FDA Briefing Document
for the
Anti-Infective Drug Products Advisory Committee

Artesunate Rectal Capsules
World Health Organization
NDA 21-242

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Food and Drug Administration
Center for Drug Evaluation and Research
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Table of Contents

Background

(The reader is referred to the briefing package provided by WHO. This document provides a detailed perspective on the problem of malaria in endemic regions of the world and the potential role of rectal artesunate in malaria control).

Rationale for developing rectal artesunate:
In malaria endemic regions of the world, *Plasmodium falciparum* is responsible for hundreds of thousands of deaths, especially among children. One of the most important reasons for the very high malaria mortality is the delay in receiving effective medication. Unfortunately, many patients with acute malaria are unable to tolerate oral therapy. Consequently, parenteral treatment is usually necessary, and this may only be available at clinics or hospitals. These facilities are often difficult to reach because of long distances and poor infrastructure in terms of roads and transport.

The aim of the applicant was to develop a product that could be administered on an emergency basis to patients too ill to take anything orally.

The goal was to reduce mortality by early intervention in the field, providing some cover over the first 24 hours to allow patients to reach definitive treatment.

An artemisinin derivative for rectal administration was selected as a suitable candidate.

Efficacy
Artemisinin derivatives have been used for many years as antimalarial agents in Asia. Published literature indicates that artemisinins given orally or parenterally are effective in causing rapid decreases in the parasite count in smears of peripheral blood. They are used extensively in areas of the world where *P. falciparum* shows resistance to quinine, and they are reportedly effective in quinine-resistant infections.

Artesunate is rapidly biotransformed to the principle metabolite: dihydroartemisinin (DHA). Both the parent drug and metabolite are active against the malaria parasite. The plasma half-life of these products (both parent-drug and metabolite) is relatively short compared with other antimalarial agents and frequent dosing has been used. Following a single rectal dose, artesunate (AS) and dihydroartemisinin (DHA) are detectable in plasma beginning about 30 minutes after dosing in most adult and pediatric patients. Concentrations of artesunate and DHA become undetectable in plasma after about 5 hours and 12 hours, respectively.

Despite their efficacy in reducing parasite counts, these products have been associated with high rates of recrudescence. To address this, they are often used in combination with other antimalarial agents, and are given repeatedly over several days.

Safety

Literature reports indicate that certain artemisinins have been associated with neuropathological changes in a variety of animal species (mice, rats, dogs, and rhesus monkeys). These changes include chromatolysis and gliosis in the brainstem nuclei associated with auditory, vestibular and muscle-control functions. They have been most strongly associated with the oil-based artemisinin derivatives, artemether and arteether.

Neurological abnormalities have not been reported in human subjects. However, the assessment of neurological changes in patients who present with acute malaria is difficult, since the disease itself may result in baseline neurological abnormalities and rarely in neurological sequelae. Further, the assessment of neurological symptoms in children may be difficult.

Alternative treatments

Drugs approved by the US Food and Drug Administration (FDA) for the treatment of malaria cause by *P. falciparum* include chloroquine (oral, IV or IM), fansidar (oral), mefloquine (oral), quinidine (IV or oral), quinine (IV IM or oral), malarone (oral) and halofantrine (oral). Since all these alternatives require oral or parenteral administration, none is suitable for patients in the bush who are unable to take orally, and who have no immediate access to trained medical personnel.

***Proposed indication*:**

"Artesunate Rectal Capsules is indicated for the initial management of acute malaria in patients who cannot take medication by mouth and for whom parenteral treatment is not available.

Warning: Artesunate rectal capsules are not intended for the initial treatment of malaria if it is possible to provide immedidate treatment of the patient with orally or parenterally (intravenous or intramuscular) administered antimalarials.

Precautions: Artesunate rectal capsules have not been evaluated as sole therapy for malaria; consequently all patient who are initially treated with artesunate rectal capsules should be promptly referred and evaluated at the nearest health care facility able to provide a full curative course of treatment for malaria.

General: Artesunate rectal capsules are indicated only for use in initial management of acute non per-os P. falciparum malaria and MUST be supplemented and/or followed by effective oral or parenteral drug therapy for malaria as soon as possible."

Issues related to the study of rectal Artesunate in patients with acute malaria

Challenge to develop appropriate studies:
Since rectal artesunate is to be used as emergency treatment for malaria when no alternatives are available, the practical question is whether the emergency use of a single dose of rectal artesunate is more effective than no treatment in reducing malaria morbidity and mortality.

Given the high mortality from untreated malaria and the dangers of delaying effective therapy, treatment cannot be ethically withheld for the first 24 hours. For these reasons, the product has been studied in trials employing active comparators. In these studies, provisions are made for the "rescue" of patients showing an unsatisfactory clinical or parasitological response. While these studies do not directly address the advantages of rectal artesunate over no treatment, they give a relative idea of efficacy versus the "standard of care".

Approach:
Three pivotal studies used a single dose of rectal artesunate alone for the first 24 hours and compared this with standard therapy for 24 hours. This was followed by a definitive or "consolidation" regimen in both treatment arms.

Shortcomings in reflecting projected use:
- The diagnosis of malaria was confirmed in study participants. This would not be the case in the bush where malaria might be confused with meningitis, typhoid and a host of other febrile illnesses.
- The studies provided ancillary treatment: fluid, transfusions, antipyretics, and anticonvulsants. These would not be available in the bush.
- Drug administration was well supervised. In the bush, supervision would depend upon family members.
- Patients in the field might get a false sense of security after rectal treatment and fail to present for definitive therapy.

Design problems:
Study populations were drawn from communities where malaria was prevalent and were likely to show various degrees of malarial immunity.

Difficulties were apparent in attempts to standardize the severity of disease.
- In some studies the inability to take orally was taken as a sign of severity.
- In others impairment of consciousness was taken as a sign of severity.
- Baseline parasitemia varied from study to study.
- Where malaria was more prevalent, subjects were likely to have some immunity and to tolerate higher parasitemias with milder clinical manifestations.

Mindful of the serious consequences if treatment failed, the applicant allowed subjects in the rectal artesunate arm of the studies to be rescued with standard therapy if the parasite count failed to reach 60% of baseline by 12 hours. (Rescue of subjects in the comparator arm was permitted in 2 of the 3 pivotal studies.)

Since the efficacy of AS is being tested for 24 hours, the evaluation of patients rescued before this time is difficult.

- Evaluating them as successes effectively ignores the contribution of the rescue therapy.
- Evaluating them as failures discounts the possibility that in some patients, the parasitological response may be a little slower
- Excluding them from the analysis relegates the efficacy analysis to "successes amongst the successes"

The problem of endpoints:
Is parasitemia an appropriate surrogate for clinical response?

Responses in peripheral parasitemia are an useful indication of the activity of antimalarial drugs for several reasons:

- Since untreated patients generally demonstrate rising levels of parasitemia with time, decreases in parasitemia are a persuasive reflection of drug activity.
- Parasite counts provide a quantifiable measurement of drug activity allowing a measurable comparison of different treatment regimens.
- The parasite count is a simple and robust test that is easy to perform and easy to interpret.

However, responses in peripheral parasitemia may not always reflect the clinical outcome and the following pitfalls should be borne in mind:

- Sequestration of parasites in the spleen and other organs has been described and may account for the morbidity and mortality in some patients with low peripheral parasite counts. Thus resolution of peripheral parasitemia may not always reflect the clinical outcome.
- Potentially fatal complications of malaria, such as renal failure or pulmonary edema, may supervene even when the peripheral parasite count is very low.
- The degree of parasitemia correlates to some extent with severity of disease. In non-immune patients, parasitemias affecting more than 5% of peripheral erythrocytes are taken to indicate severe malaria according to WHO (Trans Roy Soc Trop Med Hyg 1990, 84 Supple 2 1-65). However the parasite count may not always reflect malaria severity, as semi-immune patients may tolerate this degree of parasitemia with minimal symptoms.

Issues in selecting appropriate endpoints to reflect drug efficacy

- The rate of parasite clearance indicates one aspect of drug performance that may correlate with efficacy. However, to date, no clinical advantage (in terms of morbidity or mortality) has been identified using artemisinins (which clear parasites very rapidly) compared to quinine (which clears parasites more slowly).

- The median rate of parasite clearance in a group of patients may also fail to reflect the percentage of outlying individuals who respond poorly.
- Clinical failures may occur despite disappearance of parasites from peripheral blood.
- An evaluation several weeks after presentation generally should reflect the initial outcome of treatment but is complicated by losses to follow up, new infections and recrudescent infection. In the clinical trials reflected in this NDA, the long-term outcome depends substantially on the efficacy of the drugs used following the initial dose of rectal artesunate.
- The relative morbidity and mortality of initial attacks of malaria versus recrudescent attacks after therapy in patients without access to medical care is not clear.

Ultimately, the evaluation of efficacy depends on the aggregate of clinical and parasitological responses evaluated over several weeks.

Safety:
Safety may be difficult to assess in sick patients with malaria. Neurological symptoms are difficult to determine in young children. Malaria itself may produce neurological symptoms. Occasionally these may be severe and long lasting.

Pharmacotoxicology

Based on the known toxicity profile of the artemisinin compounds, the applicant agreed to conduct a nonclinical study to determine the neurotoxic potential of artesunate. The study was to determine the dose, duration of dosing, and possibly the total systemic exposure to artesunate needed to produce unacceptable neurotoxicity in an appropriate animal model. A subacute repeat-dose oral and intravenous toxicity study in rats was conducted to address these issues. In the original study design, rat dosing groups (16 rats/group) received oral doses of 33, 75, or 150 mg/kg/day or intravenous doses of 10 mg/kg (3 times daily), or 30 mg/kg (3 times daily), for seven days followed by either necropsy (8 rats/group) or a 14-day recovery (8 rats/group). During the study, mortality, weight loss, hypoactivity, decreased food consumption and perianal/urogenital staining were observed in the 75 and 150 mg/kg/day oral dose groups and 90 mg/kg/day intravenous group as early as Day 3.

