

FDA

Division of Anti-Infective Drug Products

ADVISORY COMMITTEE

BRIEFING DOCUMENT

ARTESUNATE RECTAL CAPSULES

For the initial management of acute malaria in patients who cannot take medication by mouth and for whom parenteral treatment is not available.

AVAILABLE FOR PUBLIC DISCLOSURE

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EXECUTIVE SUMMARY

This Briefing Document presents evidence to support the antimalarial efficacy and safety of a single 10 mg/kg dose of artesunate rectal capsules in the initial management of acute malaria in patients who cannot take medication by mouth and for whom parenteral treatment is not available.

The World Health Organization (WHO) is proposing a new drug product and indication for malaria. As an applicant, it provides data to support an argument that rectal artesunate, given in a single dose in an emergency situation when the disease is serious and potentially life-threatening, will act as an injectable to halt and reverse the progress of disease, achieving clinical stability and recovery in a severely ill patient. In so doing, it will provide meaningful therapeutic advantage in situations where at present treatment does not exist, and potentially save life. This is an unmet medical need.

The data submitted in support of the application provides plausible evidence that the drug, given as proposed, has the potential to address this unmet medical need. The application is made on the basis of an endpoint (parasite kill rate) that reasonably suggests clinical benefit. Killing parasites is how antimalarial drugs act to halt disease. The definitive evidence of life saving benefit and safety is being established in ongoing large scale Phase IV trials in 3 countries.

Malaria is a serious endemic disease in many parts of Africa, Asia, Latin America and Oceania and is estimated to affect as many as 5% of the world's population at any given time. Of the approximately 2 billion people worldwide at risk annually, an estimated 270 million are newly infected each year and it is estimated that more than 1 million deaths annually are caused by malaria. This number may be understated by a factor of three. Approximately 90% of deaths occur in children living in sub-Saharan Africa. Of the four species of *Plasmodium* causing disease in man, *P. falciparum* infections carry the highest risk of mortality.

P. falciparum malaria presents in a clinical continuum ranging from asymptomatic parasitaemia to severe malaria and death. At one end of the continuum, parasitaemia may be accompanied by no symptoms or, at most, relatively mild symptoms such as fever and malaise. A proportion of these cases may rapidly become more acute, and evolve to moderately severe malaria. The latter situation is likely to be marked by inability to take oral drugs or fluids by mouth (*non per os*) but not necessarily associated with signs of serious complications.¹ Progression from the *non per os* stage to severe malaria, with associated complications and death, can occur within hours.² Patients with malaria who are not able to take fluids and medicines by mouth, and who do not have ready access to treatment by reason of the distance at which they live from a health facility, are at considerable risk of deteriorating and dying before getting to a clinic where effective treatment is available. This is how most lives are lost from acute *Plasmodium falciparum* infection, particularly amongst infants and children, and especially in the rural areas of Sub-Saharan Africa. The risk of death is directly related to the delay in treating the acute stage of the disease. Once the opportunity for early therapeutic intervention is lost and organ failure has developed, the prognosis worsens considerably and survival depends a great deal upon the hospital's ability to manage complications.³

Antimalarial drugs save life in severe malaria by killing malaria parasites and halting progression of the disease. If no treatment is given, or if effective treatment is delayed, severe malaria leads to death. The survivors are those who have received appropriate and adequate treatment, early. Thus both in terms of mortality and morbidity, the greatest benefit is obtained by preventing progression from uncomplicated to severe disease with early administration of effective antimalarial drugs.²

Artesunate is one of a class of antimalarials, the artemisinins, which have been used in the treatment of malaria for centuries. Artesunate is a semi-synthetic derivative of the active principal of the Chinese medicinal herb *Artemisia annua*, which has been used by Chinese herbalists since 341AD for the treatment of fever associated with malaria.⁴ The modern medical use of qinghao derivatives dates from the 1970s and followed the isolation of artemisinins from the *Artemisia Annu*a plant. By 1979, over 2 000 patients with malaria due to *P. falciparum* or *P. vivax* had been cured by such treatment. By 1996, more than 2 million patients had been treated with the artemisinin derivatives.⁵ Resistance to the artemisinin drugs has not developed during clinical use.⁶

Artemisinin and its derivatives are the most rapidly acting antimalarials presently known. All reduce parasitaemia significantly faster than quinine or any other drug used for malaria.

As far as it is understood, artesunate acts by increasing oxidant stress on the intra-erythrocytic plasmodia. Its major metabolite has been shown to be dihydroartemisinin (DHA), which is concentrated to a considerable extent in erythrocytes.⁷ Compared with the other qinghaosu derivatives (arteether, artemisinin and artemether), artesunate and its metabolite, dihydroartemisinin, are the most potent *in vitro*.⁸ When administered by the oral, intravenous, or rectal route, artesunate provides rapid antimalarial action with nearly complete clearing of parasitaemia within 24 hours of initiation of dosing.

The immediate objectives of therapy in malaria when it is evolving to its severe form are to save life and to reduce the risk of serious complications. This is achieved by rapidly reducing total parasite biomass, an effect measured by reduction in circulating blood stage parasites. The artemisinin derivatives are of distinctive value as they achieve such reduction by acting principally on young parasites, preventing their development to the more mature pathological stages which adhere to the vascular endothelium and in this way sequester in the microvasculature of vital organs.⁹ Once clinical stability or recovery has been achieved the patient can move to definitive cure.

Because of its speed of action, relative bioavailability and ease of administration, the WHO undertook a clinical development program to evaluate the antimalarial efficacy and safety of a single rectal dose of artesunate to substitute for injectable therapy in conditions where access to injectable therapy is poor or does not exist. The intent of WHO was to determine the suitability of the rectal form of artesunate for use in shortening the time to achieving parasite biomass reduction and clinical stability, so as to prevent evolution of the disease to its severe form and to complications, thus saving the patient's life, and making it possible for curative therapy to be instituted.

As an applicant, WHO wishes to emphasize that registration approval of the FDA is requested for the use of rectal artesunate to initiate emergency therapy for *P. falciparum* malaria where no other therapy exists. The drug is not proposed for use as cure. For this reason, all the clinical studies initiated by WHO evaluating rectal artesunate have included additional therapy (after 24 hours) to achieve cure of the infection.

During the clinical development of rectal artesunate there was a change in formulation. The product used for Studies 1, 2, 3, and 4 was based on the F1 formulation. The product used for Studies 5, 6, 7, and 8 was based on the F2 formulation and dosed in 50 mg and 200 mg capsules. The new (F3) dosage forms (100 mg and 400 mg products) employ drug substance manufactured to GMP specifications.

Two controlled studies, comparing the non GMP (F2) and the new GMP (F3) products were conducted (studies 9 and 14) in adult healthy volunteers and in adult patients with malaria, respectively. Pharmacokinetic results from study 9 did not strictly support bioequivalence of the two new dosage forms, compared with the reference dosage forms. Yet, the key difference between the pharmaceutical products used in the clinical trials and the products proposed for the market are that the latter have the active ingredient, artesunate, produced to GMP and the encapsulated volume of 100mg and 400mg doubles that of the 50mg and 200mg capsules. Statistical testing indicated that there were no significant differences in C_{max} or AUC between these product formulations. While power calculations were not performed *a priori*, the marked intersubject pharmacokinetic variability observed in the study, which is inherent in the pharmacokinetics of the artemisinins in general (as opposed to the pharmacodynamics of this class of drugs, which are highly consistent) suggested that an exceedingly large number of subjects would be required to fully establish bioequivalence according to standard criteria if, indeed, this would be possible at all.

Study 14 used the more practical and reliable approach of demonstrating therapeutic equivalence (which serves as a direct measure of bioavailability in the patient) of the GMP product with the reference product used in the earlier phases of the clinical development of the drug. Study 14 was conducted in adult patients with acute malaria to evaluate parasitological responses to the two new dosage forms compared with the reference dosage form of rectal artesunate. Sixty-nine (69) patients were enrolled in the study (23 per group); each received a single 400 mg dose of rectal artesunate. There were no significant differences in median fractional reduction of parasitaemia between any of the three dosage forms (difference in median was less than 2%). Thus, this study established that the F3 pharmaceutical dosage forms developed under GMP were clinically and therapeutically equivalent to the F2 dosage forms of artesunate used in studies 5, 6, 7, and 8. In addition to demonstrating therapeutic equivalence, the results of this study provide additional data to support the efficacy of initial treatment with rectal artesunate in adults with acute *non per os P. falciparum* malaria.

In six key studies the antimalarial efficacy and safety of rectal artesunate have been evaluated in patients with moderately severe malaria or uncomplicated hyper-parasitaemia. Three studies (study numbers 4, 5 and 6) enrolled 211 children (208 treated) aged up to 15 years with either moderately severe malaria or uncomplicated hyper-parasitaemia. Artesunate treatment alone was not intended to

provide cure of malaria in these subjects. The study designs are summarized in the following table.

Study (site)	Treatment(s)	Dose of artesunate	Duration*	Design
4 (Ghana) 1996	Rectal then IV artesunate; or IV then Rectal artesunate; followed by chloroquine or sulfadoxine-pyrimethamine	10 and 20 mg/kg (IV, 2.4 mg/kg)	12 h	Randomized, Crossover, Open-label, Comparative, Pharmacokinetic
5 (Mae La, Thailand) 1997-1999	Rectal vs. oral artesunate, followed by mefloquine	10 mg/kg	24 h	Randomized Comparative, Open-label, Pharmacokinetic
6 (Malawi) 1997-1998	Rectal artesunate vs. IM or IV quinine, followed by sulfadoxine-pyrimethamine	10 mg/kg	24 h	Randomized, Comparative, Open-Label, Pharmacokinetic

*time from administration of rectal artesunate to administration of the follow-up antimalarial therapy

A further three studies (numbers 3, 7 and 14) enrolled altogether 152 adults aged 16 through 60 years with moderately severe malaria, and 11 patients with severe malaria (study 7). Artesunate treatment alone was not intended to result in cure of malaria in these subjects. Study designs are summarized in the following table.

Study (site)	Treatment(s)	Dose of artesunate	Duration*	Design
3 (Bangkok, Thailand) 1996	Rectal then IV artesunate; or IV then rectal artesunate, followed by oral mefloquine	10 and 20 mg/kg	12 h	Randomized, Crossover, Open-label, Comparative, In-patient 28 days
7 (South Africa) 1998-1999	Rectal artesunate vs. IM quinine, followed by sulfadoxine- pyrimethamine	10 mg/kg	24 h	Randomized, Controlled, Open-label, Comparative, Pharmacokinetic
14 (Bangkok, Thailand) 2000	Rectal artesunate F2 and F3 formulations, followed by oral or IV artesunate, then mefloquine	10 mg/kg	24 h	Single-blinded, Controlled, Randomized, Parallel Group

* time from administration of rectal artesunate to administration of the follow-up antimalarial therapy

The following two tables summarize the results obtained that demonstrate the effect of artesunate therapy on parasitaemia at either 12 hours or 24 hours after rectal dosing of artesunate in a dose of 10mg/kg in pediatric and adult studies, respectively.

Pediatric Studies: Median Percent Reduction in Parasitaemia at 12 and 24 h after a Single Dose AS 10 mg/kg (all ages < 15 years)					
Rectal Artesunate	12 h (No. of Subjects)	24 h (No. of Subjects)	Comparator	12 h (No. of Subjects)	24 h (No. of Subjects)
Study 4	85.3% (11)		IV artesunate	85.9% (11)	
Study 5	76.4% (46)	100% (28)	Oral artesunate	64.6% (17)	100% (10)
Study 6	72.3% (81)	99.9% (72)	IV or IM quinine	18% (22)	40.8% (22)

Adolescent/Adult Studies: Median Percent Reduction in Parasitaemia at 12 and 24 h after a Single Dose AS 10 mg/kg (age 16- 60 years)					
Rectal Artesunate	12 h (No. of Subjects)	24 h (No. of Subjects)	Comparator	12 h (No. of Subjects)	24 h (No. of Subjects)
Study 3	47.9% (12)		IV artesunate	46.5% (24)	
Study 7	88.1% (26)	99.4% (26)	IM quinine	35.2% (8)	72.2% (8)
Study 14	68.3% (69)	99.9% (69)	None		

These data demonstrate that rectal artesunate provides prompt antimalarial efficacy (reflected in parasite reduction) against *P. falciparum* infections in both children and adults over the 24 hours following a single rectal dose (10 mg/kg). The effect on parasitaemia was consistently superior to the effect of parenteral quinine given in no fewer than 3-7 total doses. When intravenous artesunate was used as the comparator, rectal artesunate performed in comparable manner as an effective antimalarial treatment over the first 12 hours following a single dose.

The data referred to above provide evidence of benefit based upon a surrogate endpoint that reasonably suggests clinical benefit. Therefore the definitive evidence of life saving benefit and safety is being established in ongoing large scale randomised, controlled Phase IV trials in 3 countries(57 FR 58942, December 11, 1992). These trials are intended to provide proof beyond reasonable doubt that for all patients, or for some specific types, the use of rectal artesunate is indicated or clearly contraindicated in terms of a net difference in all-cause mortality or neurological sequelae. A total of 3366 patients were enrolled by 19th March 2002, 1459 patients in Bangladesh, 991 in Ghana and 916 in Tanzania. The overall mortality rate was 3% (4% in children, 1% in adults). The Data Safety Monitoring Committee have examined the results by treatment group and have informed the WHO that there is no reason for any of the interim findings to be unblinded, or for the trial protocol to be modified.

In the submission, adverse experience data are reported from a total of 248 adults, adolescents and children who received a single dose of rectal artesunate at 10 mg/kg as initial treatment of malaria (studies 3,4,5,6,7,8,14). The overall incidence of adverse events was 33.8%, and it is noted that clinical events attributable to acute malaria cannot be distinguished from possible drug-related events. 19 adverse events were considered treatment- related: nausea in 5 patients, vomiting in 4 patients. There were no safety concerns identified in a total of 26 patients receiving a single dose of AS 20 mg/kg rectal artesunate. Safety data on 54 healthy normal volunteers enrolled in Studies 1 and 9 was also reassuring; no adverse events were reported in

these studies. In non-pivotal trials, rectal artesunate was well tolerated at a total dose of up to 1600mg administered over 3 days.^{10 11 12}

These safety data are supported by a large database of 15 567 patients, 7 006 of whom received artesunate in oral or intravenous forms, and the remaining received one of the other artemisinin congeners. Adverse drug event data were collected on 13 639 of these patients with malaria; no findings of concern were noted.¹³ Careful neurological examinations have also been made in 1 971 of 3003 patients treated with the artemisinins, 1664 in patients treated with artesunate.¹⁴ None of these studies were done with drugs in which the active ingredient was produced to GMP. Overall, the data support the remarkable safety profile of artesunate when used in the management of acute malaria.

