. falciparum*. The F32-ART strain was tolerant to artesunate, although artemisinin was used for inducing drug pressure, but not to chloroquine. A subpopulation of ring stages was in

developmental arrest that survived the higher concentrations (7000-fold initial IC₅₀) of the drug; no trophozoites or schizonts were observed. No mutations in the *PfATP6* gene and variations in the *pfmdr1* copy number were observed; the transcriptomic modifications observed include an overexpression of heat shock and RBC surface proteins and the down expression of a cell cycle regulator and a DNA biosynthesis protein. Within 24-30 hours of removal of the drug, the ring stages returned to normal cell cycle and trophozoites and schizonts developed. No quiescent rings were observed in the parent strain under drug pressure. The authors suggest increased tolerance to artemisinin to be mediated by a quiescence mechanism.

Dogovski *et al.* (2015) reported that incubation of synchronized cultures of resistant *P. falciparum* isolates, from Pailin, Cambodia, with DHA was associated with lower levels of ubiquitinated proteins and delayed onset of cell death compared to the cultures of the sensitive strains.

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

18.5.4.2 *Plasmodium vivax* drug resistance markers

Chaorattanakawee *et al.* (2017) reported *P. vivax* multi-drug resistance (*Pvmdr1*) Y976F and F1076L mutations in 78% (17/23) and 83% (19/23) of the clinical isolates, respectively, from Cambodia; of the 23 isolates tested, 74% (n=17) were double mutants, 9% (n=2) single F1076L mutants, and 17% (n=4) wild type isolates. None had *Pvmdr1* amplification. The artesunate and DHA IC₅₀ values were low (≤ 1.23 ng/mL). The clinical relevance of these findings is not known.

- ***In vivo* (Animal studies):**

Studies in rodent and nonhuman primate malaria animal models demonstrate recrudescence/recurrence after discontinuation of treatment with artesunate, DHA or other artemisinin derivatives (for details see Section 18.5.2). A potential for development of resistance in malaria animal models was measured using *Plasmodium* species that infect rodents as well as *P. falciparum*.

18.5.4.3 *Plasmodium* species infecting rodents - *Plasmodium berghei* and *Plasmodium yoelii*

Chawira *et al.* ([Chawira, Warhurst, and Peters 1986](#)) evaluated the development of resistance to artemisinin against the chloroquine-resistant NS strain of *P. yoelii*. Briefly, the TFW male mice were inoculated intravenously with 10⁷ parasitized RBCs of the NS strain of *P. yoelii* and treated with a single subcutaneous dose of artemisinin (100 mg/kg). Serial passage was performed by inoculating mice with parasitized RBCs from the artemisinin treated mice in the previous passage. After 50 passages (Day 149), about 35-fold increase (11.5 mg/kg to 400 mg/kg) in artemisinin 90% suppressive dose (SD₉₀) was observed. The resistance to artemisinin

was lost when the drug pressure was removed. However, the same level of resistance was observed by reapplying the drug pressure, in 3 passages. Resistance to artemisinin against the chloroquine-sensitive N strain of *P. berghei* was not observed when tested under similar experimental conditions.

Liu *et al.* ([Liu and Ren 1987](#)) reported the development of resistance by blood stage parasites of *P. berghei* to artesunate in mice; after 21 serial passages, the SD₉₀ increased by 29-fold. Resistance remained stable until 80 passages.

- ***Plasmodium falciparum***

Tyagi *et al.* ([Tyagi et al. 2018](#)) reported a potential for development of resistance by *P. falciparum* to artesunate in vivo, in humanized immunocompromised [NOD/SCID IL-2R γ ^{-/-} (NSG)] mice, and ex vivo. Briefly, animals were administered clodronate-containing liposomes to induce modulation of innate defenses; chimerism with human RBCs was maintained at ~78% and monitored by flow cytometry. Mice were infected with asynchronous cultures of *P. falciparum* (Uganda Palo Alto Marburg (FUP/CB or PAM) strain and parasitemia monitored by Giemsa stained smears. Artesunate was administered IV as single-doses (2.4 to 240 mg/kg) or a 2-day regimen administered 2-days apart [2.4 mg/kg/injection (total dose 4.8 mg/kg) up to 80 mg/kg/injection]; doses were progressively increased as parasites were recovered. Parasitemia was monitored every 24 hours and allowed to recover to the pre-treatment level before administering the next higher dose.

The normal day-to-day fluctuation of parasitemia in 13 untreated mice was 18.3% (95% confidence interval was 12.5–27%); based on this, the threshold for resistance, was the dose at which parasitemia failed to drop >27% the next day. The mean reduction in parasitemia of the PAM-sensitive strain, the day after a single dose administration, was 78.4 ± 18.2%; the sensitive response was defined as parasitemia greater than 60.2% that corresponded to the mean (1 SD).

A **single dose** of 0.15 mg/kg artesunate was established as the lowest effective dose; parasitemia was reduced within 24 to 48 hours and pyknotic parasites observed. However, recrudescence occurred by 72 hours post-treatment. After parasitemia recuperated back to pre-treatment levels (APC), the next higher dose of artesunate was administered. Artesunate resistant (ART-R) parasites were passaged through 7 generations. The results show, an increase in the number of mice with ART-R parasites at increasing doses. Stability of the resistant parasites was observed in increasing number of mice at a dose of 60 mg/kg in the 4th generation parasites and 120 mg/kg in the 5th generation; in the 7th generation, resistance was stable, in all 6 mice (ART-R₂₄₀) after sub-inoculation to mice treated with 240 mg/kg artesunate; this represents a 3200-fold decrease in sensitivity to artesunate in immunocompromised mice. The sensitive progenitors of the PAM strain, used as controls, remained sensitive at all doses for all the generations.

Studies for **2-day dosing** were started at a dose of 2.4 mg/kg with an ART-R₃₀ strain (i.e., resistant at a single dose of 30 mg/kg). Resistance was observed after 1 APC. The parasites were

passed through 4 generations and increasing doses administered until reproducible resistance at doses of 80 mg/kg (i.e., 160 mg/kg total) was observed.

The ex vivo sensitivity of the ART-R strains was measured by a double-site enzyme-linked pLDH immunodetection and ³H-hypoxanthine incorporation (for confirmation) assays. Briefly, the synchronous ring stage cultures with parasitemia of 0.05% and 2% hematocrit were incubated with and without drug for 72 hours. The artesunate and DHA IC₅₀ values for the sensitive strain were 10.7 nM i.e., 4.11 ng/mL and 13.8 nM i.e., 3.92 ng/mL, respectively. Although there was a trend (based on slope of the curve) towards an increase in IC₅₀ values for ART-R strains collected at lower doses, significantly higher artesunate and DHA IC₅₀ values (82.5 nM i.e., ng/mL and 54.6 nM i.e., 31.71 and 15.5 ng/mL, respectively) were reported for ART-R₁₂₀ strain (after single dose); for the ART-T₂₄₀ strain, the artesunate and DHA IC₅₀ values were 100.3 nM and 100.3 nM (i.e., 38.56 and 28.52 ng/mL), respectively. Similar shift in IC₅₀ values was observed for parasites from the 2-day dosing regimen.

The resistant parasites (ART-R) were processed for isolation of DNA, PCR/restriction fragment length polymorphism (RFLP) analyses to assess polymorphisms in the 451 PCR fragment²⁴; RFLP assessment revealed no mutation in the ART-R₂₄₀, ART-R_{DD38.4}, or the parent PAM strain.

PCR-sequencing was performed for the *RAD5*, *cNBP*, *RPB9*, *PK7*, *FP2A*, *Pfg27*, *chloroquine resistance transporter (PfCRT)*, *Na⁺/H⁺ exchanger (PfNHE)*, two fragments overlapping the *kelch13* propeller domain, and *multidrug resistant (Pfmdr1)* genes of *P. falciparum*. There was no difference in the sequencing of the putative *Kelch13* propeller domain between control (3D7, PAMwt) and ART-R strains; also, there were no sequence changes in the 611 bp *PfNHE* fragment gene, flanking the DNNND repeat, which is related to quinine resistance. The changes observed include the following:

- A mutation identified in the *RAD5* homolog, in all of the ART-R parasites recovered from mice, that were resistant to single doses of 38.4 mg/kg, 120 mg/kg, and 240 mg/kg artesunate, and to a 2-day regimen of 80 mg/kg/day artesunate; the *RAD5* mutation was not identified in the wild type parent strain and in any of 4 control clinical *P. falciparum* isolates collected from Brazil, Senegal, Comoro Islands, and Thailand in the late 1990s.
- An amplification of the *Pfmdr1* gene copy number from 1 to 2 copies as well as *N86Y* mutation after in vivo artemisinin drug pressure.

Plasma concentration of DHA, measured at 1- and 2-hour after administration of 120 mg/kg artesunate in four ART-R₁₂₀-infected mice, were between 1573-3219 ng/mL; the DHA half-life was between 20.9-53.2 minutes.

²⁴ This is the orthologous gene in *P. falciparum* (PF3D7_0104300) identified based on studies with *Plasmodium chaubaudi* (PCHAS020720), a rodent malaria parasite. A non-synonymous point mutation of *ubp1* in *P. chaubaudi* (PCHAS020720) was reported by others as being a marker of ART resistance in a rodent model.

Overall, the studies suggest a potential for development of resistance to artesunate in the blood stage parasites of *Plasmodium* species. Studies in the *P. falciparum* humanized murine immunocompromised model suggest that delay in parasite clearance time was not associated with increased IC₅₀ values; however, in stable resistant strains, there was an association with an increase in the IC₅₀ values as well as mutation in the *RAD5* but not in the *Kelch13* gene.

18.5.4.4 Clinical studies

- ***P. falciparum***

Resistance to artemisinins, including artesunate and DHA, by the *P. falciparum* parasites is clinically manifested as recrudescence and/or delay in parasite clearance time [([Bunnag et al. 1991](#)) ; ([Alin et al. 1995](#)); ([Looareesuwan et al. 1997](#)); (Krudsood *et al.*,2000); ([Krudsood et al. 2003](#)); ([Dondorp et al. 2009](#)); ([Ashley et al. 2014](#)); ([Takala-Harrison et al. 2015](#)); ([Ménard et al. 2016](#)); ([Thanh et al. 2017](#))]. This is thought to be due to short half-life of the artemisinin drugs. Therefore, artemisinin drugs are administered in combination with long-acting drugs such as lumefantrine.

There is no standardized assay to evaluate the in vitro sensitivity of the isolates to artemisinins, including artesunate and DHA. Recent studies (Witkowski *et al.*, 2013; Klonis *et al.*, 2013) suggest that a ring-stage survival assay may be useful for testing sensitivity to artemisinins; there was a good correlation between in vitro sensitivity and a delay in parasite clearance time. Thanh *et al.* (2017) reported that recrudescence was associated with a decrease in the ex vivo response to DHA as measured by the ring survival assay.

A delay in parasite clearance time and/or recrudescence has been shown to be associated with alterations in some genetic regions, such as *Kelch13* and *Pfmdr1*, of *P. falciparum* isolates from some of the regions.

- *Mutations in Kelch13*

A delay in parasite clearance time has been reported in Cambodia, Laos, Myanmar, Thailand, China, Vietnam, Bangladesh, Cambodia and Africa (Dondorp *et al.*, 2009; Ashley *et al.*, 2014; Takala-Harrison *et al.*, 2015; Ménard *et al.*, 2016; Thanh *et al.*, 2017). In some of the countries, such as Bangladesh, Cambodia, Laos, Myanmar, Thailand, and Vietnam, *P. falciparum* parasites with mutation(s) in any of the *Kelch13* domains were associated with longer parasitic clearance time compared to parasites with wild type (WT) alleles; several mutations were identified, with C580Y being the most prevalent [([Ariey et al. 2014](#)); (Takala-Harrison *et al.*, 2015); (Thanh *et al.*, 2017)]. Based on the surveillance studies in 59 countries, the *Kelch13* Artemisinin Resistance Multicenter Assessment (KARMA) consortium reported 108 non-synonymous *Kelch13* mutations with regional differences; e.g., in Cambodia-Vietnam-Laos the dominant mutation was C580Y whereas in Thailand-Myanmar-China the dominant mutation was F446I (Tilley *et al.*, 2016). In sub-Saharan Africa, however, no *Kelch13* mutations and changes in vitro sensitivity of the parasites were reported [([Taylor et al. 2015](#)); Fall *et al.*, 2015; ([Kremsner et al. 2016](#))].

Based on the surveillance studies conducted by the CDC in the US, no *Kelch13* resistant marker was reported in 77 of the 78 patients from Africa; also, no resistance marker was identified in any of the 5 patients from Central America (Mace *et al.*, 2018). Similar observations were reported recently ([Mace, Arguin, and Tan 2018](#)); of the 146 patients from Africa, A578S polymorphism was reported in 1 subject and not in any of the 2 subjects from Central America and the Caribbean.

The mechanism by which *PfKelch13* mutations/inactivation of kelch compartment proteins produce delay in parasite clearance is unclear. Studies suggest that *Kelch13* mutation may protect the resistant parasites from oxidative damage by reducing the levels of ubiquitinated and unfolded proteins [(Dogovski *et al.*, 2015); ([Mok et al. 2015](#))] and/or reduce endocytosis of hemoglobin from the host cell (Birnbaum *et al.*, 2020). Mbengue *et al.* (2015) reported an increased PfPI3K level was associated with the C580Y mutation in *Kelch13* of the resistant strain of *P. falciparum*; PfPI3K binding to C580Y *Kelch13* mutants, measured by an immunoprecipitation assay, was reduced in the resistant strains compared to the wild-type strain without *Kelch13* mutation. Mutations in *Kelch13* may decrease the degradation of PfPI3K leading to an increase in PfPI3K levels; this may allow ring-stage *P. falciparum* parasites to survive the oxidative stress induced by artemisinins by entering the quiescent stage.

Parasites may extend their ring-stage. Based on in vitro studies, Witkowski *et al.* (2010) showed that parasite is arrested at the ring-stage under drug pressure; however, cell cycle arrest was reversible after culture in drug free medium.

- *Mutations or increased copy number of pfmdr1 gene*

Treatment with a combination of artemether+ lumefantrine selected for *pfmdr1 N86* [([Somé et al. 2010](#)); ([Ngasala et al. 2011](#)); ([Malmberg et al. 2013](#))] and Y184 (Somé *et al.*, 2010) alleles. Venkatesan *et al.* ([Venkatesan et al. 2014](#)) reported that increased copy number of *pfmdr1* and the presence of *pfmdr1 N86* were a significant independent risk factors for recrudescence in patients treated with Coartem; almost 50% samples from Thailand had ≥ 2 copies of *pfmdr1*, but this is rarely observed in African samples. Kreamsner *et al.* (2016) reported that a delay in parasite clearance was associated with the N86Y *Pfmdr1* genotype in subjects treated with artesunate.

- *P. vivax, P. malariae, P. ovale and P. knowlesi*

No information available.

1.1.2. Cross-resistance

18.5.4.5 In vitro studies

In vitro activity of artesunate and DHA against chloroquine sensitive and resistant strains of *P. falciparum* was similar (Alin 1990; Le Bras, 1998; Ringwald 1999, Vivas 2008; Wongsrichanalai *et al.*, 1999; for details see Section 18.5.2).

Wongsrichanalai *et al.* (1999) reported higher artesunate IC₅₀ values for isolates collected during 1991-1997, from the Thai-Myanmar and Thai-Cambodian borders compared to those from Southern Thailand. There was a strong correlation between the artesunate and mefloquine as well as artesunate and quinine IC₅₀ values.

Brockman *et al.* (2000) reported a positive correlation between the *in vitro* sensitivity to artesunate and quinine ($r=0.43$, $P<0.001$), mefloquine ($r=0.46$, $P<0.001$), and halofantrine ($r=0.51$, $P < 0.001$). It is noted that the isolates tested were recrudescence isolates, collected between March 1995 and July 1999 from patients presenting with falciparum malaria within 42 days of treatment with artesunate + mefloquine.

18.5.4.6 *In vivo* (Animal studies)

Tyagi *et al.* (2018) reported that immunocompromised mice infected with an artesunate resistant strain of *P. falciparum* (ART-R₂₄₀) and treated with either 3 doses of quinine (73 mg/kg every 8 hours, IV), 4 doses of halofantrine (1 mg/kg, every 24 hours), and/or one dose of amodiaquine (73 mg/kg, oral) were resistant to quinine, amodiaquine, and halofantrine both *in vivo* and *ex vivo*; also artesunate plus amodiaquine were ineffective *in vivo*. Additionally, ART-R₁₂₀ and ART-R₂₄₀ strain had an amplified *pfmdr* gene. However, there was no change in IC₅₀ values for other drugs (chloroquine, mefloquine, lumefantrine, and pyrimethamine); also, artesunate plus mefloquine were effective *in vivo*. The parasites of the parent sensitive strain (PAM) were sensitive to all drugs.

18.6 Clinical Review of Specific Studies/Clinical Trials with IV Artesunate

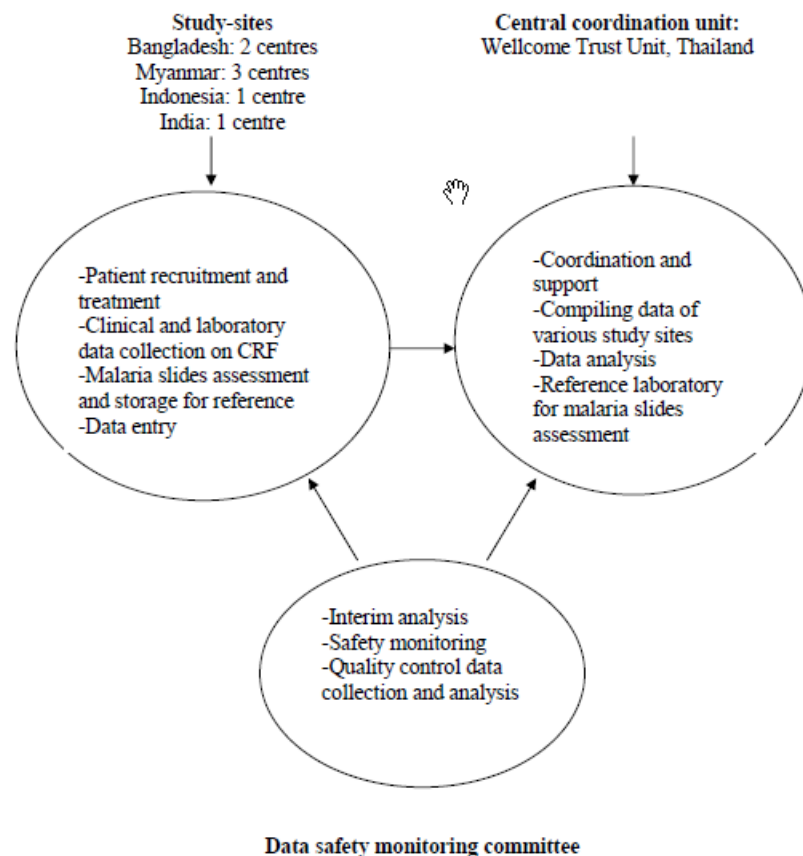
18.6.1 SEAQUAMAT

Primary outcome of mortality in SEAQUAMAT was analyzed for baseline characteristics including demographics, clinical status and laboratory evaluations for this study - See Section 8.1.1.

18.6.1.1 Study Design and Analysis

Results for SEAQUAMAT submitted in the NDA consisted of a reanalysis of legacy datasets obtained from the principle investigator, Arjen Dondorp, MD., PhD. Datasets were analyzed by the (b) (4). Because the original analysis dataset collected clinical events (adverse events) and concomitant medications in a single text field, coding was performed to split the variables into adae.xpt and adcm.xpt files. The CSR was drafted by the (b) (4).

Figure 18-12: SEAQUAMAT – Diagram of Study Structure



Source: SEAQUAMAT Protocol p. 20

18.6.1.2 Study Results – Safety

The primary objective of SEAQUAMAT was to evaluate efficacy of IV artesunate in the treatment of severe malaria, with the primary endpoint in-hospital mortality. Secondary outcomes included the incidence of neurological sequelae, combined death or neurological sequelae, recovery times (time to eat, speak, sit and discharge), and development of severe complications. Beyond these outcome measures, the CRF fields included a baseline assessment at the time of admission that assessed the presence or absence of clinical conditions associated with severe malaria, including coma, severe prostration, convulsions, severe anemia, anuria/oliguria, hypotension, suspected metabolic acidosis, respiratory insufficiency, blackwater fever, clinical jaundice, hypoglycemia, and a free text field. The CRF also collected data on Glasgow or Blantyre coma scales at baseline, vital signs and clinical history including previously administered medications. In addition to blood smears, parasite counts, limited laboratory evaluations were performed on admission, including anion gap, base excess, BUN, chloride, venous CO₂, glucose, Hb, Hct, potassium, sodium, venous pH and total CO₂. Laboratory evaluations following admission were not routinely reported.

The CRF also collected “complications not present on admission” which were coded as CETERM “After Admission” in the ADAE dataset. These clinical events included the following: ICU admission; coma, not present on admission or deterioration in GCS; convulsions not present on admission, shock (systolic blood pressure less than 80 mmHg); vasopressor drugs required, respiratory insufficiency or mechanical ventilation, renal failure (BUN > 60 mg/dL or urine output < 400 mL/dL; severe anemia (Hct < 20%); severe bleeding (requiring blood transfusion); blackwater fever/hemoglobinuria; hypoglycemia (glucose < 40 mg/dL; severe jaundice; suspected sepsis; antibiotics; in case of pregnancy: still birth or spontaneous abortion during admission; and a free text box for other complications.

The outcome was recorded as either the patient died (at home or in hospital and date of death) or survived (date of discharge). The duration of symptoms (in days) was recorded in time to speak, time to sit independently, and time to eat independently.

The following neurological sequelae were specified on the CRF, with the investigator instructed to tick if “yes”: cortical blindness, cranial nerve palsies, ataxia, loss of balance, extrapyramidal rigidity, hemiplegia/paresis/neuropsychiatric (like psychosis), and other significant neurological sequelae on discharge with a free text field to provide details.

The CRF also captured “other medical problems on discharge” in a free text field.

18.6.1.2.1 Deaths and SAEs

The table below provides mortality by malaria status on admission and by age. SAEs were not captured in SEAQUAMAT.

Table 18-30: SEAQUAMAT – Mortality by Malaria Status on Admission and Age (Safety Population)

Mortality	Artesunate (n=730)	Quinine (n=730)	Totals (n=1460)
Death Flag	107 (14.7%)	165 (22.6%)	272 (18.6%)
Severe Malaria Population Flag	509 (69.7%)	541 (74.0%)	1050 (71.9%)
Cerebral Malaria Flag	284 (38.9%)	304 (41.6%)	588 (40.2%)
Positive Blood Smear Flag	689 (94.4%)	693 (94.8%)	1382 (94.6%)
Malaria Parasites on Blood Film Flag	708 (97.0%)	716 (97.9%)	1424 (97.5%)
Death Within 48 hrs Flag	61 (8.4%)	75 (10.3%)	136 (9.3%)
Death Rate in Patients with Hyperparasitemia >10%	28/121 (23.1%)	57/108 (52.8%)	85/229 (37.2%)
Age Group 01			
>=5 years old	693 (94.9%)	698 (95.5%)	1391 (95.2%)
<5 years old	37 (5.1%)	33 (4.5%)	70 (4.8%)
Age Group 02			
>=15 years old	633 (86.7%)	626 (85.6%)	1259 (86.2%)
<15 years old	97 (13.3%)	105 (14.4%)	202 (13.8%)
Age Group 03			
<65	713 (97.7%)	710 (97.1%)	1423 (97.4%)
>=65	17 (2.3%)	21 (2.9%)	38 (2.6%)

Source: Clinical Reviewer (J Review)

18.6.1.2.2 Time to Event (Death, Discharge and Neurological Status)

See Section 8.1.1 for review of death, discharge, and neurological status.

After Admission

The following table provides clinical events observed after admission. Hypoglycemia was notably higher in the quinine group compared with the artesunate group (2.6% versus 0.8%).

Reviewer comment: The SEAQUAMAT publication (Dondorp 2005) provides some of these results in Table 3, Results by Treatment Group.

Table 18-31: SEAQUAMAT – Clinical Events After Admission¹ (Safety Population)

	Artesunate (n=730)	Quinine (n=730)	Totals (n=1460)
Antibiotics	193 (26.4%)	231 (31.6%)	424 (29.0%)
Renal Failure (BUN > 60 mg/dL) ²	140 (19.2%)	157 (21.5%)	297 (20.3%)
Acute Renal Failure Requiring Dialysis ³	65 (8.9%)	53 (7.3%)	118 (8.1%)
Coma or Deterioration in GCS	52 (7.1%)	65 (8.9%)	117 (8.0%)
Black Water Fever/ Hemoglobinuria	49 (6.7%)	33 (4.5%)	82 (5.6%)
Suspected Sepsis	45 (6.2%)	58 (7.9%)	103 (7.0%)
Other Complications	33 (4.5%)	51 (7.0%)	84 (5.7%)
Convulsions	31 (4.2%)	43 (5.9%)	74 (5.1%)
ICU Admission	28 (3.8%)	46 (6.3%)	74 (5.1%)
Respiratory Insufficiency or Mechanical Ventilation	26 (3.6%)	39 (5.3%)	65 (4.4%)

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

	Artesunate (n=730)	Quinine (n=730)	Totals (n=1460)
Shock	26 (3.6%)	36 (4.9%)	62 (4.2%)
Vasopressor Drugs Required	23 (3.2%)	24 (3.3%)	47 (3.2%)
Severe Anemia (Hct < 20%)	20 (2.7%)	20 (2.7%)	40 (2.7%)
Severe Jaundice	17 (2.3%)	13 (1.8%)	30 (2.1%)
Severe Bleeding	14 (1.9%)	11 (1.5%)	25 (1.7%)
Hypoglycemia	6 (0.8%)	19 (2.6%)	25 (1.7%)
Still Birth or Spontaneous Abortion	5 (0.7%)	5 (0.7%)	10 (0.7%)

Source: Clinical Reviewer (J Review 13.1)

¹ These events were termed “complications” in SEAQUAMAT CSR, See Table 14.2.3.1., Summary of complications after study entry. Note, CSR table did not include Renal Failure category.

² Applicant reported these numbers for renal failure after a admission but acknowledged in SN 0040, response to IR that laboratory evaluations including BUN were collected only at the time of a admission.

³ Dialysis included terms: dialysis, hemodialysis, peritoneal dialysis

Reviewer comment: SEAQUAMAT evaluated predefined clinical events after admission, including acute renal failure defined as BUN > 60 mg/dL. As the Applicant’s analysis of renal failure correlated to BUN values on admission, an alternate measure of patients experiencing acute renal failure following IV artesunate therapy was utilized by querying free text field for complications not present on admission for “dialysis” and related terms.

Table 18-32: SEAQUAMAT – Clinical Events After Admission (Severe Malaria Population)

	Artesunate n=509	Quinine n=541	Totals n=1050
Antibiotics	166 (32.6%)	198 (36.6%)	364 (34.7%)
Renal Failure (BUN > 60 mg/dL) ¹	136 (26.7%)	146 (27.0%)	282 (26.9%)
Dialysis	55 (10.8%)	44 (8.1%)	99 (9.4%)
Coma or Deterioration in GCS	43 (8.4%)	58 (10.7%)	101 (9.6%)
Suspected Sepsis	40 (7.9%)	49 (9.1%)	89 (8.5%)
Black Water Fever/ Hemoglobinuria	38 (7.5%)	25 (4.6%)	63 (6.0%)
Convulsions	28 (5.5%)	36 (6.7%)	64 (6.1%)
Other Complications	27 (5.3%)	43 (7.9%)	70 (6.7%)
ICU Admission	25 (4.9%)	43 (7.9%)	68 (6.5%)
Respiratory Insufficiency or Mechanical Ventilation	23 (4.5%)	33 (6.1%)	56 (5.3%)
Shock	23 (4.5%)	32 (5.9%)	55 (5.2%)
Vasopressor Drugs Required	20 (3.9%)	22 (4.1%)	42 (4.0%)
Severe Anemia (Hct < 20%)	19 (3.7%)	16 (3.0%)	35 (3.3%)
Severe Jaundice	14 (2.8%)	13 (2.4%)	27 (2.6%)
Severe Bleeding	13 (2.6%)	11 (2.0%)	24 (2.3%)
Hypoglycemia	5 (1.0%)	16 (3.0%)	21 (2.0%)
Still Birth or Spontaneous Abortion	5 (1.0%)	4 (0.7%)	9 (0.9%)

Source: Clinical Reviewer (J Review 13.1)

¹ Applicant reported these numbers for renal failure after admission but acknowledged that laboratory evaluations including BUN were collected only at the time of admission.

On Discharge

If the patient survived, limited information on the clinical status of the patient was collected at discharge. The CRF collected information on the duration of symptoms by recording the days required for the following:

- Time to speak
- Time to sit independently
- Time to eat independently

The CRF also captured information on neurological and other sequelae including the following tick boxes to check if present (more than one tick was permitted):

- Cortical blindness
- Cranial nerve palsies
- Ataxia
- Loss of balance
- Extrapyramidal rigidity
- Hemiplegia/paresis
- Neuropsychiatric (like psychosis)
- Other significant neurological sequelae on discharge; if ticked specify:
- Other significant medical problems; if ticked specify:
- Comments

See Section 8.1.1 for discussion of secondary endpoints including neurological outcomes. “Significant neurological sequelae” were reported in 4 (0.5%) of artesunate recipients compared with zero quinine recipients; hemiplegia/paresis and ataxia were also observed in 2 artesunate recipients each versus no quinine recipients. In addition to neurological sequelae, at the time of discharge patients were evaluated for other significant medical problems with specific information collected in a free text field. Of note, more events were observed in the “significant medical problem” category in artesunate recipients: 36 (4.6%) in artesunate group versus 28 (3.8%) in the quinine group.

When querying the “other significant medical problems” category, multiple descriptions were provided included. For artesunate recipients these included the following observations (note: some patients were reported with more than one condition):

- anemia (7 patients, Hb range 3.6 – 5%);
- left hospital against medical advice (6 patients);
- HIV positive patient- (5 patients);
- acute or persistent renal failure (4 patients);
- pulmonary TB (3 patients)
- diabetes type 1 (2 patients, 1 with diabetic ketoacidosis), Diabetes type 2 (2 patients);

- elevated BUN/Creatinine (2 patients);
- vertigo but no objective neuro deficit;
- pregnancy 24-25 weeks;
- persistent, intermittent hiccups;
- vertigo and weakness;
- keratitis due to pre-existing lagophthalmos
- benign prostrate hypertrophy;
- mitral regurgitation;
- collagen disease (? SLE);
- aplastic (hypoplastic) anemia;

The single patient reported with aplastic anemia on discharge (Subject [REDACTED]^{(b) (6)}) was a 17 years old male from Myanmar who had received four doses of artesunate. He had a positive blood smear for *P. f. malaria*, parasitemia < 10%, and discharge on day 6. No hematology labs were found.

18.6.1.2.3 Treatment Emergent Adverse Events

See Section 10.4.1 Treatment Emergent Adverse Events.

Use of Artesunate During Pregnancy

See Section 10.9.2, Human Reproduction and Pregnancy for the discussion of artesunate in pregnancy.

Subgroup safety analyses in pediatric patients in SEAQUAMAT can be found in in Section 12, Pediatrics.

18.6.2 CDC-060

Title: Retrospective Abstraction of Data Collected During IND Protocol CDC-060 – Intravenous Artesunate for the Treatment of Severe Malaria in the United States.

