Efficacy of a Novel Sublingual Spray Formulation of Artemether in African Children with *Plasmodium falciparum* Malaria

Daryl Bendel,[a] Stephen Rulisa,[b] Patrick Ansah,[c] Sodiomon Sirima[d]

Xidea Solutions Limited, Watford, Hertfordshire, England[a]; Kigali University Teaching Hospital and University of Rwanda, College of Medicine & Health Sciences, Kigali, Rwanda[b]; Navrongo Health Research Centre, Kasenna-Nankana District, Upper East Region, Ghana[c]; Centre National de Recherche et de Formation sur le Paludisme (CNRFP), Ouagadougou, Burkina Faso[d]

The efficacy of sublingual artemether (ArTiMist) was investigated in two studies. In study 1, 31 children were randomized to sublingual artemether ($n = 16$) or intravenous (i.v.) quinine ($n = 15$). In study 2, 151 children were randomized to sublingual artemether ($n = 77$) or i.v. quinine ($n = 74$). For both studies, patients weighed between 5 and 15 kg and had either severe or complicated malaria based on WHO criteria, or they had uncomplicated malaria but were unable to tolerate oral medication as a result of nausea, vomiting, or diarrhea. Patients received either 3 mg/kg of body weight of sublingual artemether or a loading dose of 20 mg/kg of i.v. quinine followed by 10 mg/kg every 8 h i.v. thereafter. The primary endpoint was parasitological success, defined as a reduction in parasite count of $\geq$90% of that at baseline at 24 h after the first dose. Other endpoints based on parasite clearance and clinical response were evaluated. In study 1, there were parasitological success rates of 93.3% (14/15) and 66.7% (10/15) for the sublingual artemether and quinine treatments, respectively. In study 2, 94.3% (66/70) of the ArTiMist-treated patients and 39.4% (28/71) of the quinine-treated patients had parasitological success ($P < 0.0001$). Indicators of parasite clearance (parasite clearance time [PCT], time for parasite count to fall by 50% [PCT_{50}], time for parasite count to fall by 90% [PCT_{90}], and percent reduction in parasitemia from baseline at 24 h [PRR_{24}]) were significantly superior for children treated with sublingual artemether compared to those treated with i.v. quinine. There were no differences between treatments for the clinical endpoints, such as fever clearance time. The local tolerability of sublingual artemether was good. Sublingual artemether leads to rapid parasite clearance and clinical recovery. (Studies 1 and 2 are registered at ClinicalTrials.gov under registration numbers NCT01047436 and NCT01258049, respectively.)

Malaria remains a major health challenge in developing countries, especially in sub-Saharan Africa (SSA). Approximately 207 million cases of malaria were reported worldwide, of which 80% were in sub-Saharan Africa. Overall, 90% of the reported 627,000 deaths, of which 77% were in children under five years of age, were in SSA (1). The overwhelming majority (98%) of malaria cases in the African region are due to *Plasmodium falciparum* (1).

In countries where the disease is highly endemic, 20% to 46% of child deaths can be attributed to malaria or febrile illness (2). In moderate- to high-transmission settings, such as is found in most SSA countries where malaria is endemic, young children are disproportionately affected by malaria (3). A child presents with an average 1.6 to 5.4 episodes of febrile malaria per year, with about 5% of malaria episodes becoming severe disease (4). Less than one-third attend clinics, and many receive malaria treatment outside the health care system. The majority of treatments are initiated on a presumptive diagnosis, with a high false-positive rate, resulting in challenges in accurate monitoring of the malaria burden in SSA (5).

Despite advances in the treatment of malaria in children, "the majority of deaths from severe malaria in childhood are caused by the delayed administration of effective antimalarial treatment. There is a relentless deterioration in the clinical condition of a young child with malaria who fails to get effective treatment, with death ensuing in a matter of hours or days. Any successful attempt to reduce mortality from malaria will have to explore novel possibilities for minimizing such delays" (6).

There is a clear need for a formulation of an artemisinin derivative that can be easily administered and adequately absorbed in a child who may be unconscious or uncooperative, or in whom nausea and vomiting preclude oral dosing. Artemether can be used as initial intramuscular (i.m.) monotherapy for severe malaria in children (7). It is also a recommended first-line oral therapy in combination with the longer half-life partner drug, lumefantrine, for uncomplicated *P. falciparum* (8) and *Plasmodium vivax* (9) infections in pediatric age groups.

A new formulation of artemether in neutral oil, ArTiMist (Suda Ltd., Perth, Australia), has been developed and has the potential to minimize the delay in the administration of an effective antimalarial agent in children with complicated or severe malaria. ArTiMist is administered as a metered sublingual spray that is more rapidly and extensively absorbed than artemether given in tablet form in healthy adult volunteers (10).

In the present study, we assessed the safety, tolerability, and clinical efficacy of ArTiMist and intravenous (i.v.) quinine for severe and complicated *P. falciparum* malaria in African children and in infected children who were unable to take oral therapy.

MATERIALS AND METHODS

Study site, approvals, and patients. The present study was conducted in two parts. Study 1 was an open-label randomized comparative trial of ArTiMist and i.v. quinine conducted in Rwanda (Rwinkwavu District Hospital) between 01 December 2009 and 19 January 2010. Study 2 was a phase III, randomized, open-label, and multicenter superiority trial of ArTiMist versus i.v. quinine conducted between 16 November 2010 and 07 September 2012 at three different sites: Rwanda (Rwinkwavu District Hospital, Eastern Province), Ghana (Navrongo Health Research Centre, Upper East Region), and Burkina Faso (Centre National de Recherche et de Formation sur le Paludisme [CNRFP], Ouagadougou). The details of the pharmacokinetic procedures and results have been published elsewhere (10, 11). The present paper provides details of the trial procedures and efficacy and safety outcomes.