Of particular concern was the failure to conduct either behavioral assessments or specific neuropathologic examinations needed to determine the parameters of interest (dose and duration of dosing needed to produce neurotoxicity). Especially troubling was the fact that rats died on study (1/16 and 2/16 receiving 75 and 150 mg/kg, respectively, after as little as three days of dosing), or were sacrificed early (90 mg/kg/day intravenous group, day 3) because of poor health. The cause of death was not determined, leaving open the possibility that the animals died due to neurotoxic effects (or possibly other toxicities). To further compound the problem, treatment was discontinued in the surviving rats in these dose groups, limiting actual exposure to artesunate. Necropsy results on these animals cannot be taken to indicate lack of neurotoxicity since these animals received much less drug than originally planned and were sacrificed as long as 10 days after the final oral doses. (In fact, in rats surviving until scheduled sacrifice, hepatic toxicity was noted in all treatment groups, consisting of centrilobular hepatocyte hypertrophy, sinusoidal inflammation cells, single cell necrosis, diffuse hepatocyte vacuolation and periportal vacuolation. Hepatic effects were not observed in recovery group animals.)

Histopathologic sectioning of CNS tissues obtained from animals sacrificed at the end of the study was of uncertain precision. The study report does not specify that brain loci known to be targets of artemisinin-induced neuronal damage were examined; it is thus possible that lesions were present but not observed. Studies have been published in which the neurotoxicity of artemisinins, including artesunate, was assessed in rodents using appropriate methods (neurohistology and functional assays); these published reports could have been used by the applicant to design an appropriate study to address the issues of concern (Kamchonwongpaisan et al, 1996; Genovese et al, 1998). These studies, as well as other published papers, can be taken to indicate that artesunate is *less* neurotoxic than other artemisinin derivatives (using both neuropathology as well as functional assays such as drug effect on balance and gait). However, in one published study (Nontprasert et al, 2000), artesunate was given to mice by daily oral gavage for 28 consecutive days followed by a 120-day recovery period; neurotoxic effects were observed at exposures similar to clinical doses

A further problem in assessing the findings in the subacute oral toxicity study in rats is that an inappropriate method of comparing rodent to human doses was used. Dose comparisons should be made based on relative body surface areas, not body weights. Using comparative body surface areas for calculations of dose equivalents, the safety margin between the proposed human dose and doses that produced adverse effects in rats (poor condition and death) is not of the same magnitude as reported by the applicant (see the table below). Based on this method, the following human equivalent doses were used in the subacute oral toxicity study in rats:

Table 1: Dose comparison based on body surface area differences

Rat dose (mg/kg), 7-day study, oral dose	Human equivalent dose (mg/kg)	Multiple of human dose	Toxicity	Mortality
33	5.5	0.55	Liver (?)	
75	12.5	1.25	Liver (?)	1/16
150	25	2.5	Liver (?)	2/16

Determination of systemic drug exposures (C_{max}, AUC) at which toxicities were observed in animals is the most useful method for establishing safe doses in humans. However, the ability to compare systemic artesunate exposure in nonclinical studies conducted by the applicant is limited by the quality of the available data. In a single dose toxicity study in rats, C_{max} of 442.3 ng/ml at 150 mg/kg , p.o., were calculated with AUC of 520 ng•hr/ml. If linear pharmacokinetics are assumed an extrapolation for the 75 mg/kg p.o. dose in rats provides a C_{max} of about 220 ng/ml and AUC of 250 ng•hr/ml. These exposures, at which deaths occurred, compare to clinical exposures following a rectal dose of 10 mg/kg producing a C_{max} of about 1100 ng/ml and AUC of 4300 ng•hr/ml. Unfortunately, pharmacokinetic data supplied by the applicant are not reliable based on careful review. The limited cumulative dose exposure from the 7-day rat study in which the dosing duration was shortened does not permit a valid toxicity comparison relevant to human exposure. Therefore, safe doses can only be determined by calculation using relative body surface areas.

Additional non-GLP studies performed in the People's Republic of China during the 1980's were included in support of the application. The studies are of uncertain value since these could not be audited and the translations from Chinese are of poor quality. However, adverse effects, such as hepatotoxicity and hematotoxicity, were reported in dogs and rats given both acute and repeated oral and intravenous doses of artesunate.

In summary, the apparent major safety concern for artesunate, neurotoxicity, remains inadequately characterized. The nonclinical study designed to assess neurotoxic potential was not performed in a way that provides useful data. Published studies provide some relevant information for determining safety. While artesunate appears to have less neurotoxic potential compared to other artemisinins, mice exhibited neurotoxicity after prolonged exposure. The issue of an unacceptably toxic dose has not been addressed and

neurotoxicity must be considered a risk following multiple-dose exposure in humans. The possibility that artesunate also produces hepatotoxicity has not been adequately addressed (this was an unanticipated finding.)

Microbiology

Biology of the parasite:

Over 100 species of *Plasmodium* have been shown to cause malaria in a wide variety of vertebrate hosts. The four species known to infect man are *P. falciparum*, *P. vivax*, *P. malaria*, and *P. ovale*. The life cycle of the malaria parasite (*Plasmodium*) is divided between 2 hosts: mosquitoes (as a vector) and vertebrates (Figure 1). Infection in humans is initiated by the bite of infected mosquitoes and sporozoites are inoculated into the blood stream. Within 2 hours, the sporozoites migrate to the liver and penetrate the hepatocytes. After 1 to 2 weeks, the sporozoites undergo nuclear division to produce thousands of merozoites that are released by the rupture of the host cell. This phase of the life cycle of *Plasmodium* is called **exoerythrocytic** or **liver schizogonic phase**. The merozoites invade the erythrocytes and progress through a series of developmental forms into mature erythrocytic schizonts. Each merozoite matures into a schizont, undergoes asexual multiplication, and forms 24 merozoites. The red blood cells rupture and the merozoites invade other erythrocytes. This part of the life cycle is called the **erythrocytic phase** and takes about 24 to 72 hours. Fever is associated with the rupture of infected red blood cells. Severe or fatal complications can occur at any time and are related to blockage of blood vessels in the internal organs such as brain, spleen, bone marrow, etc. Some merozoites mature into male and female gametocytes within the erythrocytes. The maturation of these gametes occurs once the infected blood is ingested by the mosquitoes. The gametes undergo fertilization within the vector and form a zygote. This is followed by the elongation of a zygote to form an ookinete in about 12 to 48 hours. The ookinetes invade the stomach of the mosquito and develop into oocysts. The oocyst enlarges forming over 10,000 sporozoites (in about a week after the formation of the oocyst). The sporozoites released by the rupture of the oocyst migrate to the salivary glands of the mosquitoes and are inoculated into humans the next time the mosquito feeds (i.e., during a blood meal).

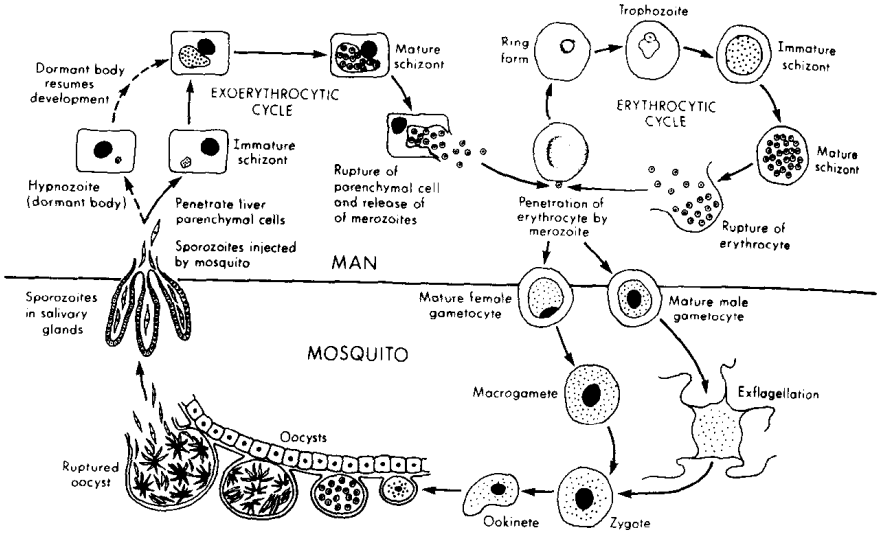


FIGURE 1 Life cycle of the malaria parasite. (Reproduced with permission from D. J. Krogstad and M. A. Pfaller, Prophylaxis and treatment of malaria, *Curr. Clin. Top. Infect. Dis.* 3:56–73, 1983.)

In patients with *P. falciparum* infection, recurrence is generally thought to be due to **recrudescence,** which is caused by residual erythrocytic forms; whereas in *P. vivax* and *P. ovale* **relapse** of an infection is considered to be due to dormant parasites (**hypnozoites**) in the liver which undergo development and release merozoites into circulation.

Mechanism of action:
Artesunate is a water soluble derivative of artemisinin, an antimalarial compound isolated from the Chinese herb Qinghao (*Artemisia annua*). Artesunate is rapidly metabolized to dihydroartemisinin (DHA) in the body. Chemically, artesunate, and its active metabolite, DHA, are sesquiterpene lactones with a trioxane ring containing a peroxide bridge. The peroxide bridge appears to be essential for the antimalarial activity of artesunate. Structure-activity relationship studies show that the deoxy derivative of DHA (that lack the peroxide bridge) was 277-fold less active than DHA. The activity of deoxyartesunate was not measured. Deoxy derivatives of other artemisinin analogs were 10- to 1000-fold less active compared to the parent compounds. Artesunate increases superoxide anion production and lipid peroxidation in falciparum-infected erythrocytes *in vitro*. However, artesunate does not suppress the activity of antioxidant enzymes (superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase) in infected or uninfected erythrocytes.

Erythrocytes infected with the ring or trophozoite forms *in vitro* accumulate 100- and 180- fold higher concentrations of DHA (12 nM i.e., 3.40 ng/ml), respectively, compared to uninfected erythrocytes. These experiments were performed in a medium containing 10% human serum. The relevance of these findings to the uptake *in vivo* is unclear. The precise mechanism by which artesunate exhibits antiplasmodial activity is not understood.

Activity *in vitro*:
Artesunate and DHA exhibit activity against the erythrocytic stages of *P. falciparum*, which includes laboratory strains and several clinical isolates from different geographical areas. The antiparasitic activity was measured by incorporation of ^{3}H-hypoxanthine or by microscopic observations. A majority of these studies were done by incubating the asynchronous parasites with the drug for 24 to 48 hours. The results expressed as 50% inhibitory concentration (IC_{50}) show the artesunate and DHA IC_{50} values against the laboratory strains or clinical isolates to be < 7 and < 24 ng/ml, respectively (Tables 2 and 3).

Table 2: *In vitro* activity against the laboratory strains of *P. falciparum*.