18.6.2.1 Study Objectives

To evaluate the safety, tolerability, and potential clinical benefits of IV artesunate for patients in United States hospitals with severe and complicated malaria who do not have access to quinidine, do not tolerate that drug, or whose infection does not respond to quinidine treatment.

18.6.2.2 Study Design and Endpoints

Data reported were retrospectively abstracted from medical records of subjects with malaria in the US enrolled in a treatment IND application from the Centers for Disease Control and Prevention (CDC) from August 2007- December 2010. Subjects were to receive four doses of

IVAS over 48 hours. Data were collected on the safety and the clinical course of the disease from administration of the first dose of IV artesunate until the subject transitioned to follow-on oral therapy (or a maximum of 7 days). These data were collected on a data abstraction template (DAT) that was developed by the US Army. The data were quality assured and then presented before a Clinical Benefits Endpoint Committee (CBEC), with a majority vote used to make decisions on inclusion of clinical benefit and safety outcomes.

Table 18-33: CDC-060 – Schedule of Events

Table 3: Time and Events Table for the Collection of Information in DATs

Event	Baseline				Release from ICU ^a
	0	1	2	3	
Study Day					Up to 7
Informed Consent	X				
IVAS Treatment		0 hr, 12 hr	24 hr	48 hr	
Start Oral Follow-on Antimalarial Medication				X	
Study Inclusion	X				
Demographics	X				
Past Medical History	X				
Daily Physical Exam	X	X	X	X	X
Malaria Microscopy Results	X	X	X	X	X
Daily Hematology	X	X	X	X	X
Daily Chemistry	X	X	X	X	X
Vital Signs	X	X	X	X	X
Clinical Benefit Outcomes		X	X	X	X
Prior & Concomitant Medications	X	X	X	X	X
Adverse Events		X	X	X	X
Subject Disposition (End of Trial)				X	X
Protocol Deviation	X	X	X	X	X
Blood and Platelet Transfusions	X	X	X	X	X
Ventilator Support	X	X	X	X	X

^a No information was recorded after Day 7.

Source: CDC-060 Protocol, Table 3, p. 30

18.6.2.3 Criteria for Evaluation (Endpoints)

Safety

- Physical examination
- Vital signs
- Clinical laboratory tests (chemistry, hematology, malaria microscopy)
- Adverse events (AEs)

Clinical Benefit (Efficacy)

- Percentage of subjects who completed IVAS therapy as defined by the protocol

- Percentage of subjects who were able to receive a follow-on oral antimalarial therapy
- Time to negative parasitology
- Time to start of a follow-on oral therapy
- Time to discharge from the intensive care unit (ICU)
- Serial parasite density
- Change in clinical signs, laboratory parameters, and symptoms of severe malaria, including an evaluation of the improvement, worsening, development of, no change, or never present status of:
 - Parasitemia
 - Impaired consciousness
 - Seizures
 - Circulatory collapse/shock
 - Pulmonary edema or acute respiratory distress syndrome (ARDS)
 - Acidosis
 - Acute renal failure
 - Abnormal bleeding or disseminated intravascular coagulation (DIC)
 - Jaundice
 - Severe anemia
 - Ability to take oral medication

18.6.2.4 Key Inclusion Criteria

Subjects eligible for IV artesunate treatment were those for whom there was a microscopy-confirmed diagnosis of severe malaria that required parenteral treatment, a strong clinical suspicion of severe malaria for which a timely, reliable microscopic diagnosis was not available, or the presence of uncomplicated malaria but an inability to take oral treatment. The administration of IV artesunate was requested because quinidine was either unavailable or could not be accessed in a timely manner, the patient experienced quinidine failure or intolerance, or the patient had contraindications to quinidine.

18.6.2.5 Statistical Analysis Plan

Continuous variables were summarized with descriptive statistics [n, mean, standard deviation (SD), median, minimum (min), and maximum (max)]. When appropriate, graphical summaries were used to describe distributions of numeric variables. Frequency tables (counts, percentages) were used to summarize categorical data. Confidence intervals (CI) (95%) for outcomes and AE rates were reported. Analysis populations were defined as follows:

- Enrolled analysis population (n=128): All subjects who had a CDC subject ID, and for whom IV artesunate was shipped.
- Safety analysis population (n=102): All subjects in the analysis population who received at least one dose of IV artesunate
- Evaluable analysis population (n=87): All subjects in the safety analysis population who met at least one of the criteria in Section A (malaria diagnosis) and Section B (need for IV treatment) of the Treatment IND.

- Inclusion criteria met and had microscopic confirmation of *P. falciparum malaria*.
- Per protocol analysis population (n=76): All subjects in the safety analysis population who met at least one criterion in Section A (malaria diagnosis), Section B (need for IV treatment), and Section C (IV artesunate desirable) of the Treatment IND Inclusion criteria.

Additional subgroup analyses included:

- Level of renal impairment, as measured by the estimated glomerular filtration rate (eGFR), calculated using the Modification of Diet in Renal Disease (MDRD) formula
- Level of hepatic impairment, as measured by Model for End-Stage Liver Disease (MELD) score
- Subjects' concurrent antimalarial use during IVAS administration
- Quinidine exposure (any time prior to or during IVAS administration)
- Presence of seizures or impaired consciousness or ventilator support at baseline

18.6.2.6 Study Results

18.6.2.6.1 Demographics and Baseline Characteristics

The following table presents the demographic characteristics of patients included in the study.

Table 18-34: CDC-060 – Demographics (Safety Population)

	Intravenous Artesunate n=102
Age	
Total subjects with data	102
mean [SD]	38.16 [16.19]
median	39.50
Range	1-72
<18 years	12 (11.8%)
>=18 years	90 (88.2%)
Sex	
M	62 (60.8%)
F	40 (39.2%)
Race	
Black or African American	64 (62.7%)
White	26 (25.5%)
Asian	9 (8.8%)
Missing	3 (2.9%)
Ethnicity	
Not Hispanic or Latino	67 (65.7%)
Missing	25 (24.5%)
Hispanic or Latino	10 (9.8%)
Weight at Baseline kg	
Total subjects with data	102
mean [SD]	78.4 (23.5%)

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

	Intravenous Artesunate n=102
median	78
Range	9.3-140.9
Pregnant	3 (2.9%)

Source: Clinical Reviewer (J Review)

Table 18-35: CDC-060 – Age Category of Enrolled Subjects (Safety Analysis)

Age Category (Years)	No. of Subjects
<2	2 (2.0%)*
2 to <5	2 (2.0%)
5 to <15	6 (5.9%)
15 to <65	89 (87.3%)
>=65	3 (2.9%)

Source: Clinical Reviewer (J Review 13.1)

*One subject was 18 months and one was 23 months of age.

Table 18-36: CDC-060 – Country of Residence and Potential Exposure (Safety Population)

	No. of Subjects n=102
Country of Residence	
USA	76 (74.5%)
Ghana	3 (2.9%)
India	3 (2.9%)
Nigeria	3 (2.9%)
France	2 (2.0%)
Haiti	2 (2.0%)
Malawi	1 (1.0%)
Thailand	1 (1.0%)
Togo	1 (1.0%)
Liberia	1 (1.0%)
Ivory Coast/Paris	1 (1.0%)
Uganda	1 (1.0%)
Ivory Coast	1 (1.0%)
S. Africa	1 (1.0%)
Past month prior to symptoms, USA	1 (1.0%)
Burkina Faso	1 (1.0%)
Countr(ies) of Potential Disease Exposure	
Nigeria	20 (19.6%)
Ghana	17 (16.7%)
Uganda	5 (4.9%)
Sierra Leone	5 (4.9%)
Haiti	4 (3.9%)
India	3 (2.9%)
Cameroon	3 (2.9%)

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

	No. of Subjects n=102
Guinea	3 (2.9%)
Sudan	3 (2.9%)
Liberia	3 (2.9%)
Ivory Coast	3 (2.9%)
Malawi	2 (2.0%)
Burkina Faso	2 (2.0%)
Guinea Bissau	2 (1.0%)

Source: Clinical Reviewer (J Review 13.1)

Table 18-37: CDC-060 – Pretreatment with Quinidine or Quinine (Safety Population)

	N=102
Quinidine – No. of subjects	47
Mean days of treatment	1.63
Range	1.0 – 4.0
Quinine – No. of subjects	24
Mean days of treatment	2.04
Range	1.00 – 5.00

Source: Clinical Reviewer (J Review 13.1)

Table 18-38: CDC-060 – Reason for Artesunate Therapy (Safety Population)

Reasons Given	No. of Subjects N=102
Number of subjects with data	80 (78.4%)
IV artesunate would be obtainable more quickly than quinidine	61 (59.8%)
Quinidine intolerance	17 (16.7%)
QTc interval is prolonged by more than 25% of the baseline value	12 (11.8%)
Development of reaction to quinidine during therapy	3 (2.9%)
Persistent hypotension unresponsive to fluid resuscitation	2 (2.0%)
Contraindications to quinidine	2 (2.0%)
Known allergy or hypersensitivity to quinidine or cinchona alkaloids (quinine)	1 (1.0%)
Left bundle-branch heart block or other severe intraventricular conduction defects	1 (1.0%)

Source: Clinical Reviewer (J Review 13.1)

Table 18-39: CDC-060 – Baseline Malaria Species (Safety Population)

Malaria Species	No. of Subjects N=102
Plasmodium species (by microscopy)	99 (97.1%)
<i>P. falciparum</i>	90 (88.2%)
<i>P. ovale</i>	2 (2.0%)
<i>P. vivax</i>	3 (2.9%)
Unknown	4 (3.9%)

Source: Clinical Reviewer (J Review 13.1)

Table 18-40: CDC-060 – Baseline Clinical Events (Safety Population)

Clinical Events	No. of Subjects (%) N=102
Parasitemia > 5%	67 (65.7%)
Jaundice	48 (47.1%)
Acidosis	37 (36.3%)
Unable to take oral Medication	36 (35.3%)
Ventilatory support	30 (29.4%)
Circulatory collapse/shock	26 (25.5%)
Acute renal failure	25 (24.5%)
Impaired consciousness	25 (24.5%)
Renal dialysis	15 (17.7%)
Pulmonary edema or ARDS	12 (11.8%)
Severe anemia with hemoglobin under 7 g/dL	10 (9.8%)
Seizures	5 (4.9%)
Abnormal bleeding or DIC	4 (3.9%)

Source: Clinical Reviewer (J Review 13.1)

Table 18-41: Follow-on Treatment (Safety Population)

Follow-on Treatment Reported Name of Drug or Therapy	Number of Subjects
Total subjects with data	67 (65.7%)
Atovaquone-Proguanil	41 (40.2%)
Doxycycline	20 (19.6%)
Clindamycin	5 (7.5%)
Primaquine	3 (4.5%)
Mefloquine	2 (2.0%)

Source: Clinical Reviewer (J Review 13.1)

Table 18-42: CDC-060 – Number of Artesunate Doses Administered (Safety Population)

Number of Doses IV Artesunate	Number of Subjects (%)
Total	102 (100.0%)
1	2 (2.0%)
2	2 (2.0%)
3	5 (4.9%)
4	92 (90.2%)
5	1 (1.0%)

Source: Clinical Reviewer (J Review 13.1)

Reviewer Comments: In CDC-060 2 of 92 (90%) patients received four 2.4 mg/kg doses of artesunate intravenously at 0, 12, 24, and 48 hours. 9 patients received less than 4 doses; 5 died before the regimen could be completed. Only one patient (# (b) (6)) received 5 doses. According to the patient narrative, this patient received 1.2 mg/kg/dose for the first 4 doses for unexplained reasons and 4.2 mg/kg/dose for the fifth dose.

Table 18-43: CDC-060 – Disposition of Subjects (Safety Population)

Disposition Status	Number of Subjects (%)
Total	102 (100%)
Completed	73 (71.6%)
Not discharged from ICU	17 (16.7%)
Death	7 (6.9%)
Other	4 (3.9%)
Unknown	1 (1.0%)

Source: Clinical Reviewer (J Review 13.1)

Table 18-44: CDC-060 – Percent Parasitemia by Study Day (Safety Population)

Visit Number	Visit Name	MICROSCOPY
1	SCREENING No. of Subjects	2
	Mean [Std. dev.]	7.0 [1.41]
	Range	6.0-8.0
2	BASELINE - No. of Subjects	102
	Mean [Std. dev.]	9.43 [13.4]
	Range	0-95.0
3	DAY 1 – Number of Subjects	87
	Mean [Std. dev.]	2.0 [3.7]
	Range	0-20.0
4	DAY 2 – No. of Subjects	72
	Mean [Std. dev.]	0.14 [0.61]
	Range	0-5.0
5	DAY 3 – No. of Subjects	49
	Mean [Std. dev.]	0.1 [0.67]
	Range	0-4.6
6	DAY 4 - No. of Subjects	30
	Mean [Std. dev.]	0.22 [0.96]
	Range	0 – 5.0
7	DAY 5 – No. of Subjects	19
	Mean [Std. dev.]	0.60 [2.26]
	Range	0-10.00
8	DAY 6 - No. of Subjects	13
	Mean [Std. dev.]	0.07 [0.26]
	Range	0-1.00

Source: Clinical Reviewer (J Review 13.1)

18.6.2.6.2 Patient Deaths and Serious Adverse Events

Cardiac arrest, felt to be due to severe malaria, occurred in two patients with severe malaria.

Table 18-45: CDC-060 – Clinical Characteristics of Patients who Died (Safety Population)

Clinical Characteristics	No. of Subjects n=7
Unable to take oral medication	7 (100.0%)
Impaired consciousness	6 (85.7%)
Acidosis	6 (85.7%)
Circulatory collapse/shock	5 (71.4%)
Parasitemia > 5%	5 (71.4%)
Abnormal bleeding or DIC	3 (42.9%)
Pulmonary edema or ARDS	2 (28.6%)
Jaundice	2 (28.6%)
Acute renal failure	2 (28.6%)
Severe anemia with hemoglobin under 7 g/dL	1 (14.3%)
Seizures	1 (14.3%)

Source: Clinical Reviewer (J Review 13.1)

Table 18-46: CDC-060 SAEs and Fatal Outcomes

Subject ID	Age/ Sex	Doses IV AS	Quinidine pretreatment Y/N	Species	Dictionary-Derived Term
(b) (6)	32 yo F	2	Y	<i>P. vivax</i>	- Acute respiratory distress syndrome - Cardiac failure congestive - Disseminated intravascular coagulation - Hypotension - Acute respiratory distress syndrome (cause of death)
	61 yo M	2	Y	<i>P. f.</i>	- Septic shock - Multi-organ failure (cause of death)
	71 yo M	3	N	<i>P. f.</i>	- Pulse absent - Cardiac arrest (cause of death)
	31 yo M	3	Y	<i>P. f.</i>	- Brain edema - Brain herniation (cause of death)
	31 yo M	3			- Acute hepatic failure - Anemia - Disseminated intravascular coagulation - Leukocytosis - Transaminases increased - Brain herniation (cause of death) - Hyperkalemia
	54 yo M	4	Y	<i>P. f.</i>	- Anemia - Brain edema - Renal failure acute - Brain edema (cause of death)
	72 yo M	4	Y	<i>P. f.</i>	Thrombocytopenia

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

Subject ID	Age/ Sex	Doses IV AS	Quinidine pretreatment Y/N	Species	Dictionary-Derived Term
					• Acute respiratory distress syndrome • Transaminases increased • Fungemia • Cardiac arrest (cause of death)

Source: Clinical Reviewer (JMP and J Review 13.1) and CDC-060 CSR Table 27

Table 18-47: CDC-060 – Serious Adverse Event by SOC/PT (Safety Population)*

SAEs	Intravenous Artesunate n=102
Subjects with at least 1 Grade 4 AE	31 (30.4%)
BLOOD AND LYMPHATIC SYSTEM DISORDERS	19 (18.6%)
Anemia	13 (12.7%)
Thrombocytopenia	6 (5.9%)
Disseminated intravascular coagulation	2 (2.0%)
Lymphopenia	1 (1.0%)
Leukocytosis	1 (1.0%)
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	9 (8.8%)
Acute respiratory distress syndrome	6 (5.9%)
Respiratory distress	2 (2.0%)
Pneumothorax	1 (1.0%)
Pulmonary hemorrhage	1 (1.0%)
Pneumonia aspiration	1 (1.0%)
HEPATOBIILIARY DISORDERS	6 (5.9%)
Hyperbilirubinemia	4 (3.9%)
Acute hepatic failure	1 (1.0%)
Cholecystitis	1 (1.0%)
RENAL AND URINARY DISORDERS	3 (2.9%)
Renal failure acute	3 (2.9%)
CARDIAC DISORDERS	3 (2.9%)
Cardiac arrest	2 (2.0%)
Cardiac failure congestive	1 (1.0%)
NERVOUS SYSTEM DISORDERS	3 (2.9%)
Brain edema	2 (2.0%)
Depressed level of consciousness	1 (1.0%)
INVESTIGATIONS	3 (2.9%)
Transaminases increased	2 (2.0%)
Pulse absent	1 (1.0%)
INFECTIONS AND INFESTATIONS	3 (2.9%)
Septic shock	1 (1.0%)
Fungaemia	1 (1.0%)
Sepsis syndrome	1 (1.0%)
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	2 (2.0%)

SAEs	Intravenous Artesunate n=102
Brain herniation	2 (2.0%)
METABOLISM AND NUTRITION DISORDERS	1 (1.0%)
Hyperkalemia	1 (1.0%)
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	1 (1.0%)
Multi-organ failure	1 (1.0%)
VASCULAR DISORDERS	1 (1.0%)
Hypotension	1 (1.0%)
PSYCHIATRIC DISORDERS	1 (1.0%)
Mental status changes	1 (1.0%)

Source: Clinical Reviewer (J Review 13.1)

*SAEs were not specifically recorded for this study. Grade 4 AEs (life-threatening AEs) were reported in lieu of SAEs.

Additional TEAEs that were considered life-threatening (coded in dataset as Grade 5) include the following: anemia (5%), elevated transaminases (3%), thrombocytopenia (2%), respiratory distress (1%), cholecystitis (1%), *C. difficile* colitis (1%), acute renal failure (1%), epistaxis (1%), petechiae (1%), mental status change (1%)

Reviewer Comments: *Per the study protocol, safety and outcome data were collected until subject's death or discharge, for a maximum of seven days. Review of patient narratives revealed an additional patient who likely experienced a poor outcome. Subject (b) (6) was a 61-year old Asian male with a history of travel to Ghana who presented with fever and headache. His clinical status worsened over the next day and he developed coma and was intubated and treated with IV antibacterial drugs for sepsis. On the day following admission, a malaria smear was positive for *P. falciparum* and he was started on IV quinidine. His clinical status continued to deteriorate with renal failure and hypotension, and he was treated with pressors and received exchange, packed RBCs and platelet transfusions. On hospital day 4 he was started on IV artesunate and received a total of 3 doses. Over the following 4 weeks the patient's neurological status did not improve and he was discharged to a palliative care facility. No further information was provided.*

18.6.2.6.3 Treatment Emergent Adverse Events

Reviewer comments: *Limited safety data were collected under the original IND so additional data were retrieved by the US Army and CDC from patient medical records. Datasets included information on mortality, adverse events, and laboratory data were reported only until death or discharge for a maximum of 7 days. Some additional information was available in patients' narratives, for example, on pregnancy outcomes.*

The lack of a comparator arm in CDC-060 makes it difficult to ascribe causality to the adverse reactions observed. Most of the reported adverse events in this study may be attributable to the condition under treatment, further complicating interpretation of safety as it was not possible to determine which AEs would have occurred more commonly with Artesunate for Injection than

with the comparator if one were used.

TEAEs by severity and totals are provided in the table below.

Table 18-48: CDC-060 – TEAE (SOC/PT ≥ 2%) by Grade (Safety Population)

TEAEs	Intravenous Artesunate n=102					All n=102
	1	2	3	4	5 (Deaths)	
Grade						
Subjects with at least 1 TEAE	51	56	39	31	7	94
BLOOD AND LYMPHATIC SYSTEM DISORDERS	27 (26.5%)	28 (27.5%)	24 (23.5%)	19 (18.6%)	0	75 (73.5%)
Anemia	14 (13.7%)	19 (18.6%)	20 (19.6%)	13 (12.7%)		66 (64.7%)
Thrombocytopenia	0	7 (6.9%)	5 (4.9%)	6 (5.9%)	0	18 (17.6%)
Leukocytosis	6 (5.9%)	3 (2.9%)	0 (0.0%)	1 (1.0%)	0	10 (9.8%)
Lymphopenia	2 (2.0%)	1 (1.0%)	3 (2.9%)	1 (1.0%)	0	7 (6.9%)
Neutropenia	2 (2.0%)	2 (2.0%)	1 (1.0%)	0	0	5 (4.9%)
Disseminated intravascular coagulation	1 (1.0%)	0 (0.0%)	0 (0.0%)	2 (2.0%)	0	3 (2.9%)
Hemolysis	1 (1.0%)	1 (1.0%)	0 (0.0%)	0	0	2 (2.0%)
INVESTIGATIONS	18 (17.6%)	22 (21.6%)	14 (13.7%)	3 (2.9%)	0	52 (51.0%)
Transaminases increased	4 (3.9%)	12 (11.8%)	10 (9.8%)	2 (2.0%)	0	28 (27.5%)
AST increased	9 (8.8%)	9 (8.8%)	4 (3.9%)	0	0	22 (21.6%)
BUN increased	1 (1.0%)	1 (1.0%)	1 (1.0%)	0	0	3 (2.9%)
ALT increased	3 (2.9%)	0	0	0	0	3 (2.9%)
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	5 (4.9%)	6 (5.9%)	1 (1.0%)	9 (8.8%)	1 (1.0%)	20 (19.6%)
Acute respiratory distress syndrome	0	1 (1.0%)	1 (1.0%)	6 (5.9%)	1 (1.0%)	8 (7.8%)
Pulmonary edema	2 (2.0%)	1 (1.0%)	0	0	0	3 (2.9%)
Pneumonia	0	3 (2.9%)	0	0	0	3 (2.9%)
Lung infiltration	1 (1.0%)	1 (1.0%)	0	0	0	2 (2.0%)
Respiratory distress	0	0	0	2 (2.0%)	0	2 (2.0%)
HEPATOBIILIARY DISORDERS	1 (1.0%)	1 (1.0%)	9 (8.8%)	6 (5.9%)	0 (0.0%)	17 (16.7%)
Hyperbilirubinemia	0	1 (1.0%)	9 (8.8%)	4 (3.9%)	0	14 (13.7%)
Cholecystitis	1 (1.0%)	0	0	1 (1.0%)	0	2 (2.0%)
RENAL AND URINARY DISORDERS	1 (1.0%)	3 (2.9%)	6 (5.9%)	3 (2.9%)	0	13 (12.7%)
Renal failure acute	1 (1.0%)	1 (1.0%)	5 (4.9%)	3 (2.9%)	0	10 (9.8%)
GASTROINTESTINAL DISORDERS	2 (2.0%)	6 (5.9%)	0	0	0	8 (7.8%)
Diarrhea	1 (1.0%)	2 (2.0%)	0	0	0	3 (2.9%)
VASCULAR DISORDERS	2 (2.0%)	3 (2.9%)	2 (2.0%)	1 (1.0%)	0 (0.0%)	8 (7.8%)
Hypotension	0	1 (1.0%)	0	1 (1.0%)	0	2 (2.0%)

TEAEs	Intravenous Artesunate n=102					All n=102
	1	2	3	4	5 (Deaths)	
Grade						
Peripheral ischemia	0	0	2	0	0	2 (2.0%)
METABOLISM AND NUTRITION DISORDERS	2 (2.0%)	2 (2.0%)	2 (2.0%)	1 (1.0%)	0	7 (6.9%)
Acidosis	2 (2.0%)	2 (2.0%)	0	0	0	4 (3.9%)
INFECTIONS AND INFESTATIONS	2 (2.0%)	1 (1.0%)	1 (1.0%)	3 (2.9%)	0	7 (6.9%)
CARDIAC DISORDERS	1 (1.0%)	1 (1.0%)	1 (1.0%)	1 (1.0%)	2 (2.0%)	6 (5.9%)
Cardiac arrest	0	0	0	0	2 (2.0%)	2 (2.0%)
PSYCHIATRIC DISORDERS	0	2 (2.0%)	2 (2.0%)	1 (1.0%)	0 (0.0%)	5 (4.9%)
Mental status changes	0	2 (2.0%)	1 (1.0%)	1 (1.0%)	0	4 (3.9%)
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	2 (2.0%)	2 (2.0%)	0	0	0	4 (3.9%)
NERVOUS SYSTEM DISORDERS	0	0	0 (0.0%)	3 (2.9%)	1 (1.0%)	3 (2.9%)
Brain edema	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (2.0%)	1 (1.0%)	2 (2.0%)
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	0	0	0	0	2 (2.0%)	2 (2.0%)
Brain herniation	0	0	0	0	2 (2.0%)	2 (2.0%)
EYE DISORDERS	2 (2.0%)	0	0	0	0	2 (2.0%)

Source: Clinical Reviewer (J Review 13.1)

Transfusions

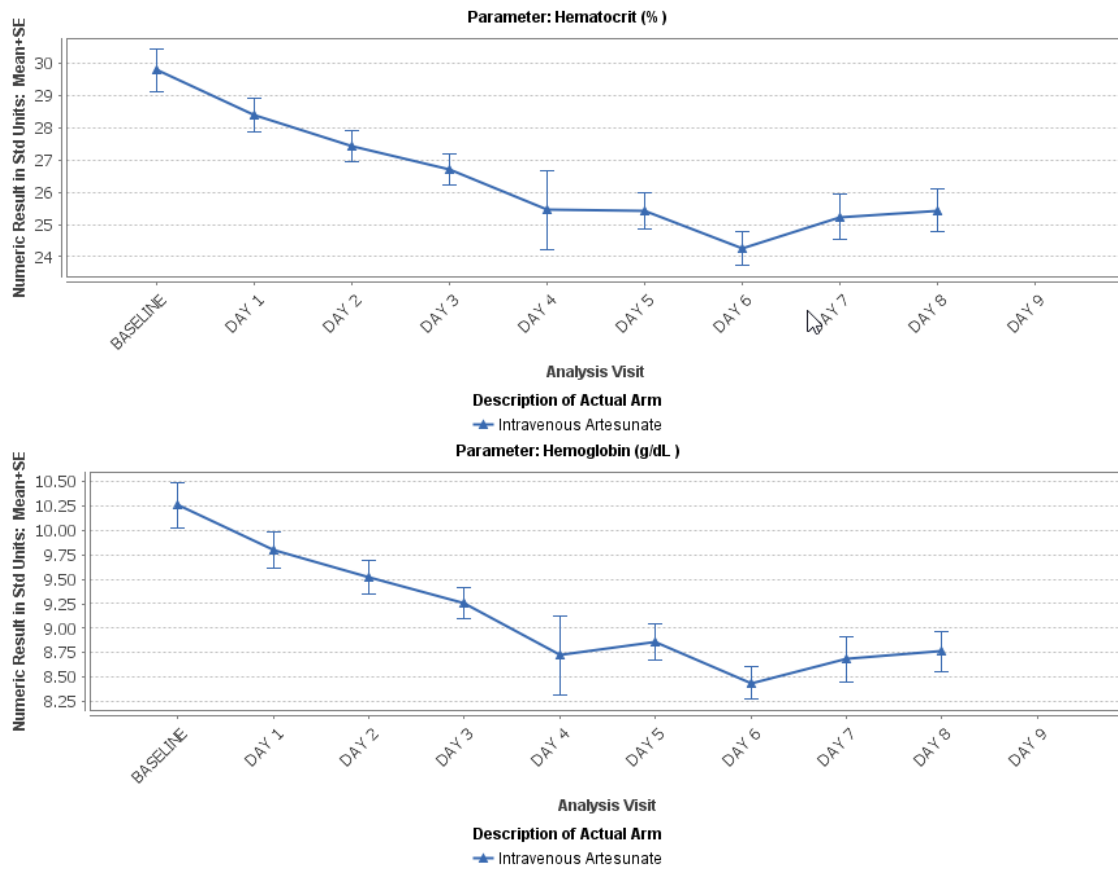
Forty-nine subjects received a total of 102 transfusions of packed RBCs from day -1 to day 8. Since data on transfusions with blood products and laboratory evaluations were provided only until hospital discharge or day 8, information on transfusions after day 8 was not reported. LDH and haptoglobin levels were not obtained; therefore, it was not possible to evaluate the occurrence of delayed hemolysis after artesunate therapy.

18.6.2.6.4 Laboratory Evaluation

Laboratory evaluations identified as TEAEs, including Hb and Hct, platelets, reticulocytes, WBCs and neutrophils, were examined for time course, see tables below.

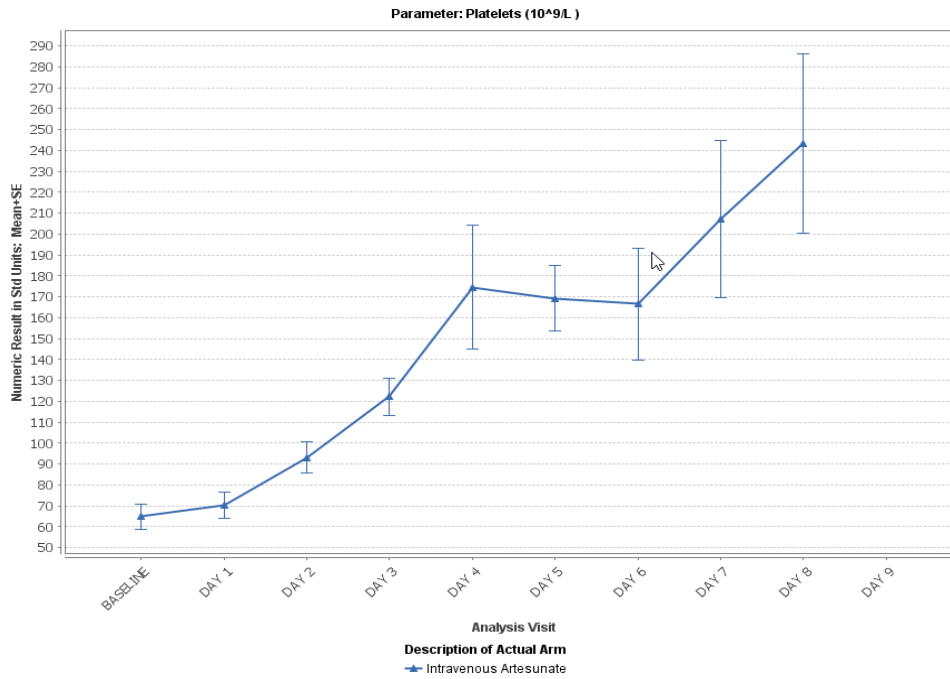
Hematology

Figure 18-13: CDC-060 Hematocrit and Hemoglobin by Day of Hospitalization



Source: Clinical Reviewer (J Review 13.1)

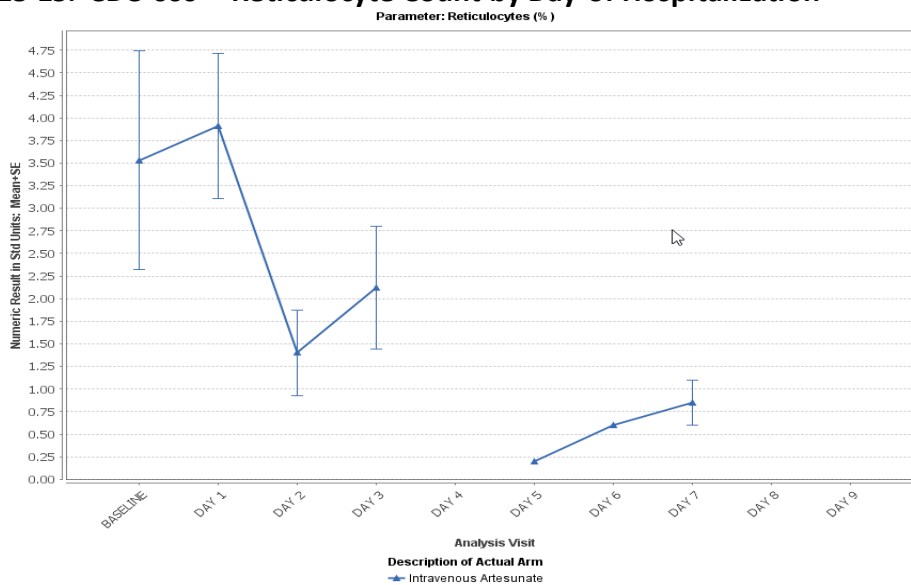
Figure 18-14: CDC-060 – Platelets by Day of Hospitalization



Source: Clinical Reviewer (J Review 13.1)

Reviewer comments: The time courses observed for anemia and platelet count were consistent with those found in patients with severe malaria. Thrombocytopenia was observed at baseline, with subsequent rise in platelet count following initiation of effective therapy.