In summary, a single 10 mg/kg dose of rectal artesunate is rapidly and consistently effective and safe, as initial antimalarial treatment for acute *non per os P. falciparum* malaria over a 24-hour period, and is highly likely to decrease significantly the risks of serious morbidity and mortality in acute malaria.

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GLOSSARY

Abbreviation	Definition
AIDAC	Anti-Infectives Drug Products Advisory Committee
AS	Artesunate
AUC	Area under the concentration-time curve
AS	Artesunate
AUC _(0-t)	Area under the concentration-time curve from time zero (dosing) until the end of the sampling schedule
CI	Confidence interval
C _{max}	Observed peak (maximum) plasma concentration of the drug
CV	Coefficient of variation
DHA	Dihydroartemisinin
DL	Deciliter
ECD	Electrochemical detection
FDA	Food and Drug Administration of the United States
GMP	Good Manufacturing Practice
H	Hour
HPLC	High performance liquid chromatography
IC ₅₀	Concentration inhibiting growth by 50%
IM	Intramuscular
IV	Intravenous
Kg	Kilogram
L	Liter
m ²	Meter squared
Mg	Milligram
Mmol	Millimole
ML	Milliliter
μL	Microliter
Ng	Nanogram
No.	Number
Non per os	Nil by mouth
<i>P. berghei</i>	<i>Plasmodium berghei</i>
<i>P. falciparum</i>	<i>Plasmodium falciparum</i>
<i>P. vivax</i>	<i>Plasmodium vivax</i>
<i>P. yoelii</i>	<i>Plasmodium yoelii</i>
RBC	Red blood cell
RI	Clearance of parasites within 7 days with recrudescence on or before day 28
RII	Significant reduction but not clearance of parasites within 7 days
RIII	No significant reduction of parasitaemia within 7 days
SD	Standard deviation
TCTP	Translationally controlled tumor protein
T _{max}	Time to the observed peak (maximum) plasma concentration of the drug
WHO	World Health Organization
vs.	Versus

**ARTESUNATE RECTAL SUPPOSITORIES -
INITIAL TREATMENT OF ACUTE *P. falciparum* MALARIA
FDA ADVISORY COMMITTEE BRIEFING DOCUMENT**

1 INTRODUCTION

Patients with malaria who are unable to take oral medication, and who live too far from a health facility for ready access to treatment, are at considerable risk of deteriorating clinically and dying during the hours that may be required to travel to a clinic and receive treatment. This is the principal reason for many of the lives lost due to acute *Plasmodium falciparum* malaria.

This document summarizes and describes the clinical, experimental, and theoretical materials submitted by the World Health Organization (WHO) in support of an application to make widely available artesunate rectal suppositories for the initial management of malaria in patients who cannot take medication by mouth and for whom parenteral treatment is not available. Once clinical stability has been achieved, definitive cure can be initiated. The data presented in these papers show a convincingly favorable benefit-risk profile for artesunate rectal suppositories that supports the proposed indication.

For patients with acute malaria in whom the disease has progressed to a state of *non per os*, oral treatment is not possible, and injections are likely to be unavailable or cannot be administered due to the lack of nearby facilities and trained health care workers. It is to meet this particular contingency that WHO has initiated and directed the development of a rectal formulation of the highly active antimalarial artesunate. The data presented show that rectal formulation of artesunate is as effective (or more so) as other oral and parenteral drugs used for acute malaria. Registration of rectal artesunate would make possible the introduction of a therapeutic (but not curative) intervention that has the realistic prospect of saving innumerable lives of infants, children, and adults with potentially life-threatening malaria in a clinical and social situation where at present no treatment is available.

The clinical evidence gathered and presented shows a reliable and consistent acute response to treatment with rectal artesunate, where speed in reduction of parasitaemia (equivalent to that which can be obtained from injectable drugs) is essential. The studies were not conducted to examine cure of malaria, where more sustained treatment and compliance are critical factors. The proposed data sheet/product information makes it clear that all patients initially treated with rectal artesunate would need to receive subsequent oral or parenteral therapy at a health care facility, according to the policies of that facility, region or country, as the case may be.

The clinical evidence submitted is on the basis of outcome of parasitaemia - a well established and generally accepted indicator of clinical effect in the evaluation of antimalarial drugs which act by killing parasites. The evidence does not establish that initial use of rectal artesunate will definitively reduce mortality from malaria. WHO have begun a program of large-scale Phase 4 studies to examine this aspect. However, given the reliability and time-tested acceptance of parasitaemia clearance as a valid

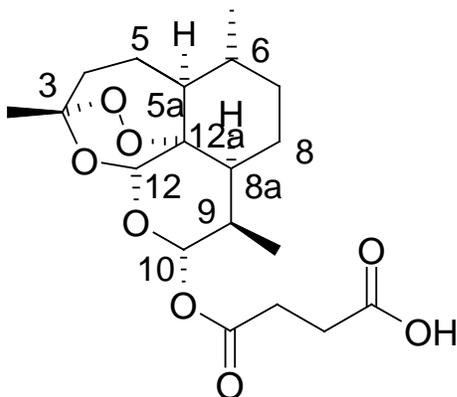
and reliable marker of prognosis in acute malaria, WHO submit that the data support the proposed strictly limited indication as stated in the data sheet/product information.

The applicant, WHO, respectfully informs the Anti-Infective Drugs Advisory Committee (AIDAC) that, in the oral presentation before the Advisory Committee, no attempt will be made comprehensively to review all the data in support of the application. Rather, a small number of special issues which required attention and explicit discussions with FDA over the past four years will be highlighted, including bioequivalence of the formulations that have been used in the development of the drug, the neurotoxic and hematotoxic potential of rectal artesunate, and the reproductive risk.

2 GENERAL *IN VITRO* INFORMATION

2.1 Mechanism of action of artesunate

Artesunate is defined as a semi-synthetic sesquiterpene belonging to the amorphane subclass of cadinane sesquiterpenes. It is the most water-soluble derivative of artemisinin, which is extracted from the leaves of *Artemisia annua* L. (qinghao). Artesunate has the following chemical structure:



The distinctive feature of artesunate (AS) and other active artemisinin-type compounds is the 1,2,4-trioxane pharmacophore (a six-membered ring containing three oxygen atoms), in which is embedded a peroxide bridge, essential for expression of its antimalarial activity. Artesunate is rapidly converted in the body into its principal metabolite, dihydroartemisinin (DHA), which is also highly active against the malaria parasite. The transformation *in vivo* occurs either chemically or enzymatically by hydrolysis of the ester linkage of the drug in position 10. Dihydroartemisinin preferentially accumulates in *P. falciparum*-infected erythrocytes *in vitro*; uninfected erythrocytes accumulate DHA less than two fold but a 300-fold concentration ratio develops between infected erythrocytes and plasma after inoculation of cells *in vitro* with 11.16 mmol/L of DHA.⁷

Two theories have been put forward for the mode of antimalarial action of the

artemisinin antimalarials, in accordance with the known properties of peroxides with medicinal activity.

The first assumes that the artemisinins must be activated by contact with either reduced haem (ferrous haem, Fe(II)PPIX) or non-haem ferrous iron (‘exogenous iron’), causing cleavage of the peroxide to generate oxygen-centred radicals (‘alkoxy radicals’) which are then presumed to be converted into ‘carbon-centred radicals’ by transfer of proximate hydrogen atoms from the periphery of the peroxide molecule. These ‘carbon-centred radicals’ are then thought to alkylate sensitive, yet unspecified, biomolecules in the parasite.^{15 16 17}

A second theory argues for a process in which the intact artemisinin binds to a site within a vital protein in the parasite. The act of binding causes the peroxide to be converted into a hydroperoxide or similar open peroxide, which in accord with known properties of such compounds, generates one or more active chemical entities, either oxidizing agents or oxygen transfer agents *per se*, or oxygen-centred free radicals.^{18,19}²⁰ This would be associated with the binding process. In such a way, the artemisinins might act as (irreversible) inhibitors. Iron may, or may not, be associated with the activation process. No specific biological target in the parasite has as yet been identified in support of this theory, but it may be membrane-bound proteins.²¹

Chemical studies involving experiments conducted with exclusion of oxygen, or in the presence of strong reducing agents, support the notion that carbon-centred radicals can form from artemisinins incubated with ferrous haem, or with exogenous ferrous iron. Only small amounts of products are formed, suggestive of the generation of free radical intermediates, although precise interpretations differ.^{21 22 23 24 25 26 27}

2.2 Spectrum of Antimalarial Activity

The asexual life cycle of the *P. falciparum* parasite is approximately 48 hours, only the first half of which is visible to the microscopist. During the exponential growth phase of *P. falciparum*, the principal parasite brood expands in rising sine wave patterns.²⁸ Sequestration in the micro-vasculature is thought to begin at 26 hours; intra-erythrocytic parasites start to induce expression of adhesins on the infected erythrocyte surface, parasitized and unparasitized erythrocytes aggregate (rosetting), and begin to adhere to the vascular endothelium (cytoadherence), leading to sequestration of parasites in the deep vasculature.³ Parasites remain sequestered for approximately 22 hours (i.e., almost half the life cycle) until merogony (schizogony) and re-invasion of erythrocytes are complete and a new cycle begins.²⁸ Sequestration impeding microcirculatory flow involves only erythrocytes containing the mature stages of the parasite (trophozoites and meronts) and not the younger, circulating parasites.³ Patients are more ill in the second half of the life cycle when the majority of the parasites are sequestered or just after schizont rupture (merogony).

The switch from asexual to sexual (transmissible) stage of the parasite appears to be triggered by a critical parasite density in the blood and requires that the infection has been present for several asexual cycles. Recrudescence infections have been present for much longer and are more likely to be associated with gametocytemia.²⁹

Of all existing antimalarials, the artemisinin compounds have the most rapid onset of action and the broadest stage specificity against *P. falciparum*. Artesunate attenuates

the growth of young parasites *in vitro* and increases the clearance of ring forms^{30 31}³² and meront (schizont) development³³ *in vivo*. Most studies confirm that artesunate has gametocytocidal activity and it is likely that it is for this reason that the drug has been effective in reducing transmission in low transmission areas of South East Asia.³⁴
35 36

Recently it has been established that after treatment with artesunate, the spleen removes the intra-erythrocytic parasite from the host red blood cells without host red cell destruction and returns the previously parasitized RBC to the circulation.^{37 38} This now explains why the haematocrit in some patients with heavy parasitaemia does not decrease to the extent that would be expected from destruction of the parasitized RBCs, and this effect is particularly evident following treatment with artesunate.³⁹

The activity of artesunate against *P. vivax* has not been assessed *in vitro*, although efficacy has been demonstrated in clinical studies.^{40 41}

2.3 Treatment outcome in severe malaria

In severe malaria, the first objective of treatment is to save life. This is achieved by speedily killing parasites and halting progression of the disease. A drug that affects a broad spectrum of parasite stages and kills a substantial number of parasites quickly should prevent both their multiplication and their sequestration. The point in the course of the infection at which the antimalarial is given (ideally before sequestration) and the rate of absorption of the antimalarial drug by the body are critical determinants of outcome. The objective in severe malaria is to achieve clinical stability and reversion to *per os* status so that curative treatment with oral or parenteral antimalarials might be commenced.

Severe malaria is characteristically associated with high parasitaemia. Parasites causing severe malaria have higher intrinsic multiplication rates, at higher parasite densities, than those causing uncomplicated malaria.⁴² In geographic areas of intense transmission, mainly in Sub-Saharan Africa, heavy parasitaemia is common in the vulnerable age group (young children), and the difference between a controlled and lethal infection may be a single multiplication cycle. Lethal infection often develops rapidly and unpredictably.²

Standard therapy for severe malaria in most countries in Africa is either intravenous or intramuscular quinine. The quinolines (quinine, quinidine) have little effect on asexual malaria parasites in the first half (24 h) of the life cycle of the parasite. As a result, exposure of ring form parasites to quinine does not significantly prevent sequestration; most circulating *P. falciparum* continue to mature, and cytoadhere despite treatment.⁴³ Exposure to quinine reduces rosetting but not cytoadherence. For these reasons, population parasitaemia time profiles following treatment with quinine show a lag phase, during which the population mean parasitaemia is unchanged, before declining.

In contrast, the artemisinin derivatives attenuate the growth of ring forms *in vivo*, with the result that there is an early decline in parasitaemia following treatment that is usually more rapid than the decline seen in treatment with quinolines. The population parasite clearance curves following artesunate show that it substantially outstrips quinine in the rate of parasite kill. The rapid and broad action of artesunate confers considerable potential to shorten the time to achieve clinical stability, making

the drug ideal for reversing a fast-developing and potentially lethal disease process.

None of the currently available antimalarial drugs, even when given intravenously, achieve both a life saving benefit *and* cure with single administration. Following immediate clinical and parasitological improvement with initial treatment, there invariably remain parasites that have to be eradicated by effective adjunctive treatment. Because severe disease is usually the result of high biomass infection,⁴² cure is determined by the number of remaining parasites to be removed and the use of a complementary drug that can effectively do so. When the eradication of parasites is incomplete, there is a higher propensity for recrudescence infections, and a greater chance of selection of resistant parasites.²⁹ The effective eradication of parasites in the early stages of high biomass, severe malaria infections is therefore important both for the clinical benefit of the patient and for reducing the potential for transmitting resistant infections.²⁹

2.4 Resistance to artesunate and to artemisinins in general

There is no convincing evidence yet of clinically relevant, stable, parasite resistance having developed to artesunate, or to the other artemisinins. Resistant strains of fresh *P. falciparum* have been established although not sustained *in vitro*.⁴⁴ Resistance of rodent malaria parasites to artesunate and regeneration of drug sensitivity can be induced experimentally in the mouse.⁴⁵ Stepwise discontinuous exposure of the parasite to increasing doses of artesunate has been reported to have generated steady state resistance.⁴⁶ Stepwise continuous exposure of *P. falciparum in vitro* to increasing concentrations of artesunate at regular intervals over 130 days is reported to result in IC₅₀ values about 3-fold higher than that of the parent isolate. When exposure of the drug-resistant strains to the drug was stopped, and the parasite was then cultured in drug-free medium for 5 weeks, its drug-resistance dramatically decreased to a level close to that of the drug-sensitive strains, suggesting that drug resistance of artesunate is unstable and dependent on presence of the drug in the *in vitro* situation.⁴⁷

In Thailand, two patients with *P. falciparum* malaria failed to respond to supervised artesunate therapy, although the *in vitro* drug susceptibility profiles showed no significant differences in IC₅₀ values for the primary and recrudescence isolates from established population values.⁴⁸ In another study of three patients who failed monotherapy with parenteral artemether⁴⁹ two may have been reinfected. A recent examination of antimalarial drug susceptibility using fresh *P. falciparum* parasite isolates from primary (268 isolates) and recrudescence (79 isolates) infections in highly multi-drug resistant areas of Thailand where the combination of artesunate-mefloquine has been deployed since 1995, demonstrated undiminished sensitivity of the parasites to artesunate; on the contrary, analyses in the areas concerned have shown a significant trend towards greater artesunate sensitivity from 1995 to 1999.⁶ However, comparison of parasite isolates from recrudescence infections with those from primary infections showed that the former had significantly higher IC₅₀ values for mefloquine, halofantrine, atovaquone and artesunate.⁶ Correlation of IC₅₀ values between the quinolines in general (quinine, mefloquine, halofantrine) and artesunate has been noted, but the Thai experience indicates that sensitivity to artesunate has not been compromised despite its large scale use together with mefloquine over a time (1995 until 1998) when there was significant resistance to mefloquine, which

would have left artesunate “unprotected” from drug pressure.