Children weighing between 5 and 15 kg were eligible for the studies if (i) they had falciparum malaria confirmed by blood film microscopy showing a *P. falciparum* density of $\geq$500/μl of whole blood (including those positive for other plasmodial species), (ii) they had severe or complicated malaria based on the WHO criteria (12) or uncomplicated malaria but were unable to tolerate oral medication as a result of nausea, vomiting, or diarrhea, (iii) they had not received any antimalarial therapy within the 7 days prior to first-study drug administration, (iv) they did not have evidence of significant comorbidity, including other infections, (v) they had no contraindication, allergy, or history of intolerance to either artemether or quinine, and (vi) their parents or attendant relatives/guardians gave witnessed informed consent and, when possible, the child assented to participation.

Study 1 was approved by the University Teaching Hospital Kigali Research Ethics Committee (EC/CHUK/002/09) and study 2 by the University Teaching Hospital Kigali Research Ethics Committee (EC/CHUK/015/10), the Navrongo Health Research Centre institutional review board (NHRCIRB107), and the Centre National de Recherche et de Formation sur le Paludisme Comité Institutionnel de Bioéthique (AEP-002/02/2011/CIB-CNRFP). Both studies were registered on ClinicalTrials.gov (under registration no. NCT01047436 and NCT01258049 for studies 1 and 2, respectively). In both studies, allocation bias was avoided by randomization using a computer-generated schedule and ensuring that the investigator remained blinded until after the randomly allocated medication was dispensed from the pharmacy.

Patients. In study 1, 31 eligible children were randomized to receive either ArTiMist ($n = 16$) or i.v. quinine ($n = 15$). There were no screening failures. In study 2, of the 180 children who were screened for study entry, 151 eligible children were randomized to receive ArTiMist ($n = 77$) or i.v. quinine ($n = 74$).

For both studies, ArTiMist (Essential Nutrition Ltd., Brough, England) was administered at a dose of 3.0 mg/kg of body weight at 0, 8, 24, 36, 48, and 60 h or until the initiation of oral antimalarial therapy. Intravenous quinine (Martindale Pharmaceuticals) was administered as a 20-mg/kg infusion over 4 h at 0 h, followed by 10 mg/kg (infusion over 4 h) every 8 h thereafter for $\geq$24 h and until the resumption of oral therapy. The exact timing of each dose was recorded.

On the resumption of oral therapy, patients could, at the discretion of the local investigator, receive further doses of oral quinine (Teva UK Limited) or of ArTiMist to complete 7 days of treatment or be converted to another suitable treatment (typically, artemisinin combination therapy [ACT]), in accordance with the respective national treatment guidelines.

Clinical procedures. Patients were initially treated as hospital inpatients and could be discharged from day 4 onwards at the investigator's discretion. Patients returned for outpatient visits on days 7, 14, 21, and 28. Vital signs (temperature, pulse rate, blood pressure, hydration status, and respiratory rate) and physical examination (including neurological examination, level of consciousness, and ability to eat, drink, and mobilize normally for age) were evaluated regularly.

The determination of parasite counts, physical examinations, and vital signs occurred as follows: day 1, predose and 3 h, 6 h, 12 h, and 18 h postdose; days 2 and 3, predose and 6 h and 12 h postdose; day 4 and discharge day, prior to discharge or prior to converting to quinine or oral therapy; every second day thereafter if prolonged hospitalization; and each of the outpatient visits on days 7, 14, 21, and 28.

Dipstix urinalysis was performed prestudy and on days 1, 2, 3, 4, 7, 14, 21, and 28 when urine was available or when clinically indicated. Adverse events were regularly elicited and concomitant medications recorded. Clinical laboratory assessments (biochemistry and hematology) were evaluated at baseline (screening or day 0), day 4, and day 21.

Parasite counts. In study 1, three independent microscopists blinded to treatment read the blood smears at the study site. For study 2, Phoenix Pharma Central Services (S) Pte. Ltd. in Singapore, which is accredited by the Ministry of Health in Singapore and the College of American Pathologists, was the central laboratory for the evaluation of parasite counts. The laboratory remained blinded to treatment status at all times. Two trained microbiologists read all slides independently, with the results averaged. A third independent microbiologist read discordant results, and the closest two parasite counts were averaged. Thick blood films were stained with Giemsa stain.

For both studies, the number of asexual parasites/microliter of blood was determined by dividing the number of asexual parasites by the number of white blood cells (WBC) counted (500) and then multiplying by an assumed WBC density of 6,000 to 8,000/μl.

Assessments of efficacy. In study 1, the primary efficacy parameters were parasitological success, defined as a reduction in parasite count of $\geq$90% of that at baseline at 24 h after the first dose, time for parasite count to fall by 90% (PCT_{90}), and time for parasite count to fall by 50% (PCT_{50}). In study 2, the primary efficacy parameter was parasitological success, defined as a reduction in parasite count of $\geq$90% of that at baseline at 24 h.

Secondary endpoints included the following parameters of parasite clearance: parasite clearance time (PCT) (the time in hours from the initiation of therapy until the first of two successive parasite-negative smears was obtained), percent reduction in parasitemia from baseline at 12 h (PRR_{12}) and 24 h (PRR_{24}), PCT_{90}, and PCT_{50}.

Early treatment failure was defined as parasitemia on day 2 above that at baseline, irrespective of axillary temperature, parasitemia on day 3 with axillary temperature of $\geq$37.5°C, parasitemia on day 3 of $\geq$25% of the count at baseline, or the requirement for rescue antimicrobial treatment. Late parasitological failure was defined as parasitemia on any day from day 7 to day 28 and tympanic temperature of $\geq$38.0°C.