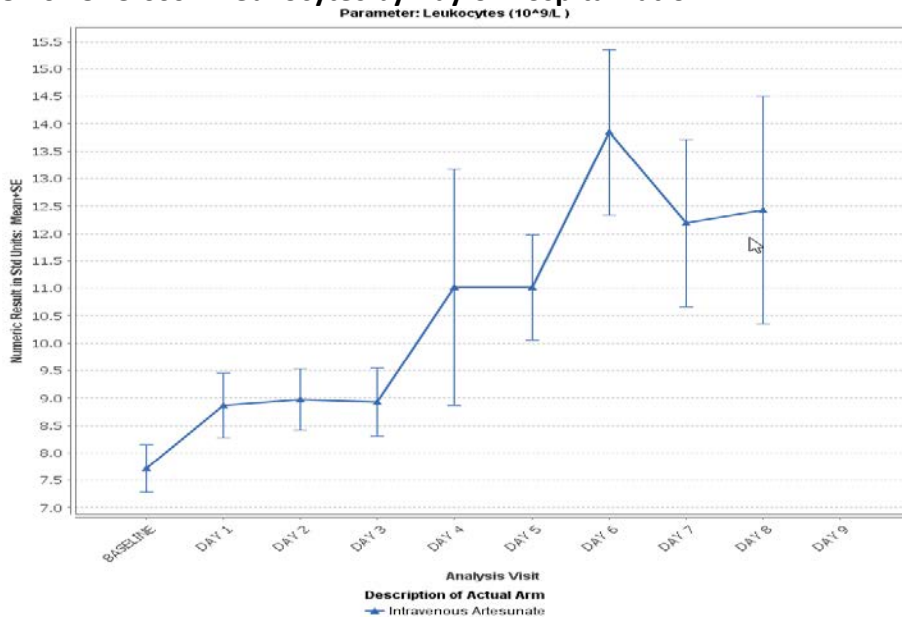
Figure 18-15: CDC-060 – Reticulocyte Count by Day of Hospitalization



Source: Clinical Reviewer (J Review 13.1)

Reviewer comments: Reticulocyte counts were not obtained on Day 4 or after day 7. Available data show the nadir for reticulocytes on day 5 with subsequent gradual rise.

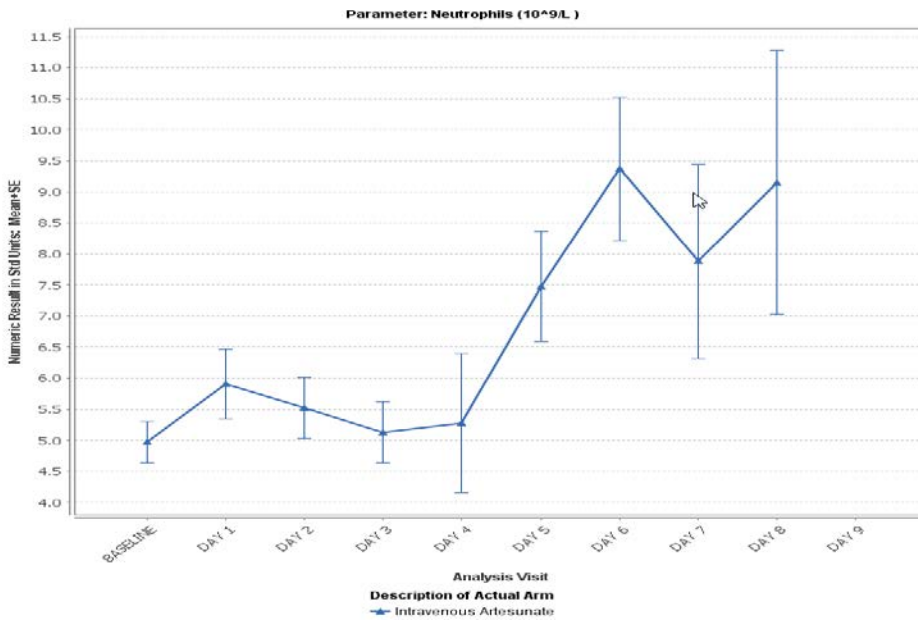
Figure 18-16: CDC-060 – Leukocytes by Day of Hospitalization



Source: Clinical Reviewer (J Review 13.1)

Reviewer comment: The observed rise in WBCs is consistent with the course of severe malaria.

Figure 18-17: CDC-060 – Neutrophils by Day of Hospitalization

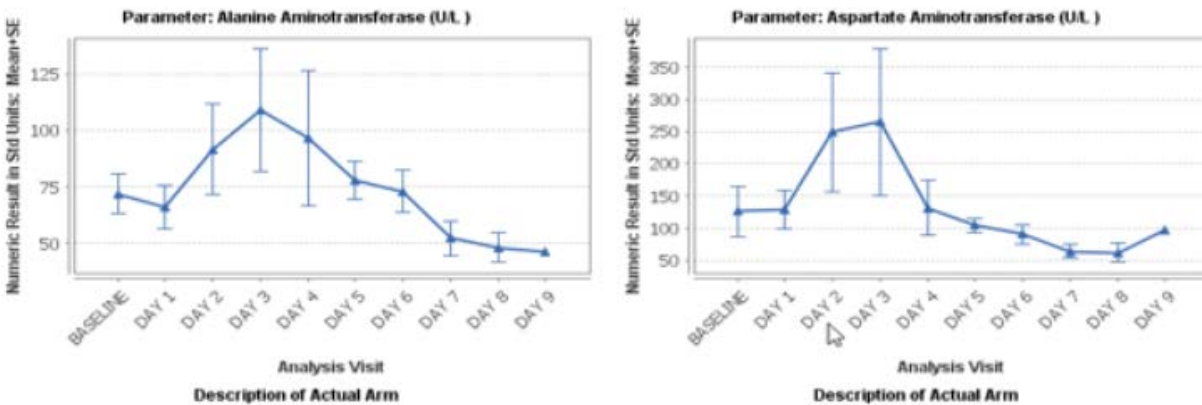


Source: Clinical Reviewer (J Review 13.1)

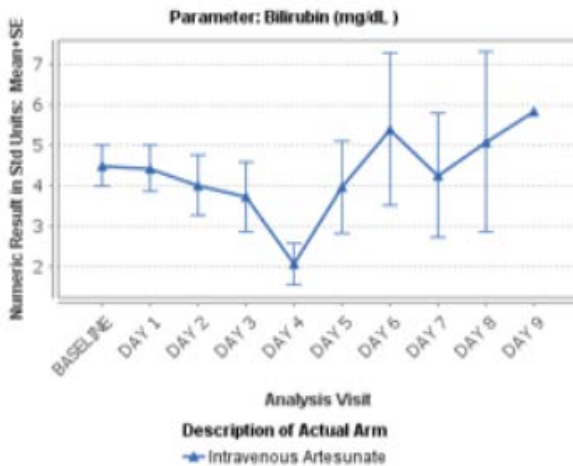
Reviewer Comment: In this study, neutrophil counts were depressed at baseline. A further reduction in neutrophils following artesunate not observed.

Transaminases and Bilirubin

Figure 18-18: CDC-060 – Transaminases and Bilirubin by Day of Hospitalization



Source: Clinical Reviewer (J Review 13.1)



Source: Clinical Reviewer (J Review 13.1)

Reviewer comments: Transaminase levels were elevated on baseline, rising until day 3 following initiation of IV artesunate. Bilirubin levels were also elevated at baseline, and after an initial decline rose after day 4.

18.6.3 WRAIR Study 1168 (Phase 2 Uncomplicated Malaria)

Title: A Phase II, Open Label, Study of the Safety, Tolerability, Efficacy and Pharmacokinetics of Intravenous Artesunate in Adults with Uncomplicated Malaria

18.6.3.1 Study Objectives

- Determine the full pharmacokinetic profile for cGMP intravenous artesunate formulation in adults with uncomplicated *falciparum* malaria
- Determine the effectiveness of the new GMP intravenous artesunate formulation to clear *Plasmodium falciparum* parasites from the blood
- Evaluate the safety of intravenous artesunate in Kenyan adult populations with uncomplicated *falciparum* malaria

18.6.3.2 Study Design

This was an unblinded, non-randomized, phase 2 clinical study of intravenous artesunate for the treatment of uncomplicated *Plasmodium falciparum* malaria in adults in Kenya. This study enrolled 30 subjects, age 18 to 65 years who received intravenous artesunate (2.4 mg/kg) once a day for 3 day, followed by Malarone for parasitologic cure. Pharmacokinetic samples were obtained on Day 0 at 0, 5 min, 10 min, 20 min, 40 min, 1 h, 2 h, 4 h, and 6 h) on Day 0. Safety laboratories were performed on Day 3 and repeated on Days 7 and 14 if abnormal. Adverse events were observed at each visit. Malaria blood films were obtained at enrollment at twice daily for the first 3 days, then once daily on Days 3, 7, and 14.

Table 18-49: Study 1168 – Schedule of Assessments

Procedure	Day 0	Day 1	Day 2	Day 3	Day 4	Day 7 ±2	Day 14±2
Informed Consent	○ ^a						
Medical History	○						
Vital Signs ^b	● ^a	●	●	○		○	○
Clinical Signs/Symptoms	●	●	●	○		○	○
Complete Physical Exam	○						
Focused Physical Exam ^c	●	●	●	○		○	○
Blood Smears (thick and thin)	●	●	●	○		○	○
Blood sample for pharmacokinetics ^d	○						
Clinical laboratory data ^e	○			○		○ ^f	○ ^f
Urinalysis							
Pregnancy Testing	○						
Assessment of Adverse Events	○	○	○	○	○	○	○
Assessment of Concomitant Medications	○	○	○	○		○	○
Intravenous Artesunate Administration	○	○	○				
Malarone Therapy ^g			○	○	○		

^a Open circles indicate one instance. Closed circles indicate twice on that study day.

^b Temperature, heart rate, blood pressure, and respiration rate.

^c A focused physical exam included general appearance, lung, and heart assessment, and neurological exam.

^d Sampled at 9 time points: at dosing (time 0) and 5 min, 10 min, 20 min, 40 min, 1 h, 2 h, 4 h, and 6 h after dosing.

^e Clinical laboratory assessments included clinical chemistry (blood urea nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase, total bilirubin, and direct bilirubin) and hematology (CBC with differential).

^f These tests may be repeated if significantly abnormal compared to previous laboratory data.

^g After the final dose of artesunate.

Source: Study 1169 CSR, Table 2, p. 12

18.6.3.3 Endpoints

- Pharmacokinetics: pharmacokinetic analysis of the 2.4 mg/kg dose in adults over 6 hours
- Efficacy: 99% parasite reduction at 48 hours, parasite clearance times (PCTs), fever clearance times (FCTs)
- Safety: the number and severity of adverse events (AE) and serious adverse events (SAE).

18.6.3.4 Statistical Analysis Plan

Efficacy

Parasite reduction ratio at 48 hours was determined using the following formula:

Peak parasitemia – Parasitemia at 48 hours / Peak parasitemia X 100%

Parasite clearance time was determined as the time (hours) parasites are first not detected if followed by 2 negative smears. Fever clearance time was the time (hours) to afebrile (<37.5°C) if sustained for 24 hours.

Safety

Safety parameters, which included vital signs, clinical signs or symptoms, and clinical laboratory measurements, were presented using counts and percentages for categorical data and continuous data were presented using the mean, SD, and number of subjects. Adverse events were coded using the Medical Dictionary of Regulatory Affairs (MedDRA) preferred terms within each system, organ, and class (SOC) grouping. Frequencies were presented by counting the number of subjects who reported the AE daily. Multiple reports in the same subject were only counted once per subject.

Efficacy results: all subjects received 100% parasite clearance by the end of Day 2.

18.6.3.5 Study Results – Safety

18.6.3.6 Key Inclusion Criteria

- Adults and non-pregnant females, 18-65 years
- Fever >37.5 C during present illness
- Falciparum malaria, greater than or equal to 200 parasites/μL

Key exclusion criteria included mixed malarial infection (malaria other than *falciparum* malaria mono-infection as detected by screening blood smear) and severe *falciparum* malaria as defined by the WHO.

18.6.3.6.1 Demographic and Baseline Characteristics

All subjects (N=30) received 3 doses of IV artesunate and completed the study. A

Table 18-50: Study 1168 Demographics (Safety Population)

Demographics	Artesunate 2.4 mg/kg n=30
Age	
mean and std. dev.	27.13 [9.49]
median	24.50
range	18-54
Sex	
F	20 (66.7%)
M	10 (33.3%)
Race	
Black	30 (100.0%)
Weight at Baseline (kg)	
mean and std. dev.	60.59 [10.36]
median	61
range	42-85
Country – count subjects and %	
Kenya	30 (100.0%)

Source: Clinical Reviewer (J Review)

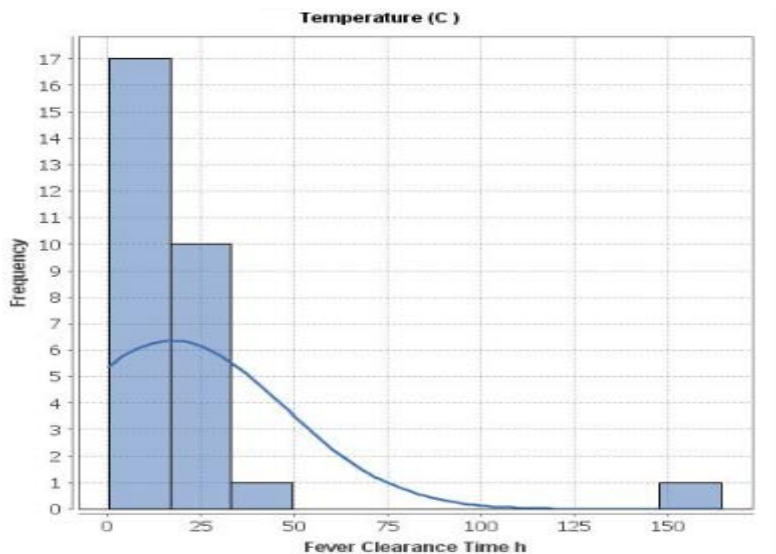
Table 18-51: Study 1168 – Baseline *P. falciparum* Asexual Parasites*

	Asexual Parasites/ μ L
Baseline- mean [SD]	23483.80 [52257.36]
median (range)	0 (0-338660)

Source: Clinical Reviewer (J Review)

*No other species identified.

Figure 18-19: 1168 Fever Histogram



Source: Clinical Reviewer (J Review)

18.6.3.6.2 Deaths and Serious Adverse Events

There were no deaths in Study 1168 and one SAE, in a 54 year old female subject (Subject # (b) (6)) who developed Stevens-Johnson syndrome (verbatim term bullous erythema multiforme) and was hospitalized. Symptoms of pruritis developed on Day 3, following completion of 3 days of IV artesunate at 2.4 mg/kg/day and within one hour 40 minutes of receiving the second dose of concomitant Malarone. The prescribing information for Malarone notes that Stevens-Johnson syndrome has been reported as a post-marketing adverse event.²⁵ Given the concomitant administration of Malarone which, it is not possible to determine an unequivocal association with IV artesunate alone. The following patient summary was provided:

After the second dose of Malarone on Day 3 ((b) (6)) the subject complained of pruritis and rash. The subject was treated with chlorpheniramine (4 mg) and prescribed a topical steroid cream (Temovate). The final dose of Malarone was not given. On Day 4, the subject had elevated temperatures (max 39.9), labile blood pressures, and a worsening rash. The subject also complained of increased pain at the rash sites which were noted to have progressed to bullous lesions with a dusky erythematous background. The subject was re-admitted to the hospital, administered IV fluids (1000 L NS) and 250 mg hydrocortisone. The subject improved over the following 4 hours (Day 5, (b) (6)). Aspiration of bullous fluid revealed mixtures of eosinophils, lymphocytes and occasional neutrophil. Gram stain was negative for bacteria. Dermatological assessments revealed lesions suggestive of bullous erythema multiforme likely resulting from Malarone administration combined with occult parasitic infection, and autoimmune disease. An allergist at Walter Reed Army Medical Center was consulted and the subject was placed on ranitidine adjunctive therapy and a tapered steroid course (oral prednisone). Tramadol was used for effective pain control, and the subject remained in the ward for rash and symptom management.

The subject improved over the following 10 days with no further progression of the bullous lesions. On Day 16 (b) (6) the patient was discharged with instructions to follow-up within 48 hours. On Day 17 (b) (6) study staff went to the home of the subject for follow up and were informed that the subject had been admitted to another hospital. The subject was finally located walking in town without difficulty. On Day 18 (b) (6) study staff once again went the subject's residence, and at that time the subject refused any further follow up. [From NDA 213035 SN1, Module 5, Section 5.3.4 2, Clinical Study Report Study 1168.]

18.6.3.6.3 Treatment Emergent Adverse Events

The table below provides the most common TEAEs by preferred term.

Table 18-52: Study 1168 TEAE by Severity, Incidence $\geq$ 5% (Safety Population)

	Artesunate 2.4 mg/kg n=30			Totals n=30
	MILD	MODERATE	SEVERE	
Subjects with at least 1 TEAE – count subjects and % with data	30 (100.0%)	12 (40.0%)	5 (16.7%)	30 (100.0%)
BLOOD AND LYMPHATIC SYSTEM DISORDERS	13 (43.3%)	10 (33.3%)	4 (13.3%)	22 (73.3%)
Anemia	8 (26.7%)	4 (13.3%)	1 (3.3%)	13 (43.3%)
Neutropenia	6 (20.0%)	7 (23.3%)	4 (13.3%)	17 (56.7%)
Eosinophilia	5 (16.7%)	0 (0.0%)	0 (0.0%)	5 (16.7%)
Leukopenia	0 (0.0%)	2 (6.7%)	0 (0.0%)	2 (6.7%)
VASCULAR DISORDERS	17 (56.7%)	0 (0.0%)	0 (0.0%)	17 (56.7%)
Hypotension	16 (53.3%)	0 (0.0%)	0 (0.0%)	16 (53.3%)
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	14 (46.7%)	2 (6.7%)	0 (0.0%)	15 (50.0%)
Pyrexia	9 (30.0%)	2 (6.7%)	0 (0.0%)	10 (33.3%)
Infusion site pain	3 (10.0%)	0 (0.0%)	0 (0.0%)	3 (10.0%)
Chills	3 (10.0%)	0 (0.0%)	0 (0.0%)	3 (10.0%)
Fatigue	2 (6.7%)	0 (0.0%)	0 (0.0%)	2 (6.7%)
CARDIAC DISORDERS	12 (40.0%)	0 (0.0%)	0 (0.0%)	12 (40.0%)
Tachycardia	6 (20.0%)	0 (0.0%)	0 (0.0%)	6 (20.0%)
Bradycardia	6 (20.0%)	0 (0.0%)	0 (0.0%)	6 (20.0%)
NERVOUS SYSTEM DISORDERS	9 (30.0%)	1 (3.3%)	0 (0.0%)	9 (30.0%)
Headache	9 (30.0%)	0 (0.0%)	0 (0.0%)	9 (30.0%)
INFECTIONS AND INFESTATIONS	9 (30.0%)	1 (3.3%)	0 (0.0%)	9 (30.0%)
Viral infection	2 (6.7%)	0 (0.0%)	0 (0.0%)	2 (6.7%)
Upper respiratory tract infection	2 (6.7%)	0 (0.0%)	0 (0.0%)	2 (6.7%)
GASTROINTESTINAL DISORDERS	8 (26.7%)	0 (0.0%)	0 (0.0%)	8 (26.7%)
Vomiting	5 (16.7%)	0 (0.0%)	0 (0.0%)	5 (16.7%)
Abdominal pain	2 (6.7%)	0 (0.0%)	0 (0.0%)	2 (6.7%)
METABOLISM AND NUTRITION DISORDERS	7 (23.3%)	0 (0.0%)	0 (0.0%)	7 (23.3%)
Hyperglycemia	3 (10.0%)	0 (0.0%)	0 (0.0%)	3 (10.0%)
Anorexia	2 (6.7%)	0 (0.0%)	0 (0.0%)	2 (6.7%)
Hypoglycemia	2 (6.7%)	0 (0.0%)	0 (0.0%)	2 (6.7%)
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	5 (16.7%)	0 (0.0%)	0 (0.0%)	5 (16.7%)
Cough	2 (6.7%)	0 (0.0%)	0 (0.0%)	2 (6.7%)
Tachypnea	2 (6.7%)	0 (0.0%)	0 (0.0%)	2 (6.7%)

Source: Clinical Reviewer (J Review 13.1)

Reviewer comments: The most common TEAEs were neutropenia (56.7%), hypotension (53.3%), and anemia (43.3%)

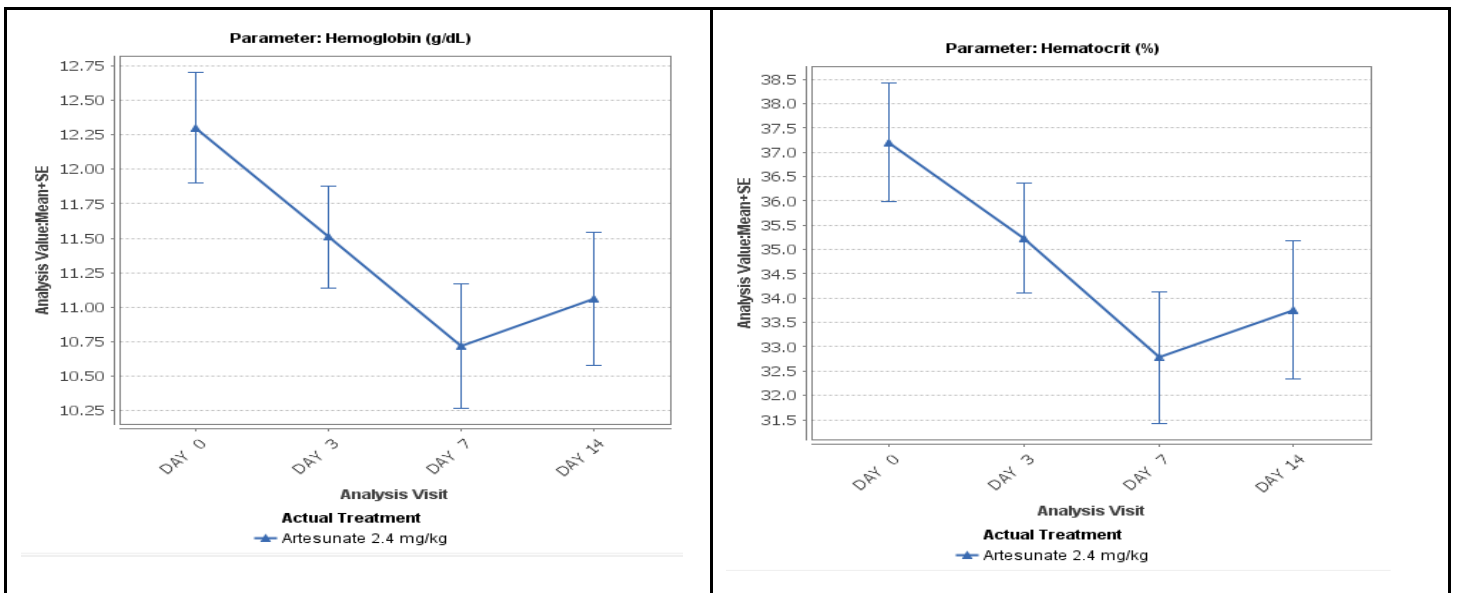
In Study 1168, the definition of neutropenia and grading scale (mild, moderate and severe) was not found in the protocol. Neutropenia in table above was according to the Applicant's coding of

this TEAE in the ADAE dataset. Based on this reviewer's analysis of neutrophil counts for patients coded as having neutropenia, the Applicant used a broad definition of neutropenia: $ANC < 2.0 \times 10^6 /L$, with occurrence on day 3 or later and severe neutropenia was generally defined as $ANC < 0.75 \times 10^6 /L$.

18.6.3.6.4 Laboratory Findings

The following graphs demonstrate the time lines for observed laboratory abnormalities.

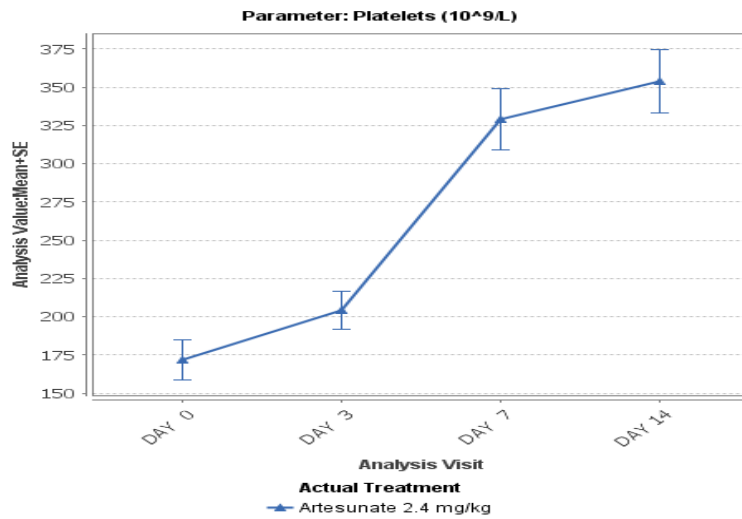
Figure 18-20: Study 1168 - Hemoglobin and Hematocrit by Study Visit



Source: Clinical Reviewer (J Review 13.1)

Reviewer comments: Hemoglobin and hematocrit demonstrated a mild decrease to Day 7 and that remained steady through Day 14.

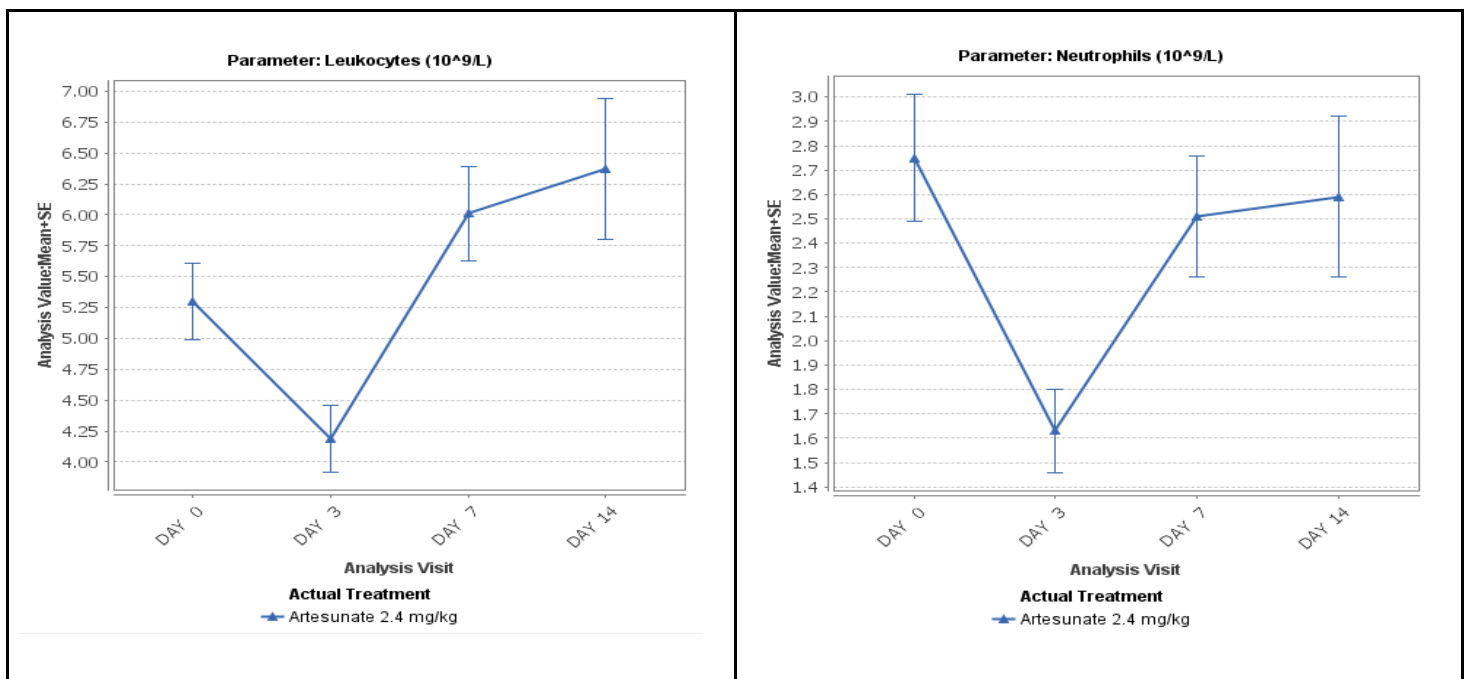
Figure 18-21: Study 1168 – Platelets by Study Visit



Source: Clinical Reviewer (J Review 13.1)

Reviewer comments: Initial platelet levels were low, most likely due to malaria disease, and rose during the course of the study. According to the annotated CRF, reticulocyte counts were performed (both absolute reticulocytes and corrected reticulocytes; however, these data were not included in the datasets and therefore could not be analyzed. The study publication reports that there was a dose-related decrease in reticulocytes, with the lowest level occurring day 4 and returning to normal in most patients by day 7. ([Li et al. 2014](#))

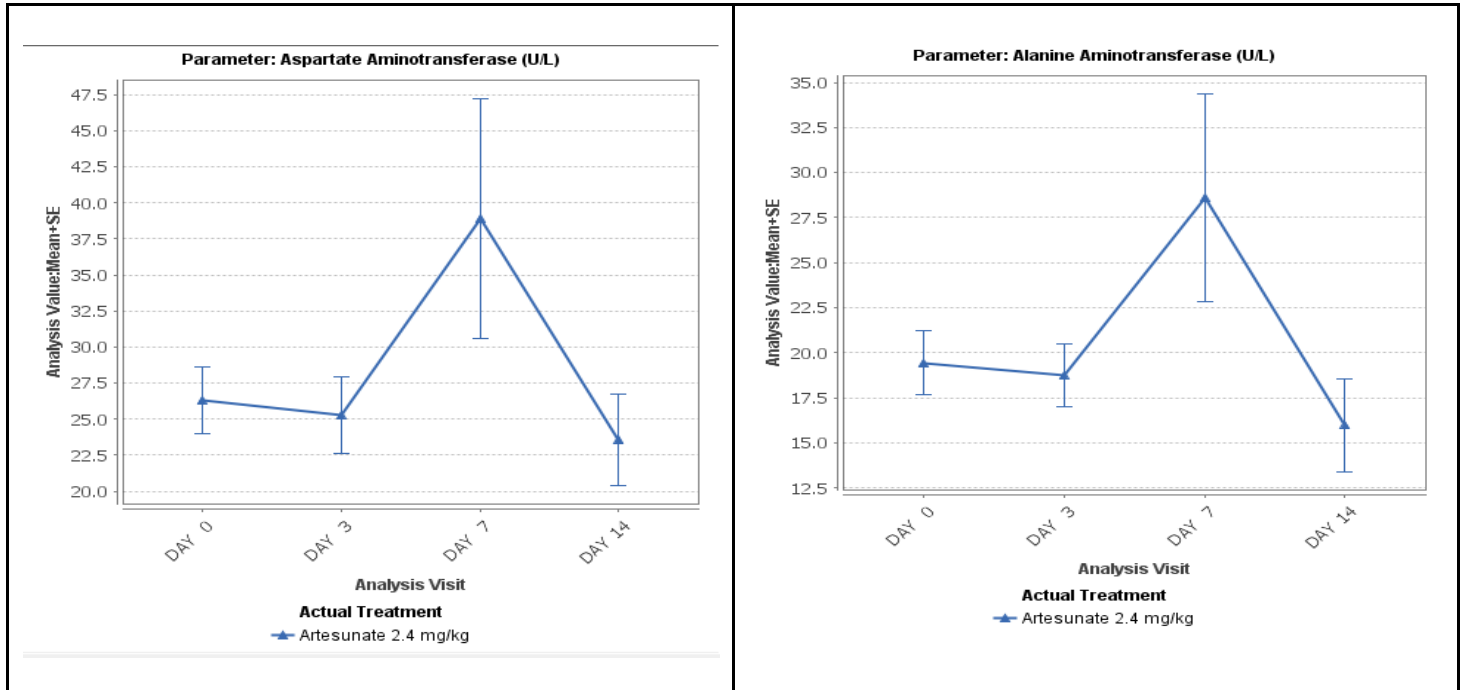
Figure 18-22: Study 1168 – WBC Parameters by Study Visit



Source: Clinical Reviewer (J Review 13.1)

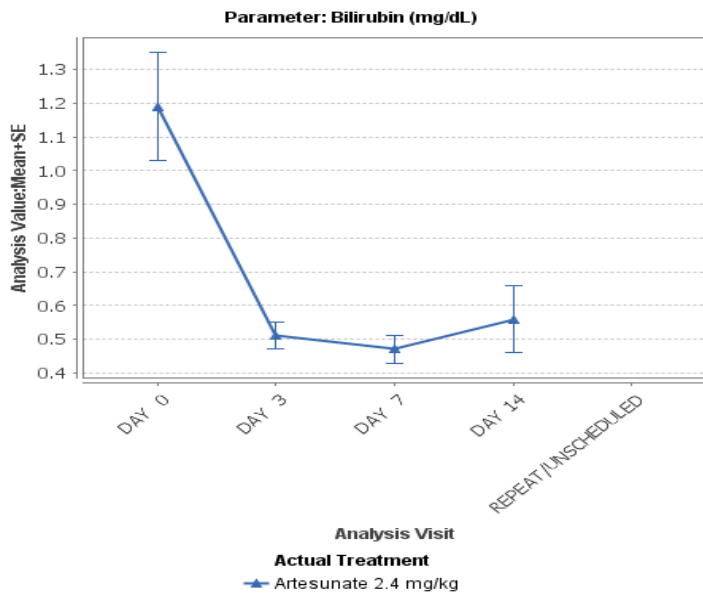
Reviewer comments: WBC and neutrophils declined until Day 3 and then rose to normal between Day 7 and 14. See additional discussion above.