As a general rule, selection for resistance happens when a number of the infecting parasite population are able to survive antimalarial drug exposure. The resistant parasite population eventually multiplies, causing recrudescence, and producing gametocytes that are then transmitted by the mosquito vector to other individuals as primary infections.²⁹

There are several reasons why the development of artesunate resistance might have been delayed. The major effect of artesunate is to reduce substantially the infecting parasite biomass by a factor of approximately 10^8 , leaving only a small number of residual parasites; the fewer parasites there are, the less likely is a resistant mutant to emerge. In general, the risk of drug resistance developing is greatest when there is prolonged exposure to sub-therapeutic levels of the drug and/or its active metabolite(s). This does not happen with rectal artesunate. Artesunate has a short half-life and the duration of its action is sufficiently brief for it to be unlikely that parasites would be exposed to subtherapeutic concentrations of the drug for significant time.³³ Moreover, artesunate has gametocytocidal activity *in vivo*.^{50 36} Parasites in patients treated with artesunate are for this reason less likely to infect mosquitoes and to be transmitted to other patients.

Now that artesunate (and the other artemisinin derivatives) are more frequently used in combination with other antimalarials such as mefloquine or benflumetol in areas with high drug resistance,^{51 52 53} they are being more generally advocated elsewhere as part of combination therapy to delay antimalarial resistance.²⁹

3 CLINICAL PHARMACOLOGY AND PHARMACOKINETICS

Following administration to humans, artesunate is rapidly hydrolyzed to its principal active metabolite, dihydroartemisinin. Data from *in vitro* studies with human liver microsomes and from clinical studies suggest that DHA-glucuronide (10-position) is the principal Phase II metabolite of DHA and that uridine diphosphate glucuronyl transferase isoforms 1A1, 1A8-9, or 2B7 may be the main conjugating enzymes.

The pharmacokinetics of artesunate and dihydroartemisinin are characterized by marked inter-subject variability. The pharmacokinetic parameters of artesunate and dihydroartemisinin differ significantly between healthy volunteers and infected patients, and among patients with different disease severity.⁵⁴ Pharmacokinetic data from unbound plasma concentrations of artesunate or dihydroartemisinin should be interpreted with caution because the drug accumulates selectively in parasitized RBCs. In *in vitro* experiments, accumulation of dihydroartemisinin in infected RBCs is in concentrations approximately 300-fold higher than those in plasma.⁷

Several methods for measurement of artesunate and dihydroartemisinin concentrations in biological fluids have been developed but none has proven entirely satisfactory. A high performance liquid chromatographic method utilizing electrochemical detection (HPLC-ECD) is presently the most accurate and sensitive (lower limit of detection of 5 ng/mL), for detection and separation of artesunate and dihydroartemisinin in plasma.⁵⁵ The method has been validated in numerous laboratories; however, it requires immediate rigorous deoxygenation of plasma samples. Degradation of artesunate occurs during deoxygenation and throughout the

period the drug is in plasma, necessitating immediate processing and rapid cold storage of plasma samples prior to analysis. Thawing further degrades the drug in plasma samples. Thus, sample preparation and handling have a marked impact on assay results.

All members of the artemisinin class of chemical compounds are highly active antimalarials that reliably cause large reductions in parasite biomass in each asexual cycle.

The studies included in the pharmacokinetic analysis of data associated with intrarectal administration of artesunate are presented in the following Table. Results from studies 1 and 2 have not been included. Results from studies 3, 4, 7, and 9 are included in this report.

Summary of Clinical Studies Including Pharmacokinetic Analysis of Data	
Study	Description
1	Comparison of oral and rectal AS in healthy volunteers using an earlier pharmaceutical product of rectal AS (designated F1)
2	Bioequivalence study involving two earlier pharmaceutical products of rectal AS (designated F1 and F2) in healthy volunteers
3	Comparison of IV AS (2.4 mg/kg) and rectal AS (10 mg/kg and 20 mg/kg) in adult patients with moderately severe malaria
4	Comparison of IV AS (2.4 mg/kg) and rectal AS (10 mg/kg and 20 mg/kg) in pediatric patients with moderately severe malaria
5	Comparison of rectal AS (10 mg/kg) and oral AS in Thai pediatric patients with uncomplicated hyperparasitaemia
6	Comparison of rectal AS (10 mg/kg) and parenteral quinine in Malawian pediatric patients with moderately severe malaria
7	Comparison of rectal AS (10 mg/kg) and parenteral quinine in South African adults with moderately severe or severe malaria
8	Comparison of rectal AS (10 mg/kg) and parenteral quinine in Brazilian adults with moderately severe malaria
9	Bioequivalence study in healthy volunteers involving the rectal AS pharmaceutical product used in pivotal studies (F2) and the proposed product for marketing (F3)

3.1 Pharmacokinetics in Healthy Volunteers

The pharmaceutical products proposed for marketing (here referred to as F3, available as 100 mg and 400 mg rectal capsules) are qualitatively identical to the product used in the pivotal WHO Phase 3 studies (referred to as F2, available as 50 mg and 200 mg rectal capsules). However, the active ingredient in F2 was not synthesized according to Good Manufacturing Practices (GMP). Therefore, Study 9 was undertaken to establish the bioequivalence of F3 (synthesized according to GMP) and F2 (used in Studies 5, 6, 7, and 8).

Study 9 was a randomized, single-blind, three-way crossover study in 42 healthy male volunteers who received each of the following single rectal doses of study medication in random order: A) 2 × 200 mg AS rectal capsules [F2, reference product]; B) 4 × 100 mg AS rectal capsules [F3, test product]; and C) 1 × 400 mg AS rectal capsule [F3, test product]. Subjects were hospitalized for 48 hours during each of three study periods separated by at least seven days. Blood samples for the measurement of AS and DHA

concentrations in plasma were collected at serial time points from pre-dose to 24 hours post-dose.

The figures on the next page depict the mean plasma concentration versus time profiles of AS and DHA, respectively. Mean maximum plasma concentrations of AS and DHA occurred earlier following intrarectal administration of 4 × 100 mg capsules of the test product compared with 1 × 400 mg of the test product or 2 × 200 mg of the reference product. This may be at least partly explained by the number of rectal capsules constituting the dose. Four rectal capsules may have been absorbed more quickly as a result of the greater surface area available to the mucosa for absorption compared to an equivalent dose administered as one or two rectal capsules. Overall, these profiles indicate that average drug exposures were similar for both test and reference products, for both the parent drug and the principal metabolite, DHA.

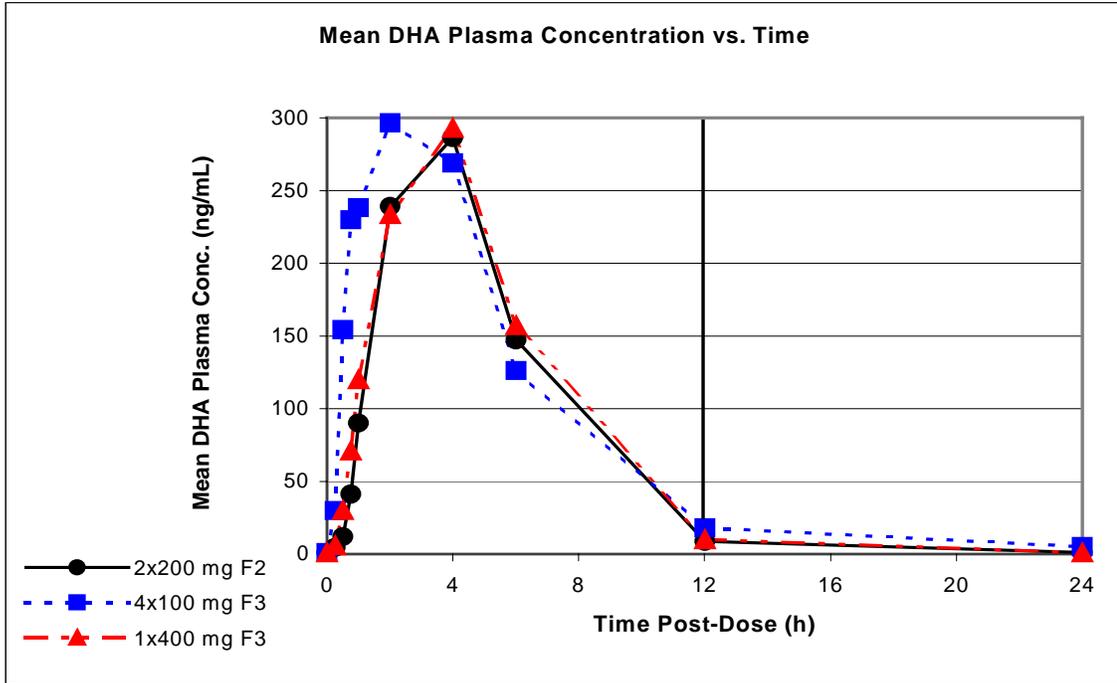
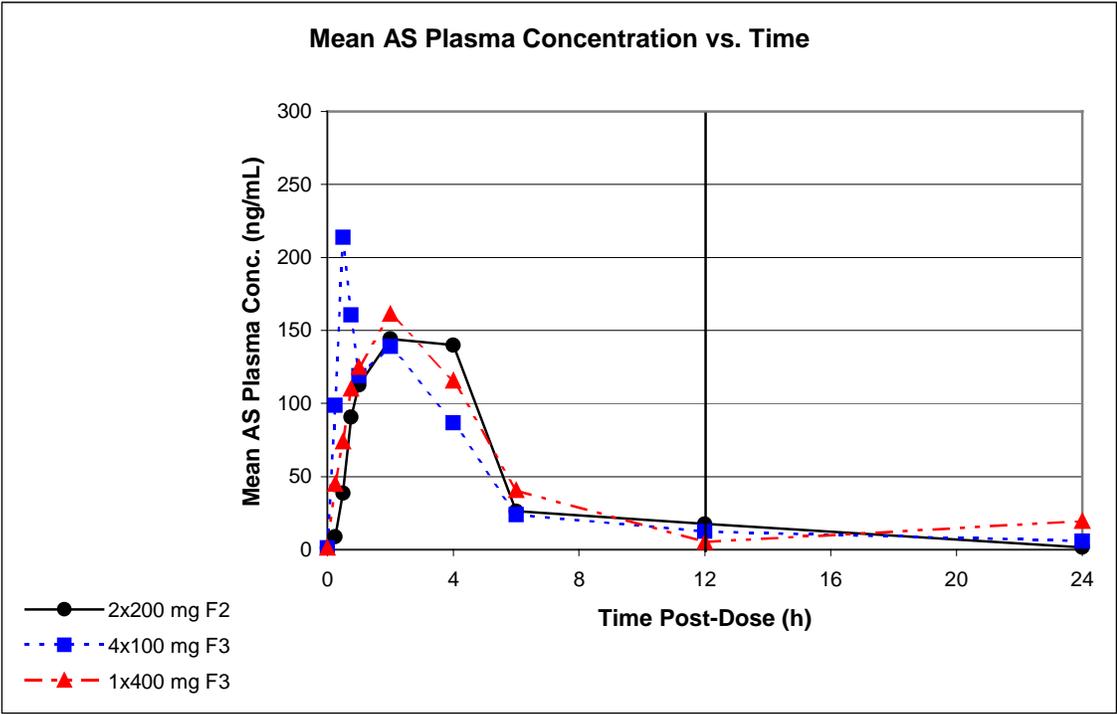
The table below presents the mean (%CV) pharmacokinetic parameters determined in 40 adult subjects with evaluable data (ages 18 to 44 years) and a mean (SD) weight of 58.5 (6.52) kg.

Mean (%CV) Cmax and AUC _(0-t) of AS and DHA in Healthy Male Volunteers ^a				
Regimen	Artesunate (AS)		Dihydroartemisinin (DHA)	
	Cmax [ng/mL]	AUC _(0-t) [ng·h/mL]	Cmax [ng/mL]	AUC _(0-t) [ng·h/mL]
A) 2 × 200 mg [F2]	257 (75.2)	845 (106)	383 (56.7)	1411 (60.1)
B) 4 × 100 mg [F3]	293 (76.5)	733 (75.8)	442 (52.5)	1692 (79.8)
C) 1 × 400 mg [F3]	261 (65.4)	1053 (130)	399 (60.2)	1374 (70.4)

^a Cmax is the observed maximum plasma concentration and AUC_(0-t) represents the area under the plasma concentration versus time curve from time zero (dosing) until the end of the sampling schedule (24 h post-dose)

No statistically significant differences in AUC_(0-t) or Cmax for either AS or DHA were noted among the three arms of the study. The data indicate that the resulting drug exposures were similar in the test and reference pharmaceutical products. In addition, ratios of geometric mean AUC_(0-t) and Cmax values in individual subjects were close to 1.00 in many cases (data not shown). Using an average bioequivalence approach, however, the data obtained in this study do not in every respect support the bioequivalence of F3 and F2, as reflected in the table below.

Bioequivalence Assessment: Ratio of Geometric Means (90% Confidence Interval)				
Comparison	Artesunate (AS)		Dihydroartemisinin (DHA)	
	Cmax [ng/mL]	AUC _(0-t) [ng·h/mL]	Cmax [ng/mL]	AUC _(0-t) [ng·h/mL]
B to A	1.22 (0.88-1.69)	0.89 (0.55-1.45)	1.22 (0.97-1.53)	1.12 (0.83-1.52)
C to A	0.92 (0.66-1.28)	0.99 (0.60-1.72)	1.05 0.83-1.32	0.98 (0.71-1.35)



3.2 Pharmacokinetics in Patients

Studies 3 and 4 were randomized, open label, crossover trials in 48 adults (16 to 50 years old) and 36 pediatric patients (2 to 7 years old), respectively, with moderately severe malaria. These studies compared single doses of intravenous (2.4 mg/kg bolus) and intrarectal (10 mg/kg or 20 mg/kg) AS administered 12 hours apart after admission to a health care facility. Study 3 was conducted in Thailand and included subsequent administration of mefloquine as definitive therapy. Study 4 was conducted in Ghana and included subsequent administration of chloroquine (or sulfadoxine/pyrimethamine if a patient had a history of chloroquine intolerance).