Clinical endpoints included fever clearance time (FCT) (time in hours from the initiation of therapy until the disappearance of fever [tympanic temperature, <38.0°C] for $\geq$24 h), time taken for patients to return to normal *per os* status, time to full consciousness in patients admitted with an impaired level of consciousness, number of patients with neurological sequelae, and number of deaths. In study 2, late clinical failure was defined as signs of severe malaria on any day between day 4 and day 28 in the presence of parasitemia without previously meeting any of the criteria of early treatment failure, or the presence of parasitemia and tympanic temperature of $\geq$38.0°C (or history of fever) on any day between day 4 and day 28 without previously meeting any of the criteria of early treatment failure.

Complete cure was defined as the complete resolution of clinical signs, symptoms, malaria-related laboratory abnormalities, and elimination of asexual parasites by day 7, with no recurrence up to day 28 ($\pm$ 2 days), and a 48-h parasite count of <25% of that at baseline with no clinical deterioration.

Adverse events and concomitant medications were recorded throughout both studies. For patients allocated to the ArTiMist treatment, the investigator evaluated local tolerability.

Statistical analysis. Statistical programming and analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC). For study 1, the sample size was not formally calculated. For study 2, sample size was calculated using the primary efficacy endpoint data from study 1, in which

the parasite success rate for quinine was 67.7%. On the assumption of a parasite success rate of 70% for quinine in study 2, and to demonstrate that ArTiMist was superior to quinine by ≥20%, the parasite success rate for ArTiMist should be ≥90%. Assuming a power of 80%, an alpha of 0.05 (two-sided) and equal allocation to the ArTiMist and quinine treatment arms, the number of evaluable patients (n) required for each treatment was 59.

Efficacy analysis was based on a modified intent-to-treat (MITT) population (full analysis set [FAS] in study 1), which included all randomized patients receiving at least one dose of study medication with evaluable parasite counts at 12 h (study 1) and 24 h (studies 1 and 2). A second efficacy population, which for study 1 was the modified FAS (MFAS), was defined as all patients in the FAS receiving all 6 doses of ArTiMist or the equivalent of quinine and who had a parasite density count performed after the final dose. For study 2, the per-protocol (PP) population included patients in the MITT population receiving ≥80% of their doses at discharge from hospital, provided evaluable data up to and including day 28, and had no major protocol violations. Efficacy analyses were conducted and presented for both populations. The safety analysis population, which included all randomized patients receiving at least one dose of study medication, was used for all safety analyses. The intention-to-treat (ITT) population, which included the set of enrolled patients who were assigned to a treatment group (randomized), regardless of whether or not they took any study drug, was used for summarizing the demographic and baseline data.

For parasitological success, each patient was defined by whether or not they had success by treatment. Parasitological success was appropriately summarized using descriptive statistics by treatment. The difference between treatments and the 95% confidence interval were determined using a logistic model with site as a prognostic factor.

The secondary endpoints and safety data were appropriately summarized using descriptive statistics by treatment, both overall and by site.

For both studies, the statistical analysis was prespecified in a statistical analysis plan, which for study 2 was finalized prior to parasite count data being received from the central laboratory. In study 2, data were analyzed overall and by site. There were no changes to the planned analysis for either study.

RESULTS

Patient characteristics. In study 1, one patient (ArTiMist) was withdrawn after the second dose due to a protocol violation (incorrect drug storage) and was replaced. The data for this patient were included in all analysis populations, apart from the PP population.

The patient disposition for study 2 is provided in Fig. 1. The baseline characteristics of participants in each study are summarized in Table 1 and are well balanced between treatments for the two studies. For study 2, treatment allocation by site is provided in Table 2.

One patient allocated to ArTiMist treatment had mixed infection with *Plasmodium ovale*.

Efficacy analysis and parasite response. The efficacy endpoint parameters are presented in Table 3 for the main analysis population in the two studies.

Primary efficacy endpoint. In study 1, the parasitological success rates were 93.3% (14/15) and 66.7% (10/15) for the ArTiMist and quinine treatments, respectively, with no difference between the FAS and MFAS. The difference between treatments was not statistically significant.

In study 2, ArTiMist demonstrated superiority over i.v. quinine in both efficacy populations. For the MITT population, 94.3% (66/70) of the ArTiMist-treated patients and 39.4% (28/71)

of the quinine-treated patients had parasitological success, which was statistically significant ($P < 0.005$).

For the PP population, 95.6% (65/68) of the ArTiMist-treated patients and 40.6% (28/69) of the quinine-treated patients had parasitological success ($P < 0.005$).

Table 2 provides further details regarding the parasite success rate at each site for both studies. By site, the parasite response differed, and site was a statistically significant factor in the logistic model for both populations ($P = 0.034$ for MITT; $P = 0.008$ for PP). At each study site, the difference between treatments was statistically significant.

The study site in Rwanda was the same for studies 1 and 2. The differences in parasite success rates between the studies, i.e., 2.4% (-12.1 to 16.9%) and 19.1% (-13.8 to 52.0%) for the ArTiMist and quinine treatments, respectively, were not statistically significant.

Secondary parasitological efficacy endpoints. As there was no difference in outcome for any of the secondary endpoints in either study between the MITT/FAS and the PP/MFAS populations, only the details of the MITT/FAS populations are described below.

In study 1, the PCT_{90} was 17.6 ± 7.3 h and 19.8 ± 13.6 h (means ± standard deviations [SD], unless otherwise noted), and the PCT_{50} was 12.0 ± 6.5 h and 10.8 ± 7.4 h for the ArTiMist and quinine treatments, respectively; the differences between the survival curves were not statistically significant. There was no difference in the PRR_{12}, PRR_{24}, or PCT between treatments.