Figure 18-23: Study 1168 - Transaminases by Study Visit



Source: Clinical Reviewer (J Review 13.1)

Figure 18-24: Study 1168 - Total Bilirubin by Study Visit



Source: Clinical Reviewer (J Review 13.1)

Reviewer comments: A mild transient rise in transaminase levels was observed following day 3. No rise in total bilirubin was observed in this study.

18.6.4 WRAIR Study 1263ab (Phase 2 Uncomplicated Malaria)

Study Title: A Phase II, Randomized, Open-label, Dose-ranging Study of Intravenous Artesunate Therapy for the Treatment of Acute, Uncomplicated *Plasmodium falciparum* Malaria

18.6.4.1 Study Objectives

Primary:

- Compare 4 regimens of WRAIR formulation of intravenous artesunate for the treatment of uncomplicated *Plasmodium falciparum* malaria using the pharmacodynamic endpoints of parasite clearance to identify the most efficacious treatment regimen.

Secondary:

- Evaluate the tolerability of these different intravenous artesunate regimens, in order to gain information to support dose selection for future studies of intravenous artesunate

18.6.4.2 Study Design

This was a randomized, open-label, dose-ranging study of four treatment regimens of intravenous artesunate in male and non-pregnant female subjects with symptomatic uncomplicated *Plasmodium falciparum* malaria. The study was conducted in 200 subjects, 100 from each study site (Thailand and Kenya). Fifty subjects were initially randomly placed in treatment cohorts 1 and 2 – Cohort 1: 2.4 mg/kg dose IV artesunate once daily for 3 days, followed by 25 mg/kg oral mefloquine (Thailand) or Malarone (Kenya); and Cohort 2: 2.4 mg/kg/dose of IV artesunate initially, 12 hours later on the same day (Day 0) and then once daily on Days 1 and 2, followed by oral mefloquine (Thailand) or Malarone (Kenya). After these cohorts were treated and the safety data from the 8 mg/kg dose given in the Phase I study 1142 were reviewed, 50 additional subjects were randomly placed into Cohorts 3 and 4 – Cohort 3: 1.2 mg/kg IV artesunate given once daily for 3 days followed by mefloquine (Thailand) or Malarone (Kenya); Cohort 4: 4.8 mg/kg of IV artesunate given once daily for 3 days followed by oral mefloquine (Thailand) or Malarone (Kenya).

The following table lists the baseline and screening assessments and the study schedule through Day 28.

Table 18-53: Study 1263 ab – Schedule of Events (Thailand)

Schedule of Activities	Screening/ Day (Hospital)	Day 1 (Hosp)	Day 2 (Hosp)	Day 3 (Hosp)	Day 7 (Outpt)	Day 14 (Outpt)	Day 28 (Outpt)
Informed Consent	X						
History	X						
PE	X	X	X	X	X	X	X

Schedule of Activities	Screening/ Day (Hospital)	Day 1 (Hosp)	Day 2 (Hosp)	Day 3 (Hosp)	Day 7 (Outpt)	Day 14 (Outpt)	Day 28 (Outpt)
Vital Signs (HR, BP, RR, T)	X	X	X	X	X	X	X
Malarial smears until negative	X	X	X	X	X	X	X
Hematology (cbc with diff)	X	X		X	X		X
UA	X						
Chemistry (Total bili/ALT/BUN/Cr Glu)		X		X (Creat/ALT)	+/-	+/-	+/-
Urine Pregnancy Test	X					X	
PK							
Adverse Events	X	X	X	X	X	X	X
IV artesunate	X	X	X				
Mefloquine 6-24 hr after last dose IV AS				X			

Source: Adapted from Study 1163ab CSR Appendix 11.1.1.1, 7.1 Study design and plan

18.6.4.3 Endpoints

Efficacy:

- Efficacy was defined by parasite clearance quantified using 3 outcome measures: the ability to clear at least 90% of asexual parasites from the peripheral blood by 48 hours after commencing IV artesunate treatment using the Giemsa-stained blood films for malaria as the diagnostic method of parasitemia quantification, the time to 90% and 100% parasite clearance denoted as parasite clearance times (PCT90 and PCT100) and the parasite reductions ratios at 12 and 24 hours after initial drug treatment (PRR12h and PRR24h), and the area under the curve (AUC) of quantifiable parasitemia.

Safety:

- Safety and tolerability of the treatment regimens were assessed through rates of adverse events and laboratories (hematology and chemistry). Fever clearance time, defined as the time from study drug administration until a subject's oral temperature first falls and then falls below 37.5°C and remains there for more than 24 hours, was also evaluated.

18.6.4.4 Key Inclusion Criteria

Thailand:

- Acute symptomatic *Plasmodium falciparum* malaria infection with a parasite density of ≥ 5000 asexual parasites/ μL as determined by malaria smear
- Male and non-pregnant female subjects from 18 to 65 years of age

Kenya:

- Acute symptomatic *Plasmodium falciparum* malaria infection with a parasite density of ≥ 5000 asexual parasites/ μL as determined by malaria smear

- Male and non-pregnant female subjects from 5 to 65 years of age.

Exclusion criteria included pregnancy, clinical evidence of severe malaria, mixed malaria infection by malaria smear.

18.6.4.5 Statistical Analysis Plan

The primary endpoint was a discrete variable (yes/no) denoting the efficacy to clear at least 90% of asexual *P. falciparum* parasites from the peripheral blood by 48 hours after administration of IV artesunate. A point estimate (Chi-Square or similar discrete variable analysis) and 95% confidence interval was constructed, and pair-wise comparisons were made employing a Bonferroni adjustment for the multiple comparisons across the groups. Summary statistics (mean, standard deviation, minimum, maximum, n, and coefficient of variation) were calculated for parasite clearance times (PCT90 and PCT100) and parasite reduction ratios (PRR12h, PRR24h, PRR48h, and PRR 72h). Comparisons between groups were made using an ANOVA or a similar non-parametric approach if data were not normally distributed. Secondary outcomes included determination of fever clearance times, and descriptive summary of adverse events, especially hematologic, associated with intravenous administration of artesunate. A temperature versus time curve was produced for each subject, and summary statistics were calculated for fever clearance time

18.6.4.6 Study Results

18.6.4.6.1 Demographic Characteristics

The following table provides the demographics of the study population, by dose of IV artesunate (Thailand and Kenya combined). Male enrollment was significantly higher than female (overall 67% to 33% male to female).

Table 18-54: Study 1262ab Demographics (Safety Population)

	1.2 mg/kg Once Daily for Three Days n=25	2.4 mg/kg Once Daily for Three Days n=25	2.4 mg/kg Twice on Day 0 and Once Daily for Two Days n=25	4.8 mg/kg Once Daily for Three Days n=25	Totals n=100
Age					
No. of Subjects	25	25	25	25	100
mean [std.dev]	18.64 [13.28]	17.24 [9.53]	18.96 [12.81]	15.08 [11.09]	17.48 [11.69]
median	15	15	13	14	14
range	5-53	5-47	6-47	5-53	5-53
Sex					
M	17 (68.0%)	19 (76.0%)	14 (56.0%)	17 (68.0%)	67 (67.0%)
F	8 (32.0%)	6 (24.0%)	11 (44.0%)	8 (32.0%)	33 (33.0%)
Race					
Black	19 (76.0%)	14 (56.0%)	15 (60.0%)	19 (76.0%)	67 (67.0%)

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

	1.2 mg/kg Once Daily for Three Days n=25	2.4 mg/kg Once Daily for Three Days n=25	2.4 mg/kg Twice on Day 0 and Once Daily for Two Days n=25	4.8 mg/kg Once Daily for Three Days n=25	Totals n=100
Asian	6 (24.0%)	11 (44.0%)	10 (40.0%)	6 (24.0%)	33 (33.0%)
Baseline weight (kg) mean[std.dev.]	39.96 [16.13]	41.88 [14.61]	37.08 [14.73]	36.12 [16.22]	38.76 [15.38]
Ethnicity					
Not Hispanic or Latino	25 (100.0%)	24 (96.0%)	25 (100.0%)	25 (100.0%)	99 (99.0%)
Hispanic or Latino	0 (0.0%)	1 (4.0%)	0 (0.0%)	0 (0.0%)	1 (1.0%)
Country					
Kenya	19 (76.0%)	14 (56.0%)	15 (60.0%)	19 (76.0%)	67 (67.0%)
Thailand	6 (24.0%)	11 (44.0%)	10 (40.0%)	6 (24.0%)	33 (33.0%)

Source: Clinical Reviewer (J Review 13.1)

A total of 59 pediatric subjects 5 to < 18 years old (59% of total subjects) were enrolled in the study. The pediatric subjects were evenly distributed among the dosage groups.

Table 18-55: Study 1263ab Demographics Pediatric Subjects (< 18 years old) (ITT)

	1.2 mg/kg Once Daily for Three Days n=15	2.4 mg/kg Once Daily for Three Days n=14	2.4 mg/kg Twice on Day 0 and Once Daily for Two Days n=14	4.8 mg/kg Once Daily for Three Days n=16	Totals n=59
Age					
No. subjects with data	15	14	14	16	59
mean and std. dev.	10.67 [3.81]	10.79 [3.29]	9.43 [3.18]	8.56 [4.00]	9.83 [3.63]
median	11	10.50	9	7.50	9
range	5-17	5-16	6-17	5-17	5-17
Sex					
M	10 (66.7%)	9 (64.3%)	5 (35.7%)	10 (62.5%)	34 (57.6%)
F	5 (33.3%)	5 (35.7%)	9 (64.3%)	6 (37.5%)	25 (42.4%)
Race					
Black OR African American	15 (100.0%)	14 (100.0%)	14 (100.0%)	16 (100.0%)	59 (100.0%)
Weight at Baseline kg – mean and std.dev.	30.80 [12.59]	34.93 [16.23]	26.29 [9.29]	27.56 [12.83]	29.83 [13.05]
Country -					
Kenya	15 (100.0%)	14 (100.0%)	14 (100.0%)	16 (100.0%)	59 (100.0%)

Source: Clinical Reviewer (J Review 13.1)

No patients died and none withdrew due to adverse events, although three patients were lost to follow up. See table below.

Table 18-56: Study 1263ab – Disposition

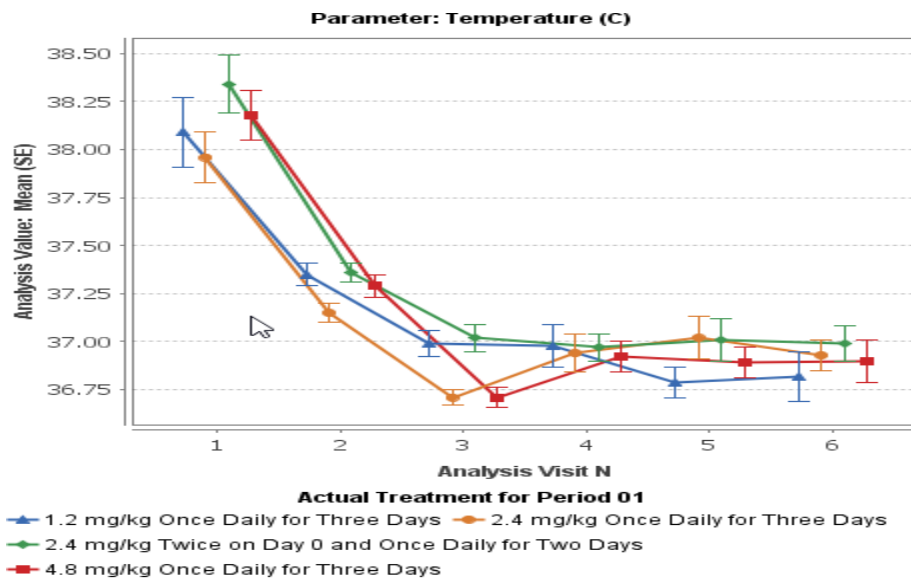
	1.2 mg/kg Once Daily for Three Days n=25	2.4 mg/kg Once Daily for Three Days n=25	2.4 mg/kg Twice on Day 0 and Once Daily for Two Days n=25	4.8 mg/kg Once Daily for Three Days n=25	Totals n=100
Completed	24 (96.0%)	23 (92.0%)	25 (100.0%)	25 (100.0%)	97 (97.0%)
Early withdrawal/ Lost to follow up	1 (4.0%)	2 (8.0%)	0 (0.0%)	0 (0.0%)	3 (3.0%)

Source: Clinical Reviewer (J Review 13.1)

Efficacy Results: There were no subject deaths in this study.

All dose groups had 90% parasite clearance by 48 hours and 100% clearance by 72 hours. In Kenya, parasite clearance was significantly reduced in the 4.8 mg/kg once daily for three days compared to the 2.4 mg/kg twice on day 0 and once daily for 2 more days. The Applicant postulates that this effect may have been due to artemisinin resistance, as this effect was not observed in Kenya, which at the time of the study was considered artemisinin-naïve.

Figure 18-25: Study 1263ab – Fever Clearance by Study Dose



Source: Clinical Reviewer (JReview)

All patients enrolled in this study had *P. falciparum*. Despite an exclusion criterion of mixed infection, two subjects in Kenya were enrolled having a mixed infection with *P. falciparum* and *P. ovale*. Patient # (b) (6) was a 13-year old male who received IV artesunate at 1.2 mg/kg once daily for three days. Patient # (b) (6) was a 15-year old female who received IV artesunate at 4.8 mg/kg once daily for three days. Both patients, became smear negative for *P. falciparum* by Day

3 of treatment. Clearance of *P. ovale*, occurred by Day 2. See discussion of Study 1263ab in Section 9.2.

Safety Results

No serious adverse events occurred in this study.

18.6.4.6.2 Treatment Emergent Adverse Events

Table 18-57: Study 1263ab – TEAE $\geq$ 5% Total Incidence by SOC/PT According to Treatment Arm (Safety Population)

	1.2 mg/kg Once Daily for Three Days n=25	2.4 mg/kg Once Daily for Three Days n=25	2.4 mg/kg Twice on Day 0 and Once Daily for Two Days n=25	4.8 mg/kg Once Daily for Three Days n=25	Totals n=100
Subjects with at least 1 TEAE	25 (100.0%)	24 (96.0%)	25 (100.0%)	24 (96.0%)	98 (98.0%)
BLOOD AND LYMPHATIC SYSTEM DISORDERS	20 (80.0%)	15 (60.0%)	19 (76.0%)	23 (92.0%)	77 (77.0%)
Neutropenia	12 (48.0%)	1 (4.0%)	0 (0.0%)	15 (60.0%)	28 (28.0%)
Anemia	8 (32.0%)	5 (20.0%)	5 (20.0%)	5 (20.0%)	23 (23.0%)
Thrombocytopenia	5 (20.0%)	2 (8.0%)	3 (12.0%)	11 (44.0%)	21 (21.0%)
Leukopenia	8 (32.0%)	4 (16.0%)	8 (32.0%)	0 (0.0%)	20 (20.0%)
Lymphocytosis	2 (8.0%)	4 (16.0%)	4 (16.0%)	4 (16.0%)	14 (14.0%)
Leukocytosis	0 (0.0%)	6 (24.0%)	3 (12.0%)	1 (4.0%)	10 (10.0%)
Lymphadenopathy	2 (8.0%)	3 (12.0%)	1 (4.0%)	1 (4.0%)	7 (7.0%)
Eosinophilia	0 (0.0%)	3 (12.0%)	1 (4.0%)	1 (4.0%)	5 (5.0%)
Lymphopenia	0 (0.0%)	3 (12.0%)	0 (0.0%)	2 (8.0%)	5 (5.0%)
Thrombocytopenia	0 (0.0%)	0 (0.0%)	5 (20.0%)	0 (0.0%)	5 (5.0%)
GASTROINTESTINAL DISORDERS	10 (40.0%)	8 (32.0%)	12 (48.0%)	8 (32.0%)	38 (38.0%)
Abdominal pain	2 (8.0%)	4 (16.0%)	6 (24.0%)	3 (12.0%)	15 (15.0%)
Diarrhea	1 (4.0%)	2 (8.0%)	4 (16.0%)	2 (8.0%)	9 (9.0%)
Vomiting	2 (8.0%)	2 (8.0%)	3 (12.0%)	1 (4.0%)	8 (8.0%)
Nausea	1 (4.0%)	2 (8.0%)	3 (12.0%)	2 (8.0%)	8 (8.0%)
INFECTIONS AND INFESTATIONS	8 (32.0%)	9 (36.0%)	9 (36.0%)	11 (44.0%)	37 (37.0%)
Rhinitis	0 (0.0%)	4 (16.0%)	4 (16.0%)	0 (0.0%)	8 (8.0%)
Oral herpes	2 (8.0%)	2 (8.0%)	0 (0.0%)	3 (12.0%)	7 (7.0%)
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	9 (36.0%)	6 (24.0%)	11 (44.0%)	9 (36.0%)	35 (35.0%)
Pyrexia	4 (16.0%)	3 (12.0%)	8 (32.0%)	5 (20.0%)	20 (20.0%)
NERVOUS SYSTEM DISORDERS	5 (20.0%)	5 (20.0%)	10 (40.0%)	9 (36.0%)	29 (29.0%)
Dizziness	3 (12.0%)	4 (16.0%)	6 (24.0%)	3 (12.0%)	16 (16.0%)
Headache	4 (16.0%)	1 (4.0%)	6 (24.0%)	3 (12.0%)	14 (14.0%)

	1.2 mg/kg Once Daily for Three Days n=25	2.4 mg/kg Once Daily for Three Days n=25	2.4 mg/kg Twice on Day 0 and Once Daily for Two Days n=25	4.8 mg/kg Once Daily for Three Days n=25	Totals n=100
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	7 (28.0%)	7 (28.0%)	7 (28.0%)	6 (24.0%)	27 (27.0%)
Cough	1 (4.0%)	3 (12.0%)	5 (20.0%)	2 (8.0%)	11 (11.0%)
Rhinorrhea	3 (12.0%)	3 (12.0%)	2 (8.0%)	1 (4.0%)	9 (9.0%)
Epistaxis	2 (8.0%)	0 (0.0%)	1 (4.0%)	1 (4.0%)	4 (4.0%)
INVESTIGATIONS	3 (12.0%)	0 (0.0%)	3 (12.0%)	1 (4.0%)	7 (7.0%)
Blood bilirubin decreased	2 (8.0%)	0 (0.0%)	1 (4.0%)	0 (0.0%)	3 (3.0%)
METABOLISM AND NUTRITION DISORDERS	1 (4.0%)	3 (12.0%)	2 (8.0%)	1 (4.0%)	7 (7.0%)
Hyperglycemia	0 (0.0%)	3 (12.0%)	0 (0.0%)	0 (0.0%)	3 (3.0%)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	2 (8.0%)	1 (4.0%)	1 (4.0%)	2 (8.0%)	6 (6.0%)
PSYCHIATRIC DISORDERS	1 (4.0%)	1 (4.0%)	1 (4.0%)	2 (8.0%)	5 (5.0%)
Insomnia	1 (4.0%)	0 (0.0%)	1 (4.0%)	2 (8.0%)	4 (4.0%)
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	0 (0.0%)	2 (8.0%)	2 (8.0%)	0 (0.0%)	4 (4.0%)
Rash	0 (0.0%)	0 (0.0%)	2 (8.0%)	0 (0.0%)	2 (2.0%)
EYE DISORDERS	2 (8.0%)	0 (0.0%)	2 (8.0%)	0 (0.0%)	4 (4.0%)
EAR AND LABYRINTH DISORDERS	1 (4.0%)	0 (0.0%)	1 (4.0%)	1 (4.0%)	3 (3.0%)
Tinnitus	0 (0.0%)	0 (0.0%)	1 (4.0%)	1 (4.0%)	2 (2.0%)
HEPATOBIILIARY DISORDERS	1 (4.0%)	0 (0.0%)	2 (8.0%)	0 (0.0%)	3 (3.0%)
Jaundice	1 (4.0%)	0 (0.0%)	1 (4.0%)	0 (0.0%)	2 (2.0%)
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	1 (4.0%)	0 (0.0%)	1 (4.0%)	0 (0.0%)	2 (2.0%)

Source: Clinical Reviewer (J Review 13.1)

Reviewer Comments: Definition of neutropenia was not found in the study protocol. Based on the reviewer's analysis of neutrophil count associated with TEAE coding of neutropenia, neutropenia was assessed at any time point after screening and neutrophil values were generally less than $2.0 \times 10^6/L$; however, 3 patients coded as having mild neutropenia had neutrophil counts above this level.

Table 18-58: Study 1263ab TEAE by SOC/PT Categorized as Severe

	1.2 mg/kg Once Daily for Three Days n=25	2.4 mg/kg Once Daily for Three Days n=25	2.4 mg/kg Twice on Day 0 and Once Daily for Two Days n=25	4.8 mg/kg Once Daily for Three Days n=25
Subjects with at least 1 TEAE	5 (20.0%)	2 (8.0%)	2 (8.0%)	7 (28.0%)

	1.2 mg/kg Once Daily for Three Days n=25	2.4 mg/kg Once Daily for Three Days n=25	2.4 mg/kg Twice on Day 0 and Once Daily for Two Days n=25	4.8 mg/kg Once Daily for Three Days n=25
BLOOD AND LYMPHATIC SYSTEM DISORDERS	5 (20.0%)	1 (4.0%)	0 (0.0%)	6 (24.0%)
Anemia	1 (4.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Thrombocytopenia	0 (0.0%)	1 (4.0%)	0 (0.0%)	1 (4.0%)
Neutropenia	4 (16.0%)	0 (0.0%)	0 (0.0%)	5 (20.0%)
GENERAL DISORDERS	0 (0.0%)	1 (4.0%)	0 (0.0%)	1 (4.0%)
Pyrexia	0 (0.0%)	1 (4.0%)	0 (0.0%)	1 (4.0%)
NERVOUS SYSTEM DISORDERS	0 (0.0%)	0 (0.0%)	1 (4.0%)	0 (0.0%)
Headache	0 (0.0%)	0 (0.0%)	1 (4.0%)	0 (0.0%)
INFECTIONS AND INFESTATIONS	0 (0.0%)	0 (0.0%)	1 (4.0%)	0 (0.0%)
<i>P. falciparum</i> infection	0 (0.0%)	0 (0.0%)	1 (4.0%)	0 (0.0%)

Source: Clinical Reviewer (J Review 13.1)

Reviewer comments: Definition of severity scale for neutropenia was not found in the study protocol. Based on this reviewer's analysis of neutrophil counts and assessment of severity in the dataset, severe neutropenia was generally less than $0.75 \times 10^6/L$, see patient details in table below.

Table 18-59: Study 1263ab – TEAE Categorized as Severe (Patient Details)

Subject Number	Site	Dose Regimen ¹	AE (Verbatim Term)	Description (Day)	Treatment Required	Outcome
(b) (6)	Kenya	1.2 mg/kg qdx3d	Anemia	Hct 24.6 (Day 1)	Yes (Transfusion)	Unknown
	Kenya	1.2 mg/kg qdx3d	Neutropenia	0.51×10^6 (Day 1)	No	Recovered
	Kenya	1.2 mg/kg qdx3d	Drop in neutrophil count	0.94×10^6 (Day 1)	No	Recovered
	Kenya	1.2 mg/kg qdx3d	Drop in Neutrophil count	0.95×10^6 (Day 1)	No	Recovered
	Kenya	1.2 mg/kg qdx3d	Drop in Neutrophil count	0.97×10^6 (Day 2)	No	Ongoing
	Thailand	1.2 mg/kg qdx3d	Thrombocytopenia	47,000 (Day 1)	No	Recovered
	Kenya	2.4 mg/kg qdx3d	Pyrexia	T 39.4°C (Day 18)	Yes (antipyretics)	Recovered
	Thailand	2.4 mg/kg qdx3d	<i>P. falciparum</i> infection	Smear neg by Day 3, Recrudescence Day 4	Yes (quinine and doxycycline Day 5-12)	Recovered

Subject Number (b) (6)	Site	Dose Regimen ¹	AE (Verbatim Term)	Description (Day)	Treatment Required	Outcome
	Kenya	2.4 mg/kg bid on Day 0 then qdx2d	Headache	(Day 4)	Yes	Recovered
	Thailand	4.8 mg/kg qdx3d	Pyrexia	(Day 0)	Yes	Recovered
	Thailand	4.8 mg/kg qdx3d	Thrombocytopenia	31,000 (drop of 12,000) (Day 1)	No	Recovered
	Kenya	4.8 mg/kg qdx3d	Neutropenia	0.95×10^6 (Day 3)	No	Recovered
	Kenya	4.8 mg/kg qdx3d	Neutropenia	0.98×10^6 (Day 3)	No	Recovered
	Kenya	4.8 mg/kg qdx3d	Neutropenia	0.51×10^6 (Day 1)	No	Recovered
	Kenya	4.8 mg/kg qdx3d	Drop in Neutrophil Count	1.08×10^6 (Day 1)	No	Unknown
	Kenya	4.8 mg/kg qdx3d	Neutropenia	0.66×10^6 (Day 1)	No	Recovered

Source: Clinical Reviewer, Adapted from Study 1263ab Clinical Study Report Table 12, p. 33-34, J Review, and JMP

¹No dose adjustments were made for TEAEs

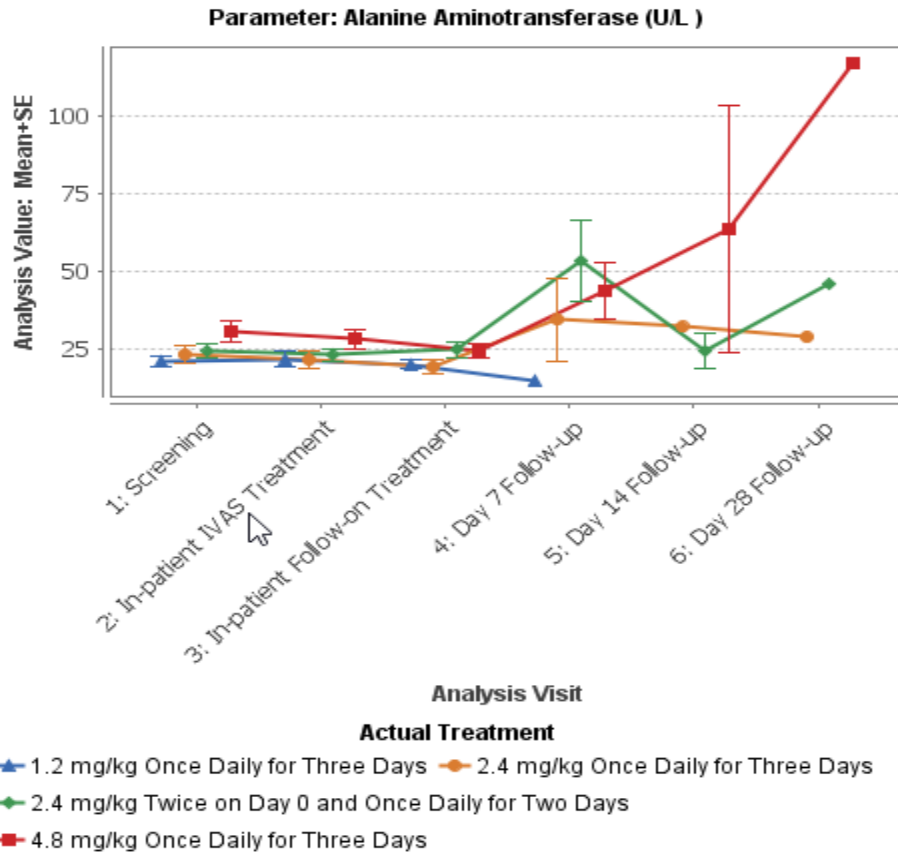
Reviewer comments: Cases classified as severe neutropenia occurred on Day 1 -3. There was not a strict dose-response relationship. Note that cases classified as severe either had an ANC $<0.75 \times 10^6/L$ or a significant drop in neutrophil count. (One patient classified as having severe neutropenia had a neutrophil count of 0.98×10^6).