Study 7 was a randomized, open label comparison between rectal AS and parenteral quinine in South African adults with moderately severe or severe malaria. In this study, a subset of 27 patients (16 to 58 years old; mean weight of 62 kg) with moderately severe malaria received a single intrarectal dose of AS 10 mg/kg followed by sulfadoxine/pyrimethamine (or 10 mg/kg intramuscular quinine if unable to tolerate oral medication) 24 hours later.

The table below summarizes the results of non-compartmental analyses that were performed using serial plasma concentration data (pre-dose through 12 hours post-dose in studies 3 and 4; pre-dose through 8 hours post-dose in study 7) associated with intrarectal administration of AS in these studies.

Mean (SD) Pharmacokinetic Parameters of AS and DHA Obtained Using Non-compartmental Methods (Studies 3, 4, and 7)						
Study Group	Artesunate (AS)			Dihydroartemisinin (DHA)		
	Cmax [ng/mL]	Tmax [h]	AUC [ng·h/mL]	Cmax [ng/mL]	Tmax [h]	AUC [ng·h/mL]
Study 3 10 mg/kg	794 (1011)	2.25 (1.89)	3071 (3970)	1097 (741)	2.80 (1.71)	4296 (4594)
Study 3 20 mg/kg	982 (1044)	3.39 (2.28)	3776 (3464)	2018 (1620)	4.19 (2.10)	11424 (11911)
Study 4 10 mg/kg	367 (339)	1.15 (0.77)	837 (854)	808 (406)	1.80 (0.89)	2783 (2010)
Study 4 20 mg/kg	536 (493)	1.51 (0.88)	1693 (2071)	1133 (845)	2.29 (1.22)	5275 (6876)
Study 7 10 mg/kg	380 (386)	3.5 (2.3)	1672 (1226)	857 (681)	4.4 (2.3)	4056 (3073)

Caution should be exercised in interpreting the results from study 7 and in comparing them with results from other studies since the sampling scheme was abbreviated in study 7. Artesunate and DHA pharmacokinetic parameters exhibited wide variability in each of the studies, with coefficients of variation often exceeding 100%. On average, maximum plasma concentrations of AS and DHA occurred later in adults than in pediatric patients. Mean drug exposures were greater in adults than in pediatric patients. In studies 3 and 4, the 20 mg/kg dose resulted in larger average AS and DHA exposures than the 10 mg/kg dose; however, there was considerable overlap in drug exposure between the doses. In study 4, pharmacokinetic/pharmacodynamic analysis did not identify any significant correlation between pharmacokinetic parameters and

age, body mass index, axillary temperature, parasite count at 12 hours, parasite clearance time, and the time for baseline parasite counts to decrease by 50% and 90%. Results from these studies also demonstrate that AS and DHA plasma exposures (i.e., C_{max} and AUC, taking actual dose administered and body weight into consideration) were greater in patients infected with *P. falciparum* than in healthy volunteers (study 9).

Compartmental modeling (utilizing a population approach) was also performed using data from studies 3 and 4. An inadequate number of measured plasma concentrations of AS precluded analysis of these data; therefore, the analysis was limited to DHA data associated with intrarectal administration of AS. The most robust structural model to fit the data was a one-compartment model with first order absorption and elimination including parameters for lag time and bioavailability. The influences of age, weight, body surface area, gender, and selected clinical chemistry results on clearance and volume of distribution were evaluated. In addition, the effects of the number of suppositories administered, age, gender, and selected clinical chemistry results on bioavailability were determined, and the influences of the number of suppositories administered, age, gender, and weight on the absorption rate constant were evaluated. The following table presents the DHA pharmacokinetic estimates that were obtained from this analysis in which data from 10 mg/kg and 20 mg/kg intrarectal doses of AS were pooled within each patient population.

DHA Pharmacokinetic - DHA (Studies 3 and 4)	Estimates Obtained From Study 3 (Adult)	Compartmental Analysis Study 4 (Pediatric)
Number of evaluable patients	48	34
Clearance	40 L/h (0.83 L/h/kg)	23.3 L/h (1.93 L/h/kg)
Volume of distribution	58.9 L (1.22 L/kg)	29.3 L (1.69 L/kg)
Plasma half-life	61 minutes	52 minutes
Bioavailability	43%	45%
Absorption rate constant	0.0864 h ⁻¹	0.254 h ⁻¹
Absorption lag time	12 minutes	5.6 minutes

In adults, clearance of DHA was linearly related to hemoglobin levels below 10 mg/dL with clearance decreasing as hemoglobin levels decreased. Adult females had a volume of distribution significantly lower than that in adult males; however, the wide therapeutic index of AS and lack of a clear dose-response relationship in this dosing range make special dosing adjustments in women unnecessary. Bioavailability (overall estimate of 43%) decreased as the number of rectal capsules constituting a dose increased; however, interindividual variability in bioavailability was high (%CV=63%).

In pediatric patients, the effects of weight, body surface area, age, and hematocrit on DHA clearance were evaluated in addition to the influences of weight, body surface area, and age on the apparent volume of distribution of DHA. None of these covariates had a statistically significant influence on clearance or apparent volume of distribution of DHA. In contrast to adults, the typical value for bioavailability of a 10mg/kg dose given in one rectal capsule (31.1%) was lower than that for the same dose given in two rectal capsules (45.5%). The coefficients of variation associated with bioavailability estimates were approximately 35%.

3.3 Discussion

Results from study 9 do not support in every respect bioequivalence between the pharmaceutical products that are proposed for marketing and the products used in pivotal Phase 3 studies. However, statistical testing shows that there were no significant differences in C_{max} or AUC between the products. While power calculations were not performed, the marked intersubject pharmacokinetic variability observed in healthy volunteers would require an exceedingly large number of subjects to fully establish bioequivalence according to standard criteria. Furthermore, the significant differences in pharmacokinetics of AS and DHA between malaria patients and healthy volunteers make data extrapolation from healthy subjects to infected patients imprudent. Reasons for these differences between healthy volunteers and infected patients may include: alterations in protein binding; the likelihood that parasitized red blood cells become an additional compartment for drug distribution (at least for DHA); alterations in hepatic and renal function; dehydration, fever, and other common clinical features associated with malaria.

Study 14 was conducted in Thailand to examine the therapeutic equivalence of the pharmaceutical products using as a primary endpoint fractional reduction in parasitaemia 24 hours after admission to the research ward. Results from this study of 69 adult patients with moderately severe malaria make it clear that the pharmaceutical products are equivalent. The reduction in parasite counts (fractional reduction in parasitaemia) at 12 and 24 hours in the groups receiving the products proposed for marketing was equivalent to the change obtained with the reference product (difference in median based on the 87.5% CI was less than 2%). In addition, there were no differences between groups receiving the products proposed for marketing and the reference product with respect to median parasite clearance times, time to resolution of fever, mean temperature, percentage of patients returning to *per os* status at 24 hours, percentage of patients able to take the first oral dose of mefloquine, nor the percentage of patients requiring rescue antimalarial treatment prior to 24 hours post-dosing of rectal artesunate. Pharmacokinetic data were not obtained in this study.

In study 14, as in all the pivotal clinical studies conducted, rectal administration of AS has resulted in predictable, reproducible, substantial and rapid reductions in parasitaemia. However, the pharmacokinetics of AS and DHA do not predict parasite clearance or clinical response at the doses used (i.e., 10 mg/kg and 20 mg/kg intrarectally). Indeed, impressive reductions in parasitaemia were observed in patients with the lowest plasma drug exposures. None of the studies has provided an indication of a minimal parasiticidal plasma concentration or drug dose. The most likely explanation is that parasite clearance rates were maximal in all treatment groups. Since a concentration-dependent killing of parasites has not been demonstrated either for AS or DHA, it is not surprising that an identifiable drug exposure-response relationship for rectal artesunate remains elusive.

A study designed to characterize the dose-response relationship of oral artesunate in Thai patients with acute uncomplicated *P. falciparum* malaria suggested that no further acceleration of parasite clearance can be achieved at single doses greater than 2 mg/kg and that this dose reflects the average lower limit of the maximally effective dose in an average patient.⁵⁶ In uncomplicated malaria, it is the extent of the absorption of the antimalarial drug, rather than the rate of its absorption, which is

important. In severe malaria, when the objective of treatment is to save life, speed is of the essence, and the rate of drug absorption from an extravascular site can be a critical determinant of outcome. It has been deemed important to decide on a dose that minimises the possibility that a patient will fail to absorb the necessary amount of drug to save their life.

It is particularly important that sufficient dose of artesunate should be given since there is no convincing evidence in any of these studies of greater drug toxicity in patients with the highest plasma drug exposures. Indeed, AS has a remarkably wide therapeutic index. In summary, despite marked intersubject variability in plasma concentrations of AS and DHA, rectal administration of AS has consistently been shown to be safe and effective at all measured levels of plasma drug exposure, which are notably higher than those needed to reduce parasitaemia. For these reasons it is argued that comparable reductions in parasitaemia and improvements in markers of clinical response are the most appropriate for demonstrating the equivalence of the pharmaceutical products in question, and on these grounds it is submitted that the requirements for demonstrating therapeutic equivalence have been fully met.

4 RECTAL ARTESUNATE SINGLE-DOSE CLINICAL PROGRAMME: EFFICACY

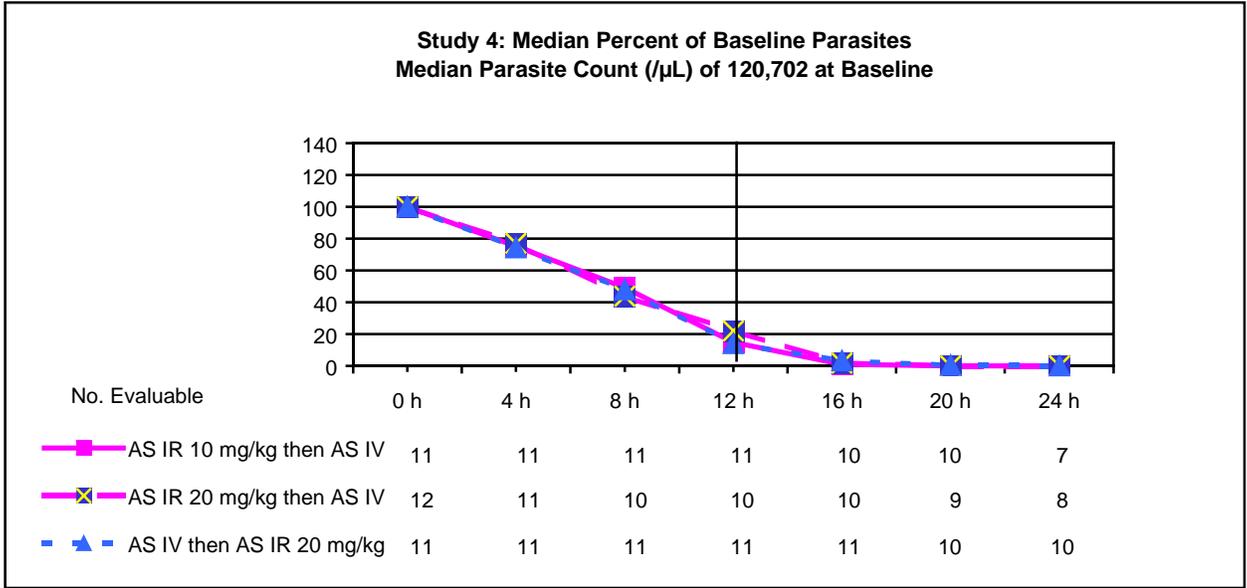
4.1 Summaries of Individual Studies in Children in Support of Efficacy of Rectal Artesunate in Acute Malaria

4.1.1 Study 4

Study 4 was a Phase 2 randomized trial of artesunate suppositories and intravenous artesunate in Ghanaian pediatric patients with moderately severe malaria (*non per os* and having asexual *P. falciparum* parasitaemia >10,000 parasites/ μ L). The trial was conducted within a pediatric ward at Komfo-Anokye Teaching Hospital, Kumasi, Ghana, in an endemic area with perennial malaria, between August and September 1996. There is limited information on the prevalence of *P. falciparum* resistance to chloroquine and sulfadoxine-pyrimethamine in Kumasi, Ghana. A total of 36 patients aged 18 months to 7 years were enrolled. Thirty-four (34) patients were randomized to three treatment groups: AS rectal 10 mg/kg; AS rectal 20 mg/kg; and AS intravenously 2.4 mg/kg. After 12 hours, all patients receiving rectal AS were given intravenous AS and the patients initially receiving intravenous AS were given rectal doses of 20 mg/kg AS. At 24 hours, a 3-day course of chloroquine was given as the follow-up treatment of choice, with sulfadoxine-pyrimethamine for chloroquine-intolerant patients. Drug-free follow-up took place 7 and 21 days after treatment, and patients with persistent parasitaemia at the follow-up visit received quinine for 7 days.⁵⁷

As shown in the figure below, all patients receiving artesunate treatment had prompt decreases in parasite counts at 4, 8, and 12 hours after treatment with either of the formulations. At 12 hours, 29 of 34 patients had greater than 50% reduction from baseline in absolute parasite count.