For study 2, 53% (37/70) of the patients treated with ArTiMist cleared their parasites within 24 h. In comparison, 4% (3/71) of the quinine-treated patients cleared their parasites within 24 h (Fig. 2). No ArTiMist-treated patient was parasitemic at 72 h, whereas 13% (9/71) of the quinine-treated patients remained parasitemic at 72 h. As indicated in Table 3, the secondary efficacy parameters relating to parasite clearance, PCT, PRR_{24}, PCT_{50}, and PCT_{90}, demonstrated a statistically significant difference overall between the treatments ($P < 0.005$) for both populations. Although the parasite counts had almost halved by 12 h (PRR_{12}) for the ArTiMist-treated patients, they increased for quinine-treated patients ($P = 0.064$).

In study 1, there were no early treatment failures. In study 2, there were no early treatment failures for the ArTiMist-treated patients; for the quinine group, 14.1% (10/71) were early treatment failures. Eight were due to parasite counts on day 2 being greater than the baseline parasite count, and two were due to the day 3 parasite count being ≥25% of the baseline parasite count. One patient developed cerebral malaria, severe anemia, and convulsions following 24 h of i.v. quinine treatment. Quinine study treatment was discontinued, and rescue treatment (i.v. artesunate) was administered, following which the patient recovered.

In study 2, 17.1% (12/70) and 19.7% (14/71) of the patients had late parasitological failures in the ArTiMist and quinine treatment groups, respectively.

For both studies, after the initial treatment with either ArTiMist or i.v. quinine, patients were given a full course of oral therapy in accordance with the applicable treatment guidelines in effect in the country at the time. For study 2, 73.5% (111/151) of the patients received a full course of artemether-lumefantrine, 8.5% (13/151) continued with oral quinine for ≥7 days, and 17.9% (27/151) continued with ArTiMist for ≥7 days.

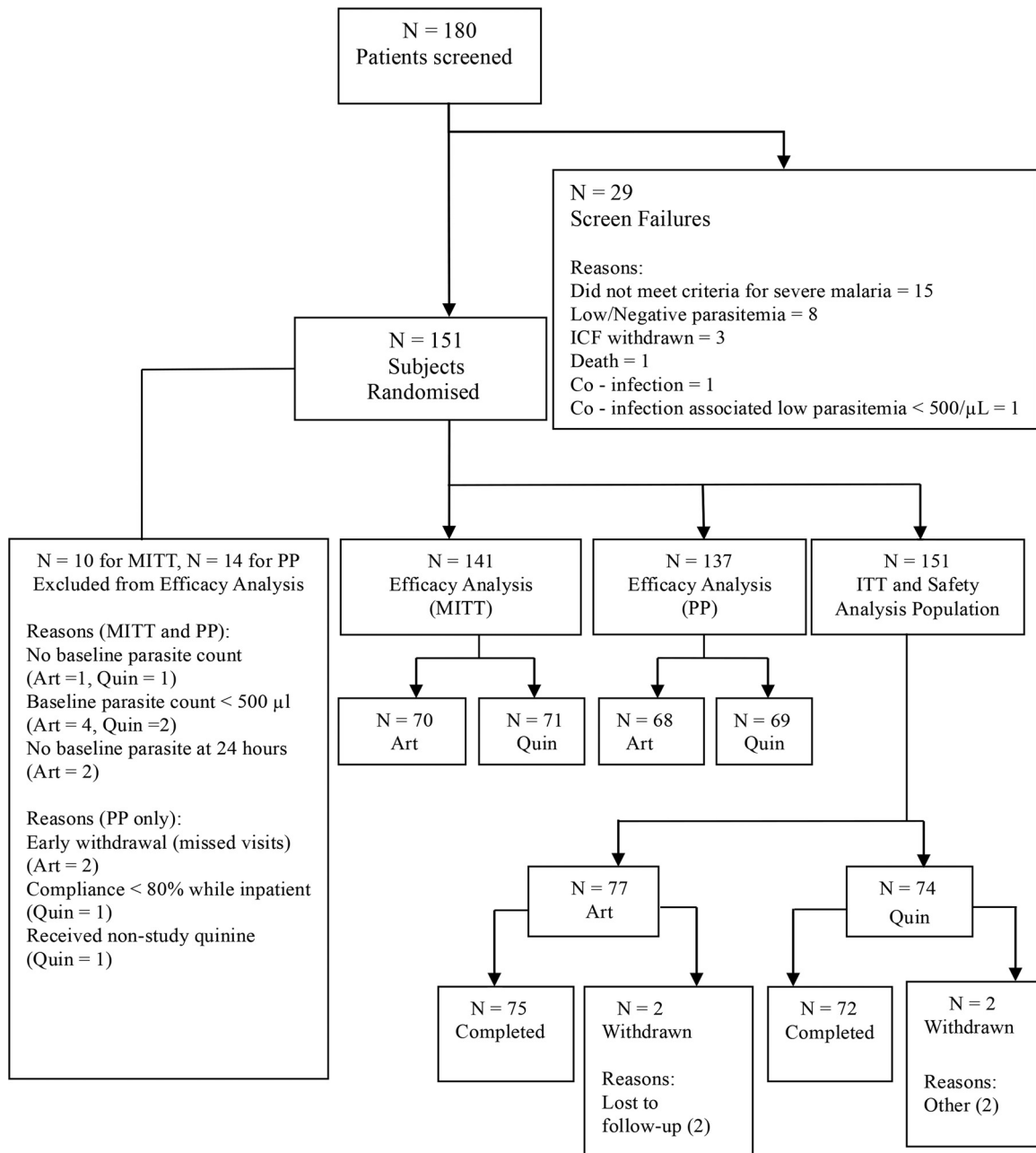**Secondary clinical efficacy endpoints.** In both studies 1 and 2,

FIG 1 Flow chart of patient disposition for the treatment of African children with malaria using sublingual artemether (ArTiMist [Art]) or i.v. quinine (Quin) in study 2. ICF, informed consent form.

there was no difference between treatments for FCT or the time to normal *per os* status. The time to return to full level of consciousness (LOC) (for patients with decreased LOC prior to treatment) was evaluated in study 2 only. All 17 ArTiMist and 21 quinine patients with a reduced level of consciousness before dosing were at the Burkina Faso study site. The times to return to full consciousness were 20.8 ± 9.6 h and 23.0 ± 16.3 h for the ArTiMist and quinine treatments, respectively, which was not statistically significant.