Strain	Resistance	Artesunate IC_{50} (ng/ml)	DHA IC_{50} (ng/ml)
FCC-1/HN	Chloroquine sensitive	1.04	ND
D10	Chloroquine-sensitive	2.14 (0.12 - 2.46)*	(0.28 - 1.15)*
3D7	Chloroquine-sensitive	(0.12 - 0.20)*	(0.28 - 1.15)*
RSA11	Chloroquine-resistant	1.32 (1.01 - 1.63)*	ND
FCD-3	Chloroquine-resistant	ND	2.70
K1	Chloroquine-resistant	5.60 (4.70 - 6.90)* 4.60 (4.00 - 5.50)*	3.60 (2.30 - 3.80)* 3.10 (2.90 - 3.60)*
W2/ Indochina	Chloroquine-resistant and Mefloquine-sensitive	0.64	0.68
D6/Sierra Leone	Chloroquine-sensitive and Mefloquine-resistant	0.84	0.70

*Represents the results of multiple testing. ND = not done.

Table 3: *In vitro* activity against *P. falciparum* isolates from different geographical regions.

Region	N	Resistance	Artesunate Mean (range) IC_{50} ng/ml	DHA Mean (range) IC_{50} ng/ml	Reference
Philippines	24	Chloroquine and amodiaquine	0.35 (0.12 - 3.15)	ND	Bustos *et al.,* 1994
Vietnam	10	Chloroquine, amodiaquine, mefloquine, and sulfadoxine/pyrimethamine	1.24 (1.13 - 1.36)	0.92 (0.84 - 1.01)	Wongsrichanalani *et al.,* 1997
Vietnam and China	4	Chloroquine	NA (0.08 - 0.24)	NA (0.23 - 0.85)	Skinner *et al.,* 1997
China-Mynamar	51	Chloroquine	2.30 (NA)	3.70 - 3.98 (NA)[a]	Yang *et al.,* 1997
China-LaoPDR	42	Chloroquine	1.92 - 2.69 (NA)	1.14 - 1.42 (NA)	Yang *et al.,* 1997
Thailand	86	Chloroquine and mefloquine	NA (0.36 - 1.65)	NA (0.34 - 0.98)[b]	Wongsrichanalani *et al.,* 1999
Africa	79	Chloroquine	ND	NA (0.25 - 0.43)	Le Bras *et al.,* 1998
Thai-Burmese Border	178 72	Chloroquine, amodiaquine, sulfadoxine-pyrimethamine and mefloquine	1.62 (0.19 - 13.30)[c,e] 2.35 (0.34 - 23.40)[d,g]	1.22 (0.15 - 6.60)[c,f] 1.43 (0.24 - 8.10)[d,h]	Brockman *et al.,* 2000
		IC_{50} Range (ng/ml)	0.08 - 23.4 (n = 467)	0.15 - 8.10 (n = 322)	

NA = Not available;
ND = not done;
[a]14 isolates were tested;
[b] 45 isolates were tested;
[c] primary infection isolates
[d] recrudescent isolates [after treatment with mefloquine (25 mg/kg) + artesunate (12 mg/kg x 3 days)]
[e] 95% CI (1.4 - 1.8 ng/ml)
[f] 84 isolates, 95% CI (1.9 - 2.9 ng/ml)

[g] 95% CI (1.0 - 1.5 ng/ml)
[h] 44 isolates, 95% CI (1.1 - 1.8 ng/ml)

Studies using synchronous culture show a rapid inhibition of the ring, trophozoites, and schizont forms of *P. falciparum* within 2 hours of exposure to DHA at a concentration equivalent to the IC_{90} value (Figure 2).

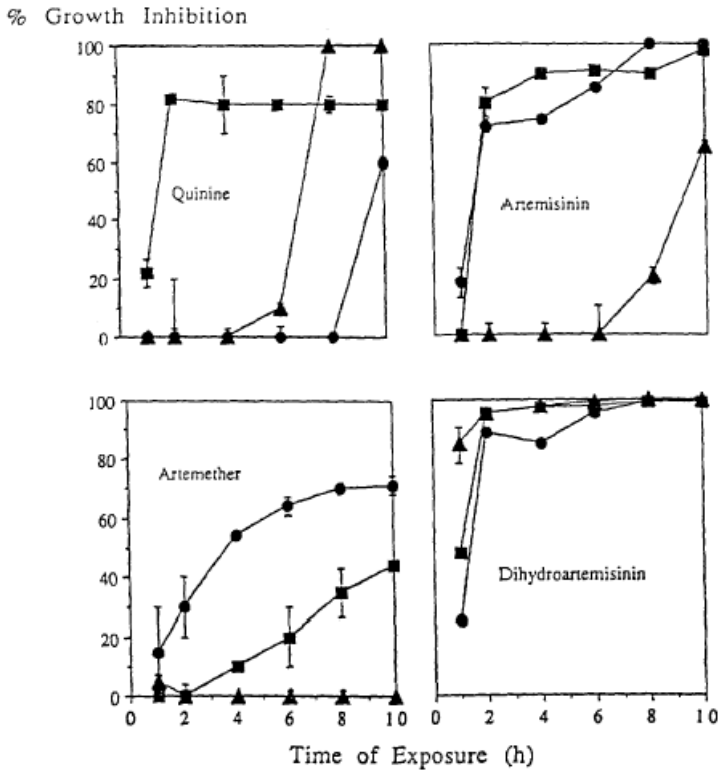


Figure 2: Stage-specific activities of quinine, artemisinin, artemether, and DHA on culture-adapted parasites of *Plasmodium falciparum*. Synchronized cultures of rings (0 h; •), trophozoites (20-24 h; ▲) and schizonts (36 h; ■) were exposed to the IC_{90} concentration (not specified) of each drug up to 10 hours. The data are presented as percentage growth inhibition ± S.D. with time of drug exposure for the 3 asexual stages compared with untreated controls. The IC_{50} values were as shown below:

Drug	IC_{50} values (M)
DHA	$0.8 - 3.0 \times 10^{-9}$ (0.28 - 0.85 ng/ml)
Artemisinin	$2.0 - 30.0 \times 10^{-9}$
Artemether	$7.0 - 30.0 \times 10^{-12}$
Quinine	$3.0 - 10.0 \times 10^{-8}$

(Skinner *et al.,* 1996)

Studies using predominantly gametocytes in culture showed that the DHA IC_{50} value to be 6.6 ng/ml. Time to gametocyte kill was not measured.

The activity of artesunate may vary with the source of erythrocytes. For example, artesunate was 3- to 10-fold less active against the K1 strain of *P. falciparum* using genetically variant erythrocytes from patients with α-thalassemia (hemoglobin Hb[H] and Constant spring, HbCS) than normal RBCs. The activity of chloroquine was similarly altered whereas that of pyrimethamine was not altered by the source of the erythrocytes.

No studies were done to demonstrate the *in vitro* activity of artesunate or DHA against the hepatic stage of the *Plasmodium* species.

Activity *in vivo*:
The activity of artesunate and DHA was measured against the erythrocytic forms of *P. falciparum, P. berghei, P. knowlesi*, and *P. coatneyi* in mice or monkeys. In a preliminary study conducted in immunocompromised mice infected with the T24 strain (chloroquine and quinine resistant) of *P. falciparum*, a complete clearance of parasite from the blood was observed on Day 2 of treatment with DHA (50 mg/kg for 2 days) by the oral route. Microscopic observations at 24 hours showed predominantly pycnotic forms (76%), some altered trophozoites (10%), few trophozoites (1%), and schizonts (3%). At 48 hours, only pycnotic forms were observed. Chloroquine and quinine were not effective in clearing the parasitemia in mice infected with the T24 strain. Treatment with chloroquine induced minimal alterations in morphology (11 - 16% pycnotic forms). However, against a chloroquine sensitive strain (NF54) morphological changes after treatment with chloroquine were similar to that of DHA against the chloroquine-resistant strain. The activity of artesunate was not measured.

In mice infected with *P. berghei (*173N strain) and treated with intravenous artesunate or chloroquine, a 50% and 90% reduction in parasitemia was observed by 18 to 24 hours, and 24 to 30 hours, respectively. Recrudescence was observed on Day 28 in mice treated with artesunate (110 mg/kg) for 5 days by the intravenous route. However, chloroquine (14.9 mg/kg for 5 days) completely cured the mice on Day 28. In another study, mice infected with another strain of *P. berghei* (ANKA strain) showed complete cure on Day 60 after intramuscular treatment with ≥ 56 mg/kg artesunate. Complete clearance of the parasitemia was observed within 2 days of treatment. Similar observations were made with DHA. The variation in the activity of artesunate in the different studies may be due to the different strains of *P. berghei* used for infection, the route of drug administration or severity of infection.

In monkeys infected with *P. knowlesi*, the intravenous administration of artesunate (10 mg/kg for 7 days) reduced the parasitemia by 90% at 13 hours. The parasite clearance time (PCT) was 42 hours and all 3 monkeys remained negative for 28 days of observation. However, at the lower dose (3.16 mg/kg), although the PCT was 40 hours, 1/3 monkeys showed recrudescence on Day 15. At a higher dose (31.6 mg/kg), the mean parasite clearance time was 56 hours and no recrudescence was observed. Quinine at 10 mg/kg dose was effective in reducing the parasitemia by 50% in 3.3 hours, however, a 90% reduction of parasitemia was not attained. At a higher dose (31.6 mg/kg), the mean time to parasite clearance was 104 hours and recrudescence was observed 2 to 10 days after parasite clearance.

In another study, normal and splenectomized monkeys infected with *P. coatneyi* were treated with artesunate. The clearance of parasitemia was slower in splenectomized animals compared to normal controls. The reduction in parasitemia at 24 hours in the splenectomized and non-splenectomized animals was 86% and 99%, respectively. Infected erythrocytes showed ultrastructural changes such as enlargement of food vacuoles and ribosomal clumping 4 hours after administration of artesunate.

The activity of artesunate against hepatic stages of the *Plasmodium* species was not examined.

Resistance:
A potential for resistance development by *Plasmodium* species to artesunate was examined *in vitro* and *in vivo*. In a preliminary study, the erythrocytic forms of the FCR3 strain of *P. falciparum* were passaged by exposure to increasing concentrations of artesunate (starting with 4 ng/ml) for 48 hours each, followed by growth in drug-free medium for 18 days (equivalent to 9 life cycles). The artesunate IC_{50} value increased by 3-fold after 6 to 7 passages over a period of 130 days. Such an effect was reversible when the parasites were grown in drug free medium for 5 weeks.