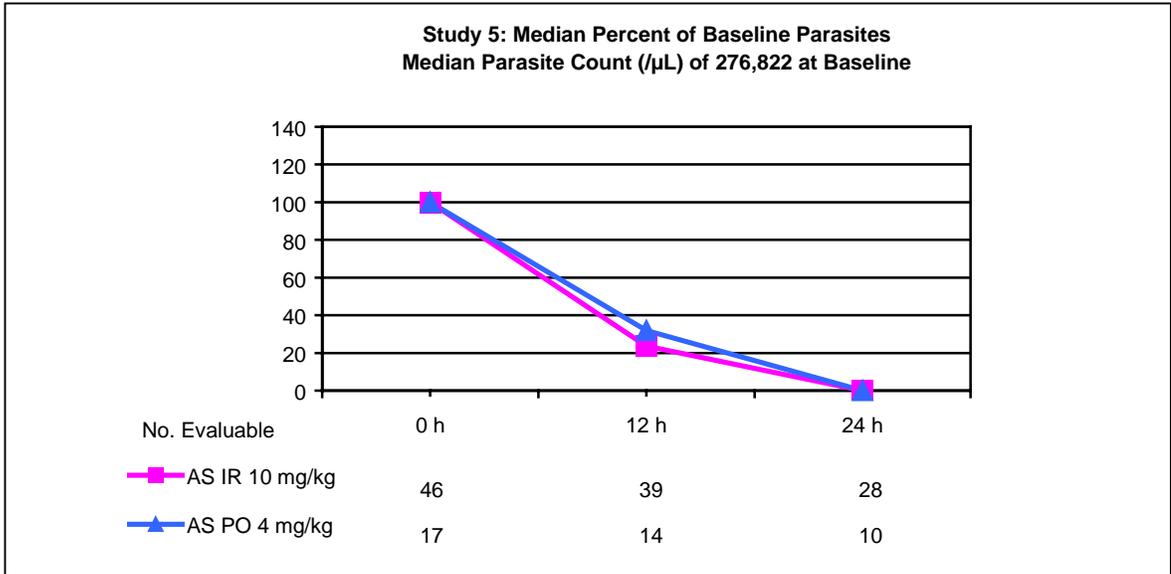
Twenty-three (23) patients received chloroquine (for 3 days) after artesunate and received sulfadoxine-pyrimethamine (a total of 32 patients). Twenty-one (21) of the initial 34 patients were examined between 14 and 28 days after treatment. Nine (9) patients failed treatment: 6 after chloroquine and 3 after sulfadoxine-pyrimethamine. All received quinine and were without parasitaemia at subsequent follow-up.



4.1.2 Study 5

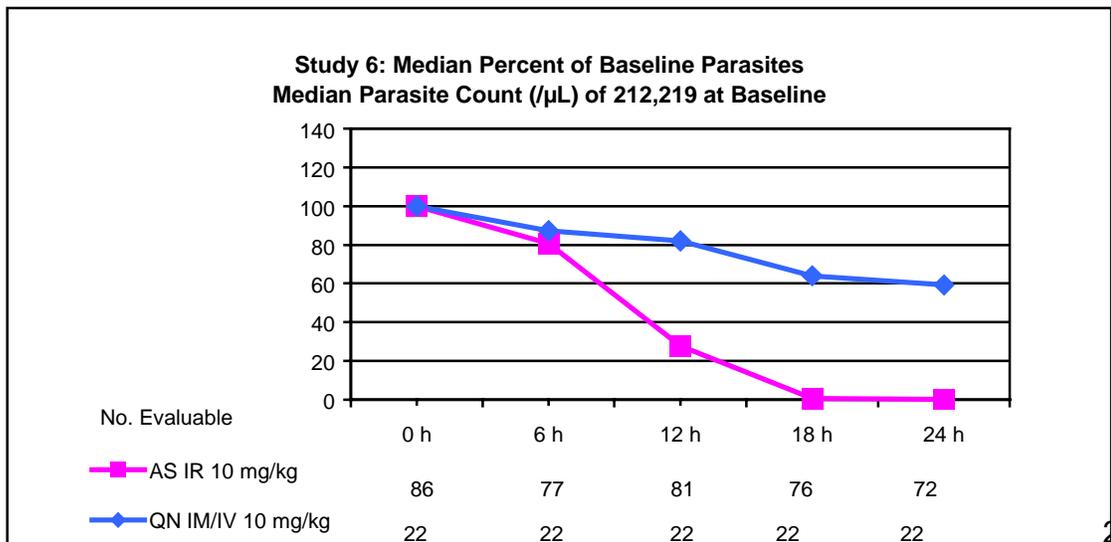
Study 5 was a Phase 3 open-label, randomized, pharmacokinetic-pharmacodynamic comparison between rectal and oral artesunate therapy in Thai children with *P. falciparum* hyperparasitaemia (>4% of peripheral red blood cells with asexual stages) who were *per os* but where previous data indicated that hyperparasitaemic children have a higher risk of mortality.⁵⁸ The median parasite count at baseline was 276,822/ μL . The study was conducted at the Shoklo Malaria Research Unit, Mae La, Thailand, from 1997 to 1999. This area has the highest levels of multi-resistant malaria worldwide, and patients with hyperparasitaemia were previously required to be treated with intravenous quinine, until decreasing efficacy of quinine was documented and oral artesunate became the treatment of choice. Transmission occurs throughout the year, with two seasonal peaks in May-July and December-January. A total of 63 children, aged 6 months to 15 years, were enrolled. Forty-four (44) patients received rectal artesunate 10 mg/kg and 17 patients received oral artesunate 4 mg/kg. Patients receiving either of these dose schedules received oral artesunate 24 hours after initial dosing followed by mefloquine initiated at 48 hours. Any patient failing to respond or deteriorating during the first 24 hours was to receive parenteral artemether.

Assessments of parasite counts and clinical outcome occurred at 12 and 24 hours and at either 28 or 35 days after treatment. Fractional reduction in parasitaemia was 76.4% at 12 hours and 100% at 24 hours in all patients receiving rectal artesunate. Decline in parasitaemia was slightly less at 12 hours (68.1%) in patients receiving oral artesunate, but was 100% at 24 hours. No treatment failures were documented following sequential treatment with oral artesunate and mefloquine of those patients who were evaluated (28 of 44 receiving rectal artesunate and 9 of 17 receiving oral artesunate). The figure below illustrates the decline in parasitaemia in both treatment groups over 24 hours.



4.1.3 Study 6

Study 6 was a Phase 3 open-label, randomized, pharmacokinetic-pharmacodynamic comparison between rectal artesunate and parenteral quinine in children aged 1 to 10



years with moderately severe malaria (defined as presence of asexual *P. falciparum* parasitaemia >20,000 and <500,000 parasites/ μ L) who were *non per os*. The trial included children with a Blantyre coma score of 3-4. The trial was conducted at the Malaria Project and Pediatrics Department, Queen Elizabeth Central Hospital, Blantyre, Malawi from July 1997 through June 1998. This area is endemic for perennial malaria, with most transmission occurring in July and August.

Patients were randomized to two treatments. One group (n=86) received a single rectal dose of artesunate 10 mg/kg followed by oral sulfadoxine-pyrimethamine at 24 hours. The second group (n=22) received parenteral quinine (intramuscular or intravenous) at 0 and 4 hours and at 12 hours (and every 12 hours thereafter); however, if the child regained *per os* status, oral sulfadoxine-pyrimethamine could be substituted for quinine. Patients in the artesunate group were withdrawn if the parasite count at 12 hours was >60% of the admission count or if signs or symptoms of severe malaria were present; 10 patients were withdrawn at 12 hours.

Among the 86 patients receiving artesunate, 74 received sulfadoxine-pyrimethamine as follow-up treatment, 9 received quinine (one with incomplete record of SP administration), 2 had incomplete treatment records, and 1 patient absconded. 39 of 86 patients treated with rectal artesunate (45.3%) had a positive parasite smear on follow-up (RI). Among the 74 patients receiving artesunate followed by sulfadoxine-pyrimethamine, there were 33 RI responses (33/74, 44.6%). Of the 8 patients receiving artesunate followed by quinine and then sulfadoxine-pyrimethamine, there were 4 RI responses (4/8, 50%). Among the 22 patients treated with quinine (and not artesunate) there were 5 RI responses (5/22, 22.7%) (OR 0.35 [95% CI 0.22-1.15]). Most patients receiving quinine (68%) received a total of 4 doses. There were no RII or RIII responses in any of the treatment groups.

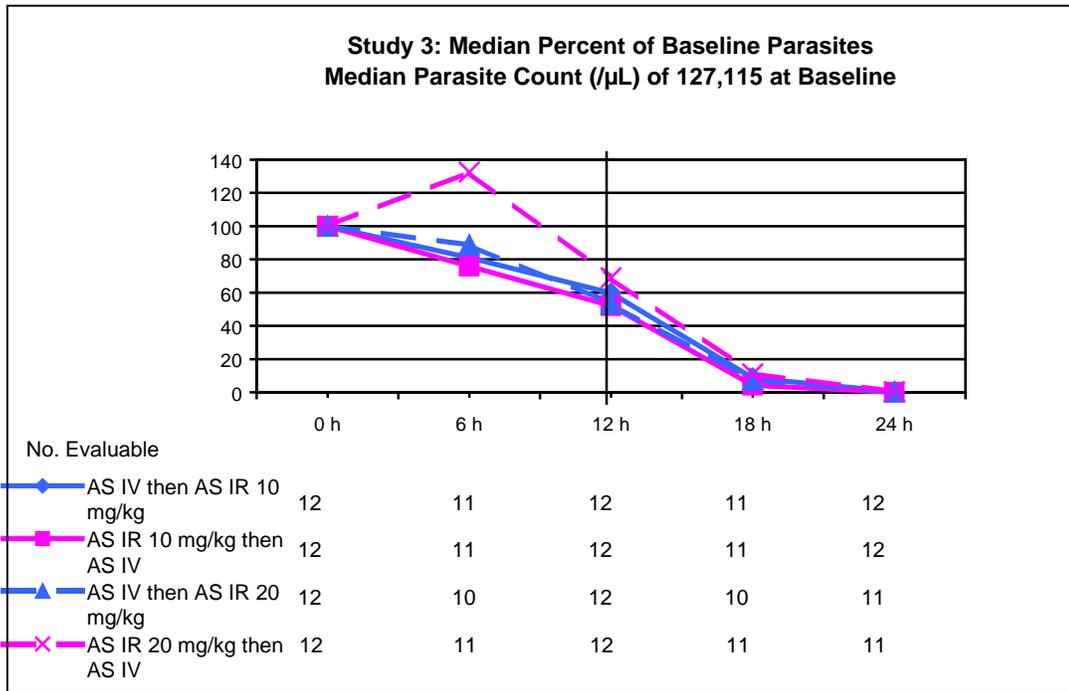
The figure above illustrates the more rapid decrease in parasite count observed over the first 24 hours in the group of children receiving rectal artesunate compared with parenteral quinine. The median fractional reduction in parasite count at 12 hours was nearly 80% in the artesunate group compared with approximately 20% in the quinine group. At 24 hours, the fractional reduction was 100% with artesunate and 40% with quinine.

4.2 Individual Studies in Adults in Support of Efficacy of Rectal Artesunate in Acute Malaria

4.2.1 Study 3

Study 3 was a Phase 2 open-label, randomized, crossover pharmacokinetic comparison between rectal artesunate and intravenous artesunate in Thai adults who had moderately severe malaria and were *non per os*. The trial was conducted from June through December 1996 at the Bangkok Hospital for Tropical Diseases, Mahidol University, Bangkok, Thailand. A total of 48 adults aged 16 to 50 years were enrolled. Two of the treatment groups received rectal artesunate (either 10 mg/kg or 20 mg/kg) followed at 12 hours by intravenous artesunate (2.4 mg/kg). Two groups received intravenous artesunate initially followed at 12 hours by one of the two doses of rectal artesunate. All patients received mefloquine at 36 and 48 hours after initial treatment and all were hospitalized for the 28 days of observation.

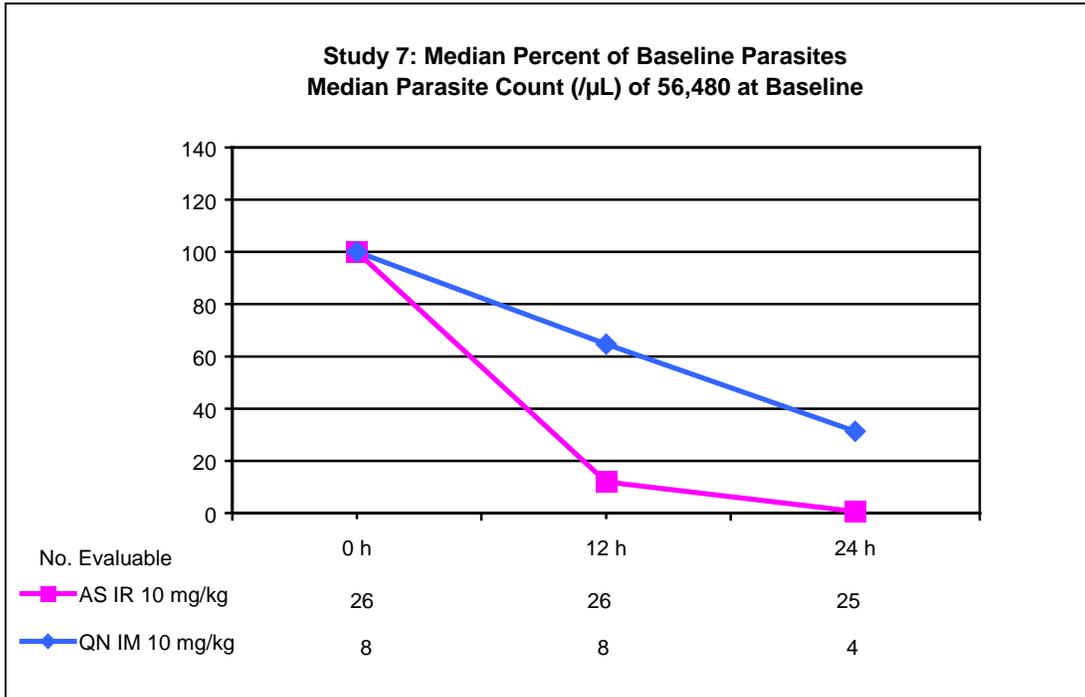
As shown in the figure below, there were comparable reductions in parasitaemia with the rectal and intravenous treatments over the first 12 hours of observation.