Of the patients treated with ArTiMist and quinine, 4.3% (3/70) and 1.4% (1/71) had late clinical failures, respectively, which was not statistically significant.

Cure rates. Cure rates were evaluated for study 2 only. Overall, the complete cure rates were similar for ArTiMist- and quinine-treated patients for both the MITT and PP populations.

One patient with a mixed infection cleared parasites of both *Plasmodium* species (*P. falciparum* and *P. ovale*) within 24 h of starting ArTiMist treatment and had a complete cure at day 28.

Safety evaluation and adverse events. There were no deaths in either of the studies. One patient (quinine) in study 1 had a serious adverse event (SAE), with malaria recrudescence/reinfection at the day-28 visit requiring inpatient treatment with i.v. quinine. Of the 14 SAEs reported in study 2, 71.4% (10/71) were in quinine-treated patients, and the remaining 28.6% (4/70) were in ArTiMist

TABLE 1 Demographics and baseline characteristics of African children in studies 1 and 2 who were treated for malaria using sublingual artemether (ArTiMist) or i.v. quinine

Characteristic	Study 1		Study 2	
	ArTiMist	Quinine	ArTiMist	Quinine
No. of patients	16	15	77/70[a]	74/71[a]
Age (mean ± SD) (yr)	3.0 ± 1.5	3.6 ± 2.5	2.8 ± 1.3	2.5 ± 1.2
Male sex (no. [%])	9 (56.3)	7 (46.7)	37 (48.1)	35 (47.3)
Disease definition				
Severe or complicated malaria (no. [%])	10 (62.5)	12 (80.0)	49 (63.6)	51 (68.9)
Uncomplicated malaria with GI[b] complications (no. [%])	6 (37.5)	3 (20.00)	28 (36.4)	23 (31.1)
Mixed, with ≥500 *P. falciparum* parasite count/μl (no. [%])			1 (1.3)	0 (0)
Vital signs				
Wt (mean ± SD) (kg)	11.2 ± 2.5	11.4 ± 3.4	11.7 ± 2.4	11.2 ± 2.5
Respiratory rate (mean ± SD) (breaths/min)	31.9 ± 9.1	32.8 ± 10.7	36.7 ± 11.0	35.9 ± 11.3
Pulse rate (mean ± SD) (beats/min)	142.4 ± 24.7	144.3 ± 15.1	146.5 ± 20.2	147.5 ± 26.3
Tympanic temp (mean ± SD) (°C)	38.2 ± 1.3	37.8 ± 0.8	38.6 ± 1.1	38.6 ± 1.0
Systolic blood pressure (mean ± SD) (mm Hg)	87.5 ± 10.0	84.3 ± 6.5	99.3 ± 12.3	99.4 ± 11.6
Diastolic blood pressure (mean ± SD) (mm Hg)	50.5 ± 10.0	50.7 ± 7.3	58.7 ± 10.8	58.5 ± 11.4
Blantyre Coma Scale score <5 (no. [%])	3 (18.7)	3 (20.0)	17 (22.1)	21 (28.4)
Parasite count (/μl)				
Rwanda				
Mean ± SD	63,430 ± 173,551	30,989.7 ± 33,841	69,752 ± 94,146	65,537 ± 63,357
Median (range)	19,660 (1,480–712,307)	21,800 (1,120–109,440)	28,958 (933–374,248)	51,061 (1,067–216,601)
Ghana				
Mean ± SD			151,014 ± 124,850	190,723 ± 243,425
Median (range)			138,021 (8,067–515,556)	128,686.0 (1,225–843,746)
Burkina Faso				
Mean ± SD			123,905 ± 137,880	125,557 ± 151,246
Median (range)			62,431 (581–494,500)	48,415 (6,679–551,067)

[a] Patient numbers are for the safety analysis population, except for the parasite counts, which are for the MITT population.
[b] GI, gastrointestinal.

patients. There were 9 reports (9 patients) of anemia, all of which were unrelated to study medication, that either was life-threatening or required prolonged hospitalization. All required blood transfusion, following which all events resolved; 77.8% (7/9) of those were in quinine-treated patients, and the remaining 22.2% (2/9) were in ArTiMist-treated patients. There was 1 report of bronchopneumonia (1 patient) and 1 report of sepsis (1 patient) in patients allocated to ArTiMist treatment that either required or prolonged inpatient hospitalization. Both are common comorbidities in children with severe malaria and were considered to be unrelated to ArTiMist treatment.

One patient allocated to quinine treatment failed to respond to i.v. quinine therapy and developed cerebral malaria. Quinine treatment was stopped, and the patient received i.v. artesunate as rescue therapy,

TABLE 2 Primary efficacy endpoint of African children in studies 1 and 2 who were treated for malaria using sublingual artemether (ArTiMist) or i.v. quinine by site

Study (population)	Site	Treatment	No. of patients	No. (%) with parasitological success[b]	No. (%) without parasitological success[b]	Δ (95% CI)[c] (%); P value
1 (MFAS)	Rwanda[a]	ArTiMist	15	14 (93.3)	1 (6.7)	26.7 (−0.3–53.7); 0.17
		Quinine	15	10 (66.7)	5 (33.3)	
2 (MITT)	Rwanda[a]	ArTiMist	23	22 (95.7)	1 (4.3)	48.1 (21.8–74.4); <0.05
		Quinine	21	10 (47.6)	11 (52.4)	
	Ghana	ArTiMist	23	23 (100.0)	0 (0.0)	88.0 (59.8–116); <0.05
		Quinine	25	3 (12.0)	22 (88.0)	
	Burkina Faso	ArTiMist	24	21 (87.5)	3 (12.5)	27.5 (2.8–52.2); <0.05
		Quinine	25	15 (60.0)	10 (40.0)	

[a] Study site was the same in study 1 and study 2.
[b] Parasitological success was defined as a reduction in parasite count of ≥90% of baseline at 24 h after the first dose.
[c] Differences between groups (Δ) and their 95% confidence intervals (CI) are shown.