Studies *in vivo* show that serial passage of the N strain of *P. berghei* in mice treated with escalating doses of artesunate for 4 days each, increased the 90% effective dose (ED_{90}) by about 29-fold after 21 passages (85 days). The effect was reversible after discontinuation of therapy. However, the clinical significance of these observations is unknown.

In patients with severe or moderate malaria, treatment with a single dose of rectal artesunate decreased the parasite count by > 90% in 24 hours. In patients treated with multiple doses of artesunate (oral, rectal or IV) the parasite clearance time was 16 to 56 hours. However, recrudescence was reported on Days 10 to 28 after discontinuation of therapy. In the absence of genotyping and phenotyping it is unclear whether the recrudescence is due to resistance. Also, the presence of dormant parasites cannot be ruled out.

Cross-resistance:
P. falciparum strains with chloroquine resistance (IC_{50} > 100 nM) and mefloquine resistance (> 20 nM) were susceptible to artesunate *in vitro*. However, a positive correlation was observed between the *in vitro* activity of artesunate and those of halofantrine, mefloquine, quinine, or atovaquone, suggesting cross-resistance. The clinical significance of the *in vitro* observations is unclear.

Although no correlation was observed between the *in vitro* activity of artesunate and chloroquine against *P. falciparum*, a 16 - 17 fold higher concentration of artesunate was required to suppress 90% of asexual forms of *P. berghei* chloroquine-resistant RC strain than the chloroquine-sensitive N strain in mice, suggesting likelihood of cross-resistance. However, in another study DHA was effective in clearing parasitemia in mice infected with a chloroquine-resistant T24 strain of *P. falciparum.*

In clinical studies, artesunate was effective in the treatment of *P. falciparum* infections from regions known to be resistant to chloroquine.

Drug combination:
The *in vitro* activity of artesunate in combination with other drugs against *P. falciparum* was measured. A combination of artesunate with mefloquine or quinine was synergistic

against *P. falciparum*. However, a combination of artesunate with pyrimethamine was antagonistic. The combination of artesunate with chloroquine varied from additive to anatgonistic against the different *P. falciparum* strains tested. The oxidant drugs (miconazole and doxorubicin) also show a synergistic effect in combination with artesunate. The *in vivo* activity of artesunate in combination with other antimalarials was not examined.

The activity of artemisinin in combination with other antimalarial drugs against *P. falciparum* was measured *in vitro* and against *P. berghei in vivo*. A combination of artemisinin with mefloquine was synergistic whereas that with pyrimethamine was antagonistic *in vitro* and *in vivo*. A combination of artemisinin with other antimalarials (sulfadiazine, sulfadoxine, sulfadoxine-pyrimethamine, cycloguanil, and dapsone) was also shown to be antagonistic *in vivo*.

Clinical Issues

Efficacy
An overview of the studies supporting clinical efficacy is provided below.

Table 4: Synopsis of data supporting the efficacy of rectal artesunate in NDA 21-242

Study number	Clinical Site Location	# Allocated to Rectal Artesunate	# Allocated to Comparator	Type of Patient
003	Thailand	24	24	Adults
004	Ghana	24	12	Children
005	Thailand	46	17	Children
006	Malawi	87	22	Children
007	South Africa	27	8	Adults
014	Thailand	69	0	Adults
009	(literature)			
010	(literature)			
011	(literature)			

Studies in patients with clinical malaria
Six studies were performed on patients with clinical malaria.

Three comparative studies (005, 006, and 007) employed the projected dose of rectal artesunate given alone for the first 24 hours of therapy in the experimental arm. These were regarded as pivotal studies.

A bioequivalence study (Thailand 014) comparing 3 formulations of rectal artesunate in the projected dosing regimen provided further non-comparative data on the safety and efficacy of rectal artesunate, when used as proposed.

Two further studies (Thailand 003, Ghana 004) employed regimens for rectal artesunate that differed either in dosage or duration from the projected use and provided supportive information.

Data from three older published studies (009, 010, and 011) were reanalyzed by the applicant and presented as supportive data. The dose of rectal artesunate in each of these studies was twice the proposed clinical dose. As such, these studies did not support the efficacy of the proposed dose, although the information provided some evidence of safety.

Pivotal studies
FDA considered three studies, performed in Thailand, Malawi and South Africa, as pivotal in supporting this indication. In each of these studies the experimental arm involved treatment with rectal artesunate 10mg/kg as a single dose for the first 24 hours,

followed by one of several follow-up or "consolidation" antimalarial regimens. All three studies were randomized, comparative and unblinded.

The studies differed in several ways from the envisaged use of the product. In all three studies, patients were admitted to hospital. The diagnosis of malaria was confirmed on smear, and the progress of parasitemia was monitored. Permitted ancillary treatment including transfusions, parenteral fluids, anticonvulsants, antipyretics, and glucose given at the clinicians' discretion.

The study designs are compared below

Table 5: Drug regimens used in pivotal studies

	Location (Study No)		
	Thailand (005)	**Malawi (006)**	**South Africa (007)**
Experimental arm	Rectal AS (10mg/kg) single dose	Rectal AS (10mg/kg) single dose	Rectal AS (10mg/kg) single dose
Comparator	Oral AS (4 mg/kg) single dose	Quinine 10mg/kg IM or IV at 0, 4 hrs then 12 hourly till oral treatment tolerated	Quinine 10mg/kg IM or IV at 0, 4 and 12hrs
Consolidation regimen	**24 hrs**: Oral AS (2mg/kg) **48 hrs**: Oral AS (2mg/kg) + MQ (15mg/kg) **72 hrs**: Oral AS (1mg/kg) + MQ (10mg/kg) **Daily for 6 more days**: Oral AS (1mg/kg)	**24 hrs**: Oral SP* (25mg/kg SDX) (or "standard dose" parenteral quinine if unable to take orally) SP given at 24 hours in experimental arm SP given after a minimum of 2 doses of quinine in comparator arm	**24 hrs**: Oral SP (3 tablets) single dose (or IM quinine 10mg/kg if unable to take orally)

*SP - sulfadoxine-pyrimethamine

[The raw pharmacokinetic data from Study 07 have not been submitted and therefore a detailed review has not been performed to verify the pharmacokinetic parameters obtained in this study.]

Comment on treatment regimens:
The final treatment outcome, several weeks after completing therapy, is a reflection of the efficacy of the total treatment regimen. Recurrences of parasitemia shortly after finishing treatment may reflect a treatment regimen that is inadequate in terms of dose or duration, or infection with a parasite that has some degree of resistance to the drug.

SP is a long-acting agent and the recommended regimen for the treatment of acute malaria in adults is a single dose of 3 tablets. (In children, an appropriate single dose is calculated on a mg/kg basis.) Malaria that is resistant to SP is common, and may vary by geographic region. Thus, studies performed in different locations may show different long-term outcomes when SP is used. In parts of Africa, resistance to SP may be present in up to 60% of cases.

Mefloquine is a long-acting agent and the recommended regimen for the treatment of acute malaria in adults is a single dose of 1250mg. Mefloquine resistance appears less prevalent than SP resistance. Mefloquine has been used in combinations with artemisinin derivatives for the clinical management of malaria in parts of Thailand where multidrug resistant *P falciparum* is prevalent.

Quinine sulphate is used to treat malaria in adults at a dose of 600 mg tid for 7 days, often in combination with doxycycline. Shorter courses are prone to result in recrudescences.

The entry criteria aimed to capture "moderately severe" malaria.

Table 6: Inclusion and exclusion criteria for pivotal studies

	Location (Study No.)		
	Thailand (005)	**Malawi (006)**	**South Africa (007)**
Age	6 months-15 years	1 – 10 years	16 years – 65 years
Eligible parasitemia	>4% (200,000/ : l)	>0.4% (<20,000/ : l)	-
Clinical eligibility	-	Unable to eat or drink or Impaired consciousness	Unable to eat or drink
Exclusion	Diarrhea	Diarrhea	Diarrhea
	Unable to eat or drink	-	-
	Previous antimalarial in past 24 hrs	Previous antimalarial in past 24 hours	Previous antimalarial in past 24 hours
	Severe malaria: • Acidotic • Hct < 15% • Jaundice • Bleeding • Shock • Decreased consciousness	Severe malaria: • Deep breathing • Hct < 18% • Jaundice • Bleeding • Shock • Stupor or coma • Convulsions	Severe malaria*: • Respiratory distress • Jaundice • Bleeding • Shock • Coma • >1 Convulsion • Renal failure • Hypoglycemia • Lactate < 5 mmol/l
	Parasitemia >20%	Parasitemia >10%	Parasitemia >10%

* Two additional arms were originally included in this study to incorporate patients with the features of severe malaria. Since the treatment in all these patients was to be quinine

with or without artesunate, the results in these arms were not considered pivotal to this application. During the study, it was determined that facilities were inadequate for such sick patients and these arms were discontinued after recruiting a total of 11 patients. Among the 11 patients there were 3 deaths. Ultimately all 3 deaths were attributed to the complications of severe malaria.

Results

Characteristics of the study populations are described below:
(In each row of the table, results are listed for the rectal artesunate-arm above and the comparator-arm below)

Table 7: Baseline characteristics of patients in pivotal studies

	Location (Study No.)		
	Thailand (005)	**Malawi (006)**	**South Africa (007)**
Number of patients Artesunate Comparator	 46 17	 87 22	 27 8
Mean Age Artesunate Comparator	 7 years 7 years	 4 ½ years 4 years	 29 yrs 25 yrs
% Males	63.5%	61.5%	51%
Entry parasitemia Artesunate Comparator	Geom mean 260,600 409,900	Geom mean 180,000 217,000	Median 55,000 101,000
Platelet count Artesunate Comparator	Median 85,000 129,000	Geom mean 74,500 67,000	Mean 75,000 72,500

In all three studies, the patient demographics, the severity of disease, and clinical laboratory results at baseline were balanced between the treatment arms.

Protocol defined criteria for "rescue" with other antimalarial agents included:

- A parasite density $60% of the admission parasite density after 12 hours.
- Clinical deterioration with the development of features of severe malaria, repeated convulsions or coma.

In each study, "rescue" was permitted as shown:

Table 8: Provisions for rescue therapy in pivotal studies

Thailand (005)	Malawi (006)	South Africa (007)
Rescue in both arms	Rescue only in rectal artesunate arm	Rescue in both arms

Once a patient was rescued with a non-study drug, any clinical or parasitological finding subsequent to rescue could not be clearly attributed to the study drug. The FDA regarded rescued patients as clinical failures.