4.2.2 Study 7

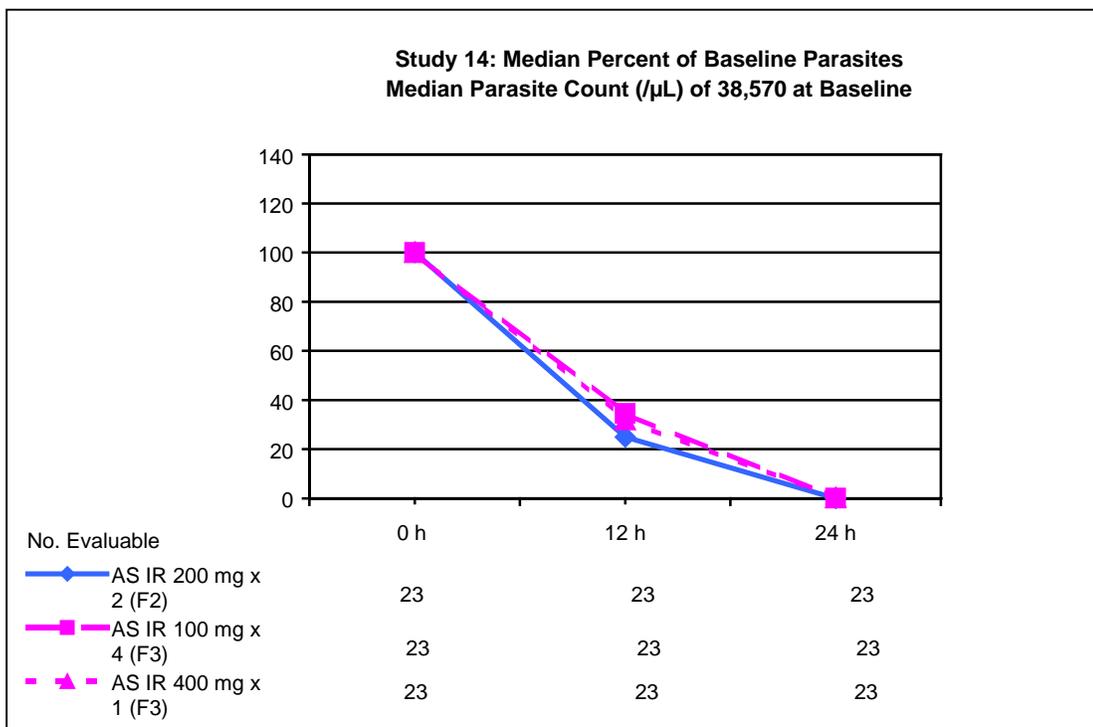
Study 7 was a Phase 3 open-label, randomized, pharmacokinetic and pharmacodynamic comparison between rectal artesunate and parenteral quinine in South African adults with moderately severe malaria. The trial was conducted at Mosvold District Hospital, Northern KwaZulu Natal, South Africa, an area with low seasonal malaria transmission which peaks between February and May. A total of 35 adults were treated; 27 received rectal artesunate 10 mg/kg followed by oral sulfadoxine-pyrimethamine at 24 hours, and 8 received intramuscular quinine (10 mg/kg) at 0, 4, and 12 hours followed by oral sulfadoxine-pyrimethamine at 24 hours. Patients receiving either treatment who developed any signs or symptoms of severe malaria or who had parasite density at 12 or 24 hours of >60% of baseline density were to be rescued immediately with intravenous quinine. Follow-up examinations were conducted 7, 14, and 42 days after therapy.

As shown in the figure below, there was a more rapid decline in parasitaemia in the group receiving treatment with rectal artesunate than in the group receiving parenteral quinine. At 24 hours, artesunate had caused a 100% median decrease in parasitaemia, whereas quinine resulted in approximately a 70% median decrease in parasitaemia.



4.2.3 Study 14

Study 14 was a three-arm randomized, single-blinded comparison of the pharmacodynamics of two new dosage formulations of rectal artesunate (F3 100 mg and F3 400 mg) with the reference formulation (F2 200 mg) that had been used in previous clinical studies.



The study was conducted in the research ward of the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. The trial was conducted from June through November 2000. A total of 69 adult patients with moderately severe malaria (presence of asexual *P. falciparum* parasitaemia >100,000 parasites/ μ L and/or *non per os* status with no vital organ dysfunction) were enrolled. Twenty-three (23) patients were randomly assigned to each treatment arm. Rectal artesunate was given in a dose of 10 mg/kg with all formulations. At 24 hours, all patients who had returned to *per os* status received oral artesunate and those who were still *non per os* received intravenous artesunate.

As shown in the figure above, the median fractional reductions in parasitaemia in all groups were similar at 12 and 24 hours. At 12 hours the median fractional reductions ranged from 65.5% to 75%, and all were 100% at 24 hours.

4.3 Overall Summary of Efficacy Studies

There are no data, except from the pivotal trials commissioned by WHO, that supports the indication for the drug, dose and route.

The data on efficacy of rectal artesunate given as a single 10mg/kg dose over 24 hours support the indication achieving 99-100% reduction of parasitaemia at 24 hours. This compares favourably with the median reduction of parasitaemia for parenteral quinine of 42-72% at 24 hours. The following two tables summarize the effect of artesunate therapy on parasitaemia at both 12 hours and 24 hours after rectal dosing of artesunate at a dose of 10 mg/kg in pediatric and adult studies, respectively.

Pediatric Studies: Median Percent Reduction in Parasitaemia at 12 and 24 h after a Single Dose AS 10 mg/kg (all ages < 15 years)					
Rectal Artesunate	12 h (No. of Subjects)	24 h (No. of Subjects)	Comparator	12 h (No. of Subjects)	24 h (No. of Subjects)
Study 4	85.3% (11)		IV artesunate	85.9% (11)	
Study 5	76.4% (46)	100% (28)	Oral artesunate	64.6% (17)	100% (10)
Study 6	72.3% (81)	99.9% (72)	IV or IM quinine	18% (22)	40.8% (22)

Adolescent/Adult Studies: Median Percent Reduction in Parasitaemia at 12 and 24 h after a Single Dose AS 10 mg/kg (age 16- 60 years)					
Rectal Artesunate	12 h (No. of Subjects)	24 h (No. of Subjects)	Comparator	12 h (No. of Subjects)	24 h (No. of Subjects)
Study 3	47.9% (12)		IV artesunate	46.5% (24)	
Study 7	88.1% (26)	99.4% (26)	IM quinine	35.2% (8)	72.2% (8)
Study 14	68.3% (69)	99.9% (69)	None		

These data demonstrate that rectal artesunate provides prompt and consistent antimalarial efficacy against *P. falciparum* infections in children and adults over the 24 hours immediately following a single rectal dose (10 mg/kg). The effect on parasitaemia of a single dose of rectal artesunate was superior to that of parenteral quinine given in 3-7 doses to patients unable to take oral drugs. When intravenous artesunate was used as the comparator, rectal artesunate given in a single dose performed as effectively as the intravenous drug over the first 24 hours.

The data referred to above provide evidence of benefit based upon a surrogate endpoint that reasonably suggests clinical benefit. Therefore the definitive evidence of life saving benefit and safety is being established in ongoing large scale randomised, controlled Phase IV trials in 3 countries (57 FR 58942, December 11, 1992). These trials are intended to provide proof beyond reasonable doubt that for all patients, or for some specific types, the use of rectal artesunate is indicated or clearly contraindicated in terms of a net difference in all-cause mortality or neurological sequelae. A total of 3366 patients were enrolled by 19th March 2002, 1459 patients in Bangladesh, 991 in Ghana and 916 in Tanzania. The overall mortality rate was 3% (4% in children, 1% in adults). The Data Safety Monitoring Committee have examined the results by treatment group and have informed the WHO that there is no reason for any of the interim findings to be unblinded, or for the trial protocol to be modified.

5 SAFETY OF ARTESUNATE

In the clinical context for which rectal artesunate is being proposed in this submission the risk of death ranges between 4-46% (weighted mean 18.6%¹) if the patient reaches a hospital where treatment is given, and it is virtually certain if not. The safety of rectal artesunate given in the manner proposed would need to be considered in relation to the sequelae of severe malaria when not treated in the first few hours of illness. It should also be compared with the safety of alternative drugs such as quinine, halofantrine or mefloquine when given in the acute phase of the illness, when it is possible to do so. The disease and its complications advance rapidly and the severely ill patient not able to take oral medications, without immediate access to

parenteral medication, has no other drug therapy available. The balance of risk and benefit is presented in the light of these considerations.

The assessment of safety draws heavily on the large body of clinical safety information available for artesunate. This compound - in different formulations - is in widespread use and the human safety database is extensive. These data include pivotal and non-pivotal trials with rectal artesunate, as well as information on the clinical use of the artemisinin derivatives (15 567 patients) including artesunate (7006 patients) amassed over the past 15 years in South East Asia.¹³ Adverse drug event data were collected on 13,639 of these patients with malaria; no findings of concern were noted.¹³ Careful neurological examinations have also been made in 1 971 of 3003 patients treated with the artemisinins, 1664 in patients treated with artesunate.¹⁴ Apart from Study 9 and Study 14, the active ingredient upon which the safety data derive has not been produced to GMP. These data support the remarkable safety profile of artesunate when used in the management of acute malaria.

The assessment of safety also refers to the pre-clinical evidence. In this respect, three special concerns are considered: neurotoxicity, reproductive safety, and hematological effects. The NDA draws attention to limits to present knowledge regarding the safety of artesunate and describes what has still to be done before full understanding of its safety can be achieved.

5.1 Safety Events of Interest in Pivotal Trials

In the studies reported in the artesunate NDA, 4 patients died and an additional 11 patients had non-fatal serious adverse events. One patient receiving rectal artesunate died following iatrogenic water intoxication, which was attributed to be the cause of death. The remaining deaths occurred in patients with severe and complicated malaria enrolled in Study 07 (1 patient was treated with concurrent artesunate and quinine and 2 with intravenous quinine) - all deaths were judged to be related to the severity of the disease and its complications. The information about patients who died is summarized in the following table:

Code	Study Number	Treatment Group	Summary
AS9	Study 5 (Thailand)	Rectal AS 10 mg/kg	<p>3 year-old, admitted with parasitemia 4%. An infusion of 1L of D5W was started at 15:20 at a rate of 20 drops/min. About 4 hours after admission, it was noticed that 650 cc were infused over a period of 77 minutes, but the child seemed well. After 15 minutes, the child had repeated episodes of vomiting and loss of consciousness. Glucose was 14.7mmol/L. Unsuccessful attempts were made at resuscitation. The child died 4h55min after enrolment of sudden respiratory arrest. Facilities were not available for post-mortem examination.</p> <p>PC before death was PF 65/500WBC; sodium levels dropped from 139 to 117; potassium levels from 3.5 to 2.5 and albumin from 3.8. to 3.2 over a period of approximately 2 hours.</p> <p>Cause of death was judged probably related to water intoxication.</p>

S24	Study 7 (S. Africa)	Group 3 Rectal AS 10mg/kg + Quinine IV	52 year-old, admitted with severe malaria (altered mental status, dyspnea, lactate 8.2, and parasite count 7,120/uL). Clinical status rapidly deteriorated following admission and patient required transfer to another institution for ventilation and dialysis. Patient died approximately 36 hours after enrollment into the study. Clinical picture was judged related to severe malaria complicated by acute tubular necrosis, marked metabolic acidosis, cerebral malaria and pneumonia. Post-mortem findings were judged consistent with severe and complicated malaria.
S23	Study 7 (S. Africa)	Group 4 Quinine IV	29 year-old, admitted with severe malaria (jaundice, oliguria, and parasite count 412,800/uL). Deteriorated clinically 24-36 hours after admission with worsening renal function requiring peritoneal dialysis and development of pneumonia. Patient continued to deteriorate despite IV quinine, broad-spectrum antibiotics. Patient died 5 days after enrollment. Clinical picture was judged related to severe malaria complicated by acute tubular necrosis, cerebral malaria and pneumonia/ Acute Respiratory Distress Syndrome (ARDS).
S31	Study 7 (S. Africa)	Group 4 Quinine IV	49 year-old, admitted with severe malaria (profoundly dehydrated, anuric, Glasgow Coma Score 8/15, and parasite count 31,085/uL). Initial improvement of mental status, however with subsequent development of diffuse, bilateral pulmonary infiltrates and worsening respiratory status. Broad spectrum antibiotics and anti-tuberculous medications were prescribed, but to no avail. Patient died 6 days after enrollment into the study. Clinical picture was judged related to severe malaria complicated by pneumonia/ARDS.

Nine (9) patients treated rectal artesunate and 2 patients treated with quinine experienced a serious adverse event. Of patients treated with artesunate, 4 experienced convulsions, 3 developed impairment of consciousness, 2 had a liver abscess, 1 had pneumonia and an additional patient had severe anaemia. Among patients treated with parenteral quinine, 1 patient had pneumonia and 1 patient developed severe anaemia. A number of patients experienced more than one serious adverse event. None of these events were judged related to the study medications. Impairment of consciousness, convulsions and severe anaemia are all known complications of severe malaria.

5.2 Adverse Events in Children and Adults

Adverse experience data are reported from a total of 248 adults, adolescents and children who received a single dose of rectal artesunate at 10 mg/kg as initial treatment of malaria (studies 3,4,5,6,7,8,14). All patients received subsequent treatment with other antimalarials, in some cases beginning 12 hours after administration of rectal artesunate. Adverse experiences reported may therefore reflect the effects of the underlying disease (malaria), subsequent antimalarial treatments or rectal artesunate.

Among adults who received rectal artesunate for initial treatment of malaria, adverse

experiences that occurred in >5% of patients were abdominal pain / tenderness.

Among pediatric patients who received rectal artesunate for the initial treatment of malaria, no adverse experiences were reported to occur in >5% of patients.

Table 2: Adverse Experiences in Clinical Trials of Artesunate Rectal Capsules for Initial Treatment of Malaria

ADVERSE EXPERIENCES REPORTED IN CLINICAL TRIALS OF Artesunate Rectal Capsules FOR THE INITIAL TREATMENT OF MALARIA IN CHILDREN AND ADOLESCENTS AGED 2-15 YEARS AND ADULTS AGED 16-60 YEARS

Adverse Experience	Percent (no.) of subjects with Adverse Experiences	
	2 - 15 YR (n=132)	16 - 60 YR (n=107)
Headache	3.8 (5)	0.9 (1)
Abdominal Pain	0.8 (1)	9.3 (10)
Nausea	2.3 (3)	2.8 (3)
Vomiting	3.8 (5)	4.7 (5)
Gastroenteritis		1.9 (2)
Diarrhea		1.9 (2)
Liver Abscess	1.5 (2)	
Skin infection	0.8 (1)	0.9 (1)
Upper or Lower Respiratory infection	1.5 (2)	3.7 (4)
Fever, chills and rigors		0.9 (1)
Conjunctivitis		0.9 (1)
Cough		1.9 (2)
Rhinitis		3.7 (4)
Proteinuria / hematuria / cystitis	1.5 (2)	1.9 (2)
Decreased creatinine clearance		0.9 (1)
Impaired consciousness	2.3 (3)	
Seizure	1.5 (2)	
Abnormal reflexes		0.9 (1)
Tinnitus / Impaired hearing		2.8 (3)
Vertigo / Dizziness		3.7 (4)
Thrombophlebitis		0.9 (1)
Systolic murmur		0.9 (1)
Pelvic Inflammatory Disease		0.9 (1)
Vaginitis		0.9 (1)
Jaundice		0.9 (1)
Anemia	0.8 (1)	3.7 (4)
Hypoglycemia	0.8 (1)	

A total of 26 patients received a single dose of AS 20 mg/kg with no significant safety concerns identified. Data on 54 healthy normal volunteers enrolled in Studies 1 and 9 was also reassuring. No adverse events were reported in these studies. In non-pivotal trials of rectal artesunate at a total dose of up to 1600mg over 3 days, the drug was well tolerated.^{10 11 12}

5.3 Issues of Safety in the Use of Rectal Artesunate

In the clinical development program for rectal artesunate, WHO has taken special steps to explore three aspects of the safety of rectal artesunate: the potential for neurotoxicity, hematologic toxicity, and effects on outcomes of pregnancy. In each of these three areas, judgment has been reached by evaluation of both human experience and specially designed animal toxicity studies using doses relevant to the proposed human exposures and the envisaged conditions of administration of the drug.