TABLE 3 Parasitological and clinical endpoints for African children who were treated for malaria using sublingual artemether (ArTiMist) or i.v. quinine for study 1 (MFAS population) and study 2 (MITT population) by study and treatment group

Endpoint	Study 1			Study 2		
	ArTiMist	Quinine	Δ (95% CI) or hazard ratio (95% CI)[a]	ArTiMist	Quinine	Δ (95% CI; P) or hazard ratio (95% CI; P)[a]
No. of patients	15	15		70	71	
Parasitological success (no. [%])	14 (93.3)	10 (66.7)	26.6 (−0.3 to 53.7)	66 (94.3)	28 (39.4)	54.9 (42.25 to 67.45; <0.005)
PCT (h)						
Mean ± SD	35.7 ± 42.0	51.2 ± 79.0	NA	30.3 ± 13.2	68.3 ± 98.0	39.0 (−62.2 to −15.72; <0.005)
Median (range)	24 (12 to 182)	30 (12 to 331)	NA	24.0 (12 to 72)	47.8 (3 to 526)	NA
PCT_{90} (h)						
Mean ± SD	17.6 ± 7.3	19.8 ± 13.6	1.05 (0.5 to 3.0)	15.0 ± 5.8	27.93 ± 18.0	−12.9 (−17.4 to −8.4; <0.005)
Median (range)	18 (6 to 36)	18 (3 to 60)	NA	15.8 (3 to 32)	26.4 (3 to 156)	0.2 (0.1 to 0.3; <0.005)
PCT_{50} (h)						
Mean ± SD	12.0 ± 6.5	10.8 ± 7.4	0.9 (0.3 to 2.2)	9.4 ± 5.7	18.6 ± 9.2	−9.2 (−11.7 to −6.6; <0.005)
Median (range)	12 (3 to 24)	12 (3 to 24)	NA	10.5 (2 to 22)	20.1 (1 to 42)	0.2 (0.1 to 0.3; <0.005)
PRR_{12} (%)						
Mean ± SD	45.1 ± 85.1	58.5 ± 53.1	−13.5 (−66.0 to 39.1)	47.6 ± 70.3	−132.2 ± 765.9	174.1 (−10.4 to 358.6; 0.064)
Median (range)	79.6 (−220 to 100)	75.9 (−58 to 100)	NA	72.3 (−287 to 100)	−10.3 (−635 to 100)	NA
PRR_{24} (%)						
Mean ± SD	97.7 ± 6.0	89.1 ± 16.1	8.7 (−0.4 to 17.7)	98.2 ± 6.1	44.5 ± 114.3	54.0 (27.1 to 81.0; <0.005)
Median (range)	100 (77 to 100)	96.7 (53 to 100)	NA	100.0 (69 to 100)	80.6 (−664 to 100)	NA
Time to normal *per os* (h)						
Mean ± SD	17.7 ± 11.3	20.7 ± 9.4	NA	22.1 ± 12.9	25.3 ± 16.3	−3.2 (−8.2 to 1.8; 0.20)
Median (range)	17.2 (0 to 48)	18.0 (6 to 36)	NA	23.8 (2 to 50)	23.9 (1 to 64)	0.9 (0.7 to 1.3)
FCT (h)						
Mean ± SD	89.9 ± 72.8	86.1 ± 25.8	NA	42.6 ± 34.5	41.6 ± 22.7	1.0 (−10.2 to 12.1; 0.86)
Median (range)	75.9 (30 to 331)	91.7 (30 to 126)	NA	38.1 (3 to 229)	42.6 (3 to 96)	0.9 (0.6 to 1.3)
Time to return to full consciousness (h)[b]						
Mean ± SD	9.0 ± 6.5	11.0 ± 6.2	NA	20.8 ± 9.6	25.3 ± 16.3	−3.2 (−8.2 to 1.8; 0.62)
Median (range)	6 (3 to 18)	12 (3 to 18)	NA	23.8 (3 to 36)	17.8 (3 to 60)	0.8 (0.4 to 1.6)
Early treatment failure (no. [%])	0 (0.0)	0 (0.0)	NA	0 (0.0)	10 (14.1)	NA
Late clinical failure (no. [%])	NA	NA	NA	3 (4.3)	1 (1.4)	2.6 (0.3 to 26.6; 0.41)
Late parasitological failure (no. [%])	NA	NA	NA	12 (17.1)	14 (19.7)	1.0 (0.4 to 2.6; 0.95)

[a] Differences between groups (Δ) and their 95% confidence intervals (CI) are shown. Hazard ratio (95% CI) and difference in survival curves were compared by log rank (study 1) and Cox regressions analysis (study 2). NA, not applicable, as was not assessed or calculated.
[b] For patients whose level of consciousness was reduced prior to dosing.

following which the patient recovered. The investigator considered the SAE not to be related to the study medication.

Local tolerability was assessed by regular physical examination of the mouth and documentation of local adverse events. There were no adverse events reported or physical/oral examination observations noted that related to local tolerability in any of the patients treated with ArTiMist.