Patient # (b) (6) enrolled at the Thailand site, was smear negative on Day 3 but experienced recrudescence of *P. falciparum* malaria on Day 4 and required secondary treatment with quinine and doxycycline.

18.6.4.6.3 Laboratory Findings

White blood cells and neutrophils showed a general decline during IV artesunate therapy and then showed a strong response on Day 3, recovering to normal by Day 7. Higher dose groups appeared to take longer to recover to baseline.

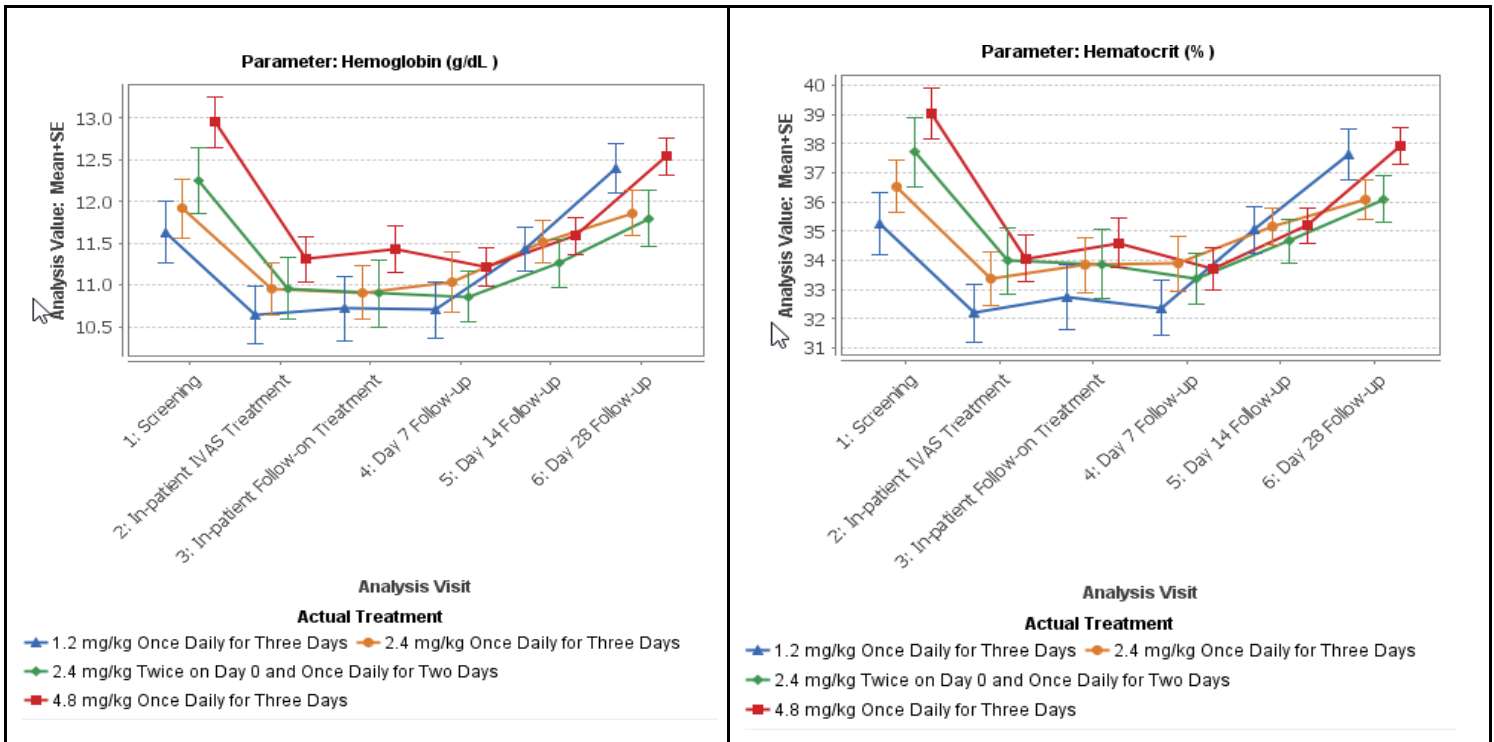
Figure 18-26: Study 1263ab – ALT by Study Visit and Dose



Source: Clinical Reviewer (J Review 13.1)

Reviewer comments: ALT levels appeared to show a mild elevation between Day 3 and Day 7 and then returning to baseline, with the exception of the 4.8 mg/kg dose group which continued to rise through Day 28 of follow up.

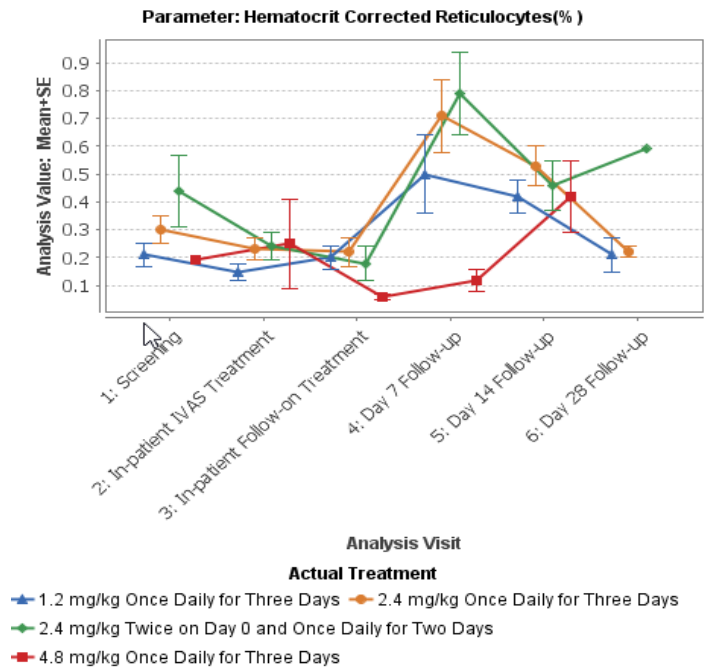
Figure 18-27: Study 1262ab – Hematocrit and Hemoglobin by Study Visit and Dose



Source: Clinical Reviewer (J Review 13.1)

Reviewer comments: Hemoglobin and hematocrit started out low and began to rise slowly through Day 28. Platelets levels were depressed initially, most likely due to malaria disease, increased sharply after IV artesunate administration, and then leveled off with slight decline after day 7.

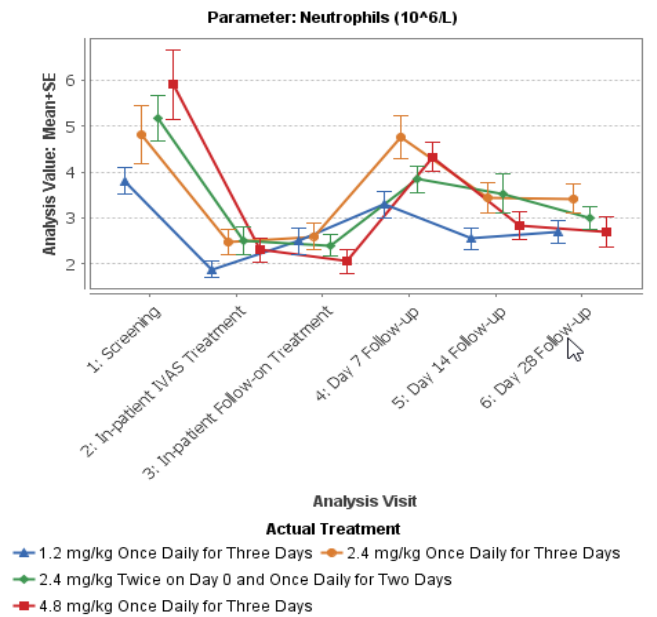
Figure 18-28: Study 1263ab – Reticulocytes by Study Visit



Source: Clinical Reviewer (J Review 13.1)

Reviewer comments: Reticulocytes declined through day 3 and all dose groups rose steadily until Day 7, with the exception of the 4.8 mg/kg dose which remained depressed until day 7 then rose to meet other dose groups by Day 14.

Figure 18-29: Study 1263ab – Neutrophils by Study Visit



Source: Clinical Reviewer (J Review 13.1)

Reviewer comments: *Neutropenia following IV artesunate was observed but there was not a strict dose-response relationship. For example, 4/25 patients in the 1.2 mg/kg/dose group, 0/50 in the 2.4 mg/kg/dose group and 5/25 patients in the 4.8 mg/kg/dose experienced severe neutropenia ($ANC < 0.75 \times 10^6$).*

18.6.5 WRAIR Study 1128

Title

A Phase 1a Randomized, Double-Blind, Placebo-Controlled Dose Escalation Study of the Safety, Tolerability, and Pharmacokinetics of Single Intravenous Doses of Artesunate Administered to Healthy Subjects

18.6.5.1 Study Objectives

Primary:

- To determine the safety of a single dose administration of artesunate in doses that bracket the anticipated compassionate use dose of 2.4 mg/kg of artesunate by measuring adverse events (AEs) and cardiovascular responses [heart rate (HR), blood pressure (BP), and electrocardiogram parameters (ECG)].

Secondary:

- To determine the safety and tolerability of the compassionate use anticipated dose of 2.4 mg/kg of artesunate given intravenously (IV).
- To assess the pharmacokinetics of artesunate and its major metabolite Dihydroartemisinin (DHA) in healthy volunteers.

18.6.5.2 Study Design

This study was a Phase 1a, randomized, double-blind, placebo-controlled, inpatient, ascending single IV dose study to determine the safety, tolerability, and pharmacokinetics of Intravenous artesunate in healthy male and non-pregnant, non-lactating female subjects aged 18 to 55 years old.

Five cohorts of 8 volunteers per cohort were studied. Within each cohort, 6 subjects received a single dose of Intravenous Artesunate infused over a period of 2 minutes at one of 5 specified dose levels, 0.5 mg/kg, 1.0 mg/kg, 2.0 mg/kg, 4.0 mg/kg, and 8.0 mg/kg, and 2 subjects in each cohort received a similar infusion of placebo (mannitol in phosphate buffer). Subjects underwent an outpatient screening visit for medical history, vital signs, physical exam (with a standardized neurologic assessment), hematology parameters including complete blood cell (CBC) count with platelets and differentials, coagulation indices, blood chemistries, urinalysis, urine drug screen, and, if female, a serum pregnancy test. Within 21 days of initial screening visit, eligible subjects were scheduled for a 6-hour outpatient visit in which

pre-dose ECGs and vital signs were obtained over the same period of time to compare with those obtained during investigational product dosing on Day 1.

On Day 0, subjects were admitted to the CPU to begin the inpatient phase of the study, and underwent a brief physical exam. On Day 1, pre-dose vital signs and ECG were performed. Subjects then received investigational product. Subjects were closely monitored by evaluating vital signs (pre-dose, approximately 5 min, 20 min, 40 min, 1 h, 2h, 4 h, 6h, 8 h, and 24 h after completion of infusion), ECG (pre-dose, and approximately 5 min, 20 min, 40 min, 1 h, 2 h, 4 h, 6 h, and 24 h after completion of infusion, and assessment of spontaneously reported AEs over the next 24 hours. Blood was drawn for pharmacokinetic pre-dose, approximately 5 min, 20 min, 40 min, 1 h, 2 h, 4 h, 6 h, and 24 h post-dose, and when directed by AEs. Blood for hematology, chemistry, and coagulation marker analysis was collected 6 h and 24 h post dose. Urinalysis was also performed 6 h and 24 h post dose. On Day 2, subjects were discharged. Outpatient follow-up included Day 4 vital signs, ECG, hematology, blood chemistry, and urinalysis, Day 8 hematology, vital signs, and ECG, and Day 15 hematology, vital signs, ECG, urine pregnancy test, and physical exam.

Table 18-60: Study 1128 – Schedule of Events

Schedule of Events	Day -1	Day 0	Day 1											Day	Day 4 (+/-1)	Day 8 (+/- 1)	Day (+/-)
	Screen	Pre-Admit	Admit	0	5 min	20 min	40 min	1h	2h	4h	6h	8h	24h	72h	--		
Informed Consent	X	X	X														
History and Demographics	X	X	X														
Physical Examination	X		X ^e										X			X	
Hematology ^a	X										X		X	X	X	X	
Coagulation Studies (PT/PTT) ^b	X										X		X				
Clinical Chemistry	X ^c										X		X	X			
Serology	X																
Urinalysis	X										X		X	X			
Urine Pregnancy Test [*]		X	X												X		
Serum Pregnancy	X																
Urine Drug Screen	X		X														
Alcohol Breath Test			X														
ECG (12 lead)	X	X		X	X	X		X	X	X	X		X	X	X	X	
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Infusion Checks				X	X												
PK Blood ^d				X	X	X	X	X	X	X	X		X				
Adverse Events (AE)	←																
Drug Administration			X														

33 of 73

Source: Study 1128 Protocol, p. 33

Note: Day 15 information is cut off in the original.

18.6.5.3 Criteria for Evaluation (Endpoints)

Pharmacokinetics

The following pharmacokinetic parameters were calculated for both artesunate and DHA: area under the plasma concentration curve (AUC) from time 0 to the time of the last quantifiable concentration (AUC_{0-last}), AUC extrapolated to infinity ($AUC_{0-\infty}$), terminal half-life ($T_{1/2}$), plasma clearance (CL), and volume of distribution (Vd). The predicted concentration at the end of infusion (C_{033}) was calculated for artesunate only, and the C_{max} and T_{max} were calculated for DHA only.

Safety

Safety was assessed through AEs, cardiovascular assessments primarily ECG, vital signs, clinical laboratory assessments (blood chemistry, hematology and urinalysis), and physical exams.

Cardiac – See QT-IRT review (Section 10.4.9)

18.6.5.4 Key Inclusion Criteria

Healthy adult males and non-pregnant, non-lactating females 18 to 55 years of age (inclusive).

18.6.5.5 Statistical Analysis Plan

Safety was analyzed using the safety population which consisted of all randomized subjects who received study drug and had any post-baseline data. Pharmacokinetic analyses were done using both the intention-to-treat (ITT) population. The ITT population was all randomized subjects who received study drug. The evaluable population was subject who completed the study through Day 2. All subjects met this requirement. The ECG data set consisted of all ECGs from all subjects who were dosed. All available post-treatment QTc data with a corresponding baseline value on Day -1 was used.

Summary statistics (n, mean, median, minimum, and maximum) for demographics were to be presented including age, sex, race, weight, height, tobacco history, and BMI for each treatment group. Individual subject data listings were to be generated for medical history and physical examination.

Safety Analysis

AEs were coded according to the Medical Dictionary for Regulatory Affairs (MedDRA) version (8.0). Treatment emergent AEs are summarized by System Organ Class (SOC) and MedDRA preferred term for each treatment group. Summaries are presented for AEs by seriousness, severity, and relation to study drug. A data listing of all information relating to AEs is provided. Vital sign data (pulse rate, BP, temperature, respiratory rate, height, and weight) is summarized, and the number of subjects with substantial increases or decreases in BP (20 mmHg) and HR (>15 bpm) is tabulated.

Laboratory data were summarized and presented with descriptive statistics (n, mean, median, standard deviation, minimum, and maximum) were to be calculated for each laboratory parameter at baseline and each scheduled time point, as well as for change from baseline. Time to greatest change and greatest change from baseline regardless of time was also summarized

See Section 9 – Clinical Pharmacology for SAP for pharmacokinetics.

See QT-IRT for Review of Cardiac Data Analysis

18.6.5.6 Study Results (Safety)

18.6.5.6.1 Demographics Characteristics

Demographics of subjects enrolled in Study 1128 are found in the Table below. Two subjects were lost to follow up following Day 4 of study, one in the placebo group and one receiving artesunate. This study had a predominance of male subjects enrolled in the study (80%) with female enrollment at 20%.

Table 18-61: Study 1128 – Demographics Pooled Artesunate vs Placebo (Safety Population)

	Artesunate Pooled n=30	Placebo n=10	Totals n=40
Age			
mean [SD]	39.73 [11.18]	40.00 [11.24]	39.80 [11.06]
Median	38.50	45.50	38.50
Range	19-55	19-54	19-55
Sex – count subjects and %			
M	23 (76.7%)	9 (90.0%)	32 (80.0%)
F	7 (23.3%)	1 (10.0%)	8 (20.0%)
Race – count subjects and %			
White	11 (36.7%)	4 (40.0%)	15 (37.5%)
Black or African American	11 (36.7%)	4 (40.0%)	15 (37.5%)
Multiple	7 (23.3%)	2 (20.0%)	9 (22.5%)
Asian	1 (3.3%)	0 (0.0%)	1 (2.5%)
Weight at Baseline kg			
mean [SD]	79.35 [11.69]	76.62 [8.02]	78.67 [10.86]
Median	81.45	79.55	80.05
Range	50-93.7	63.6-88.6	50-93.7
Height at Baseline cm			
mean [SD]	172.63 [9.11]	175.14 [7.38]	173.26 [8.69]
Median	172.70	179.15	172.85
Range	152.4-195.6	165.1-185	152.4-195.6
BMI at Baseline kg m ²			
mean [SD]	26.39 [2.44]	24.99 [1.90]	26.04 [2.37]
Median	26.45	25.50	26.20
Range	20-29.3	21.1-27.9	20-29.3

	Artesunate Pooled n=30	Placebo n=10	Totals n=40
Country – count subjects and %			
USA	30 (100.0%)	10 (100.0%)	40 (100.0%)

Source: Clinical Reviewer (J Review 13.1)

18.6.5.6.2 Treatment Emergent Adverse Events

There were no SAEs observed in this study.

The most common AEs, occurring in $\geq 5\%$ of artesunate subjects, were dysgeusia, dizziness, headache, nausea, vomiting, fatigue, myalgia, and anemia. Of these, only dysgeusia was dose-dependent and was only observed in 5 of 6 (83%) patients in each of the 4.0 and 8.0 mg/kg dose groups and one of the placebo control subjects. The dysgeusia was described consistently as a transient metal, bitter, or garlic taste that started within a few minutes of the IV artesunate infusion and lasted 10 to 25 minutes. There were no subjects in the lower artesunate dose groups that experienced this AE. Three subjects receiving artesunate were reported to have mild anemia. All the TEAEs were considered mild or moderate and resolved without treatment.

Reviewer comments: *In the CSR the Applicant asserts that the three cases of anemia may have been in part due to the volume of blood collected for pharmacokinetic and safety studies. Plot of hematocrit and hemoglobin over time does show a decrease following study Day 1 for both the placebo and artesunate groups. See results of Laboratory Analysis, below.*

One subject, a 38-year old male who received artesunate at 8.0 mg/kg experienced urticaria that started on the day of dosing which was considered moderate in severity and resolved in one day after receiving Benadryl.

TEAEs by pooled artesunate are listed in the table below.

Table 18-62: Study 1128 – TEAE by Pooled Artesunate vs Placebo by SOC/PT, Incidence PT $\geq 5\%$ in Either Group or Total (Safety Population)

TEAEs	Artesunate Pooled n=30	Placebo n=10	Totals n=40
Subjects with at least 1 TEAE	26 (86.7%)	6 (60.0%)	32 (80.0%)
NERVOUS SYSTEM DISORDERS	18 (60.0%)	2 (20.0%)	20 (50.0%)
Dysgeusia	10 (33.3%)	1 (10.0%)	11 (27.5%)
Dizziness	10 (33.3%)	1 (10.0%)	11 (27.5%)
Headache	5 (16.7%)	1 (10.0%)	6 (15.0%)
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	9 (30.0%)	0 (0.0%)	9 (22.5%)
Erythema	2 (6.7%)	0 (0.0%)	2 (5.0%)

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

TEAEs	Artesunate Pooled n=30	Placebo n=10	Totals n=40
Ecchymosis	2 (6.7%)	0 (0.0%)	2 (5.0%)
Hyperhidrosis	2 (6.7%)	0 (0.0%)	2 (5.0%)
GASTROINTESTINAL DISORDERS	7 (23.3%)	2 (20.0%)	9 (22.5%)
Vomiting	3 (10.0%)	0 (0.0%)	3 (7.5%)
Nausea	3 (10.0%)	0 (0.0%)	3 (7.5%)
Diarrhea	1 (3.3%)	1 (10.0%)	2 (5.0%)
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	5 (16.7%)	1 (10.0%)	6 (15.0%)
Fatigue	3 (10.0%)	1 (10.0%)	4 (10.0%)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	4 (13.3%)	0 (0.0%)	4 (10.0%)
Myalgia	3 (10.0%)	0 (0.0%)	3 (7.5%)
BLOOD AND LYMPHATIC SYSTEM DISORDERS	3 (10.0%)	0 (0.0%)	3 (7.5%)
Anemia	3 (10.0%)	0 (0.0%)	3 (7.5%)
VASCULAR DISORDERS	3 (10.0%)	0 (0.0%)	3 (7.5%)
Hot flush	2 (6.7%)	0 (0.0%)	2 (5.0%)
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	3 (10.0%)	0 (0.0%)	3 (7.5%)
Cough	2 (6.7%)	0 (0.0%)	2 (5.0%)
PSYCHIATRIC DISORDERS	2 (6.7%)	0 (0.0%)	2 (5.0%)
Depression	1 (3.3%)	0 (0.0%)	1 (2.5%)
Insomnia	1 (3.3%)	0 (0.0%)	1 (2.5%)
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	2 (6.7%)	0 (0.0%)	2 (5.0%)
Excoriation	1 (3.3%)	0 (0.0%)	1 (2.5%)
Arthropod bite	1 (3.3%)	0 (0.0%)	1 (2.5%)
INFECTIONS AND INFESTATIONS	2 (6.7%)	0 (0.0%)	2 (5.0%)
Vaginal mycosis	1 (3.3%)	0 (0.0%)	1 (2.5%)
Upper respiratory tract infection	1 (3.3%)	0 (0.0%)	1 (2.5%)
Gastroenteritis	1 (3.3%)	0 (0.0%)	1 (2.5%)
EYE DISORDERS	2 (6.7%)	0 (0.0%)	2 (5.0%)
Photopsia	1 (3.3%)	0 (0.0%)	1 (2.5%)
Lacrimation increase	1 (3.3%)	0 (0.0%)	1 (2.5%)
METABOLISM AND NUTRITION DISORDERS	1 (3.3%)	1 (10.0%)	2 (5.0%)
Anorexia	1 (3.3%)	0 (0.0%)	1 (2.5%)
Hyperkalemia	0 (0.0%)	1 (10.0%)	1 (2.5%)
RENAL AND URINARY DISORDERS	1 (3.3%)	1 (10.0%)	2 (5.0%)
Hematuria	1 (3.3%)	1 (10.0%)	2 (5.0%)

Source: Clinical Reviewer (J Review)

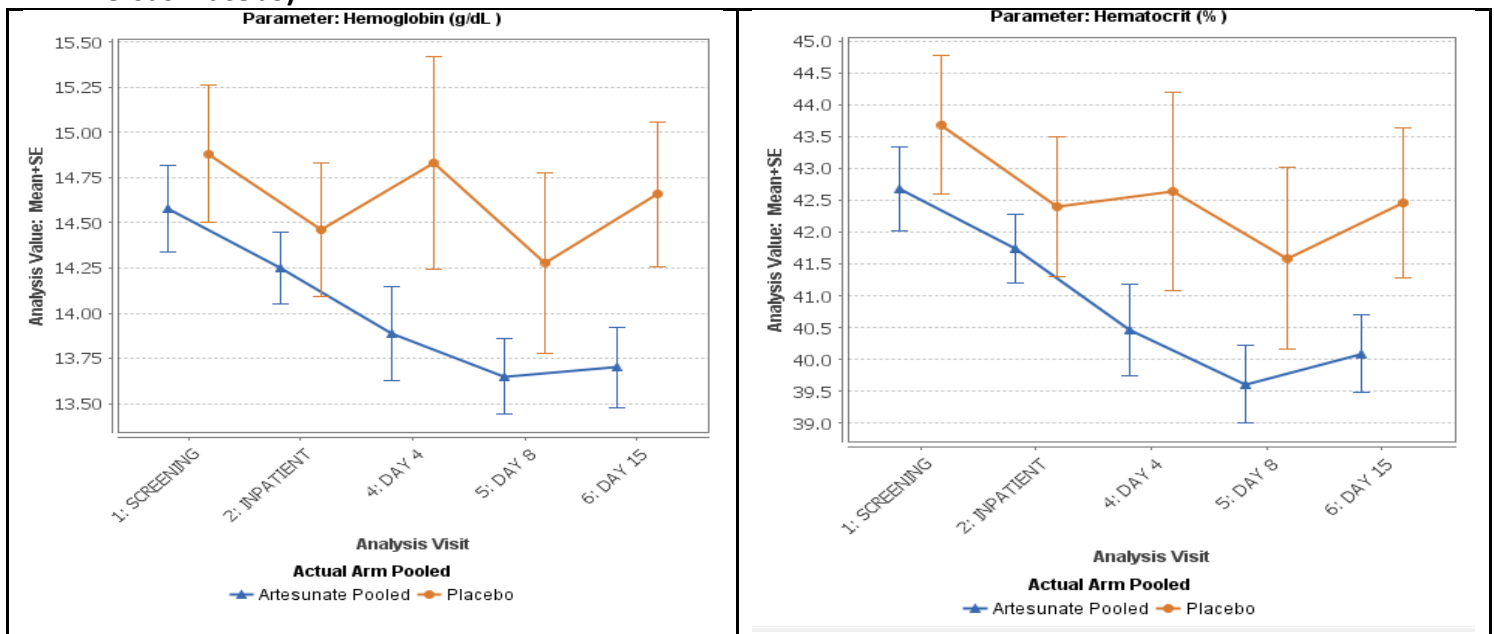
Reviewer comment: Dose-response relationship for TEAEs was not observed but numbers of subjects per dose (n=6) were too small to have a meaningful comparison.

No cases of neutropenia were coded as TEAE. Analysis of neutrophil counts following admission showed a mild transient drop in neutrophil count in artesunate recipients, see graphical depiction below. One placebo recipient and two artesunate recipients (one at 4.0 and one at 8.0 mg/kg/dose) experienced a transient drop in neutrophil count less than 1.5×10^6 /L with return to baseline by day 15.

18.6.5.6.3 Laboratory Findings

A transient decrease in the hematocrit and hemoglobin were observed and were noted to be outside the normal ranges for hematocrit in 11 subjects receiving artesunate (3 placebo) and hemoglobin in 9 subjects receiving artesunate (2 placebo).

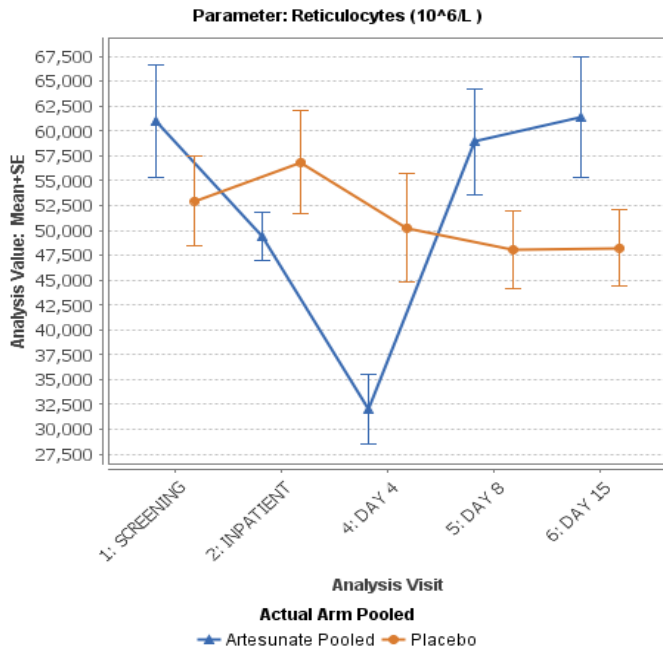
Figure 18-30: Study 1128 - Hemoglobin and Hematocrit by Study Visit (Pooled Artesunate versus Placebo)



Source: Clinical Reviewer (J Review 13.1)

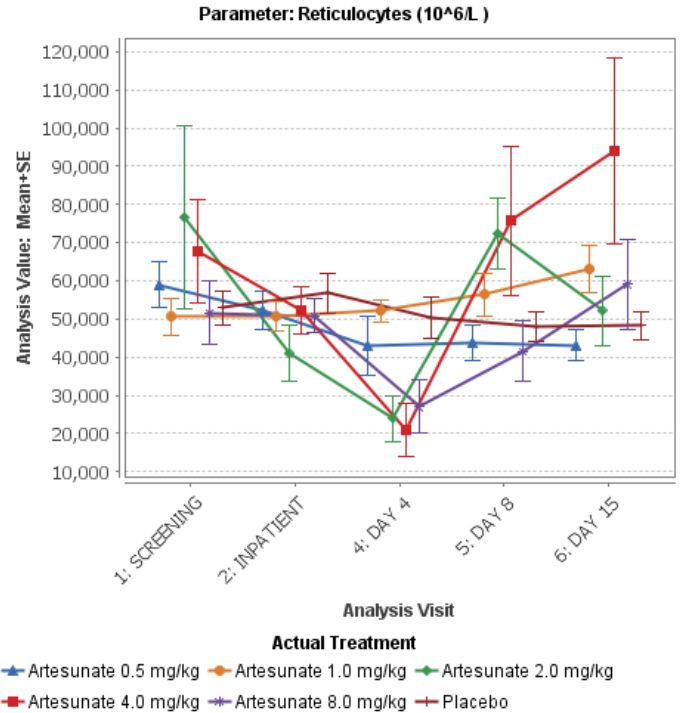
Reviewer comment: Mild anemia was considered a TEAE in three subjects receiving artesunate, one receiving 1.0 mg/kg (Subject (b) (6) a 19 year old male HCT on Screening = 41.7, Day 8 = 36.5, Day 15 = 42.0) and 2 receiving 2.0 mg/kg (Subject (b) (6) a 33 year old female, HCT on Screening=34.9, Day 1 = 29.9, Day 8 = 32.4, Day 15 = 32.6; and ART 3008, a 30 year old male, HCT on Screening =39.4, Day 1 = 32.7, Day 8=34.4, Day 15 = 37.5).

Figure 18-31: Study 1128 – Reticulocyte Count by Study Visit (Pooled Artesunate versus Placebo)



Source: Clinical Reviewer (J Review 13.1)

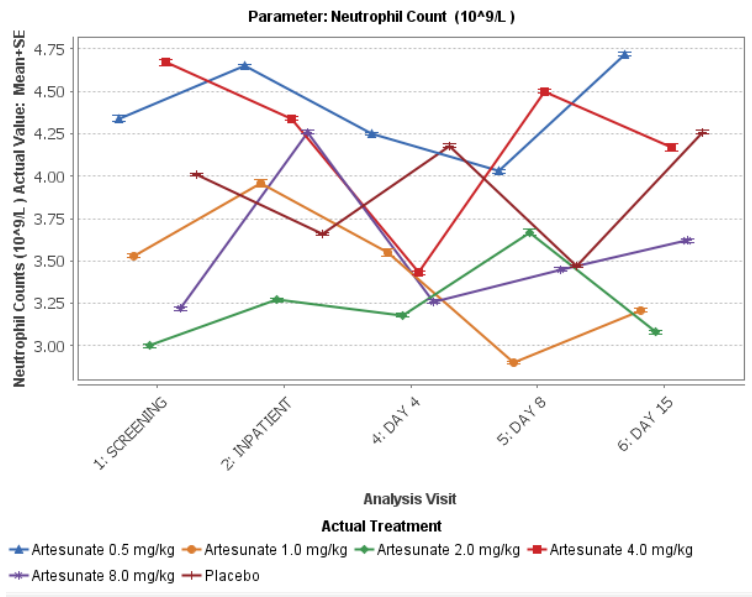
Figure 18-32: Study 1128 – Reticulocyte Count by Dose Regimen and Study Visit



Reviewer comments: Following artesunate infusion, there was a decrease in reticulocyte counts which dropped until Day 4 and returned to normal by Day 8. When examining the different dose regimens, a dose-dependent drop in reticulocyte count following IV artesunate is evident.