It is recognized that this approach erred on the side of stringency since many patients treated for malaria do not reach a parasite density <60% of baseline at 12 hours, despite which they proceed to recover clinically.

The applicant used the fractional reduction in parasite counts at 12 and 24 hours as the primary basis for the determination of comparative drug efficacy. In this analysis, clinical and parasitological failures who received non study-therapy were treated as "withdrawals". Clinical failures and those patients receiving rescue therapy were separately presented.

The FDA defined the following clinical and parasitological endpoints to incorporate data from all patients randomized to treatment, including those who were rescued because of clinical or parasitological deterioration.

Table 9: FDA defined endpoints

24-hour clinical success	All treated patients: • who were evaluated after 24 hours on study drug • who had not received rescue therapy or alternative antimalarial therapy • who neither died nor deteriorated clinically since the baseline evaluation
24-hour parasitological success	All 24-hour clinical successes whose 24 hour parasite count was #10% of the baseline count*
28-day recrudescence/ re-infection	Any patient who received study drug and was found to have a recurrence of parasitemia between the time that therapy was stopped and day 28.

* This composite endpoint was created since it represented the outcome just before administration of the follow-up regimen and was considered to reflect the activity of rectal artesunate when used as proposed while taking into account prior rescue therapy. Since parasite clearance >90% of baseline was a parameter that the applicant determined in all studies, and was a clear indicator of a favorable treatment response, it provided a suitable representation of the parasitological component in the 24-hour efficacy assessment.

Study-related events with an impact on the clinical results are described in the table below.

Table 10: Study related events with an impact on clinical results for pivotal studies
(In each row of the table, results are listed for the rectal artesunate-arm above and the comparator-arm below)

	Location (Study No.)		
	Thailand (005)	**Malawi (006)**	**South Africa (007)**
Number of patients Artesunate Comparator	 46 17	 87 22	 27 8
Exclusions Artesunate Comparator	 5 3	 3 0	 1 1
Rescued for 12 hour parasitemia $60% of baseline Artesunate Comparator	 7 4	 3 0 (no provision for rescue)	 1 2
Clinical deterioration Artesunate Comparator	 1 0	 4 0	 0 0
Death Artesunate Comparator	 1 0	 0 0	 0 0
Other failures Artesunate Comparator	 1 (expelled supps) N/A	 1 N/A	 0 0

As detailed above, there was one death in a patient in study 005. The description of this case is provided below.

Case report of death on study

This 3-year-old boy was admitted following a 24-hour history of fever. He was able to sit, stand, drink and eat unaided. Admission parasite count was 40/1000 RBC (4%) HCT was 34% WBC 15.3, and platelet count 211K. Glucose was 3.3mmol/L. The child was given 3 x 50mg rectal capsules.

As the child was not drinking and IV infusion was started at 20 drops per minute. It was later noted that 650cc were accidentally infused over 77 minutes. Despite this, the child seemed well and was eating cake. Two and a half hours after starting therapy the child vomited and died suddenly. A parasite count shortly before death was 65/500 WBC. (Since the RBC was not given, only an approximate comparison of this count and the admission count was possible. The follow up count (~0.01%) was

substantially lower than the admission count. It was found that sodium levels had fallen from 139 to 117, potassium levels from 3.5 to 2.5 and albumin from 3.8 to 3.2 over 2 hours. The consensus of the investigators and expert consultants was that the cause of death was water intoxication, and not malaria. Plasma levels of dihydroartemisinin were noted to be high in this patient although this was not thought to have accounted for his death. (This is further addressed in the section on safety.)

The final clinical outcome at 24 hours, according to the FDA-defined endpoints is summarized in the table below.

Table 11: Clinical and parasitological outcomes at 24 hours, according to FDA defined endpoints

	Location (Study No.)		
	Thailand (005)*	**Malawi (006)****	**South Africa (007)***
Randomized			
Artesunate	46	87	27
Comparator	17	22	9
Excluded			
Artesunate	5	3	1
Comparator	3	0	1
Clinical Failures			
Artesunate	11	8	1
Comparator	4	0	2
24-hr clinical success rate			
Artesunate	30/41 (73%)	76/84 (90.5%)	25/26 (96%)
Comparator	10/14 (71.4%)	22/22 (100%)	6/8 (75%)
95% CI Artesunate – Comp.	(-26.9, 36.9)	(-38.6, 14.3)	(-16.5, 95.9)
*P –value****	1.0000	0.2006	0.1310
24-hr parasitological success rate			
Artesunate	30/41 (73%)	74/84 (88%)	22/26 (84.6%)
Comparator	10/14 (71.4%)	3/22 (13.6%)	2/8 (25%)
95% CI Artesunate – Comp	(-26.9, 36.9)	(42.1, 80.2)	(15.3, 87.9)
*P –value****	1.0000	<0.0001	0.0034

* Rescue was permitted in both arms in these studies
** Rescue was only permitted in the AS arm in this study.
*** Exact confidence interval and Fishers Exact Test

Following completion of treatment, patients were to be followed for 28 days from the time of study entry. During this time, weekly visits were scheduled and in addition, patients with a recurrence of clinical symptoms were seen at the time when they presented to study centers. The attendance at these follow-up visits was poor. For example, in Study 006, 55% of the patients in the artesunate arm and 73% of patients in

the control arm did not attend the 28-day follow-up visit. For Studies 005 and 007 the number of patients lost to follow-up by Day 28 was not available.

Since the study centers also served as the local clinical treatment centers, we assumed that in general, subjects who did not report to the clinic between the day that therapy was completed and Day 28 were clinically well. However, we can not exclude the possibility that several of the patients lost to follow-up may have developed malaria.

The 28-day recrudescence/new infection rate was calculated based on the number of patients initially enrolled, and the number of patients with a laboratory confirmed recurrence of parasitemia.

Table 12:
28-day recrudescence/reinfection rates per number of patients enrolled in the study:

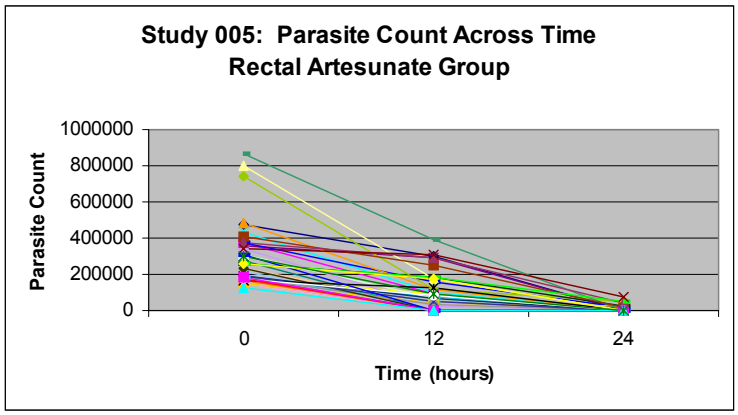
	Location (Study No.)		
	Thailand (005)*	**Malawi (006)***	**South Africa (007)***
28-day recrudescence/ reinfection rate			
Artesunate	0/41 (0%)	39/86 (45.3%)	2/26 (8%)**
Comparator	0/14 (0%)	5/22 (22.7%)	2/8 (25%)**

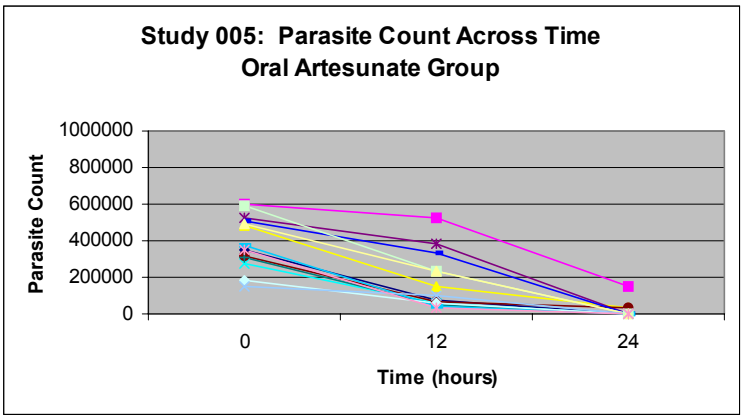
* Number of patients lost to follow-up is not available, thus the denominator represents the number of patients randomized. Patients with missing data are assumed not to have developed recrudescent malaria for the purpose of this analysis.
** Recrudescence diagnosed by PCR only, smears negative

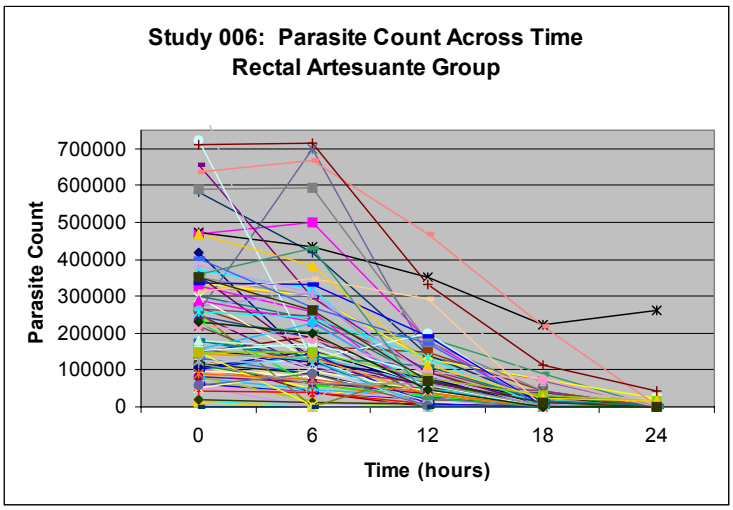
These rates are presumed to reflect the combined efficacy of rectal artesunate or the comparator drug plus the efficacy of the follow up regimen. This is turn is related to the prevalent drug sensitivity of *P. falciparum* in the region where the study was conducted. In general, resistance to Fansidar is more common than resistance to mefloquine. This may account in part for the low recrudescence rates in study 005 compared to studies 006 and 007.