Neurotoxicity

Brewer *et al* have described a progressive syndrome of clinical neurological defects with cardio-respiratory collapse and death. The findings, initially in dogs, and subsequently in rats and rhesus monkeys, were based on experiments lasting from 8 to 28 days, in which small groups of animals were injected IM with Arteether or Artemether in doses up to about 50mg/kg/d in various lipid solvents. Detailed neurological examination was not done on any animals, but there are reports in monkeys of jerking limbs and generalised tremor. In dogs and rats findings have included gait disturbance, loss of spinal and pain response reflexes, and loss of brain stem and eye reflexes. Microscopic neuropathic lesions have been seen in the pons and medulla of dogs, rats, and monkeys.

In view of the concern raised by these reports WHO commissioned independent review of material. Existing clinical evidence of neurotoxicity in areas of the world in which the artemisinins constitute first line treatment of malaria was examined. Five independent experts reviewed slides and experimental protocols.⁵⁹ The conclusion with regard to neurotoxicity of the artemisinins, when given in sufficiently high doses and under specified testing conditions to experimental animals, is that they induce dose-dependent toxic neuronal injury, mainly confined to the brainstem, associated with functional impairment of auditory, vestibular, cerebellar, motor and reticular activating systems. The NOEL for Arteether/Artemether lies between 45 and 75mg/kg total dose in the rat, and below 100mg/kg IM in rhesus monkeys. The same observations are suspected but not proven in mice⁶⁰ and hamsters. Reviews of the limited material on artesunate show that total doses of 210 and 420mg/kg given over 14 days to rats do not result in neuropathological lesions. Nevertheless, neuronal pathology in the brain has been described for at least three artemisinin compounds, so it should probably be regarded as a class effect.

WHO also commissioned a 7-day toxicity and toxicokinetic study in the rat using artesunate as the test substance with a 14-day treatment free period. The unique neuronal lesions were looked for in this study designed to identify a dose effect with high doses of artesunate (10 mg/kg tid intravenous, 30 mg/kg tid intravenous, 33 mg/kg *per os* daily, 75 mg/kg *per os* daily, 150 mg/kg *per os* daily); these doses are

significantly greater than the proposed dose of 10mg/kg to be given once or at the most twice for the indication. After dosing over the time periods specified, the animals were killed by perfusion fixation with Karnofsky's fixative (phosphate buffered 4% gluteraldehyde). The brain, brain stem, spinal cord, liver, adrenals, kidneys and heart were removed from the perfusion fixed animals for microscopic examination. Detailed clinical and neurological examinations were made, followed by autopsy, perfusion fixation of the brain and neuropathological examination of multiple sections of all levels of the brain. Oral doses levels of 75 mg/kg or 150 mg/kg and 90 mg/kg were not sustainable due to clinical effects (death, weight loss, hypoactivity, loose faeces, urogenital staining and reduced staining). There was no histopathological evidence of neurotoxicity among the examined animals. Mild reversible hepatotoxicity was observed at all dose levels. 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 210-300mg/kg (21-30 fold greater than the total proposed human dose) did not result in clinical or neuropathological findings, nor indicate any neurotoxic action.

Daily dose (mg/kg)	0	30**	33	75	150	90**
Method of administration	Oral (Gavage)	Intravenous	Oral (Gavage)	Oral (Gavage)	Oral (Gavage)	Intravenous
Vehicle/formulation	PEG 400*	0.5% w/v NaHCO ₃	PEG 400*	PEG 400*	PEG 400*	0.5% w/v NaHCO ₃
No of Animals	M16	M16	M16	M16	M16	M14
Died or sacrificed moribund	0	0	0	1	2	5

* Polyethylene glycol

** Divided in three doses

Comparative neurotoxicity studies in a murine model suggest that parenteral artesunate is significantly less neurotoxic than intramuscular artemether⁶⁰ and that a once a day oral administration of artesunate which transiently exposes the central nervous system is safe. Neurotoxic potential appears to be enhanced by constant exposure either from the depot effect of an oil based drug or repeated intake.⁶¹ In a new study using the same animal model oral artesunate (AS), dihydroartemisinin (DQHS) and artemether (AM) were administered once or twice daily at different doses ranging from 50 to 300 mg/kg/day for 28 days.⁶² The dose regimens were: oral DQHS at 50, 100, 150, 200, 250, 300 mg/kg daily for 28 days (20 animals per group); and oral DQHS, AS and AM at 150 mg/kg twice-daily for 28 days (20 animals per group). There was no evidence of clinical or neuropathological toxicity at doses below 200 mg/kg/day. No differences were noted between the different artemisinin derivatives. Clinical observation was more sensitive than pathological examination as mice with obvious balance and gait abnormalities did not have evidence of neuronal death when examined at 120 days. Artesunate was safe in doses up to 300mg/kg/day which is considerably higher than that proposed for use in the treatment of malaria.

In humans treated with artemisinin drugs, adverse effects attributed to neurotoxicity have not been reported. 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.¹⁴ Additional studies evaluated auditory evoked potentials in 242 patients repeatedly exposed to artesunate and artemisinin (produced in China and Vietnam, total doses 150mg-600mg artesunate, 750mg-3000mg artemisinin), and no evidence of neurotoxicity was found.⁶³

An independent case-control study evaluated 79 patients treated with at least 2 courses of oral artesunate or artemether within the previous 3 years and 79 age- and sex- matched subjects living in the same area at the Thai-Burmese border. Clinical neurological examination, audiometry and early latency auditory evoked responses testing were performed. No consistent differences between cases and controls were identified. The authors note that neither auditory evoked potentials nor other neurophysiologic assessment have been validated as sensitive predictors of neuronal damage in humans by these drugs.⁶⁴

Neuropathological examination of brainstems of 21 adults who died of severe malaria after receiving treatment including artemisinins did not reveal any of the neuropathological changes observed in experimental animals. Half of the patients had received conventional treatment with quinine intravenously, and the other half artemether intramuscularly. In histological examination of the brains, there was no evidence of neurotoxicity of the kind described with the artemisinins in experimental animals.⁶⁵ Within the limits of the material and the circumstances possible for this investigation, the pathologists have concluded that there was no evidence in these fatal cases of drug-induced neurotoxicity. It was judged that the doses of rectal artesunate proposed for initial, limited therapy should not pose a risk for patients.⁶⁶

Nevertheless, it is improbable that artesunate, as a member of the class, is exempt from the toxicity seen in experimental animals with the artemisinin derivatives. It is probably a matter of dose, duration of administration, and total cumulative amount administered that is likely to determine the expression of artemisinin-related neurotoxicity. Therefore, further investigation of the potential neurotoxicity of artesunate, under the strict conditions of use in practice for which it is proposed, is now being conducted as part of the protocol of the Phase IV studies according to the simple and achievable programme identified by a WHO Informal Consultation. Patients aged greater than 6 months with a clinical diagnosis of malaria are randomised to receive a single capsule of artesunate or placebo and are immediately referred to hospital for further treatment. Children are given a single capsule of 100mg and adults are given a single capsule of 400mg. All randomized individuals are evaluated between 7 days and 4 weeks after enrollment for mortality and, if they have survived, for any neurological sequelae. There are currently three study sites: Bangladesh, Ghana and Tanzania. Bangladesh is the only site with malaria that affects both adults and children and therefore enrolls both adults and children; in Ghana and Tanzania young children are affected and these sites enroll only children below 6 years of age. A total of 3366 patients have been enrolled in the multi-country study as of March 19, 2002. Sixteen (16) patients (0.47%) were identified with neurological sequelae on scheduled follow-up, 13 of whom were reported in Ghana and 3 in Tanzania. Among the patients with documented neurological sequelae, all received follow-up treatment at the health facility. 7/16 patients (43.8%) were unconscious at baseline. Eight (8) patients (50%) had a positive malaria blood smear at the time of recruitment. Four (4) patients had bacterial meningitis confirmed on cerebrospinal fluid (CSF) culture, but it should be noted that lumbar puncture was not performed in all patients.

With increasingly reassuring evidence from detailed and large-scale studies it seems likely that in conditions of clinical practice there is a considerable margin of safety with artesunate with regard to neurotoxicity.

Hematologic Toxicity

The mechanism of action of the antimalarial endoperoxides, including artesunate, and the concentration of the principal metabolite of artesunate in infected erythrocytes raises the question as to whether artesunate might be toxic to the haemopoietic system. It has been demonstrated that oxidative stress in malaria-infected human erythrocytes is augmented and the anti-oxidant system is attenuated, compared with normal red blood cells.⁶⁷ Sodium artesunate markedly increases the levels of active oxygen species and production of malonyl dialdehyde in normal red blood cells and, to an even greater extent, in malaria-infected red blood cells. Augmentation by artesunate of intra-cellular oxygen and hydrogen peroxide production may account, at least in part, for the antimalarial action. High doses of artesunate administered to animals, 30-times greater than the proposed human dose, causes a dose-related depression of reticulocytes and white cells. At the highest dose (90mg/kg/day x 28 days) there is evidence of depression of red blood cells in the peripheral blood, "maturation hindrance" of normoblasts, and histological change in the bone marrow, all suggesting bone marrow depression.

In human clinical trials evaluating artesunate and other artemisinins in the treatment of malaria, involving more than 10 000 patients, no pattern of clinically significant hematologic toxicity has been noted. RN Price and others have reported on factors contributing to anemia following uncomplicated *P falciparum* malaria.¹⁴ Of 4 007 cases of *P falciparum* malaria on the western border of Thailand, 727 (18%) had anemia defined as a haematocrit less than 30%. 55 of the patients (1%) required blood transfusion. Following treatment of malaria, regardless of the antimalarial regimen used (there were 4 categories of treatment groups altogether of which artesunate alone was one), there was a mean fractional fall in haematocrit of 14.1% from the baseline reading. This fall was greatest in young children (<5 years), and in association with prolonged illness, high parasitaemia and delayed parasite clearance. It includes loss of parasitised red blood cells, which would account for less than 10% of the loss. Recovery was generally complete by 6 weeks. Anaemia was not attributable to any of the treatment categories.

In WHO-supported Phase II clinical trials of rectal artesunate a modest but statistically significant drop in haematocrit was observed. In Study 3 among the 48 adults treated with 2.4 mg/kg intravenous, 10 mg/kg and 20 mg/kg rectal artesunate there was a slight decrease in haematocrit from baseline in all treatment groups over the initial 7 days (mean difference -3.51; p=0.002). This effect was reversible - there was no statistical difference in haematocrit from baseline on Days 14 and 21 (mean difference from baseline was -1.55 and +0.68, respectively), with a significant increase from baseline and a trend towards normalisation on Day 28 (mean difference +6.40; p=0.011). No dose-effect and no statistical difference between the four drug regimens were demonstrated.

In the same study, there was also a small and transient decrease in neutrophil count from baseline on Days 7 and 14. Neutrophil counts were not significantly changed from baseline on Days 21 and 28. Twenty (20) of 48 patients had a low neutrophil count and

11 of 48 had an elevated neutrophil count on admission. On day 7, twenty-four of 47 patients with available data had a low neutrophil count and 3 of 47 patients had a high neutrophil count. On day 28, twelve of 33 patients with data had a low neutrophil count and 3 of 33 patients had an elevated neutrophil count. There was no significant difference between the four treatment groups and routes of administration.

In study No 4, among the 36 children treated with 2.4 mg/kg artesunate intravenously, and 10 mg/kg and 20 mg/kg rectal artesunate, there was a small decrease in haematocrit levels over the first 24 hours in the three treatment groups. The mean difference was -2.73 at 4 hours ($p < 0.0001$), increasing to -4.2 at 24 hours ($p < 0.0001$). There was no significant difference between the treatment regimens.

In study No 6, in Malawi in children with moderately severe malaria, there was no significant difference in haematocrit levels between quinine and artesunate-treated patients at 12 (28.4 ± 5.89 and 27.4 ± 4.6) and 24 hours (26.8 ± 5.14 and 25.8 ± 4.2). At discharge, haematocrit levels were significantly lower in the quinine group (26.5 ± 4.81 and 24.1 ± 4.0 for artesunate). There was a significant drop in haematocrit levels over the initial 24 hours in both treatment groups.

As reported in section 2.2 above, there is now evidence that after treatment with artesunate the spleen removes the intra-erythrocytic parasite from the host red blood cells returning the intact RBC into circulation.^{37 38} In principle this explains why the haematocrit in some patients with heavy parasitaemia does not decrease to the extent that might be expected from destruction of parasitized RBCs following treatment with artesunate.³⁹

With the doses of rectal artesunate proposed for use as initial treatment of malaria, it is concluded that the chances of hematologic toxicity are remote.

Risk in Pregnancy

Reproductive studies performed in rats and rabbits show dose-dependent loss of implantation without major congenital abnormalities with the artemisinins, including artesunate. Doses within the therapeutic range have abortive effects. The dose, and presumably exposure, of the artemisinins that causes reduced fetal survival and increased abortion rates in experimental animals are similar to the proposed human dose. Thus, in respect of fetal injury (survival) there is no margin of safety. Despite the consistent finding of impaired fetal survival following exposure to artemisinins in the first trimester in animals, none of the studies in any animal species has shown teratogenicity. Neither is there evidence of mutagenicity, cytotoxicity, or immunosuppressant activity attributable to any member of the artemisinin class of drugs. The pharmacokinetics of the artemisinins in human pregnancy, including women with malaria, are unknown.

Notwithstanding the consistent finding of impaired fetal survival following exposure to artemisinins, 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.

A prospective study in humans has not confirmed risk of teratogenicity attributable to artesunate, and neither has evidence been demonstrated of other fetal injury or

impairment of maternal health beyond that attributable to malaria alone. There are data on the outcome of more than 500 pregnancies in women with malaria who received treatment with artesunate or another of the artemisinin derivatives. There have been no congenital malformations in these infants, and a significant number also underwent neurological examination at 1 year of age without abnormal findings.

Most of the studies in pregnant women have been conducted at the Shoklo Malaria Research Unit in the Thai-Burmese border, an area with the highest levels of antimalarial-drug resistance yet described⁶ where no antimalarial is currently either demonstratively effective or safe for use in pregnancy.⁶⁸ Three new studies using oral artesunate have recently been published.