No significant changes were observed in leukocytes during the study period.

Figure 18-33: Study 1128 -- Neutrophils by Study Visit and Dose Regimen versus Placebo

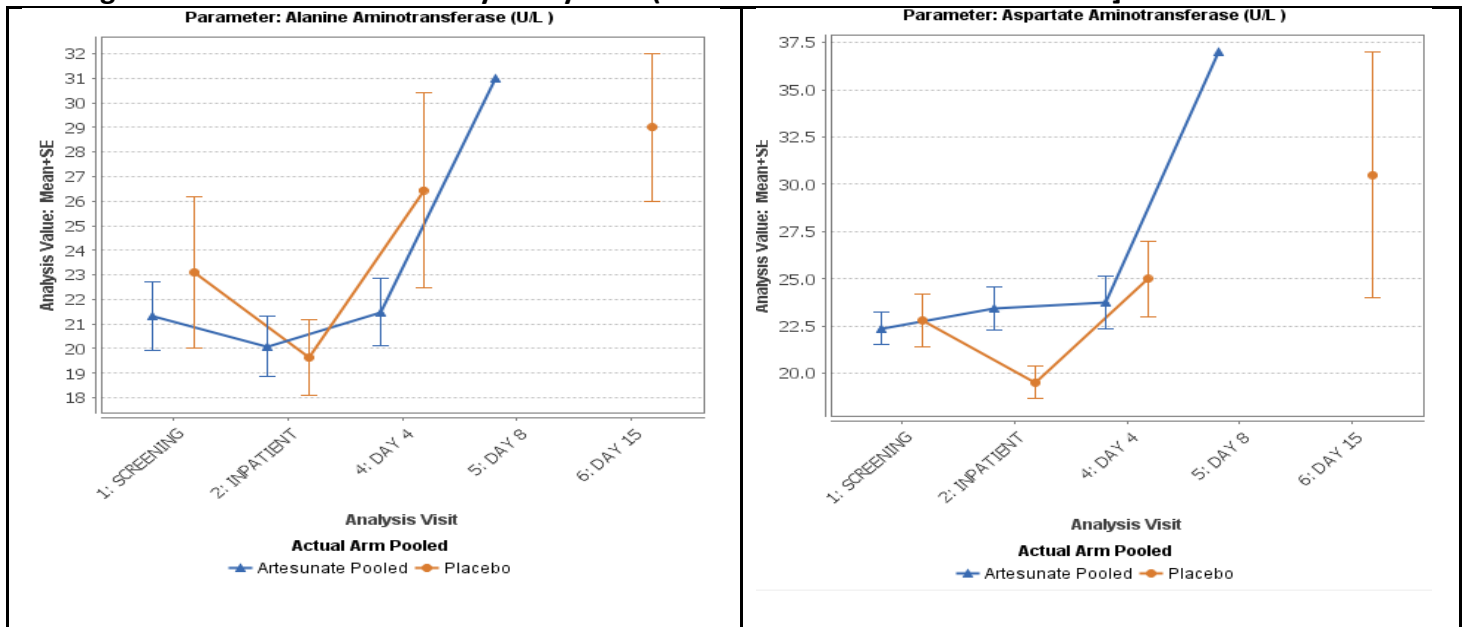


Source: Clinical Reviewer (J Review 13.1)

Reviewer comments: Following infusion on Day 1, a slight decrease in neutrophils were observed from inpatient dosing with Artesunate for Injection until day 4 and returning to baseline by Day 15. The decrease was steepest for the highest dose regimens (4.0 and 8.0 mg/kg/dose.)

Transaminase levels remained stable through Day 4, the last scheduled day of testing.

Figure 18-34: Transaminases by Study Visit (Pooled Artesunate versus Placebo)



Source: Clinical Reviewer (J Review 13.1)

Reviewer comments: *No artesunate-related rise in transaminases was observed compared with placebo.*

18.6.5.6.4 Vital Signs

Transient increases in systolic blood pressure of more than 29 mmHg was observed in 2 subjects in the control group as well as two subjects in each of the following artesunate dose groups: 0.5 mg/kg, 1.0 mg/kg, and 2.0 mg/kg and one subject in the 8.0 mg/kg group. One subject in the placebo group and 4 subjects receiving artesunate (1 in the 0.5 mg/kg group and 3 in the 1.0 mg/kg group) had systolic blood pressure increases > 140 mmHg in the 8 hour post infusion period. In addition, 2 subjects receiving 1.0 mg/kg and 1 subject receiving 4.0 mg/kg had diastolic blood pressure changes greater than 90 mmHg. All changes returned to <140 (systolic) and < 90 (diastolic) within 24 hours post dose.

Changes in HR $\geq$ 15 bpm during the first 8 hours after infusion occurred in 6 artesunate recipients, and 2 placebo controls. No episodes of tachycardia (HR >101 bpm) were noted.

18.6.6 WRAIR Study 1142

Title: A Phase 1 Double-Blind, Placebo-Controlled, Randomized, Multiple Dose Escalation Study to Evaluate the Safety, Tolerance, and Pharmacokinetics/Pharmacodynamics of a New GMP Formulation of Intravenous Artesunate in Healthy Subjects

18.6.6.1 Objectives

Primary:

- To determine the safety of a single dose administration of artesunate in doses that bracket the anticipated compassionate use dose of 2.4 mg/kg of artesunate by measuring adverse events (AEs) and cardiovascular responses [heart rate (HR), blood pressure (BP), and electrocardiogram parameters (ECG)].

Secondary

- To determine the safety and tolerability of the compassionate use anticipated dose of 2.4 mg/kg of artesunate given intravenously (IV).
- To assess the pharmacokinetics of artesunate and its major metabolite dihydroartemisinin (DHA) in healthy volunteers.

18.6.6.2 Study Design

This study was a Phase 1a, randomized, double-blind, placebo-controlled, inpatient, ascending single IV dose study to determine the safety, tolerability, and pharmacokinetics of Intravenous Artesunate in healthy male and non-pregnant, non-lactating female subjects aged 18 to 55 years old. Five cohorts of 8 volunteers per cohort were studied. Within each cohort, 6 subjects

received a single dose of Intravenous Artesunate infused over a period of 2 minutes at one of 5 specified dose levels, 0.5 mg/kg, 1.0 mg/kg, 2.0 mg/kg, 4.0 mg/kg, and 8.0 mg/kg, and 2 subjects in each cohort received a similar infusion of placebo (mannitol in phosphate buffer). Subjects underwent an outpatient screening visit for medical history, vital signs, physical exam (with a standardized neurologic assessment), hematology parameters including complete blood cell (CBC) count with platelets and differentials, coagulation indices, blood chemistries, urinalysis, urine drug screen, and, if female, a serum pregnancy test. Within 21 days of initial screening visit, eligible subjects were scheduled for a 6-hour outpatient visit in which pre-dose ECGs and vital signs were obtained over the same period of time to compare with those obtained during investigational product dosing on Day 1.

On Day 0, subjects were admitted to the CPU to begin the inpatient phase of the study and underwent a brief physical exam. On Day 1, pre-dose vital signs and ECG were performed. Subjects then received investigational product. Subjects were closely monitored by evaluating vital signs (pre-dose, approximately 5 min, 20 min, 40 min, 1 h, 2h, 4 h, 6h, 8 h, and 24 h after completion of infusion), ECG (pre-dose, and approximately 5 min, 20 min, 40 min, 1 h, 2 h, 4 h, 6 h, and 24 h after completion of infusion, and assessment of spontaneously reported Aes over the next 24 hours. Blood was drawn for pharmacokinetic pre-dose, approximately 5 min, 20 min, 40 min, 1 h, 2 h, 4 h, 6 h, and 24 h post-dose, and when directed by Aes. Blood for hematology, chemistry, and coagulation marker analysis was collected 6 h and 24 h post dose. Urinalysis was also performed 6 h and 24 h post dose. On Day 2, subjects were discharged. Outpatient follow-up included Day 4 vital signs, ECG, hematology, blood chemistry, and urinalysis, Day 8 hematology, vital signs, and ECG, and Day 15 hematology, vital signs, ECG, urine pregnancy test, and physical exam.

Table 18-63: Study 1142 Schedule of Events

Schedule of Events		Day 0	Days 1, 2, 3											Day 4	Day 7 (+/-1)	Day 10 (+/- 1)	Day 15 (+/- 1)
	Scr	Pre-Admit	Admit	pre dose	0 min	5 min	20 min	40 min	1h	2h	4h	6h	8h	24h / 72h ^o	--	--	--
Informed Consent	X																
History and Demographics	X																
Physical Examination	X													X			X
Brief Physical Exam		X	X			X											
Hematology ^a	X		X									X		X	X	X	X
Coagulation Studies (PT/PTT) ^b	X		X									X		X			X
Clinical Chemistry	X ^c		X ^c									X		X	X	X	X
Serology	X																
Urinalysis	X		X									X		X	X	X	X
Serum Pregnancy Test ^d	X																
Urine Pregnancy Test ^d		X	X														X
Urine Drug Screen	X		X														
Alcohol Breath Test			X														
ECG (12 lead) ^e	X	X	X	X		X	X	X	X	X	X		X	X	X	X	X
Vital Signs	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X
Infusion Checks				X													
PK Blood ^f				X		X	X	X	X	X	X		X	X			
Adverse Events	←																
Drug Administration					X												

Source: Study 1142 CSR Protocol Version 2.7 p.36

18.6.6.3 Key Inclusion Criteria

Healthy adult males and non-pregnant, non-lactating females 18 to 55 years of age (inclusive).

18.6.6.4 Endpoints

Pharmacokinetics:

The following pharmacokinetic parameters were calculated for both artesunate and DHA: area under the plasma concentration curve (AUC) from time 0 to the time of the last quantifiable concentration (AUC_{0-last}), AUC extrapolated to infinity (AUC_{0-∞}), terminal half-life (T_{1/2}), plasma clearance (CL), and volume of distribution (Vd). The predicted concentration at the end of infusion (C_{0.033}) was calculated for artesunate only, and the C_{max} and T_{max} were calculated for DHA only.

Safety:

Safety was assessed through AEs, cardiovascular assessments primarily ECG, vital signs, clinical laboratory assessments (blood chemistry, hematology and urinalysis), and physical exams.

18.6.6.5 Statistical Analysis Plan

Safety was analyzed using the safety population which consisted of all randomized subjects who received study drug and had any post-baseline data. Pharmacokinetic analyses were done using both the intention-to-treat (ITT) population. The ITT population was all randomized subjects who received study drug. The evaluable population was subject who completed the study through Day 2. All subjects met this requirement. The ECG data set consisted of all ECGs from all subjects who were dosed. All available post-treatment QTc data with a corresponding baseline value on Day-1 was used. Summary statistics (n, mean, median, minimum, and maximum) for demographics were to be presented including age, sex, race, weight, height, tobacco history, and BMI for each treatment group. Individual subject data listings were to be generated for medical history and physical examination.

Safety Analysis

TEAEs were coded according to the Medical Dictionary for Regulatory Affairs (MedDRA) version (8.0). TEAEs are summarized by System Organ Class (SOC) and MedDRA preferred term for each treatment group. Summaries were presented by seriousness, severity, and relation to study drug. Vital sign data (pulse rate, BP, temperature, respiratory rate, height, and weight) were summarized, and the number of subjects with substantial increases or decreases in BP (20 mmHg) and HR (>15 bpm) was tabulated.

Laboratory data were summarized and presented with descriptive statistics (n, mean, median, standard deviation, minimum, and maximum) were to be calculated for each laboratory parameter at baseline and each scheduled time point, as well as for change from baseline. Time to greatest change and greatest change from baseline regardless of time was summarized.

See Section 6 – Clinical Pharmacology for discussion of analysis of PK.

See QT-IRT report for discussion of ECG and QT analysis.

18.6.6.6 Results – Safety

18.6.6.6.1 Demographics at Baseline

Demographics among the artesunate and groups appeared evenly distributed. Of note, 24/26 (92.3%) of subjects were male.

18.6.6.6.2 Treatment Emergent Adverse Events

There were no SAEs observed in this study.

The most common TEAE observed in recipients of artesunate were dysgeusia (16 subjects or 80%), headache (6 subjects or 30%), anemia (5 subjects or 25%), dizziness (3 subjects or 15%).

All of these occurred more commonly in subjects administered artesunate compared with those receiving placebo (16 to 1 for dysgeusia and 3 to 0 for dizziness). Dysgeusia appears to be dose dependent, with 4 of 6 patients (67%) in the 2 mg/kg group and 5 of 6 patients (87.7%) of subjects in both the 4 mg/kg and 8 mg/kg groups experiencing this TEAE.

The table below provides the TEAEs by System Organ Class and Preferred Term, for both the pooled artesunate groups and each dose group;

Table 18-64: Study 1142 TEAE (SOC/PT), Incidence $\geq$ 5% in Pooled Artesunate (Safety Population)

TEAEs	Artesunate Pooled n=20	Placebo n=6	Totals n=26
Subjects with at least one TEAE	19 (95%)	6 (100%)	25 (96.2%)
NERVOUS SYSTEM DISORDERS	18 (90.0%)	2 (33.3%)	20 (76.9%)
Dysgeusia	16 (80.0%)	1 (16.7%)	17 (65.4%)
Headache	6 (30.0%)	1 (16.7%)	7 (26.9%)
Dizziness	3 (15.0%)	0 (0.0%)	3 (11.5%)
Paresthesia	2 (10.0%)	0 (0.0%)	2 (7.7%)
Somnolence	2 (10.0%)	0 (0.0%)	2 (7.7%)
Parosmia	1 (5.0%)	0 (0.0%)	1 (3.8%)
Syncope vasovagal	1 (5.0%)	0 (0.0%)	1 (3.8%)
BLOOD AND LYMPHATIC SYSTEM DISORDERS	6 (30.0%)	3 (50.0%)	9 (34.6%)
Anemia	5 (25.0%)	3 (50.0%)	8 (30.8%)
Leukopenia	1 (5.0%)	0 (0.0%)	1 (3.8%)
Neutropenia	1 (5.0%)	0 (0.0%)	1 (3.8%)
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	3 (15.0%)	1 (16.7%)	4 (15.4%)
Erythema	1 (5.0%)	0 (0.0%)	1 (3.8%)
Dermatitis	1 (5.0%)	0 (0.0%)	1 (3.8%)
Pruritis generalized	1 (5.0%)	0 (0.0%)	1 (3.8%)
Rash pruritic	1 (5.0%)	0 (0.0%)	1 (3.8%)
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	3 (15.0%)	0 (0.0%)	3 (11.5%)
Venipuncture site inflammation	1 (5.0%)	0 (0.0%)	1 (3.8%)
Fatigue	1 (5.0%)	0 (0.0%)	1 (3.8%)
Tenderness	1 (5.0%)	0 (0.0%)	1 (3.8%)
INFECTIONS AND INFESTATIONS	2 (10.0%)	1 (16.7%)	3 (11.5%)
Upper respiratory tract infection	2 (10.0%)	0 (0.0%)	2 (7.7%)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	2 (10.0%)	0 (0.0%)	2 (7.7%)
Joint stiffness	1 (5.0%)	0 (0.0%)	1 (3.8%)
Arthralgia	1 (5.0%)	0 (0.0%)	1 (3.8%)
Back pain	1 (5.0%)	0 (0.0%)	1 (3.8%)
METABOLISM AND NUTRITION DISORDERS	2 (10.0%)	1 (16.7%)	3 (11.5%)
Decreased appetite	1 (5.0%)	0 (0.0%)	1 (3.8%)

NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

TEAEs	Artesunate Pooled n=20	Placebo n=6	Totals n=26
Anorexia	1 (5.0%)	0 (0.0%)	1 (3.8%)
EYE DISORDERS	2 (10.0%)	0 (0.0%)	2 (7.7%)
Photophobia	1 (5.0%)	0 (0.0%)	1 (3.8%)
Eye pain	1 (5.0%)	0 (0.0%)	1 (3.8%)
Asthenopia	1 (5.0%)	0 (0.0%)	1 (3.8%)
INVESTIGATIONS	2 (10.0%)	0 (0.0%)	2 (7.7%)
Body temperature increased	1 (5.0%)	0 (0.0%)	1 (3.8%)
Aspartate aminotransferase increased	1 (5.0%)	0 (0.0%)	1 (3.8%)
Alanine aminotransferase increased	1 (5.0%)	0 (0.0%)	1 (3.8%)
VASCULAR DISORDERS	2 (10.0%)	0 (0.0%)	2 (7.7%)
Hot flush	1 (5.0%)	0 (0.0%)	1 (3.8%)
Flushing	1 (5.0%)	0 (0.0%)	1 (3.8%)
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	1 (5.0%)	0 (0.0%)	1 (3.8%)
Nasal congestion	1 (5.0%)	0 (0.0%)	1 (3.8%)
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	1 (5.0%)	0 (0.0%)	1 (3.8%)
Muscle strain	1 (5.0%)	0 (0.0%)	1 (3.8%)

Source: Clinical Reviewer (J Review 13.1)

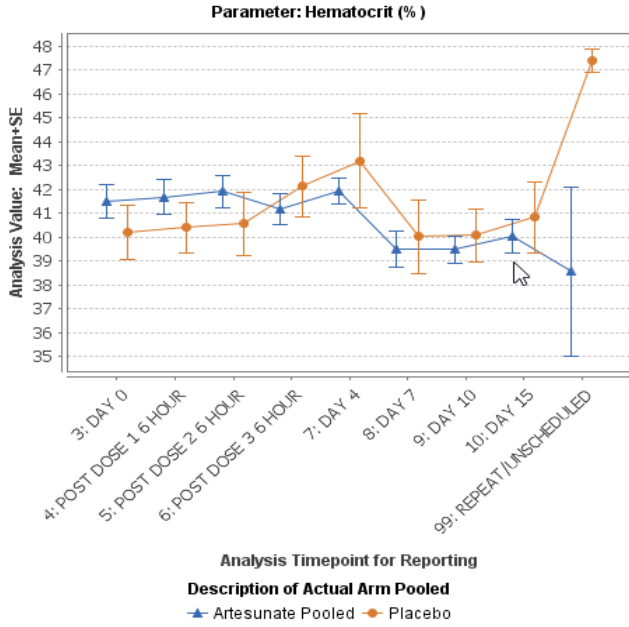
Reviewer comments: TEAEs were analyzed by dose regimen and no dose-response relationship was observed; however, it was difficult to make meaningful comparisons due to the small numbers of subjects.

No definition of neutropenia was found in the study protocol. One subject who received Artesunate for Injection at 8.0 mg/kg/dose was coded as having neutropenia classified as mild on Day 3 ($1.3 \times 10^6/L$) with a further drop in on day 4 to $0.9 \times 10^6/L$. Analysis of the full dataset showed an additional 3 subjects with neutrophil counts between 1.0 and $1.5 \times 10^6/L$: two subjects received 8.0 mg/kg/dose and one subject received placebo.

18.6.6.6.3 Laboratory Evaluation

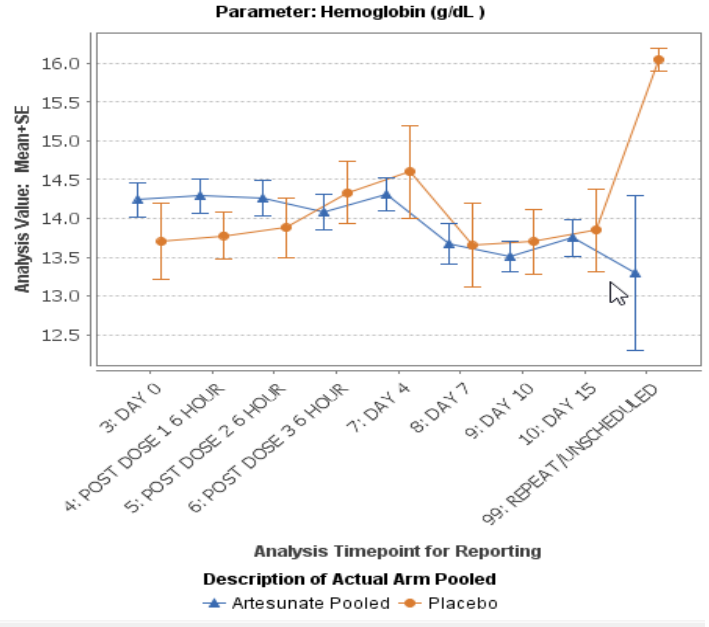
The figures below illustrate the time course for laboratory evaluation.

Figure 18-35: Study 1142 – Hematocrit by Study Visit (Pooled Artesunate vs. Placebo)
 Parameter: Hematocrit (%)



Source: Clinical Reviewer (J Review 13.1)

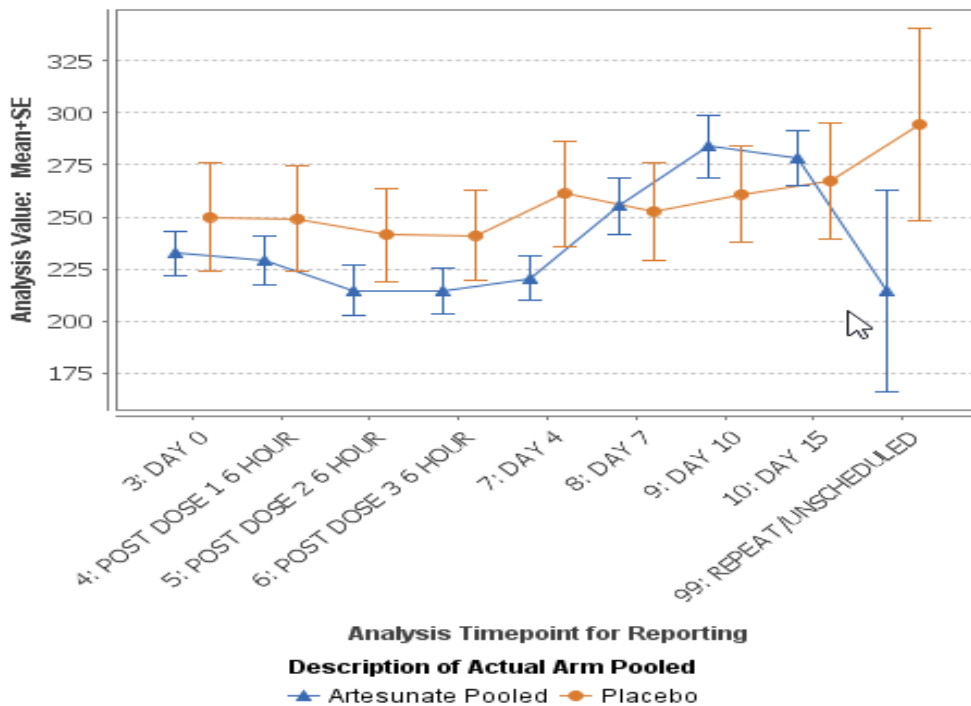
Figure 18-36: Study 1142 – Hemoglobin by Study Visit (Pooled Artesunate vs. Placebo)
 Parameter: Hemoglobin (g/dL)



Source: Clinical Reviewer (J Review 13.1)

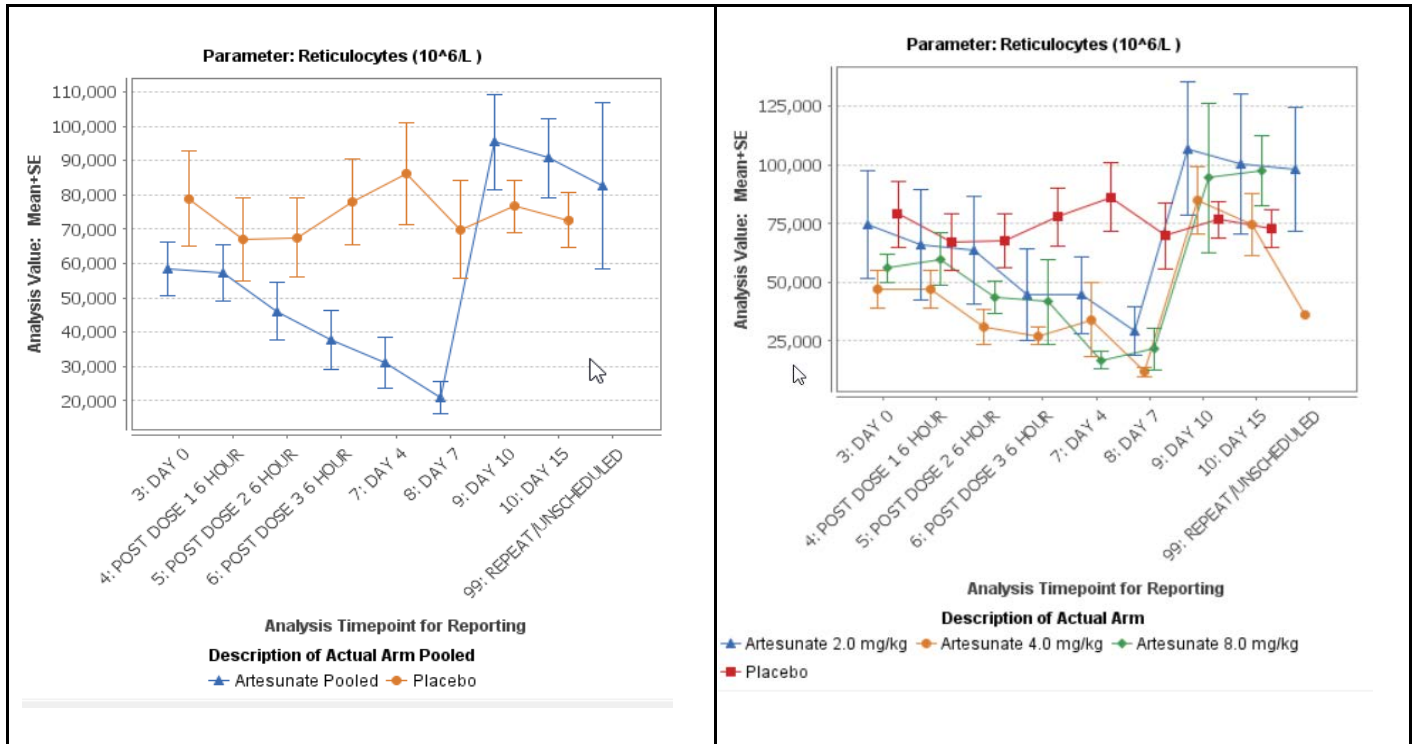
Reviewer comments: As in Study 1128, transient decreases in hemoglobin and hematocrit were observed in both the placebo and artesunate groups, following Day 1 (PK evaluation).

Figure 18-37: Study 1142 – Platelets by Study Visit (Pooled Artesunate vs. Placebo)
 Parameter: Platelets (10⁹/L)



Source: Clinical Reviewer (J Review 13.1)

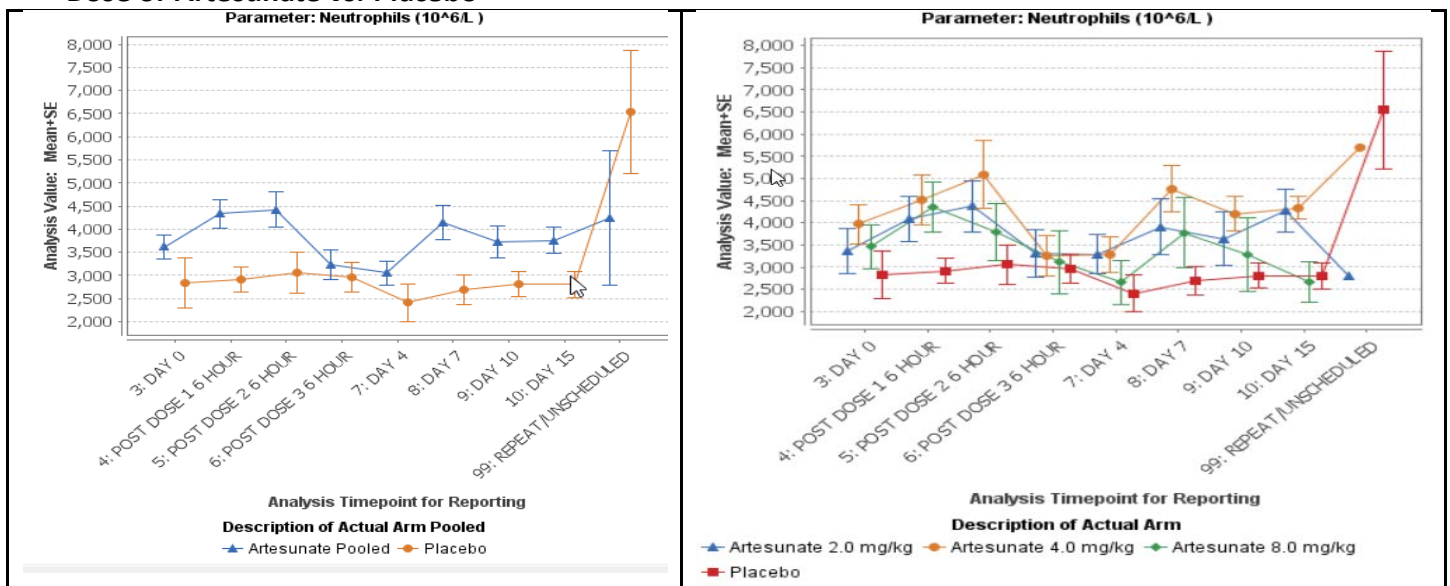
Figure 18-38: Study 1142 – Reticulocytes by Study Visit (Pooled Artesunate vs. Placebo and Dose of Artesunate vs. Placebo)



Source: Clinical Reviewer (J Review 13.1)

Reviewer comment: As was observed in Study 1128, a dose-dependent decrease in the reticulocyte count was observed, with lowest levels at 7 days.