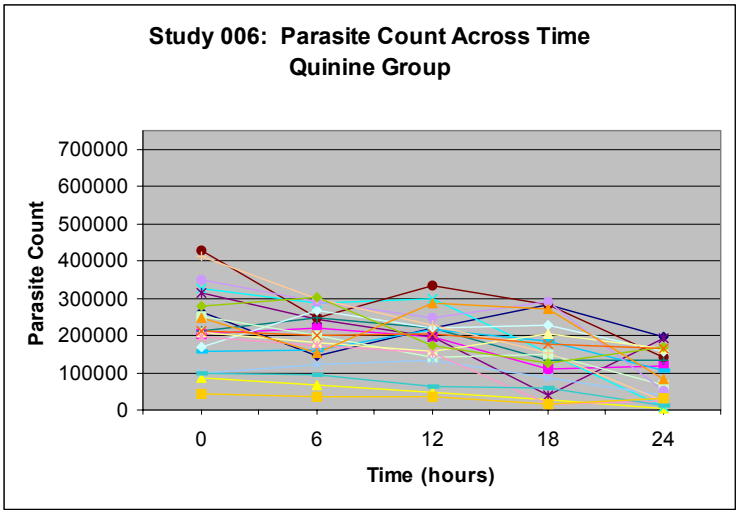
Parasitological clearance

The figures below show individual subject-level parasite counts over time for each of the pivotal studies. Results for the experimental arm and the control arm are show separately.



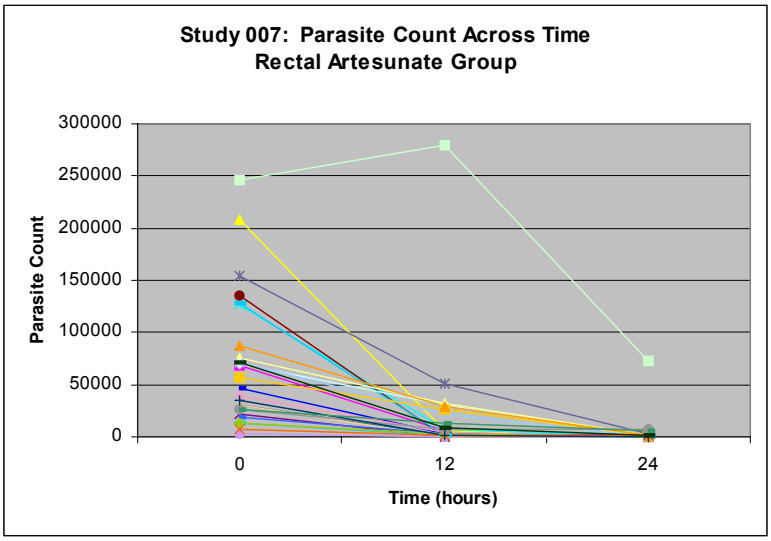


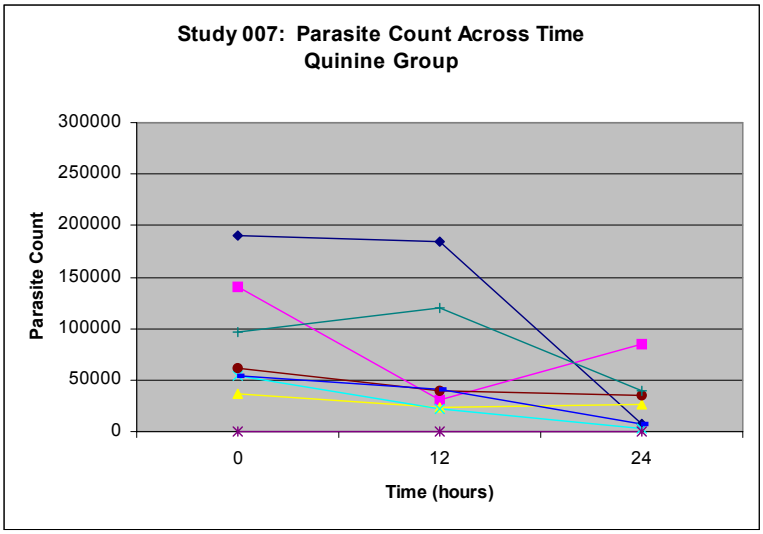




* The following patients (and their parasite counts across time) were not included in these figures since their parasite count in at least one time point exceeded a reasonable range for the y-axis:

Group	Subj #	Baseline	6 hrs.	12 hrs.	18 hrs.	24 hrs.
Rectal	60	169694	missing	791534	373499	149361
Artesunate	106	312352	5230003	35119	17	37
Quinine	39	376692	1036878	785148	1087549	567025
	83	701784	561660	584496	552380	844660





While these results demonstrate the rapid parasite clearance in most subjects treated with artesunate, a small number of patients, described previously, who failed to reach 60% of baseline parasitemia at 12 hours were given rescue therapy. In study 005 there were 7 (17%) and 4 (29%) rescued subjects in the Artesunate and Quinine groups, respectively. Study 006 had 3 (4%) Artesuante subjects rescued and no provisions for rescue in the Quinine group. Finally, Study 007 had 1 (4%) and 2 (25%) rescued subjects in the Artesunate and Quinine groups, respectively. Parasite counts obtained in these subjects after rescue are included in the figures above. Although this analysis represents an unfairly favorable parasitological response to therapy for the rescued subjects, the fact that rescue was performed in both the arms of studies 005 and 007 permits some comparison between the arms.

Additional supportive studies

Study 14
Study 14 was a bioequivalence study to demonstrate the parasitological efficacy of three different formulations of rectal artesunate and did not provide comparative data with other antimalarial agents.

This three-arm study compared the formulation used in phase I and II studies, the formulation used in phase III studies, and the formulation proposed for marketing.

> (A prior bioequivalence study in healthy male volunteers [Study 009] comparing these formulations failed to show equivalence for both AS and DHA. The intrasubject variability in C_{max} and AUC for both AS and DHA was relatively high and ranged from 60 to 70%. The lack of adequate sensitivity of the assay resulted in a large number of non-measurable samples and lost data points, limiting the power for the analysis. Other potential reasons for variability in the study include: inconsistencies in rectal absorption, variability in the amount of drug substance absorption in the dosage form, variability in drug product quality, dissolution rate limitations from the formulation, and individual variability in drug metabolism. Study 14 was designed to address these difficulties.)

Table 13: Initial treatment in Study 14:

Moderately severe malaria	Group A	Rectal AS 2 x 200mg suppositories
	Group B	4 x 100mg suppositories
	Group C	1 x 400mg suppository

Table 14: Consolidation regimens in Study 14

All groups (if able to take orally) 24 hours after entry, oral artesunate (4 x 50mg tablets) for 3 days 24 hours after last dose of oral AS, 3 x 250mg tablets of mefloquine 48 hours after last dose of oral AS, 2 x 250mg tablets of mefloquine
All groups (if unable to take orally) 24 hours after entry, IV artesunate (120mg) then IV artesunate 60mg 12 hourly till able to tolerate oral medications and complete a total dose of 480-600mg artesunate 24 hours after last dose of oral AS, 3 x 250mg tablets of mefloquine 48 hours after last dose of oral AS, 2 x 250mg tablets of mefloquine

The study was performed on 69 hospitalized adult patients (23 per arm) with moderately severe malaria.

Inclusion criteria required a baseline parasitemia $100,000/:l and/or non-per os status. Exclusion criteria resembled those for studies 005, 006, and 007 where patients with complicated malaria or decreased level of consciousness were not enrolled.

All 69 patients completed the study successfully without the need for rescue therapy. Parasitemia was rapidly cleared as shown below:

Table 15: Median parasite counts /:l at 0, 12, 24 and 48 hours

	Baseline	**12 hours**	**24 hours**	**48 hours**
Group A	46,720	3,890	30	1
Group B	30,960	9,980	86	0
Group C	40,680	13,080	62	0

The mean fractional reduction in parasitemia at 24 hours for the 3 groups ranged from 83.1% to 97.3% and by 48 hours, all patients had effectively cleared parasites.

Fever clearance time was similar for all three groups with a mean of 21.6 hours in Group A, 21.9 hours in Group B and 24.8 hours in group C.

Data beyond 7 days were not described and recrudescence/new infection rates for this population are not known.

Studies 003 and 004

These were two randomized, open-label, crossover studies between IV and rectal artesunate performed on hospitalized patients with "moderately severe" malaria. These studies were designed to provide both clinical and pharmacokinetic data. The WHO conducted population pharmacokinetic analyses using pharmacokinetic data from both of these studies. The review of these analyses is currently ongoing; therefore, the WHO's conclusions cannot be verified at this time.

The clinical data from these studies are summarized in the following pages.

The treatment arms involved crossover between rectal and intravenous artesunate at 2 dosing strengths as shown in the table.

Table 16: Treatment arms in Studies 003 and 004

	Location (Study No.)	
	Thailand (003)	**Ghana (004)**
IV AS 2.4mg/kg After 12 hrs, Rectal AS 10mg/kg	12 patients (Group 1)	0 patients
Rectal AS 10mg/kg After 12 hours IV AS 2.4mg/kg	12 patients (Group 2)	12 patients (Group 1)
IV AS 2.4mg/kg After 12 hrs, Rectal AS 20mg/kg	12 patients (Group 3)	12 patients (Group 3)
Rectal AS 20mg/kg After 12 hours IV AS 2.4mg/kg	12 patients (Group 4)	12 patients (Group 2)

Notably none of these arms included initial rectal artesunate according to the proposed regimen (Rectal artesunate, 10mg/kg alone during the first 24 hours of therapy).

Table 17: Consolidation regimen for studies 003 and 004

	Location (Study No.)	
	Thailand (003)	Ghana (004)
"consolidation therapy"	Mefloquine at 36 and 48 hours	Chloroquine over 3 days (sulfadoxine-pyrimethamine if intolerant of chloroquine)

Table 18: Inclusion and exclusion criteria for studies 003 and 004

Thailand (003)	**Ghana (004)**
Inclusion criteria	
Adults 16-50 years	Children 18 months to 7 years
Entry parasite count ≥ 100,000 / :1	Entry parasite count ≥ 10,000 / :1
Non per-os patient	Non per-os patient
No vital organ dysfunction	
Exclusion criteria	
Cerebral malaria or complicated malaria (e.g. pulmonary edema, renal failure, shock)	Cerebral malaria (coma ≤ 2 on Blantyre scale) hypoglycemia, blood lactate ≥ 5 mmol/l or Hct < 15%
Rectal abnormalities/acute diarrhea	Rectal abnormalities/acute diarrhea
	Previous antimalarial treatment

Short term (24-hour) clinical outcome:
Patients given the same initial treatment regimens were pooled and the clinical outcome at 24 hours was determined as successful if the patient completed treatment and did not require "rescue" therapy with additional antimalarial drugs.