The first presents an open randomized comparison of supervised quinine 10 mg/kg for 7 days (QN7) versus the combination of oral artesunate 4 mg/kg/d for 3 days plus mefloquine (25 mg base/kg total dose) (MAS3) in the treatment of 108 women in the second and third trimester with uncomplicated malaria.⁶⁹ The 108 eligible women delivered 91 (84.3%) singletons and 1 (0.8%) set of twins. In the remaining 16 pregnancies, there were 13 (12%) lost to follow-up, 2 (1.9%) mid-trimester abortions and 1 (0.9%) maternal death unrelated to malaria. The 2 mid-trimester abortions both occurred in the MAS3 group in primigravidae on their first documented *falciparum* malaria episode. No stillbirths or congenital abnormalities occurred in the 92 documented outcomes. In the study, MAS3 was more effective, associated with less gametocyte carriage and significantly better tolerated than QN7. The findings need to be balanced against a possible increased risk of stillbirth with the use of mefloquine in pregnancy, and the risk of untreated malaria in pregnancy.

Results of an open randomized comparison of the combination of quinine-clindamycin (QC7) (quinine 10mg salt/kg and clindamycin 5 mg/kg q8 hours for 7 days) versus artesunate 2 mg/kg/ day for 7 days (AS7) in 129 women with acute uncomplicated malaria in the 2nd and 3rd trimester of pregnancy are now available.⁷⁰ Both regimens were very effective with cure rates of 100%, confirmed by parasite genotyping. The AS7 regimen was associated with less gametocyte carriage rate. There were no differences in the incidence of gastrointestinal symptoms, but there was significantly more tinnitus in the QC7 group (44.9% vs. 8.9%; RR 5.1; 95% CI 1.9 - 13.5; p<0.001). For the singleton pregnancies, there was one stillbirth in each group and one congenital abnormality in the QC7 group. The stillbirths were considered unlikely to be related to the drugs: one resulted from abruptio placenta in the QC7 group and the other from prolonged labour in a primiparum who had a home birth. The congenital abnormality was a midline epidermoid cyst superior to the nose bridge. There were no significant differences in mean birth weight, placental weight, estimated gestational age, proportion of low birth weight between the groups. There were 3 neonatal deaths, 2 QC7 and one AS7, all of whom died in the first week of life of causes unrelated to malaria. Developmental milestones were similar for both groups.

A prospective treatment study of artemisinin antimalarials in pregnancy where artesunate (n=528) or artemether (n=11) was used to treat 539 episodes of acute *P. falciparum* malaria in 461 pregnant women, including 44 first-trimester episodes has been reported.⁷¹ It was not possible to distinguish between failure to respond to treatment and re-infection and hence treatments were classified as primary or re-treatment. The majority were re-treatments (310 [57.5%]). Using survival analysis, the cumulative artemisinin failure rate for primary infections was 6.6% (95% confidence interval, 1.0-12.3), compared with the re-treatment failure rate of 21.7% (95%

confidence interval, 15.4-28.0; p=.004). Pregnancy outcomes were not significantly different from the community rates for abortion, stillbirth, congenital abnormality, and mean gestation at delivery (see table 4 of the referred paper).

Plasmodium falciparum malaria in pregnancy is associated with deleterious consequences to the mother and fetus. Maternal and fetal mortality, abortion, stillbirth, premature labour, birthweight reduction, maternal and fetal anemia are all known complications of falciparum malaria. Whatever the risk to reproductive toxicity of artesunate might be, it would need to be balanced against the risk to the fetus of untreated malaria, and the known dangers to the fetus of other antimalarials such as quinine, mefloquine and chloroquine compared with those of artesunate.

Human studies of the safety of artesunate in pregnancy are encouraging so far. The fetal resorption seen in animal studies may not apply to humans given therapeutic doses of artemisinins. However, the numbers examined may be too few to rule out rare adverse effects in pregnancy of artesunate. Because studies in humans cannot rule out the possibility of harm, WHO advises that artesunate should be used during pregnancy only when there is no safe and effective alternative.

Other: Allergic Reactions

There have been no allergic reactions documented in the clinical trials conducted by WHO with rectal artesunate. However, a recent report from Leonardi et al.⁷² describes two cases of severe, potentially life-threatening allergic reactions to oral artesunate. Both required administration of adrenaline, high dose antihistamines and steroids. The authors suggest that artemisinin congeners should not be given to patients with a previous history of an allergic reaction following their consumption or if an urticarial rash develops during treatment. Patients with a history of hypersensitivity reaction to one of the artemisinins should be advised not to take any of the derivatives again.

5.4 Overall Summary of Safety of Single-Dose Use of Artesunate Rectal Suppositories

It is apparent that artesunate, whether given as a rectal dose of 10mg/kg, 20mg/kg (pivotal studies) or in a total rectal dose of 1200mg or 1600mg over 3 days followed by mefloquine (non pivotal studies) or in different formulations (oral or intravenous), has a highly favourable 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.

6 ADVANTAGES AND PUBLIC HEALTH IMPORTANCE OF ARTESUNATE RECTAL SUPPOSITORIES

Patients presenting with severe malaria have relatively short histories of illness, emphasizing and confirming that the disease progresses rapidly.⁷³ To survive, a patient with severe illness must get access rapidly to a health facility where injectable treatment can be given safely. It is rare that safe, parenteral antimalarial treatment is

available at primary health care level (that is, at the point of first contact with health care personnel) when parents arrive with their sick child. Parenteral treatment is usually available only in hospitals. In malaria endemic countries, with their poor road infrastructure, safe injectable treatment at tertiary care facilities may not be achievable for many hours or even days after initial symptoms. This is the cumulative result of the delays of arduous travel, the need to care for the remaining children in the family, consultation with traditional healers, and lack of money. The interval between the onset of severe symptoms and arrival at the hospital may average 2.5 days (Bangladesh); in Tanzania it is 40 hours. The patient might die before getting to the hospital, or be admitted with advanced disease and complications, and then die.¹ Even after admission to hospital, and with appropriate antimalarial and supportive treatment, the risk of death for severe malaria is highest within the first 24 hours.¹

If no treatment is given, severe malaria is fatal. The survivors are those who have rapidly received appropriate and adequate treatment. Treatment of severe malaria infection reduces the chance of dying by up to fivefold.² Of those referred to hospital for treatment, only 18% reach hospital; there is documented evidence that in some parts of rural Africa the remaining proportion of those referred for treatment - 82% - either die en route to hospital or at home

Severe malaria with organ failure and cerebral malaria are the most important of the manifestations of *P. falciparum* infection, accounting for 80 per cent of malaria deaths. Severe malaria is associated with high parasitaemia, and parasites in patients with severe malaria have high intrinsic multiplication rates, often making the course of lethal disease swift - a single multiplication cycle of 48 hours.

The pathophysiological processes in *falciparum* malaria are thought to be attributable to the sequestered forms of the parasite; mechanical obstruction to the microcirculation of the brain and the vascular endothelium by cytoadherent, parasitised red cells considered to be the principal mechanism leading to organ dysfunction and coma. The purpose of rapid and effective therapeutic intervention is to prevent this cascade of pathophysiological events, or to abort it as early as possible, thus reducing the complications and potentially fatal risks associated with severe malaria, returning the patient to a clinically stable position from which curative therapy can be safely instituted.

If one could develop an ideal drug to treat severe malaria, it would have rapid and precise action against the parasite, broad spectrum of activity (clearing young ring forms and inhibiting them from developing to sequestered forms, with gametocytocidal action to prevent transmission of resistant parasites), precise toxic action against the parasite without impairing the body's repair process, substantial and sustained bioavailability with a single dose, a wide safety margin, close correlation between blood concentration levels and minimum inhibitory activity *in vivo*, low potential for influencing the activity of other drugs or being adversely affected by them, low potential for resistance, and ease of administration.

There is now a considerable body of evidence to suggest that artesunate, and the class of compounds to which it belongs, consistently has a faster onset of action and a broader parasite stage specificity against *P. falciparum* than all other known antimalarial drugs, including quinine. Artesunate increases the clearance of ring forms, inhibits meront development and has gametocytocidal action. It is highly focused in toxic action, targeting the parasitized red blood cell with 300-fold affinity

compared with the non-parasitized RBC, removing the parasite from the host red blood cells without red cell destruction, and returning the unparasitized RBC to the circulation. In severe disease, where speed of therapy and rapid and sustained elimination of the high parasite biomass is required, artesunate has a superior therapeutic response to quinine.

The drug has a wide safety margin. Given in conjunction, or in sequence, with other drugs, artesunate appears to retain efficacy, not interfering with the activity of the other agents, nor influencing adversely tolerance of the other agents. There is no evidence that artesunate impairs the repair processes in malaria (on the contrary, it is now thought it may inhibit the development of anemia associated with malaria). Neither does artesunate influence adversely the common complications of severe malaria such as hypoglycaemia, fetal toxicity when administered to the pregnant woman, thrombocytopenia, impaired liver function, renal toxicity or neurotoxicity. The drug does not overlap in toxicity with the adverse effects of other antimalarial agents with which it might be used either concurrently or sequentially, nor is there evidence that other drugs potentiate the toxicity of artesunate. Fetal toxicity seen in pre-clinical studies has not been found in clinical exposure in pregnancy to the drug of more than 500 women so that the drug appears to be safe for administration in pregnancy where there are few safe drugs presently available for treatment and where the risks are high and the prognosis poor for both the mother and fetus if malaria is not treated.

Although artesunate is not neurotoxic in clinical practice, it belongs to a class which is neurotoxic in experimental animals at high doses, when exposure is sustained. The WHO has addressed the neurotoxicity issue comprehensively. All the data upon which the concerns of neurotoxicity of the artemisinins are based have been thoroughly evaluated, including critical analysis of the published literature, review of the extensive unpublished clinical experience, preclinical studies of artesunate neurotoxicity with artesunate, and a prospective clinical safety programme to quantify rare and adverse events (occurring at a rate of 1/2000). Neurotoxicity has not been seen in clinical practice. It is concluded that administration *per rectum* of one or even two doses of artesunate in a dose of 10 mg/kg to humans, adults and children, poses no safety risk.

In one respect artesunate does not meet the ideal characteristics of an antimalarial: there is no validated pharmacokinetic-pharmacodynamic relationship. The pharmacokinetics of artesunate cannot be used to predict clinical outcome or dose. In all pharmacokinetic-pharmacodynamic studies carried out on artesunate, published and unpublished, the *clinical and parasitological* response has been immediate, sustained and predictable. In contrast, the pharmacokinetic data are subject to considerable variance that cannot be explained. In severe malaria, when the objective of treatment is to save life, speed is of the essence, and WHO has considered it necessary to decide on a dose that minimises the possibility that a patient will fail to absorb the necessary amount of drug necessary to save their life.

The data on efficacy of rectal artesunate given as a single 10mg/kg dose support the indication. A 99-100% reduction of parasitaemia at 24 hours is achieved without risk of accumulation in the body or plasma of artesunate or dihydroartemisinin. This compares most favourably with the median reduction of parasitaemia for parenteral quinine of 42-72% at 24 hours, given in 3-7 doses. There is no indication that decreasing the dose or increasing the frequency of dosing would convey further

benefit within the initial 24-hour period. In the absence of a known pharmacokinetic-pharmacodynamic relationship for artesunate the empirical regimens derived and used with rectal artesunate remain unchallenged.

Despite years of use in South East Asia, resistance to artesunate has not developed and stable resistance is difficult to induce. Since resistance to antimalarials is associated with high biomass infections, the effective and substantial reduction in parasite numbers in severe malaria infection is important both for clinical benefit of the patient and for reducing the potential for transmitting resistant infections.

Since 1985 the efficacy of artesunate in a suppository formulation has been public knowledge.⁷⁴ However, the WHO pivotal studies demonstrate that artesunate in a rectal dose of 10mg/kg can effectively substitute for an injectable drug, by reducing parasitaemia sufficient to achieve clinical stability, thus reversing a potentially fast-developing and lethal disease process in patients who have no alternative therapy. Since ease of administration is imperative for a drug intended to be used where there is no treatment alternative and health facilities are rudimentary, registration of rectal artesunate would make possible the introduction of a therapeutic (but not curative) intervention that has the realistic prospect of saving innumerable lives of infants, children, and adults. Rectally administered artesunate appears to have many of the ideal characteristics of an antimalarial therapeutic agent, including ease of administration (and suitability for *non per os* status), rapid onset of antiparasitic action, favourable bioavailability after administration, no evidence of cross-resistance with other antimalarials, a reassuring safety and efficacy profile compared with other available oral and parenteral treatments, and no evidence of antagonism of the beneficial effects of other therapy when they are given concurrently.

7 CONCLUSIONS

The outcome of administration of artesunate rectal suppositories for the initial treatment of malaria has been thoroughly studied by WHO within a strictly focused clinical development plan aimed at providing data on safety and efficacy in the early stages of treatment of malaria for a patient population that does not have access to parenteral therapy and whose disease state precludes oral treatment. The efficacy data in this submission have been confined to the data from the pivotal trials commissioned by WHO supporting the indication. The pivotal trials consistently show the compound to be safe, compared with the outcome of the disease and with alternative antimalarial treatment for severe malaria - intravenous quinine.

The safety data depend on a large series of patients (>10 000) treated with different formulations of artesunate and other artemisinins studied in South East Asia. No data are presented to support the use of artesunate for longer-term or curative therapy of malaria, and no claims have been made in this regard in the submission.

The studies show that artesunate does not have a predictable correlation between its pharmacokinetics and those of its principal active metabolite, dihydroartemisinin, and its pharmacodynamic activity reflected in parasite clearance in acute malaria. This precludes formulaic derivation of optimum dosage or reliance on blood levels to predict or to judge likely clinical response. It is probable that further clarification of the optimum dose will be obtained through clinical experience. However, for the

limited indication proposed, rectal artesunate in a dose of 10 mg/kg given to *non per os* patients with acute *P. falciparum* malaria who are at high risk of progression to severe complications and mortality without treatment, makes possible a novel therapy with a highly favorable, predictable and convincing benefit-risk profile.

The applicant, WHO, during its presentation to the AIDAC, will draw attention to the issues which WHO regards as unresolved to date, but which WHO believes should not preclude or delay regulatory approval of this new medicine. These include determination of the optimum minimum dose and the critical need for administration of rectal artesunate to be supported in the community by a thorough educational programme. If the initial dose of rectal artesunate is not followed by other curative therapy, the outcome for the individual patient would be poor. For this reason, it is imperative that the health system should ensure that the patient receives appropriate treatment at the correct time and is well informed of the consequences of the intervention. The clinical development program for rectal artesunate that has been conducted by WHO has been designed and executed with this goal in mind.

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