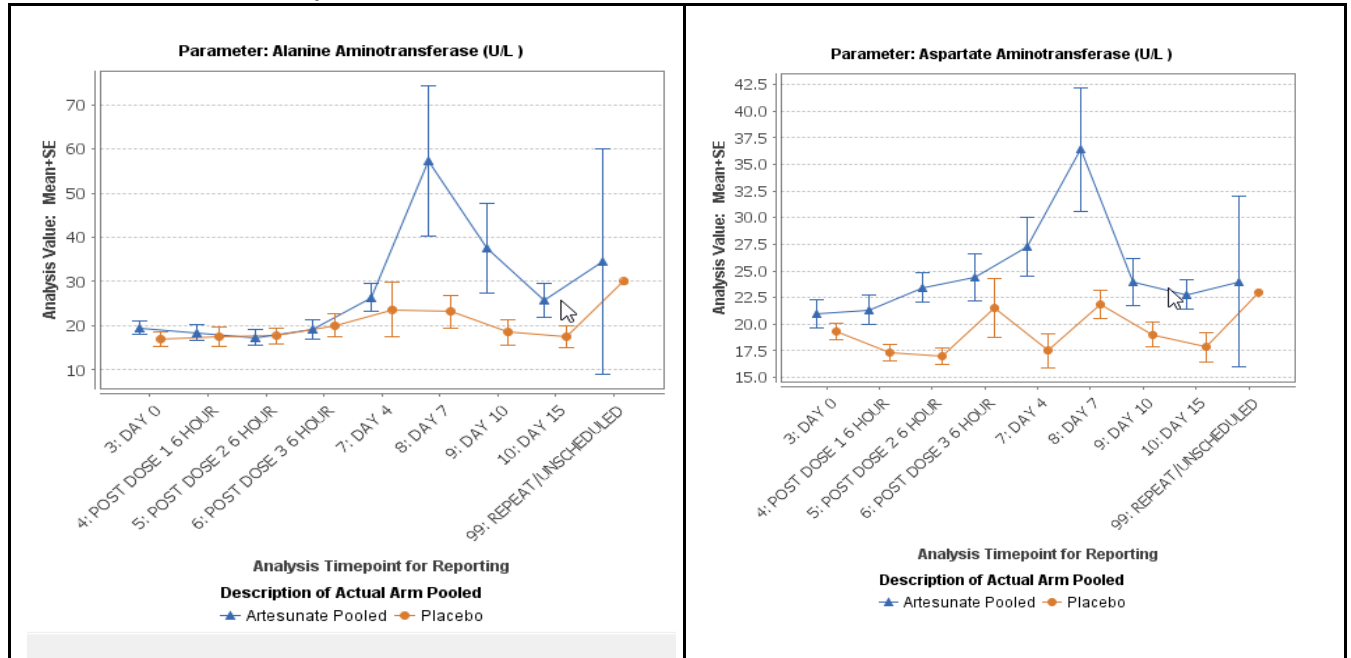
Figure 18-39: Study 1142 – Neutrophils by Study Visit (Pooled Artesunate vs. Placebo and Dose of Artesunate vs. Placebo)



Source: Clinical Reviewer (J Review 13.1)

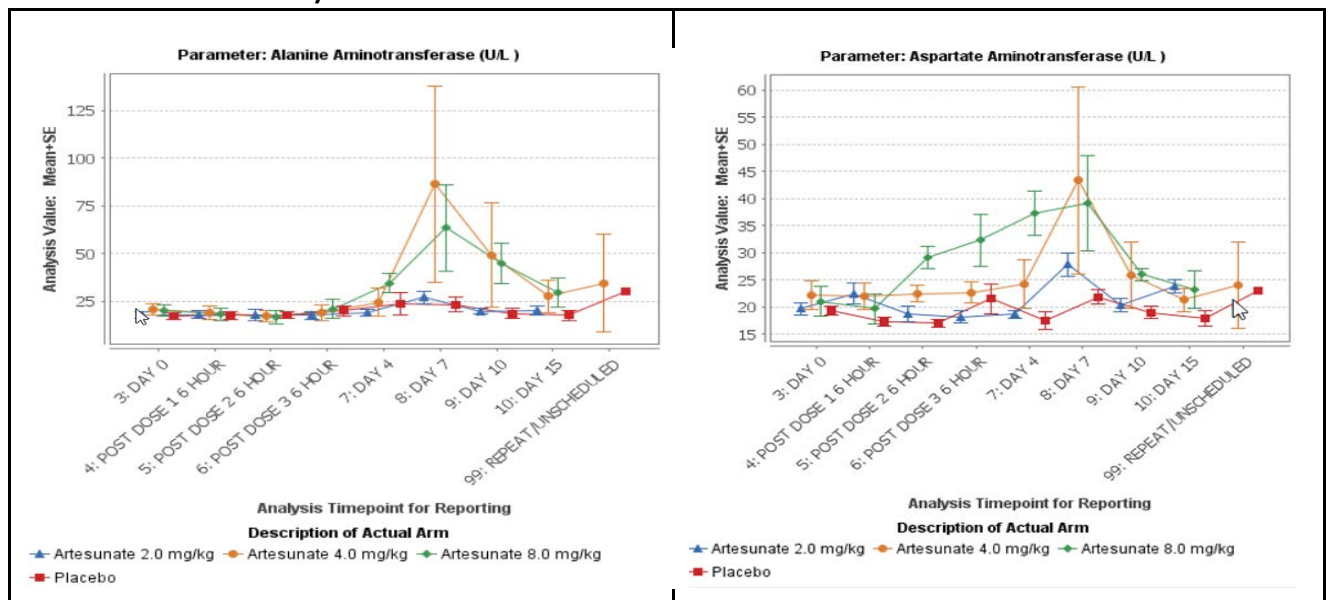
Reviewer comments: A transient drop in neutrophils was observed following the second dose of IV artesunate with the nadir on day 4 with rise to approximately baseline by day 7. No trends in total WBCs or monocytes were observed following IV artesunate administration.

Figure 18-40: Study 1142 – ALT by Study Visit (Pooled Artesunate vs. Placebo and Dose of Artesunate vs. Placebo)



Source: Clinical Reviewer (J Review 13.1)

Figure 18-41: Study 1142 - AST by Study Visit (Pooled Artesunate vs Placebo and Dose of Artesunate vs. Placebo)



Source: Clinical Reviewer (J Review 13.1)

Reviewer comments: *Mild, transient increases in ALT and AST were observed after dosing with Artesunate for Injection with peaks between days 4 and 7. Two patients experienced elevations rated as mild TEAEs: Subject 4007 (4 mg/kg/dose) had elevation of ALT and AST on day 7 of 292 U/L and 112 U/L respectively; and Subject 5030 (8 mg/kg/dose) had elevation of ALT and AST on day 7 of 166 U/L and 70 U/L respectively. Elevations declined by day 15 for both patients.*

Vital Signs

Transient increases in systolic blood pressure > 20 mmHg and heart rate were noted for each of the dose groups as well as placebo recipients. No dose related changes in vital signs were noted.

18.6.7 AQUAMAT

See Section 8.1.2 – Statistical and Clinical Evaluation of AQUAMAT for information on study design and analysis; baseline characteristics, mortality and complications. See Section 10.5.1 for discussion of neurological sequelae.

18.6.8 EDCTP-MMV07-01

See Section 8.1.3 – Statistical and Clinical Evaluation of EDCTP-MMV07-01 for additional baseline characteristics including demographics, clinical status and laboratory evaluations for this study and efficacy analysis.

The Safety population included all subjects who were randomized and received study drug (n=194). 100 patients received dosing regimen A (2.4 mg/kg at 0, 12, 24, 48 and 72 hours), and 94 patients received dosing regimen B (4.0 mg at 0, 24 and 48 hours). (Note: In the population that the Sponsor called ITT population, n=182, a total of 12 subjects were excluded from the Safety Population, 7 subjects due to missing time for parasitemia at 24 hours, 3 patients for “serious” protocol violations, one for withdrawal of consent, one for aparasitemia on Day 0.

18.6.8.1 Study Design

The schedule of study events is summarized on the table below.

Table 18-65: Study EDCTP-MMV07-01 – Schedule of Events

Table 1. Study Activities

Activity	Screening/ Day 0	Day 1	Day 2	Day 3	Day 7	Day 14	Day 28
Written Informed Consent	X						
Demographic Information and Medical History	X ^a						
Vital Signs	X ^b	X ^b	X ^c	X ^c	X	X	X
Assessment of Clinical Signs and Symptoms	X ^d	X ^d	X ^d	X ^d	X	X	X
Complete Physical Examination	X ^a						
Limited Physical Examination	X ^e	X ^e	X ^e	X ^e	X	X	X
Hematology	X		X		X		X
Biochemistry	X		X		X ^f		X ^f
Parasitological Assessments							
Thick and Thin Blood Smears	X ^g	X ^g	X ^g	X ^g	X	X	X
Plasma for HRP2 Estimate of Parasite Biomass	X						
Whole Blood for Parasite Testing	X						
Blood Sampling for Pharmacokinetic Analysis ^h	X		X				
Blood Sampling for PCR genotyping on filter paper	X	X			X ^k	X ^k	X ^k
Admission to Hospital	X						
Review Inclusion/Exclusion Criteria	X						
Randomization	X						
Study Drug Administration	X	X	X	X ⁱ			
Administer Oral Sulfadoxine/Pyrimethamine				X ^j			
Monitor Adverse Events	X	X	X	X	X	X	X
Recording of Prior and Concomitant Medication	X	X	X	X	X	X	X
Discharge from Hospital				X			

Source: EDCTP-MMV07-01 Protocol, Table 1, p. 24.

Safety endpoints included the incidence of any adverse event or clinically significant changes in laboratory parameters, mortality rate, neurological sequelae in patients with cerebral malaria at inclusion or vital signs

18.6.8.2 Study Results – Safety

18.6.8.2.1 Deaths and Serious Adverse Events

There were two patients (# (b) (6) and (b) (6) who died during the study. Both had cerebral malaria. Patient # (b) (6) died on day 2 after experienced seizures and coma; patient (b) (6) died on day 1 following a grand mal seizure progressing to coma. Both patients received the 5 dose regimen. See further details in Section 10.4.1.

A total of 23 patients experienced 31 SAEs, 13 in Group A and 18 in group B. The most common SAE was anemia in 5 patients and seizures (Preferred Term grand mal convulsions or convulsions) in 4 subjects.

Table 18-66: EDCTP-MMV07-01 – Number of SAEs per Treatment Group (Safety Population)

	Treatment Group		All N=194	Test
	A N=100	B N=94		
Any SAE				Fisher Exact
NO	90 (90.0%)	81 (86.2%)	171 (88.1%)	P = 0.507
YES	10 (10.0%)	13 (13.8%)	23 (11.9%)	
Nb of SAE events				
YES	13	18	31	

Source: EDCTP- MMV Clinical Study Report – Table 8.2-1

Table 18-67: EDCTP-MMV07-01 – SAEs by SOC/PT (Safety Population)

SYSTEM ORGAN CLASS	PREFERRED TERM	A		B		All		Fisher P=
		Subjects N=10	Events N=13	Subjects N=13	Events N=18	Subjects N=23	Events N=31	
BLOOD AND LYMPHATIC SYSTEM DISORDERS	ANAEMIA	2 (20.0%)	2	3 (23.1%)	3	5 (21.7%)	5	1.000
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	DEATH	0 (0.0%)	0	1 (7.7%)	1	1 (4.3%)	1	1.000
INFECTIONS AND INFESTATIONS	BACTERIAL INFECTION	7 (70.0%)	9	4 (30.8%)	4	11 (47.8%)	13	
	BRONCHOPNEUMONIA	1 (10.0%)	1	0 (0.0%)	0	1 (4.3%)	1	0.435
	CELLULITIS	0 (0.0%)	0	1 (7.7%)	1	1 (4.3%)	1	1.000
	LUNG INFECTION	1 (10.0%)	1	0 (0.0%)	0	1 (4.3%)	1	0.435
	MALARIA	2 (20.0%)	4	0 (0.0%)	0	2 (8.7%)	4	0.178
	PLASMODIUM FALCIPARUM INFECTION	0 (0.0%)	0	1 (7.7%)	1	1 (4.3%)	1	1.000
	SALMONELLA SEPSIS	1 (10.0%)	1	0 (0.0%)	0	1 (4.3%)	1	0.435
	SEPSIS	1 (10.0%)	1	0 (0.0%)	0	1 (4.3%)	1	0.435
	STAPHYLOCOCCAL SEPSIS	1 (10.0%)	1	1 (7.7%)	1	2 (8.7%)	2	1.000
NERVOUS SYSTEM DISORDERS	CONVULSION	0 (0.0%)	0	3 (23.1%)	7	3 (13.0%)	7	
	GRAND MAL CONVULSION	0 (0.0%)	0	2 (15.4%)	2	2 (8.7%)	2	0.488
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	SKIN SWELLING	1 (10.0%)	1	0 (0.0%)	0	1 (4.3%)	1	0.435
SURGICAL AND MEDICAL PROCEDURES	HOSPITALISATION	0 (0.0%)	0	1 (7.7%)	1	1 (4.3%)	1	1.000
VASCULAR DISORDERS	PHLEBITIS	1 (10.0%)	1	2 (15.4%)	2	3 (13.0%)	3	1.000

Source: EDCTP-MMV-01-07 Clinical Study Report – Table 8.3-2

18.6.8.2.2 Treatment Emergent Adverse Events

During the trial, 133 subjects (68.6%) experienced at least one adverse event, 68 (68%) in the 3-dose group, and 65 (69.1%) in the 5-dose group.

The most common TEAEs by preferred term were anemia in a total of 30 of 194 patients (15.4%), cough in 28 (14.4%), abdominal pain in 20 (10.3%), and pyrexia in 18 (9.3%).

Neurological outcomes

While EDCTP-MMV included neurological outcomes as a secondary endpoint, limited information was found regarding neurological follow up in the clinical study report. Of the four patients with SAEs of SOC nervous system disorders and Preferred Term “convulsions” or “grand mal convulsions”, two died (Subjects # (b) (6) and (b) (6)). In the remaining two patients,

the SAEs were considered resolved: Subject # (b) (6) on day 2, and Subject # (b) (6) by the outpatient follow up visit on day 28.

18.6.8.2.3 Laboratory Evaluation

The CSR reported “clinically significant” changes for the following laboratory parameters: hematocrit, hemoglobin, MCV, platelets, RBCs and WBCs. The highest percentage of patients with clinically significant changes was found on day 0 and decreased with subsequent visits, consistent with the severity of malaria. The CSR reported on the number of subjects with clinically significant results for each lab test. The CSR also provided the mean, median and range for each parameter. The investigators concluded that changes in laboratory values were most likely associated with the underlying malarial disease.

18.7 References

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NDA Multi-disciplinary Review and Evaluation NDA 213036
Artesunate for Injection

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DIVISION OF ANTI-INFECTIVE PRODUCTS
PHARMACOLOGY/TOXICOLOGY REVIEW AND MEMO TO FILE

DATE: 2/11/19

Application number: 64,769
Supporting document/s: 138
Sponsor's letter date: 12/10/2018
CDER stamp date: 12/10/2018
Product: IV Artesunate
Indication: Initial treatment of severe (b) (4) malaria
in adults and children
Sponsor: Amivas, LLC (co-development partner for US
Army Medical Material Development Activity,
USAMMDA)
Review Division: Division of Anti-Infective Products
Reviewer: Kelly Brant, MPH, Ph.D., DABT
Supervisor/Team Leader: Terry J. Miller, Ph.D.
Division Director: Sumathi Nambiar, MD
Project Manager: Gregory DiBernardo

1. BACKGROUND

The Sponsor is intending to apply for a marketing authorization of IV artesunate under section 505(b)(2) of the 21 U.S.C. 9 FD&C Act and has received a Breakthrough Therapy Designation (9/11/18) and Orphan Drug Designation. Artesunate has marketing authorization in 32 countries (28 African and 4 Asian) for treatment of severe malaria in adults and children. Artesunate is also available in several European countries and Canada through the Named Patient Programmes. The proposed dosing regimen for IV Artesunate is 2.4 mg/kg at 0, 12, 24, (b) (4)

(b) (4) The parenteral AS manufactured by Guilin Pharmaceutical Company used to treat malaria outside the US lacks approval by the FDA. To meet the need for a safe and effective antimalarial for the treatment of severe (b) (4) (b) (4) malaria in patients in the US, a formulation of IV artesunate has been manufactured by the US Army under the current IND (64, 769). The Surgeon General, Department of the Army requested a Type C meeting to obtain Agency concurrence on the non-clinical studies to be included in the NDA (a separate pre-NDA Clinical and CMC meeting have also been requested).

The nonclinical studies were conducted with either Guilin or the US Army formulations of artesunate (manufactured by (b) (4)) and the Sponsor submitted bioequivalence information (study reports and/or published literature) of the two IV formulations carried out in rats and dogs. The Sponsor indicated that the (b) (4) API used in the nonclinical and clinical studies under IND 64, 769 will no longer be manufactured by (b) (4) as they will only manufacture the semi-synthetic produced API in the future with a DMF registered with the Agency. A Letter of Authorization has been granted for

this DMF. The change from (b) (4) to semi-synthetic API was subject of a separate Type A meeting request (IND 64, 769, [SN 0128](#)). Comparative data (b) (4) will be presented in the pre-NDA CMC briefing document when the data are available. The Sponsor intends to use the semi-synthetic source of API for registration batch manufacturing and has proposed (b) (4)

2. STUDIES SUBMITTED TO IND 64, 769:

2.1 Studies Reviewed¹:

Study No.	Species/ Test System	Study Title	GLP	API Manufacturer	
Safety Pharmacology					
1466-103	Dog (beagle)	Cardiovascular Safety Pharmacology Study of Artesunate in Beagle Dogs	Yes	(b) (4)	
PN02-01	Monkey (rhesus)	(b) (4) Pathology Report	No		
Repeat-Dose Toxicity					
WRAIR 8H	Rat (SD)	The LD50, vascular irritation, hemotoxicity and bone marrow depression evaluation of intravenous artesunate and artesunate in <i>P. berghei</i> -infected and non-infected rats	No		
AQ0008_02_AF	Monkey (rhesus)	Toxicity of Subacute, 7-Day Dosing of Candidate Intravenous Artemisinins in Rhesus Monkey	No		
1466-102	Dog (Beagle)	14-Day Toxicity Study of Artesunate in Beagle Dogs	Yes		
Genotoxicity					
G305-08	In vitro (<i>S. typhimurium</i> , <i>E. coli</i>)	Evaluation of Artesunic Acid in the <i>Salmonella-Escherichia coli</i> /microsome plate incorporation assay	Yes		
G306-08	In vitro (CHO cells)	Evaluation of Artesunic Acid in the Chinese Hamster Ovary (CHO) Chromosome Aberration Assay	Yes		
G307-08	Mouse (Swiss Webster)	Evaluation of Artesunic Acid in the Mouse Bone Marrow Micronucleus Assay	Yes		
Reproductive and Development Toxicity ²					
CD2006/00795/00	In vitro (Cynomolgus monkey blood cells)	Artesunate: In Vitro Effects on Embryonic and Fetal Blood Cells of Cynomolgus Monkeys	No		
CD2004/00606/01	Rat (SD)	Artemisinins: Oral Embryo-Fetal Development Comparison Study in Rats	Yes		

¹ See Section 5. for full reviews of submitted study reports

² Previously reviewed:

https://darts.fda.gov/darts/faces/ViewDocument?documentId=090140af800c69eb&_afRedirect=4658590351298442

CD2004/00258/00	Rat (SD)	Oral Dose-Range Embryo-Fetal Development Study in Rats	No
CD2004/00353/00	Rat (SD)	Oral Embryo-Fetal Development Study During Critical Developmental Windows in the Rat	Yes
CD2005/00559/00	Rat (SD)	Artesunate: Oral (Gavage) Study of Embryo-Foetal Development in the Rat	Yes
CD2005/00560/00	Rabbit (NZW)	Artesunate: Oral (Gavage) Study of Embryo-Foetal Development in the Rabbit	Yes
CD2005/01092/00	Monkey (cynomolgus)	Fourteen-Day Repeated Oral Dose Range-Finding Study of SB-714702 (Artesunate) in Non-Pregnant Cynomolgus Monkeys	No
CD2006/00794/00	Monkey (cynomolgus)	A Study for the Effect of SB-714702 (Artesunate) on Embryo-Fetal Development in Cynomolgus Monkeys by Oral Administration	Yes
CD2005/00558/00	Rabbit (NZW)	Chlorproguanil/Dapsone/Artesunate: Oral (Gavage) Study of Embryo-Foetal Development in the Rabbit	Yes

(b) (4)

2.2 Studies Not Reviewed:

Study No.	Species/ Test System	Study Title	GLP	API Manufacturer
Single-Dose Toxicity				
WRAIR 8H	Rat (SD)	The LD50, vascular irritation, hemotoxicity and bone marrow depression evaluation of intravenous artelinate and artesunate in <i>P. berghei</i> -infected and non-infected rats	No	
AQ0008 01 AF	Monkey (rhesus)	Acute Toxicity of IV Artemisinins in Rhesus	No	
Other Toxicity Studies				
0725XW16.001	In vitro (rat, dog, monkey, human blood)	Evaluation of a Test Article to Induce Hemolysis in Human, Rat, Dog and Monkey Blood	Yes	
CD2005/00557/00	Rat (SD)	Chlorproguanil/Dapsone/Artesunate: Oral (Gavage) Study of Embryo-Foetal Development in the Rat	Yes	
CD2005/01454/00	Rat (SD)	SB-714703 (Chlorproguanil/Dapsone/Artesunate): Dose Range-Finding Toxicity Study in Juvenile Rats	Yes	
(b) (4) 04-06	Monkey (rhesus)	In Vivo Drug Interaction Studies of Artesunate Using Rhesus Monkeys and LC/MS Analysis	No	
Primary Pharmacodynamics				
WRAIR 8F	Rat (SD)	The Efficacy of Intravenous AL and AS in <i>P. berghei</i> -infected Sprague-Dawley Rats	No	

(b) (4)

WRAIR 8C	Rat (SD)	Pharmacokinetics and Pharmacodynamics Evaluations of Artelinate and Artesunate in Infected Rats with <i>P. berghei</i>	No	(b) (4)
WRAIR 8G	Rat (SD)	Therapeutic Indices and Definitive Efficacy of Intravenous Artelinate (AL) and Artesunate (AS) in <i>P. berghei</i> -infected Rats	No	
(b) (4) 8O	Monkey (rhesus)	Pharmacokinetics/Pharmacodynamics of Candidate IV Artemisinins	No	
(b) (4) 8P	Monkey (rhesus)	Dose Ranging in Severe Malaria in Rhesus	No	
(b) (4) 8Q	Monkey (rhesus)	Efficacy and Pharmacokinetic Profiles of Intravenous Artemisinin Candidates (Artelinic Acid (AL), Artesunate (AS) or AL-like compounds) using a <i>Plasmodium coatneyi</i> -Rhesus monkey model of severe malaria	No	
Pharmacokinetics				
1466-102 (PK Report)	Dog (Beagle)	14-Day Toxicity Study of Artesunate in Beagle Dogs: Pharmacokinetic Evaluation	Yes	(b) (4)
AQ007 04 WR	Rat (SD)	Tissue Distribution, mass balance, elimination, pharmacokinetics, protein binding, metabolic profile, and whole-body autoradiography of [¹⁴ C]-artesunate following single intravenous injection in rats.	No	
ET-05003 (Dog)	Dog (Beagle)	Pharmacokinetics and bioequivalence of new artesunate intravenous formulation compared to Chinese drug product in beagle dogs	No	
ET-05003 (Rat)	Rat	Pharmacokinetics and bioequivalence of new artesunate intravenous formulation compared to Chinese drug product in rats	No	
(b) (4) 132N-0501	Rat (SD)	Quantitative tissue distribution of drug-related material using whole body autoradiography (WBA) following a single intravenous dose of [¹⁴ C] artesunate (5 mg/kg) to 11-day pregnant Sprague Dawley rats	No	
0365-2009	In Vitro	Dihydroartemisinin (DHA): Evaluation of the <i>In Vitro</i> Cross Species Intrinsic Clearance and Metabolism with Mouse, Rat, Dog and Human Hepatocytes	No	Not specified
8740133	In Vitro, TC7 subclone of Caco-2 cell line	Study of Compounds PQP and DHA	No	Not specified
Melendez Report	In Vitro	<i>In Vitro</i> Biotransformation and Drug Interaction Studies of Artesunate and its Active Metabolite Dihydroartemisinin	No	(b) (4)

		Using Plasma and Liver Preparations by LCMS and Fluorometric Analyses		(b) (4)
AQ0004-04-WR	Rat	The Assessment of the Reproductive Toxicity of Injective AS in Rats	No	(b) (4)
(b) (4) 04-06	Rhesus Monkey	Final Report of <i>In Vivo</i> Drug Interactions Studies of Artesunate in Rhesus Monkeys	No	Not Specified
Reproductive and Developmental Toxicity				
CD2006/01142/00	Rat (SD)	SB-714702 (Artesunate): Single Dose Intravenous Embryo-Fetal Development Study in Rats	No	(b) (4)
CD2006/01570/00	Rat (SD)	SB-714702 (Artesunate): Single Dose Intravenous/Oral Toxicokinetic Study in Female Rats	No	(b) (4)
CD2005/01316/00	Rat (SD)	SB-714702: Study to Investigate the Acute Hematological Response of the Pregnant Rat to a Single Dose of Artesunate on Day 11 Post-Coitum	No	(b) (4)
CD2005/00559/00	Rat (SD)	Artesunate: Oral (Gavage) Study of Embryo-Foetal Development in the Rat	Yes	Not Specified
CD2006/01429/00	Monkey (cynomolgus)	SB-714702 (Artesunate): Effects of Reticulocytes Following Three-Day Oral Repeated Doses to Female Cynomolgus Monkeys	Yes	(b) (4)
CD2007/00245/00	Monkey (cynomolgus)	A Study for the Investigation of Developmental Toxicity of SB-714702 (Artesunate), in Two Different Treatment Periods, in Cynomolgus Monkey	Yes	(b) (4)
CD2006/01492/00	Monkey (cynomolgus)	Histopathological Investigation of the Embryonic Effect of SB-714702 (Artesunate) in Cynomolgus Monkey	No	(b) (4)

2.3 Planned Non-Clinical Studies for IV artesunate*:

(b) (4)

3. NONCLINICAL SUMMARY OF STUDIES REVIEWED AND PROPOSED TOXICOLOGY PROGRAM

Safety pharmacology studies in telemetered beagle dogs administered IV artesunate at doses up to 50 mg/kg revealed no cardiovascular or respiratory changes. An additional non-GLP study in monkey given IV artesunate did not include any neurobehavioral assessments of the animals. The Sponsor has proposed to fill this gap in safety pharmacology by including a functional observational battery (FOB) in their 4-week repeated dose toxicology study in rat.

Changes in hematological parameters were observed following repeat dosing with IV artesunate. A significant reduction in RBC, HCT, and RETIC at ≥ 20 mg/kg IV artesunate was observed in female dogs after 14 days. Male dogs also showed evidence of reduced RBC, HCT, and RETIC following administration of IV artesunate, but these findings only reached statistical significance for RETIC at doses ≥ 35 mg/kg. All noted hematological changes had resolved by end of the 14-day recovery period. A second GLP repeat-dose study was not submitted. In a non-GLP 7-day repeat-dose toxicity of IV artesunate in rhesus monkey (up to 128 mg/kg), 3 of the 4 animals treated with 128 mg/kg artesunate died prematurely. Surviving animals displayed leukopenia, neutropenia, red blood cell loss, and reticulocytopenia that resolved by the end of a 14-day recovery period. Also observed were increases in AP and BUN in animals administered ≥ 16 mg/kg artesunate, however histologic correlates cannot be determined as histopathology findings were not provided in the study report. The Sponsor has proposed a 4-week repeat dose study with 28-day recovery period in rat and dog using the semi-synthetic API to support marketing of IV artesunate.

Artesunate was negative in the Ames, mammalian chromosome aberration assay (CHO), and in vivo micronucleus assay (up to 500 mg/kg, highest concentration tested). No carcinogenicity studies were conducted with artesunate and none are proposed. Given the short duration of treatment and negative findings from the genotoxic test battery, carcinogenicity studies are not indicated to support submission of an NDA for IV artesunate.

Over 15 reproductive and developmental toxicity studies with artesunate have been submitted to the IND, including GLP-compliant embryofetal toxicity studies of artesunate conducted in rat, rabbit and monkey and embryofetal development studies with an oral combination of chlorproguanil/dapsone/artesunate (CDA) in rat and rabbit. Nine of the submitted reproductive and

developmental toxicity studies were previously reviewed³. In summary, artesunate was embryo- or fetal-toxic in all animal models tested and induced an increased number of embryo or fetal deaths and abortions compared with control animals. Main developmental findings were cardiovascular malformations (ventricular septal defects) and skeletal anomalies (ribs, long bones, and/or scapulae). These development effects were dependent on both the dose level and the day or days of administration during gestation, although NOAELs were not determined for either the fetal rat or rabbit. Artesunate also was toxic to several hematologic cell types in cynomolgus monkeys and to monkey nucleated fetal red blood cells *in vitro*. Maternal findings of weight loss and/or reduced appetite were not significant, apart from weight loss and lack of appetite following treatment with the combination product Chlorproguanil/Dapsone/Artesunate. The Sponsor noted that no ICH compliant, GLP studies assess the effects on artesunate on male or female fertility or pre-and post-natal development of offspring exposed to drug via the dams. The submitted nonclinical studies in rat did not examine the effects of artesunate on male reproductive organs, and the 14-day repeat dose study in dog showed no effect on male reproductive organs, though the pathology report notes animals were not yet sexually mature at time of necropsy (animals were 5.5 to 6.5 months old) The Sponsor provided a summary of the published literature indicating adverse effects of artesunate and other artemisinin derived compounds on spermatogenesis in male rats and mice (e.g., abnormal sperm, decreased sperm count).

The Sponsor submitted a juvenile rat study performed with the combination product Chlorproguanil/Dapsone/Artesunate (CDA) administered during day 4 to 31 postpartum. Levels of artesunate in the CDA combination product were 0.235 and 0.706 mg/kg. Study results indicate no drug-related effects on clinical signs, body weights, hematology, or macroscopic necropsy observation at any tested dose (no histopathology data was available). The Sponsor states that based on other study findings, the toxicity of the CDA combination product is largely attributable to the artesunate content, and that any toxicity in the juvenile rat with CDA is representative of the toxicity with artesunate alone. Of note, previous dose-range finding studies conducted with CDA combination at higher doses (2.35 – 23.5 mg/kg artesunate) was associated with high rate of male and female pup mortality, resulting in early termination of treatment. The cause of pup death was not determined.

Reviewer comment: The submitted and proposed safety pharmacology studies and 4-week repeat-dose toxicity study with 28-day recovery period are in accordance with M3(R2) and ICHS7A and would support marketing authorization. (b) (4)

(b) (4) It is recommended that at a minimum, an audited draft of the study report from the repeat-dose toxicity studies be available at time of NDA submission. Having the findings from this study available during NDA review will allow for assessment of any unexpected toxicities with the semi-synthetic API formulation, whether any additional nonclinical studies will be needed to bridge any reproductive safety and toxicity concerns not addressed in the clinical data and evaluate any need for juvenile toxicity studies. It is not clear from the meeting package when the Sponsor will have the semi-synthetic API available to begin conducting the 4-week repeat dose studies. Per communications with the CMC review team, it appears that the semi-synthetic API described as being manufactured in (b) (4) might not have been released yet. The project manager, Gregory DiBernardo, spoke with the Sponsor on 2/6/2019 regarding the status of their pre-NDA CMC only meeting request for this IND and was told the Sponsor plans to submit their NDA during the first week of May 2019, at which time the Sponsor

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anticipates having 3 months stability data and side-by-side comparative analysis between the (b) (4) and semi-synthetic API. It is recommended that the Sponsor begin conducting the 4-week repeat dose studies as soon as possible. Provided they have sufficient quantities of the semi-synthetic API on hand, a draft report of the study findings could conceivably be made available to the Agency within the Sponsor's projected time frame of NDA submission.

4. LIST OF SPONSOR-PROVIDED QUESTIONS FOR DISCUSSION

Question 1:

A comprehensive pharmacology, pharmacokinetic, and toxicology program has been undertaken to characterize the non-clinical efficacy, disposition, metabolism, and safety of AS. A tabular summary of nonclinical studies submitted to IND 64,769 are provided in Table 4 to Table 6 (Section 2.12.1.1). Does the Agency agree that the existing toxicology studies and the newly proposed studies (Table 7) including 4-week repeat dose rat and dog toxicity studies as per ICH M3(R2) and transporter protein studies as per FDA 2017 with the semi-synthetic drug substance is sufficient to support the NDA filing?