In study 1, there were no reported AEs that were considered by the investigator to be treatment related. In study 2, 6.5% (5/70) of the patients treated with ArTiMist experienced six related treatment-emergent adverse events (TEAEs): 2 cases of diarrhea, 2 cases of vomiting, 1 case of parotitis, and 1 case of cough.

Of the quinine-treated patients, 8.1% (6/71) had six related TEAEs, which included 1 case of anemia, 2 cases of abdominal pain, 1 case of headache, 1 case of diarrhea, and 1 case of vomiting.

In both studies, a number of patients had abnormalities in clinical status, vital signs, and laboratory parameters at baseline

(Table 1). In study 2, there were no differences between the treatment groups for the mean values (overall and by site) for body weight changes, systolic blood pressure, diastolic blood pressure, heart rate, respiratory rate, and temperature during the study. Following treatment with ArTiMist, these abnormalities resolved as expected and were consistent with the patients in the comparator treatment arm. No new abnormalities that were associated with ArTiMist treatment emerged.

DISCUSSION

Death from malaria reflects a delay in administration of effective antimalarial treatment. Immediate treatment with prereferral rectal artesunate substantially reduces the risk of death or permanent disability in severely ill young children for whom there is a delay in access to treatment (13).

The present data show that ArTiMist given at a dose of 3.0 mg/kg twice daily for 3 days to young African children who have
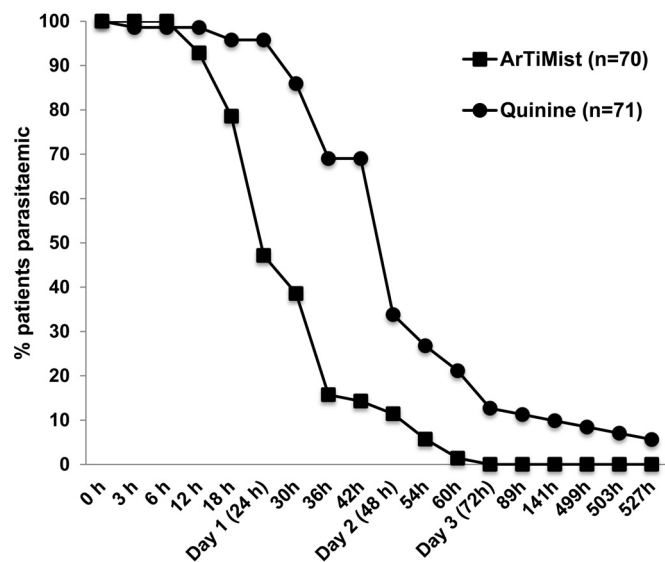
FIG 2 Percentage of patients still parasitemic over time among African children who were treated for malaria using sublingual artemether (ArTiMist) or i.v. quinine in study 2.

severe malaria or who are unable to tolerate conventional oral therapy leads to rapid clearance of parasites and clinical recovery.

Pharmacokinetic analysis shows that ArTiMist is promptly and adequately absorbed regardless of the indices of severity, including consciousness level, and even in patients with a history of vomiting at presentation (11). Simulations demonstrated that sublingual artemether bioavailability (artemether and dihydroartemisinin [DHA]) was at least equivalent to that after conventional artemether-lumefantrine oral therapy in older Melanesian children with uncomplicated malaria (11).

Intravenous quinine was chosen as the active comparator for both studies. This was the standard of care for children with severe or complicated falciparum malaria or uncomplicated falciparum malaria with gastrointestinal complications during study 1 and at the start of study 2 (14).

During the course of study 2, the treatment recommendations for severe childhood malaria were updated (15), following the publication of a large randomized controlled trial enrolling 5,425 children <15 years of age across Africa, showing a significant reduction in mortality (by 22.5%) in the artesunate group compared to mortality in the quinine group (16). Intravenous or i.m. artesunate was recommended as the treatment of choice in children with severe malaria, although i.v. quinine remained an acceptable alternative if artesunate was not available (15).

The concerned ethics committees, regulatory authorities, and investigators were notified and considered that i.v. quinine remained an acceptable comparator treatment in this study, as (i) i.v. quinine continued to be an acceptable treatment in the updated WHO treatment guidelines, (ii) i.v. artesunate was not on the national treatment guidelines for any of the concerned countries at the time, and (iii) Good Manufacturing Practices (GMP)-quality i.v. artesunate was not available to complete the trial.

The dose of 3 mg/kg of ArTiMist was based on a previous pharmacokinetic study in healthy adults (10) with extrapolation to children. The dosing regimen was the same as the twice-daily 3-day course of artemether-lumefantrine treatment.

In our studies, the baseline *P. falciparum* parasite count was required to be ≥500/μl. Although some studies of severe malaria required a higher parasite count for study entry (17, 18), this level was considered appropriate, as patients unable to tolerate oral medication could also be included. The concern of including patients with low baseline counts would be that a patient has incidental parasitemia while being ill due to a different condition. Clinically, however, as it is often difficult to differentiate the symptoms from malaria and other coinfections or conditions, patients would require treatment for malaria, in which case ArTiMist may be of potential benefit for their initial treatment.

The primary endpoint used for both studies was parasitological success, which has been used to evaluate early treatment for severe malaria following rectal artemisinin treatment (19). Although the PCT is the most commonly used primary endpoint in the reported literature, it is a function of the pretreatment parasite count (20) that might be biased over the 24-h period, detracting from the study objectives. Once parenteral antimalarials had been started, it was required to administer them for ≥24 h before converting patients to suitable oral treatment, irrespective of the ability of the patient to tolerate oral medication earlier (14). Therefore, it was essential to have the primary endpoint evaluated at 24 h, as the quinine-treated patients could then be changed to oral therapy, potentially confounding the PCT thereafter. The PCT, PCT_{50}, PCT_{90}, PRR_{12}, and PRR_{24} were evaluated as secondary endpoints for parasitological clearance, with FCT, time to normal *per os*, and time to return to full consciousness as secondary endpoints for clinical response in both studies to allow wider comparability to other reported studies.