Table 19: 24-hour clinical success rates

Regimen	Short term clinical success
IV AS 2.4 mg/kg, Rectal AS 10 mg/kg	12/12
Rectal AS 10 mg/kg, IV AS 2.4 mg/kg	23/24
IV AS 2.4 mg/kg, Rectal AS 20 mg/kg	22/23
Rectal AS 20 mg/kg, IV AS 2.4 mg/kg	22/24 *

*In this treatment group, 2 patients progressed clinically to severe malaria

Short term (24 hour) parasitological outcome

Table 20: Pooled parasitological results showing the numbers of patients with ≥90% reduction of baseline parasitemia at 12 and 24 hours:

Regimen	12 hours	24 hours
IV AS 2.4 mg/kg, Rectal AS 10 mg/kg	1/12	8/12
Rectal AS 10 mg/kg, IV AS 2.4 mg/kg	5/23	21/23
IV AS 2.4 mg/kg Rectal AS 20 mg/kg	5/23	20/22
Rectal AS 20 mg/kg IV AS 2.4 mg/kg	3/24	21/23 *

*In this treatment group, 2 patients progressed clinically to severe malaria

Recrudescences or new infections

Table 21:Recurrent parasitemia during the first 2 to 3 weeks after completion of therapy

	Recrudescence rate among patients allocated to treatment
Mefloquine	7/48 15%
Chloroquine	7/23 30%
Sulfadoxine-pyrimethamine	2/9 22%

Discussion of efficacy results
In the studies described above, rectal artesunate results in a rapid clearance of parasites in most patients. The clearance of parasites is notably slower when quinine is used for treatment.

At 24 hours, the clinical outcome in the pivotal studies is similar for rectal artesunate and for comparator regimens.

It is unclear what the clinical outcome would have been without additional therapy in the small number of patients rescued for unsatisfactory parasitologic responses in the first 12 hours.

While these studies characterize the benefits of emergency rectal artesunate compared to standard therapy in the hospital setting, the benefits of emergency rectal artesunate compared to no treatment in the bush, without ancillary supportive therapy, are not directly determined. Such an evaluation should take into account the mortality of untreated, moderately severe malaria, which is estimated to be high.

By Day 28, high rates of recrudescence occur in some studies. This presumably depends in part on the follow-up regimen used and the prevalent patterns of drug resistance. However recrudescence rates appear higher for artesunate-treated patients than for comparator treated patients, even when both are given the same follow-up regimen. In evaluating the benefit of early treatment versus the risk of a higher recrudescence rate with rectal artesunate, the anticipated mortality from initial malaria versus recrudescent malaria should be considered. This may depend on several factors including access to medical attention and patients' understanding of the disease.

Safety

> *(The reader is referred to WHO's Briefing Document pages 30-39 regarding safety and the corresponding references. Direct statements from the WHO's application and safety briefing will be presented as Arial font in quotes.)*

The applicant proposes to use artesunate rectally at the dose of 10 mg/kg x 1 in the initial treatment of malaria when the patient cannot tolerate oral route medications and parenteral administration is not available. In support of safety, three sets of data have been submitted as part of NDA 21-242, and a recent safety update, which contains data from an on-going field trial (Study 13, which is not part of this NDA review).

Summary of data presented to the Chinese regulatory authorities

For the registration of artesunate as injection in 1989, there was one Phase 1 study with 26 healthy subjects, two phase 2 studies with 70 patients in total which also contained a control arm (Quinine IV) with 12 subjects, and 3 clinical studies with patients having varying degrees of falciparum malaria totaling 236 subjects. All of these studies were open-labeled and only one phase 2 study seems to have had a control arm. It appears that there were children included in the clinical studies but to what extent is unclear. There was one single-dose study, four multi-dose studies (4 dose over 3 days), and one 7-day study. The highest total dose (these are all intravenous doses) was in the 7-day study of 9.6 mg/kg. The clinical studies in ill patients all gave a total dose ranging from 4.8 to 6 mg/kg over a 3-day period. Adverse events described included bitter taste, mild pain at the injection site, bradycardia, paroxysmal ventricular premature beat, incomplete right bundle branch block, first-degree atrio-ventricular block, and urticaria. No major setbacks were due to safety issues with artesunate. The IV quinine group experienced tinnitus, deafness, nausea, dizziness, headache, and ECG abnormalities including bradycardia and incomplete right bundle branch block. Laboratory abnormalities described included decreased reticulocyte count on the 3rd day returning to baseline by the 7th day (in normal volunteers), increased SGOT, SGPT, and BUN, proteinuria, haematuria, and pyuria.

These documents with the corresponding commentaries are supportive only. There were no datasets submitted. Neither inspection nor audits are possible at this time (over a decade later). Given these faacts, the applicant's interpretation was that the data collected by the Chinese investigators provided "… a favorable tolerability profile of artesunate. The drug was given through the intravenous route on a variety of doses and up to a maximum of 7 days with no major safety setbacks. Although not all of these trials were controlled and none was blinded, they provide supportive data in support of the use of artesunate for a limited indication."

Summary of a systemic review of published and unpublished data

A systemic review of all available safety information from published (n=151) and unpublished (n=18) studies was the second set of supportive evidence submitted for review. The applicant's summary included a total of 15,567 patients from 169 studies exposed to artemisinin derivatives, with safety information on 13,639 patients from 130

studies across a spectrum of disease. In the artesunate safety database, there was a total of 6258 patients with the majority coming from studies in uncomplicated malaria (n=5485). There were 417 patients in studies for severe malaria, 10 in moderate malaria, and 1346 in "other". The Systemic Review does not cover the dosage used in these studies. However, from the available literature, the highest total dose used for rectal artesunate was 45.71mg/kg given over 4 days divided in 8 doses in adult patients and 57 mg/kg total dose given over 3 days in 3 divided doses in children. The number of patients exposed to these doses was small, both sets of patients being < 30. Adverse events described include few cases of bitter taste, pain at the injection site, fever and slight burning sensation as adverse events seen in studies on healthy normal volunteers. For comparative studies, the review describes the most commonly reported adverse events (in the order of <1%) to be mild gastrointestinal (nausea, vomiting, diarrhea, abdominal pain) events. In severe malaria, artemisinin derivatives had fewer incidences of hypoglycemia, skin reactions (pruritis, urticaria, rash), tinnitus, and dizziness than quinine. In uncomplicated malaria, pruritis was more common with chloroquine, nausea, dizziness, and tinnitus more common with quinine, and vomiting more common with mefloquine than artemisinin derivatives. Laboratory abnormalities described include neutropenia, reticulocytopenia, eosinophilia, anemia, transaminitis, culture-negative pyuria, hemoglobinuria, ECG abnormal-bradycardia, and prolongation of QT interval in the order of 1% of the tests done. There were a few cases of elevated bilirubin, atrial extra-systoles and T-wave abnormalities. The vast majority of the studies included in the Systemic Review did not have neurological assessments done. However of the available information, dizziness was the most common neurological adverse event. Thus far, there is a lack of evidence to suggest an association between artemisinin derivatives and increased neurological deficits/sequelae.

There are obvious methodological deficiencies of compiling published and unpublished data (with no available raw data) from various studies over a span of time. Some of those deficiencies which make the synthesis of the data problematic may include variation in study design and quality, inadequate data collection, subjects representing different disease severity (especially since many of the side effects of the drug may be similar to the actual disease manifestations of malaria) and the uncertainty of pooling adverse events from individual studies to come up with an overall incidence. Given that, this systemic review is "the most comprehensive attempt to identify all the available information on the safety of artemisinin-type compounds". There were few side effects reported with these compounds overall in the available literature and these side effects were mainly mild and transient.

Summary of WHO-specific studies in the NDA submission

This safety dataset consisted of 14 study protocols and 13 study reports of which 2 were bioavailibility studies, 2 were bioequivalency studies, 3 were pediatric "pivotal" studies, 3 adult "pivotal" studies, and 3 supportive (re-analyzed raw data from published literature) studies. The total safety database for subjects exposed to rectal artesunate was 501 subjects (all hospitalized patients mainly in Southeast Asia and Africa) which included 135 healthy volunteers, 275 patients with moderately severe disease and 91 patients with severe malaria. One hundred sixty-six of these patients were children and all of them had the diagnosis of moderately severe malaria. All pharmacokinetic and

"pivotal" studies were one rectal dose studies with a majority receiving 10 mg/kg x 1 dose with a range of 6.8 to 22.2 mg/kg. Repeated dosing with rectal artesunate was given in the supportive studies (Studies 10, 11, and 12) over 3 to 4 days with mean total doses between 25- and 32 mg/kg dose with a range of 11.3 to 45.7 mg/kg. If all formulations of artesunate administered in these studies are viewed collectively, the maximum dose exposure for adults was 45.7 mg/kg total dose given over 4 days in 8 divided doses. For children, the maximum dose exposure was 21.4 mg/kg and the longest duration of exposure was 7 days (rectal dose x 1 followed by oral dosing). In the literature, the highest total rectal artesunate dosage used was 57 mg/kg given over 3 days in daily divided doses.

The integrated adverse event count from one-dose rectal studies (this excludes multiple dose supportive Studies 10-12) yielded 63 adverse events in 219 adults receiving single dose rectal artesunate verses 34 adverse events from 14 comparator regimens (all IV quinine in Study 7: patients with severe malaria). Adverse event incidence comparison is not possible for the adult patients due to the extremely small number and type (severe disease patients only) of comparator cases. The numbers and type of adverse evnets (all children had diagnosis of moderately severe malaria) for comparison are better for the pediatric population where 33 adverse events were reported in 166 children (19.9%) receiving rectal artesunate as compared to 10 adverse events in 39 (25.6%) comparator-group children (17 with PO artesunate and 22 with IV quinine). The leading adverse events reported were gastrointestinal in all groups (mostly vomiting, and abdominal pain). Collectively, central nervous system/neurologic adverse events were 10/166 (6%) in the rectal artesunate dosed pediatric patients vs. 2/39 (5%) in the comparator group. Of the three remaining supportive studies, only one study reported adverse events. Of the 9 adverse events reported, 8 were gastrointestinal in nature and 1 case of dizziness. In all, very few events led to treatment discontinuation and were classified as treatment emergent or serious in nature.