Pharmacology/Toxicology Response: Your proposed pharmacology, pharmacokinetic, and toxicology program using the nonclinical studies submitted to IND 64769 and newly proposed 4-week repeat-dose toxicity study in rat (including neurobehavioral assessment) and dog with 28-day recovery, along with *in vitro* transporter protein studies appears reasonable. Adequacy of the newly proposed nonclinical studies to support the NDA filing will be assessed upon review of the study reports at the time of submission.

Question 2:

Amivas is acutely aware that an urgent unmet medical need exists with IV quinidine being withdrawn from the US market. Can Amivas file the current non-clinical (and clinical) data package for review in January 2019 with the clock starting for NDA review as soon as the CMC package is received by the Agency in April 2019?

Pharmacology/Toxicology Response: PharmTox is fine with a rolling review.

Question 3:

If the answers to Q1 and Q2 are yes, can Amivas file the 4-week dog and 4-week rat studies as a post-marketing commitment? Our view is that the proposed in-vitro transporter studies (Section 2.12.1.4) are useful scientifically to support the clinical data we have with severe renally and hepatically impaired ICU patients, but the 4 week repeated dose toxicity studies to comply with ICH M3(R2) will not substantially add to the existing evidence provided in the 3-day rat, 7-day monkey and 14-day dog studies (Table 6) and could be deferred.

Pharmacology/Toxicology:

- Per ICH Guideline M3(R2), repeated-dose toxicity studies of one-month duration are recommended to support marketing of an indicated treatment up to 2 weeks (b) (4). (b) (4) We recommend commencing the 4-week repeat dose studies in rat and dog as soon as the semi-synthetic API becomes available. We are willing to review audited draft reports of the study findings at time of NDA submission, with the final full study reports to be submitted within 60 days of the NDA submission. Any changes in the final study report from the audited draft should be clearly identified.

- Plan to provide a rationale for your maximum intended dosing levels and/or projected multiples of human exposure in the repeated-dose toxicity studies. Ideally, the studies should be conducted in a clinically relevant manner (i.e., IV) and the highest dose in a general toxicology study should demonstrate the maximum tolerated dose (MTD), achieve large exposure multiples above the clinical dose, or reach the maximum feasible dose (MFD).

Question 4:

The existing toxicology package with additional support from published studies have been used to identify risks in terms of potential effects on fertility and post-natal development. There is a potential for effect on male fertility, and at clinical doses, there is an apparent lack of effect on postnatal development and maturation ([Section 2.12.1.3](#)). The sponsor proposes no further reproductive nonclinical studies are needed. Does the Agency agree?

Pharmacology/Toxicology Response: The adequacy existing reproductive nonclinical studies using the (b) (4) API to support your NDA submission using the semi-synthetic API will be evaluated upon review of your 4-week repeat dose with 4-week recovery period toxicology studies in rat and dog. You may wish to consider including a sperm analysis (sperm counts, sperm motility, sperm morphology) and evaluation of male and female reproductive organs as part of the planned repeat dose toxicity studies. Typically, pre-and-post natal development studies are recommended to support marketing authorization in accordance with ICH Guidelines M3(R2) and S5A. Additional pre- and post-natal developmental toxicity assessments may be needed to address any unexpected toxicities encountered in your study findings and to bridge any reproductive safety and toxicity concerns not addressed in the clinical data.

Question 5:

Studies in adolescent to adult aged animals across species are provided by the sponsor's toxicology program, including short single/3-day studies in Sprague Dawley (SD) rats, 14-day studies in beagle dogs, and 7-day dosing in rhesus monkeys. A juvenile rat study with a combination product including AS did not show adverse effects in the developing target organs affected by AS in the older animals studied under Good Laboratory Practice (GLP) ([Section 2.12.1.2](#)). Amivas does not consider additional juvenile animal studies warranted or likely to add to the current clinical understanding of AS toxicity in pediatric patients. The sponsor does not intend to perform additional non-clinical juvenile studies to support short-term dosing in infants/children with severe malaria as part of the NDA submission. Does the Agency agree?

Pharmacology/Toxicology Response:

The conduct of any juvenile animal toxicity studies should be considered when previous animal data and human safety data, including effects from other drugs of the pharmacological class, are judged to be insufficient to support pediatric studies. Based on the studies completed to date, additional non-clinical juvenile studies to support short-term dosing in infants/children with severe malaria as part of the NDA submission may not be needed. However, a determination of the safety and efficacy of IV artesunate for treatment of malaria in children will be determined upon review of the completed 1-month GLP repeat-dose toxicology studies with a 28-day recovery period in rat (with functional observational battery) and dog using the semisynthetic drug formulation, and clinical findings. Adequacy of these findings, and identification of the need for juvenile toxicity studies, will be assessed upon review of the final study reports and clinical trial data at the time of submission.

5. REVIEW OF SUBMITTED STUDIES

5.1 Safety Pharmacology

5.1.1 Central nervous system

Study Title: The Neuropathology of Intravenous Artesunate Administration in the Rhesus monkey (Macaca mulatta) ((b) (4) Pathology Report [PN02-01](#))

Methods: Rhesus monkeys aged 5-25 years (n=2 of each sex/group) were administered once per day with IV artelinate lysine salt (AL; 5.9, 11.8, and 47.2 mg/kg), IV artesunate sodium salt (AS; 4, 8, 16, 32, and 128 mg/kg) for seven days. Lysine and sodium bicarbonate served as vehicle controls for AL and AS, respectively (n=2, one male and one female). An additional group treated intramuscularly once per day with 16 mg/kg arteether (AE) served as positive control for neurotoxicity assessment. Animals were euthanized 14 days after the last treatment and perfused with Bouin's fluid.

Results: Animals treated with AE exhibited chronic histological findings consistent with widespread neuronal necrosis in brain stem nuclei. Two females treated with 128 mg/kg AS died of GI/hematologic toxicity prior to perfusion fixation and were noted unsuitable for study given the widespread cellular damage which prevented detailed neuroanatomic analysis. One male in the 128 mg/kg AS group was prematurely euthanized on day 10 due to impending death with multi-organ failure, including neurologic effects. No evidence of neuronal necrosis was found in animals administered AL or in the surviving animals administered AS.

Reviewer comment: This study was not conducted in compliance with GLP regulations. Study report was submitted under Safety Pharmacology, but focused on histologic lesions in the medulla oblongata, pons, cerebellum, midbrain, auditory and visual systems in the brainstem; neurobehavioral assessments of the animals were not provided.

5.1.2 Cardiovascular and Respiratory system

Study Title: Cardiovascular Safety Pharmacology Study of Artesunate in Beagle Dogs ([Study Report 1466-103](#)).

Methods: Telemetered beagle dogs aged 6-7 months (2 each per sex) were administered sterile 0.3M phosphate buffer (vehicle control) via intravenous injection on study day 1. Beginning study day 2, animals received a dose of 10, 20, and 50 mg/kg via a single slow intravenous dose and 50 mg/kg administered via a single fast intravenous dose with at least a one-week wash out period between each escalating dose level.

Results: Artesunate (single slow IV dose of 0, 10, 20 and 50 mg/kg or single fast IV dose of 50 mg/kg) did not have any effects on mortality, clinical observations, body weights, ECGs, QT intervals, heart rate, blood pressure, or respiratory rate.

Reviewer comment: The study was conducted in compliance with GLP regulations. Artesunate acid (Batch No. 14290-16A; (b) (4), ≥ 99.5% purity) was dissolved in phosphate buffer prior to use.

5.2 Repeat-Dose Toxicity

5.2.1 Study Title: 14-Day Toxicity Study of Artesunate in Beagle Dogs

Study no.: [1466-102](#)
 Study report location: EDR
 Conducting laboratory and location:  (b) (4)
 Duration: 14 days
 Duration Units: days
 GLP compliance: Y
 Drug, lot #, and % purity: Artesunate
 Lot No. 090403-A/Batch No. 14290-15A
 Purity ≥ 99.5%

Methods

Doses: 10, 20, 35, or 50 mg/kg
 Frequency of dosing: Daily
 Number/Sex/Group: 4 dogs/sex/group: 2 dogs/sex/group necropsied at study day 15, 2 dogs/sex/group necropsied on study day 29 following 2-week recovery period
 Dose volume: 1 mL/kg
 Formulation/Vehicle: 0.3 M phosphate buffer
 Route of administration: INTRAVENOUS BOLUS
 Species: DOG
 Strain: BEAGLE
 Age / Sexual Maturity: 5-6 months
 Comment on Study Design and Conduct: This was a well-conducted/designed study
 Dosing Solution Analysis: Dosing solutions were 99.2-110% (10 mg/kg), 96.4-110% (20 mg/kg), 91.3-104% (30 mg/kg), and 97.0-101% (50 mg/kg) nominal concentrations

Observations and Results

Mortality (≥ 2 times/day)

All animals survived until scheduled termination.

Clinical Signs (prior to dose on SD1, weekly thereafter, and at termination)

No changes in clinical observations (evaluation of skin and fur characteristics, eye and mucus membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor and behavior patterns) were observed between artesunate-treated animals and vehicle control. Some animals exhibited erythema, diarrhea, emesis, and mucoid feces. These responses were not considered to be test-article related as they not show a dose-dependent pattern, and 2 of the 4 control-treated animals also experienced erythema.

Body Weights (prior to dose on SD1, weekly thereafter, and at termination)

No effects on body weights were observed in animals administered artesunate.

Feed Consumption (daily)

No significant differences in mean food consumption between artesunate and control-treated animals were observed.

ECG (pre-initial dose, ~2h post-dose on SD1, SD2, SD8 and SD15)

No atrioventricular conduction defects or premature atrial or ventricular complexes were observed in artesunate or control-treated animals.

Hematology (prior to initial dosing, SD15 and prior to necropsy)

No changes in coagulation parameters were observed between artesunate and control-treated animals at the end of treatment (SD15) or at the end of the recovery period (SD29).

Clinical Chemistry (prior to initial dosing, SD15 and prior to necropsy)

The following parameters were affected by artesunate administration⁴:

			SD15				SD29			
Artesunate mg/kg/day			WBC K/uL	RBC M/uL	HCT %	RETIC M/uL	WBC K/uL	RBC M/uL	HCT %	RETIC
0	M	Mean	12.30	5.69	38.3	0.02	13.10	5.35	36.3	0.09
		SD	1.08	0.24	1.48	0.01	0.82	0.06	0.21	0.01
	F	Mean	10.43	6.64	44.9	0.03	11.14	6.55	45.7	0.10
		SD	2.00	0.34	2.36	0.02	2.38	0.08	0.14	0.01
10	M	Mean	10.41	5.38	34.9	0.02	9.30	5.84	37.5	0.09
		SD	4.14	0.72	5.83	0.02	1.16	0.57	0.92	0.01
	F	Mean	21.73*	6.12	40.9	0.02	9.18	6.08	41.7	0.08
		SD	2.71	0.40	2.55	0.01	0.08	0.16	2.12	0.04
20	M	Mean	15.69	4.81	30.3	0.00	8.39	5.83	39.0	0.09
		SD	5.32	0.85	4.39	0.01	0.24	0.22	0.71	0.01
	F	Mean	14.31	5.45*	36.5*	0.00*	10.27	5.75	39.6	0.09
		SD	2.35	0.64	3.77	0.00	1.23	0.52	4.74	0.03
35	M	Mean	17.14	5.08	32.5	0.00*	12.74	5.85	38.0	0.11
		SD	3.77	0.77	5.83	0.00	2.43	0.13	0.49	0.01
	F	Mean	13.60	5.22*	33.9*	0.00*	9.52	5.36	35.1	0.10
		SD	1.17	0.78	5.42	0.00	0.28	0.01	0.28	0.00
50	M	Mean	15.41	4.84	31.3	0.00*	8.10	5.76	39.5	0.11
		SD	4.99	0.49	3.61	0.00	1.87	0.20	1.77	0.011
	F	Mean	11.64	4.75*	31.5*	0.00*	8.67	5.73	39.2	0.09
		SD	3.87	0.79	5.33	0.00	1.48	0.18	3.46	0.00

*, statistically significant difference from control group (p<0.05). WBC=white blood cell, RBC=red blood cell, HCT=hematocrit, RETIC=reticulocyte

In summary, females treated with 10 mg/kg/day artesunate showed higher WBC, with corresponding increase in absolute lymphocyte and monocyte counts. Females treated with 20 mg/kg/day artesunate

⁴ Adapted from pages 191-210 of Study Report 1466-102

also showed significantly elevated absolute lymphocyte counts compared with control treated animals. A significant reduction in RBC, HCT, and RETIC was observed in females treated with ≥ 20 mg/kg/day artesunate. A significant reduction in HGB was also observed in females treated with artesunate at doses ≥ 35 mg/kg/day. Males treated with artesunate also showed had lower levels of RBC, HGB, HCT and RETIC, with the reduction in RETIC reaching statistical significance at ≥ 35 mg/kg/day. By the end of recovery period (SD29), no statistically significant differences in clinical chemistry parameters were observed in artesunate-treated animals compared with the control group.

Variation in sodium levels in the animals prior to treatment were observed: females assigned to 20 and 35 mg/kg/day and males assigned to 35 mg/kg/day had higher sodium compared with animals assigned to the control group. On SD15, significantly higher amounts of sodium and chloride were observed in females and males, respectively, treated with 50 mg/kg/day artesunate. A decrease in aspartate aminotransferase was observed in males treated with 10 mg/kg/day. These changes were attributed to animal variation and are not considered to be test article-related.

Gross Pathology

Gross pathology included examination of external surface of the body, injection site, all orifices, cranial, thoracic and abdominal cavities and their contents. Discoloration of the injection site and enlarged testes were observed in both control and artesunate-treated animals and discoloration of the cecum was observed in 1 of 4 males dosed with 20 mg/kg/day artesunate. These findings were considered to be unrelated to test article administration.

Organ Weights

There were no statistically significant differences in mean absolute organ weights, mean organ-to-body weight ratios, or mean organ-to-brain weight ratios between artesunate and control-treated animals.

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Follicular lymphoid hyperplasia of the mandibular lymph nodes and minimal to mild hemorrhage/inflammatory responses were observed across control and treated animals. No test-article related effects on histopathology were observed at end of treatment (SD15) or recovery (SD29).

Toxicokinetics

Blood was collected (jugular vein) for toxicokinetic evaluation at the following timepoints:

SD 1	Prior to initial dosing, 5 (± 1) min, 10(± 1) min, 30(± 5) min, 2h (± 5 min), and 6h (± 5 min) post initial dose
SD 2	24h (± 30 min) post initial dose
SD 8	Pre-repeat dose
SD 15	Prior to interim termination for main group and SD 15 for recovery group
SD 29	Prior to termination

Results: Main PK parameters of artesunate and its active metabolite (DHA) on day 1 post-14 day IV administration⁵.

⁵ Table 1, pages 13 and 14 of Study Report [1466-102 \(PK Report\)](#)

Parameters	10 mg/kg Male Dogs (n = 4)	10 mg/kg Female Dogs (n = 4)	20 mg/kg Male Dogs (n = 4)	20 mg/kg Female Dogs (n = 4)
Artesunate (AS)				
C _{max} (ng/ml)	9795 ± 1653	4902 ± 2305	24696 ± 7861	15844 ± 4442
T _{max} (min)	0	0	0	0
AUC _{inf} (ng·h/ml)	1150 ± 559	471 ± 152	2254 ± 494	2282 ± 786
t _{1/2} absorption (h)	0.06 ± 0.02	0.07 ± 0.03	0.06 ± 0.01	0.09 ± 0.03
t _{1/2} elimination (h)	0.39 ± 0.10	0.41 ± 0.15	0.32 ± 0.04	0.50 ± 0.10
V _{ss} (ml/kg)	1928 ± 1075	3246 ± 1892	1073 ± 234	2131 ± 1054
V _c (V1, ml/kg)	1045 ± 192	2348 ± 909	868 ± 246	1333 ± 341
V _t (V2, ml/kg)	884 ± 145	898 ± 1065	909 ± 826	797 ± 774
CL (ml/min/kg)	169.8 ± 72.4	387.3 ± 141.0	205 ± 92	159 ± 52
MRT	0.22 ± 0.15	0.26 ± 0.14	0.12 ± 0.03	0.24 ± 0.14
Dihydroartemisinin (DHA)				
C _{max} (ng/ml)	3690 ± 802	2139 ± 291	3611 ± 1097	3910 ± 801
T _{max} (min)	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.05 ± 0.02
AUC _{inf} (ng·h/ml)	1181 ± 116	739 ± 128	2126 ± 491	2129 ± 335
t _{1/2} absorption (h)	0.06 ± 0.01	0.06 ± 0.01	0.04 ± 0.01	0.06 ± 0.05
t _{1/2} elimination (h)	0.39 ± 0.10	0.36 ± 0.02	0.48 ± 0.23	0.43 ± 0.15
MAT	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
MRT	0.44 ± 0.11	0.49 ± 0.03	0.68 ± 0.36	0.59 ± 0.15
AUC Ratio of DHA/AS	1.10 ± 0.22	1.68 ± 0.45	1.01 ± 0.29	1.03 ± 0.31
Total AUC (AS + DHA)	2309 ± 579	1213 ± 252	4402 ± 529	4416 ± 900

Parameters	35 mg/kg Male Dogs (n = 4)	35 mg/kg Female Dogs (n = 4)	50 mg/kg Male Dogs (n = 4)	50 mg/kg Female Dogs (n = 4)
Artesunate (AS)				
C _{max} (ng/ml)	67098 ± 26735	52789 ± 20690	112065 ± 31689	130249 ± 33744
T _{max} (min)	0	0	0	0
AUC _{inf} (ng·h/ml)	7351 ± 3043	5903 ± 2211	12733 ± 4834	12755 ± 3652
t _{1/2} absorption (h)	0.08 ± 0.02	0.07 ± 0.01	0.07 ± 0.02	0.06 ± 0.01
t _{1/2} elimination (h)	0.40 ± 0.05	0.41 ± 0.05	0.38 ± 0.06	0.34 ± 0.02
V _{ss} (ml/kg)	604 ± 215	1136 ± 997	651 ± 179	485 ± 150
V _c (V1, ml/kg)	582 ± 210	773 ± 392	475 ± 137	409 ± 132
V _t (V2, ml/kg)	22 ± 9	363 ± 609	176 ± 71	76 ± 56
CL (ml/min/kg)	87.7 ± 27.3	107.5 ± 31.4	71.8 ± 23.1	70.2 ± 23.4
MRT	0.12 ± 0.02	0.17 ± 0.12	0.15 ± 0.01	0.12 ± 0.02
Dihydroartemisinin (DHA)				
C _{max} (ng/ml)	5172 ± 1914	4604 ± 956	7179 ± 818	7971 ± 2583
T _{max} (min)	0.14 ± 0.11	0.09 ± 0.01	0.11 ± 0.01	0.07 ± 0.03
AUC _{inf} (ng·h/ml)	3010 ± 945	2553 ± 748	4518 ± 694	4137 ± 1236
t _{1/2} absorption (h)	0.13 ± 0.01	0.15 ± 0.01	0.16 ± 0.03	0.15 ± 0.01
t _{1/2} elimination (h)	0.40 ± 0.08	0.47 ± 0.10	0.52 ± 0.13	0.50 ± 0.14
MAT	0.09 ± 0.05	0.08 ± 0.01	0.09 ± 0.02	0.07 ± 0.01
MRT	0.60 ± 0.18	0.57 ± 0.10	0.65 ± 0.14	0.56 ± 0.10
AUC Ratio of DHA/AS	0.45 ± 0.16	0.46 ± 0.09	0.39 ± 0.08	0.34 ± 0.07
Total AUC (AS + DHA)	10377 ± 3749	8405 ± 2848	17335 ± 6009	16787 ± 4550

Reviewer comment: Ophthalmoscopic evaluations and urinalysis were not performed in this study. Toxicokinetic results were summarized in a separate study report, No. [1446-102 \(PK Report\)](#).

5.2.2 Study Title: Toxicity of Subacute, 7-Day Dosing of Candidate Intravenous Artemisinins in Rhesus Monkey

Study no.: [AQ0008-02-AF](#)
 Study report location: FDR
 Conducting laboratory and location:  (b) (4)
 Duration: Seven days
 Duration Units: days
 GLP compliance: N
 Drug, lot #, and % purity: Artesunate, Batch No. 010601

Methods

Doses:	0, 4, 8, 16, 32, and 128 mg/kg
Frequency of dosing:	Daily
Number/Sex/Group:	2 each male and female/artesunate group 1 each male and female/control group
Dose volume:	Not specified
Formulation/Vehicle:	Sodium bicarbonate (5%)
Route of administration:	INTRAVENOUS
Species:	MONKEY
Strain:	RHESUS
Age / Sexual Maturity:	4 to 25 years
Dosing Solution Analysis:	Not provided

Observations and Result

Mortality (3x/day)

All animals in the control, 4, 8, 16, and 32 mg/kg/day artesunate groups survived until the end of the study. Three of the 4 animals administered 128 mg/kg artesunate died prematurely. Two of the animals died overnight (on days 6 and 10) and the third showed signs of transient blindness, ptosis, and nystagmus prior to elective euthanasia with Bouin's fixation on day 10. Prostration was not noted initially but was noted as a pre-terminal event in all of the animals that died.

Clinical Signs (3x/day during dosing period, 2x/day during recovery period)

Depressed motor activity was observed in animals administered ≥ 8 mg/kg artesunate, with severity increasing with increasing dose. The slight depressed motor activity observed in one each of the animals treated with 8 and 16 mg/kg was transient in nature (resolving within 1h), whereas 3 of the 4 animals treated with 32 mg/kg displayed mild-to-moderate decreased motor activity that was more persistent (up to 6h). All animals in the high dose group (128 mg/kg) showed persistent depressed motor activity, and 3 of the 4 animals displayed slight truncal ataxia. Hypersalivation, prostration and ataxia were observed in animals administered ≥ 32 mg/kg artesunate. Soft stool and diarrhea were observed in animals administered ≥ 16 mg/kg. The GI distress resolved in the 16 and 32 mg/kg groups, whereas diarrhea in the 128 mg/kg treated animals was more severe and progressed to bloody mucosa.

Body Weights (Days 1, 4, and 7 prior to dosing and Days 10, 12, 15, and 22 during recovery period)

A weight loss of 5-10% occurred in animals treated with artesunate, with the exception of one animal administered 16 mg/kg that had an 11.3% weight loss.

Feed Consumption

One female in the 8 mg/kg group displayed intermittent poor appetited during the study period. The appetite of the animals in the higher dose groups were more severely affected: 2 of 4 animals in the 16 and 32 mg/kg group displaying poor appetite, requiring NG feeds up to SD22; poor appetite with severe anorexia requiring IV fluids occurred in all the animals administered 128 mg/kg artesunate.

Hematology and Clinical Chemistry (1 day prior to administration and Days 4, 8, 15, and at terminal sacrifice on day 22)

Significant leukopenia, neutropenia, red blood cell loss, and reticulocytopenia was observed in all animals administered artesunate, with thrombocytopenia observed at doses ≥ 32 mg/kg. The leukocyte,

neutrophil, RBC, and reticulocyte numbers rebounded during the recovery period (between days 15-22). One animal in the control group had a 5% loss of RBC at time of euthanasia, which was attributed to phlebotomy. No other hematologic changes were observed in the control group.

A significant total drop in protein (10-15%) was observed in animals treated with ≥ 8 mg/kg artesunate. An increase in alkaline phosphate was observed starting at 16 mg/kg, and animals in the higher dose groups (32 and 128 mg/kg) also had an increase in BUN, this increase was statistically significant in the 128 mg/kg group.

Urinalysis (baseline and days 8, 15, and 22 after start of administration)

Microscopic hemoglobinuria was noted in at least 50% of animals treated with 8, 16, and 32 mg/kg artesunate on more than one occasion. The microscopic hemoglobinuria resolved by SD22. Urine from animals in the high dose group (128 mg/kg) was red, contained bilirubin or urobilirubin. The three animals that died prematurely had ketonuria, hemoglobinuria, pyuria and proteinuria.

Gross Pathology and Histopathology

Necropsies in the control, 4, 8, 16 and 32 mg/kg were unrevealing for brain or other organ abnormalities. Animals who died prematurely had hypocellular marrow consistent with the peripheral neutropenia and reticulocytopenia. Hepatic vacuolation was observed the 3 animals that died but was not observed in the surviving animal. Moderate-to-severe congestion and hemorrhage of the stomach and intestines were also observed, and 2 of the 3 animals that died had moderate adrenocortical congestion and hemorrhage. The males in the 128 mg/kg group had noted spermatid degeneration. The surviving animal showed neuronal chromatolysis in the cranial nerve nucleus.

Reviewer comment: The protocol states that the adequate battery of tissues was collected and examined, with complete gross and microscopic analysis to be carried out by a board-certified veterinary pathologist at (b) (4) but detailed histopathological findings were not provided in this study report. The pathology report containing histopathologic findings in the brain of animals treated with artesunate was submitted as (b) (4) Pathology Report [PN02-01](#) under Safety Pharmacology and is discussed in detail under 5.1.1 above. The protocol notes that lumbar puncture for CSF was performed on day 7 (following treatment), and that blood was taken for at baseline and at days 1, 2, 4, and 9 after dosing for assessment of antimalarial activity. Activity levels in plasma were reported to be $\leq$ LOQ and no antimalarial activity was detected in the CSF.

5.3 Genetic Toxicology

5.3.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study Title: Evaluation of Artesunic Acid in the *Salmonella-Escherichia coli*/microsome plate incorporation assay

Study no.: [G305-08](#)

Study report location: FDR

Conducting laboratory and location:  (b) (4)

GLP compliance: Yes

Drug, lot #, and % purity: Artesunic acid, Lot 14462-16, purity 100%

Methods

Strains: *S. typhimurium* TA1535, TA1537, TA98, TA100 and *E. coli* strain WP2 (*uvrA*)

Concentrations in definitive study: 156.3, 312.5, 325, 1250, 2500, and 5000 mcg/plate

Basis of concentration selection: The highest dose (5000 mcg/plate) is the recommended maximum testing concentration. Dose selection was made to assess potential dose-response relationship and at least one cytotoxic dose.

Negative control: DMSO

Positive control: (-S9) TA1535, TA100: Sodium azide (5 mcg/50 mcl)
 TA1537: 9-Aminoacridine hydrochloride hydrate (50 mcg/50 mcl)
 TA98: 2-Nitroflourene (5 mcg/50 mcl)
 WP2 (*uvrA*): 4-Nitroquinoline N-oxide (2.5 mcg/50 mcl)
 (+S9) 2-Anthramine:
 2 mcg/mcl (TA98, TA100)
 4 mcg/50 mcl (TA1535, TA1537)
 20 mcg/50 mcl (WP2 (*uvrA*))

Formulation/Vehicle: Artesunic Acid/DMSO

Incubation & sampling time: Plates were incubated at 37°C for 48h

Comment on Study Validity: Experiment was considered valid: strains were analyzed to confirm genetic markers, solvent controls were within historical limits for spontaneous revertants, positive controls elicited positive response and there were at least three nontoxic dose levels.

Results

Artesunic Acid was negative in the bacterial reverse mutation assay under test conditions used in this study. Cytotoxicity, apparent by either decreased revertant counts, thinning of the background lawn, or the complete absence of revertant counts, was seen only with the *Salmonella* tester strains at 5000 mcg/plate in the presence and absence of metabolic activation. No statistically significant increases in the number of revertant colonies were seen for *S. typhimurium* TA1535, TA1537, TA98 or *E. coli* strain WP2(*uvrA*) in the presence or absence of metabolic activation (10% S9). A slight (<2-fold) but dose-dependent significant increase in revertant colonies was observed in TA100 in the presence of 10% S9. However, the increase in revertant colonies was not considered to be toxicologically significant as the number of colonies fell within the historical spontaneous range for the strain.

5.3.2 *In Vitro* Assays in Mammalian Cells

Study Title: Evaluation of Artesunic Acid in the Chinese Hamster Ovary (CHO) Chromosome Aberration Assay

Study no.:	G306-08
Study report location:	EDR
Conducting laboratory and location:	 (b) (4)
GLP compliance:	Yes
Drug, lot #, and % purity:	Artesunic Acid (Lot 14462-16, purity 100%) dissolved in DMSO

Methods

Cell line:	Chinese hamster ovary (CHO) cells
Concentrations in definitive study:	20, 40 and 80 mcg/ml (+/- S9) for 3h 0.625, 1.25 and 2.5 mcg/ml (-S9) for 21h
Basis of concentration selection:	The top recommended dose of 5000 mcg/ml was used for cytotoxicity experiment. Doses for chromosome aberration experiments were determined based on mitotic index and culture confluency results.
Negative control:	DMSO
Positive control:	(-S9) methyl methanesulfonate (25 and 50 mcg/ml) (+S9) cyclophosphamide (12.5 and 20 mcg/ml)
Formulation/Vehicle:	Artesunic acid (Lot No. 14462-16, Purity 100%) was dissolved in DMSO (1% v/v)
Incubation & sampling time:	Test and control articles were added to cells and incubated at 37°C for 3 or 21h.
Comment on Study Validity:	Study was considered valid: frequency of cells with structural chromosome aberrations in positive control was significantly elevated above that of solvent control; the highest concentration evaluated was within a factor of 2 of the level resulting in

significant effect on mitotic index or confluency (>50% reduction).

Results

Artesunic acid was negative in the *in vitro* mammalian chromosome aberration assay under test conditions used in this study. No concentration-related increases or statistically significant increases in structural chromosomal aberrations were observed at any dose level in the presence or absence of metabolic activation.

5.3.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study Title: Evaluation of Artesunic Acid in the Mouse Bone Marrow Micronucleus Assay

Study no: [G307-08](#)
 Study report location: EDR
 Conducting laboratory and location:  (b) (4)
 GLP compliance: Yes
 Drug, lot #, and % purity: Artesunic Acid, Lot No. 14462-16, 99.6% purity

Methods

Doses in definitive study: 125, 250 and 500 mg/kg
 Frequency of dosing: Single administration
 Route of administration: Oral (gavage)
 Dose volume: 10 ml/kg
 Formulation/Vehicle: Artesunic Acid suspended in 0.5% methyl cellulose
 Species/Strain: Mice/Swiss-Webster
 Number/Sex/Group: 10 mice/sex/dose group, 5 mice/sex/dose group were sacrificed at each of 2 time points (24 and 48h after treatment).
 Satellite groups: N/A
 Basis of dose selection: Doses selected based on range-finding experiment results which showed 57% suppression of polychromatic erythrocytes/red blood cell ratio in males, and 83% suppression of PCE/RBC in females at 1000 mg/kg.
 Negative control: 0.5% methyl cellulose
 Positive control: Urethane (300 mg/kg)
 Comment on Study Validity: Study is considered valid given that 1) frequency of micronucleated cells in vehicle control group was within  (b) (4) historical mean $\pm$ 2 SD, 2) administration of urethane positive control resulted in statistically significant elevation in incidence of micronucleated cells, and 3) there were at least 6 surviving animals with PCE/RBC ratio in bone marrow $\geq$ 0.1 in two or more dose groups.

Results

Artesunic Acid was negative in the mammalian bone marrow erythrocyte micronucleus assay under test conditions. No statistically significant increases in the frequency of micronucleated polychromatic erythrocytes (PCE) was observed at any dose level in either male or female mice at 24- or 48-hr post treatment.

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