There were 7 deaths (1.4%) out of the 501 patients in the total safety database or 1/275 (0.36%) in patients with moderately severe malaria and 6/91 (6.6%) in patients with severe malaria. These are within rates for patients treated in a hospitalized setting. Overall, the reported mortality rates in the literature range from 0.1% in the uncomplicated malaria diagnosis to up to 40% for severe disease with cerebral malaria in areas with poor access to care. The single pediatric death occurred in a 3-year old boy in Study 5 who received rectal artesunate (11.5 mg/kg). The probable cause of death was determined by the study investigators and the WHO reviewers to be due to "water intoxication". The narrative and the chemistry laboratory values (i.e., his serum sodium decreased from 139 to 117 over 2 hours) corroborate this as likely. Although the applicant surmised that the PK analyses "indicated that rectal AS administration was felt not to be likely related to the death of the patient", his dihydroartemisin (DHA) level at both 2 hours and 4 hours post dose was over 2 to 3 times the mean dose of children from Ghana (subject SA09: 2002 ng/ml at 2 hrs and 977 at 4 hrs compared to Ghana children 652 ± 353.2 ng/ml at 2 hrs and 396.8 ± 545.2 at 4 hrs) Since the metabolite DHA is considered to be the most neurotoxic of all the artemisinin derivatives and in light of the our lack of knowledge regarding the PK/PD of this drug in humans, the

high levels of DHA seen in this child at the time of death is concerning. Nevertheless, the investigator's alternate explanation of death was satisfactory. Three other deaths occurred in another pivotal trial (Study 7) and all three patients were in the group of patients diagnosed with severe malaria. One death was in the rectal artesunate arm and two deaths were in the IV quinine arm. The investigator's explanation for these three deaths was underlying malarial disease with inadequate supportive care in all three cases. The narratives are consistent with the investigator's assessments. The last three death reports come from Study 10 (a supportive study), which also had patients with severe malaria. Here however, the investigators were unable to offer a clear explanation of the deaths since in all three cases, the patients had cleared their parasitemia at the time of death.

Laboratory monitoring was limited. Only Study 3 recorded had comprehensive CBC, Chemistry and LFTs. Overall, there was a transient decrease in hematocrit at 12-24 hours with return to baseline levels by day 7 and reaching normal values by Day 28. Previous healthy human studies had shown mild reticulocytopenia in patients receiving artesunate however, reticulocyte levels were not measured in these studies. The transient decrease in hematocrit was not attributable to any treatment categories. Post-treatment, platelet counts, bilirubin levels, glucose and lactate levels gradually normalized. There were three patients with normal baseline levels whose SGOT and SGPT rose over 3 times above the upper limits of normal (not clinically symptomatic), peaked at Day 7-14, and started to come back down on day 21. Increased transaminases have been reported in the literature with many studies confounded by mefloquine co-administration. A preclinical toxicology study submitted with this application (Study No. BDC/002) did show liver toxicity (centrilobar hepatocyte hypertrophy, sinusoidal inflammatory cells, single cell necrosis and diffuse hepatocyte vacuolation) and it is known that artesunate's major metabolite DHA is mainly cleared via glucuronidation. Thus, increased liver enzymes may be a related event with artesunate. However, this event has not translated thus far to symptomatic liver toxicity in known clinical experience. Only in Study 3 was ECG monitored. No significant abnormalities were noted.

Special populations

With regards to pediatric patients, there were too few patients < 2 years old in these studies (8 out of 166 pediatric subjects) and the applicant is limiting the indication of this drug to patients older than 2 years of age at this time. Also, the applicant has not shown any data for the geriatric patient population (6 out of 335 adult subjects were above the age of 50). Specific studies in patients with hepatic and renal impairments were not performed. Pregnant patients were not included in any of the WHO-specific studies. Consistent findings of impaired fetal survival (but no evidence of teratogenicity) following first trimester exposure to artemisinins in animal studies are of concern. However, the applicant states:

> "carefully conducted human studies have not confirmed a level of toxicity or risk of teratogenicity attributable to the artemisinins, and neither has evidence been demonstrated of other fetal injury or impairment of maternal health over and above the effects on reproductive health of malaria itself".

Neurotoxocity

Since neurotoxicity is considered a serious class effect for artemisinin derivatives (dose-related unique pattern of neuropathology showing necrosis of some neurons in vestibular, cochlear, olivary, red and other nuclei in the brainstem of animals), the applicant has attempted to address this issue by providing:

1) Non-clinical summary data from the Chinese studies,
2) Non-clinical WHO-commissioned toxicology studies performed by Quest of United Kingdom,
3) A 1998 WHO-sponsored expert review of all available pathological material as well as published and unpublished information,
4) Neurological assessments in clinical Studies 5, 6 and 7, and
5) Literature sources citing human clinical experience regarding neurological adverse events/sequelae.

The Chinese data (as per the FDA Toxicology Review) was mainly not interpretable and could not be validated. The WHO-commissioned toxicology data submission was disappointing (FDA had asked for a specific neurotoxicity study; however the submitted study was a 7-day rat toxicology study without specific neurotoxicology assays, staining, or behavioral assessments) and concerning (the high-dose arms were prematurely discontinued due to unexplained deaths; 1 /16 rat died on day 4 of 75 mg/kg dosing and 2/16 rats died on day 3 of 150 mg/kg dosing). The 1998 WHO sponsored expert review discussed neurotoxic effects of artemisinin derivatives in 3 animal species. The NOAEL for arteether and artemether in rats was thought to be at 45 – 75 mg/kg IM x 1 or over 7 days; in dogs at 3 – 6.25 mg/kg/day IM x 7 days; and in monkeys 100 mg/kg IM x 1 or over 7 days. The limited information for Artesunate did not show neuronal necrosis at 420 mg/kg x 1 IM or 200 mg/kg/day PO x 5-7 days.

The WHO briefing document states:

> "The conclusion is that the administration of artesunate by the oral or intravenous route to the study animals at dose levels and duration that gave a total dose of 210-300 mg/kg (21-30 fold greater than the total proposed human dose) did not result in clinical or neuropathological findings, nor indicate any neurotoxic action".

This direct dose comparison between animal and clinical doses relating to safety margin (not accounting for body surface area) is inappropriate. The body surface area conversion of the rat dosages of 210-300 mg/kg provides equivalent human doses of approximately 35-50 mg/kg, which is only 3 to 5 times the proposed 10 mg/kg x 1 proposed human dose, not "21-30 fold greater" as claimed by the applicant. Body surface area conversion of the rat dosages at which deaths occurred in the WHO-commissioned studies (75 and 150 mg/kg PO) provides equivalent human doses of approximately 12 and 25 mg/kg, respectively, which considerably narrows the margin of safety.

Neurological assessments in WHO-specific clinical studies were performed for Studies 5, 6, and 7 only. This should provide data on approximately 164 rectal artesunate recipients tested (neurological assessments consisting of cranial nerves, nystagmus, hearing, tandem walking, the Romberg test, line drawing, picking tablets, rapid alternating movements, and finger tapping) out of the 501 total patients. However, missing data was extremely common and, according to the applicant, many of the young patients did not cooperate with the assessments. Nevertheless, no pattern of neurological abnormalities was concerning. The WHO's main argument for the safety of artesunate and the lack of human neurotoxicity draws upon the large body of literature and the actual usage experience. The applicant states:

> "careful neurological examinations have also been made in 1303 patients treated with the artemisinins, 806 in patients treated with artesunate….The analysis of a large body of clinical data by Price et al to assess patients treated with artemisinins, including dosing by the oral, intravenous, and rectal routes, did not identify any neurotoxic effects".

Unfortunately, a closer look at the reference that the applicant is citing in the Briefing Document (Reference 14; Price et al 1999), identifies that it discusses patients with acute uncomplicated malaria, which is not the population that the applicant proposes to treat. Furthermore, the authors of the article write that "neurologic examinations could be performed reliably only in patients >5 years old". It is very young children who are not able to take PO that the proposed indication will most likely include.

This leads us to a brief look at the **120-day Safety Update** which contained data from Study 13 (a field study that is on-going in Bangladesh, Ghana, and Tanzania) where the proposed indication is being studied in placebo-controlled fashion and with patients as young as 6 months of age. This study remains blinded at this time. The update highlights the fundamental differences in the populations between the WHO-specific studies submitted to the NDA (e.g., all hospitalized patients, all with proven malaria disease, mainly moderately-severe malaria disease, only 8 patients < 2 years of age) versus the on-going field study (patients at remote sites, 74% of available malaria smears positive, 12% of patients unconscious at baseline, 23% with repeated convulsions, and 1093/1947 (56%) of patients enrolled in Ghana and Tanzania aged <2 years). Moreover, of the 16 patients thus far being followed for neurological sequelae, only 50% had a positive malaria smear at baseline and 25% had confirmed bacterial meningitis by cerebrospinal fluid analysis (although not all had a lumbar puncture). The low incidence of neurological sequelae thus far (0.47%) is reassuring. However, this safety update calls attention to the likelihood that the population that the proposed indication will actually serve will be very young children with severe malaria disease and/or other severe disease such as bacterial meningitis. This is a population in whom little information is available (even in the large body of clinical experience, and certainly none in the actual WHO-specific studies of this NDA) to draw upon for an adequate safety evaluation of artesunate.

Although we are in agreement with the applicant's overall summary which stated:

> "it is apparent that artesunate, whether given as a rectal dose of 10 mg/kg, 20 mg/kg (pivotal studies) or in a total rectal dose of 1200 mg

> or 1600 mg over 3 days followed by mefloquine (non pivotal studies) or in different formulations (oral or intravenous), has a highly favorable safety profile. The number of adverse events is small and, even if they were drug-related in such a severely ill group of patients, the incidence is very low. No consistent pattern of toxicity has been identified",

we are mindful of the many uncertain safety issues including the as-of-yet undetermined safety margin for neurotoxicity in particular, and the as-of-yet undetermined safety parameters for the population that will be treated with the proposed indication in general. Nevertheless, as we consider the risk/benefit ratio, the cost of the *disease* malaria (especially with rising multi-drug resistant malaria) to individual human suffering and global public health becomes the paramount challenge to overcome. In this light, the use of artesunate at the dose and formulation proposed (10 mg/kg x 1) is within the safety limits of the collective currently available knowledge. However, the uncertainties and our concerns regarding safety would drastically expand if repeated dosing with rectal artesunate becomes an issue.