Comparisons with other published data. In both studies, children had less-severe malaria than that reported in some other studies of children with severe malaria; notably, there were no deaths or patients with neurological sequelae in either of our studies. In the AQUAMAT study, for example, the mortality rate was 10.9% for the quinine treatment (control). At baseline, 35% of the patients in the quinine treatment arm had coma (16), whereas 20% and 28.1% of children receiving quinine treatment had a reduced level of consciousness in our studies 1 and 2, respectively.

ArTiMist has been developed to minimize the delay in the initial administration of an effective antimalarial in children with severe malaria. In this regard, the most important consideration is the rapid clearance of parasites with corresponding improvement of clinical signs or a delay in disease progression while the patient is transported to a suitable facility for further treatment.

As the clearance of parasites is a surrogate endpoint, it cannot be assumed that greater parasite clearance results in improved clinical outcome and lower mortality. However, both the AQUAMAT (16) and SEAQUAMAT (21) trials demonstrated that more rapid parasite clearance with artesunate resulted in significantly lower mortality rates. Without direct evidence, the same cannot be inferred for other artemisinin derivatives, such as artemether.

A comparison of our results to those in the literature for both the ArTiMist and quinine treatments, with respect to parasite clearance and clinical response, contributes to the validity of our studies and the weight of evidence of the proposed intervention.

The parameters for PCT are consistent with those reported in the literature for both the ArTiMist (artemether) and quinine treatment arms. For ArTiMist-treated patients, the mean (35.7 ± 42.0 h and 30.3 ± 13.2 h) and median (24 h [range, 18 to 182 h] and 24.0 h [12 to 72 h]) PCT for studies 1 and 2, respectively, are

in accord with data from other studies, ranging from 16.0 ± 9.2 h to 54.2 ± 33.6 h (17, 18, 22, 23) and 32 h (24 to 52 h) to 48 h (36 to 60 h) (24–28).

Similarly, the corresponding parameters for quinine-treated children (51.2 ± 79 h and 68.3 ± 98.0 h, 30 h [12 to 331 h], and 47.8 h [3 to 526 h]) were in accord with the reported PCT for the quinine control groups, ranging from 22.4 ± 11.5 h to 55.0 ± 24.3 h (22, 23, 28) and 40 h (32 to 48 h) to 60 h (48 to 72 h) (24, 25, 27).

Gomes et al. (19) pooled individual patient data from 1,167 patients in 15 clinical trials of rectal artemisinin derivative therapy (artesunate, artemisinin, and artemether) to compare the rapidity of clearance of *P. falciparum* parasitemia. The reported parasite reduction ratio at 12 h (PRR_{12}) ranged from 32.7% to 73.5%. In comparison, for studies 1 and 2, the PRR_{12} values for ArTiMist were $45.1\% \pm 85.1\%$ and $47.6\% \pm 70.3\%$, respectively. Similarly, for the PRR_{24}, the reported parameters ranged from 83.1% to 96.7% (19), and the respective parameters for studies 1 and 2 were $97.7\% \pm 6.0\%$ and $98.2\% \pm 6.1\%$. The reported values for the i.v. quinine arm for PRR_{24} ranged between 63.7% and 68.7% (19), in comparison to $89.1\% \pm 16.1\%$ and $44.5\% \pm 114.3\%$ for studies 1 and 2, respectively.

In a Cochrane review conducted by Sinclair et al. (29), eight trials enrolling 1,664 adults and 5,765 children comparing i.v. or i.m. rectal artesunate with i.v. or i.m. quinine for treating adults and children with severe malaria who are unable to take medication by mouth were included (29). In this analysis, it was reported that artesunate appeared to be superior to quinine at reducing the PCT_{50} by a median of 8.14 h (range, -11.55 to -4.73 h) (292 participants in three trials), the PCT_{90} by 18.50 h (-24.13 to -12.87 h) (61 patients in one trial), and PCT by 9.77 h (-18.11 to -1.44 h) (419 patients in four trials) (29). For study 2, the corresponding parameters were -9.2 h (-11.71 to -6.61 h), -12.1 h (-17.38 to -8.44 h), and -39.0 h (-62.2 to -15.7 h). There was no difference in coma recovery time or FCT (29).

In a similar review by McIntosh and Olliaro (30), 11 studies compared artemether (1,069 patients) with quinine (1,073 patients). The median PCT values from the three largest studies were 32 to 72 h with artemether, compared with 40 to 90 h for quinine (30).

Five studies showed no difference in FCT, three showed that artemether was faster, and one showed that quinine was faster than artemether (30). In both of our studies, there was no difference in FCT.

Only one study reported fully on the time to drink, eat, sit, stand, and walk. As in our studies, there was no significant difference shown between the treatment groups. Another study reported no significant difference in the time to walk (30).

Cure rates were secondary endpoints of lesser importance in this study, as they are not a reflection of the evaluation of the treatment intervention under investigation. There was no difference in cure rates between the ArTiMist- and quinine-treated patients.

ArTiMist treatment was well tolerated. Most of the clinical abnormalities reported were due to malaria and not the study treatment; no local adverse events were reported.

Given the WHO recommendations regarding the need for artemisinin drugs to be administered in combination with a longer half-life partner (such as lumefantrine), except in situations in which initial oral therapy cannot be given safely and reliably (14), ArTiMist is likely to have application as one- or two-dose prereferral treatment as sick children are transferred for parenteral ar-

temisinin therapy or oral ACT. Given potential pharmacokinetic (31), cultural (13), and efficacy (32) concerns regarding artesunate suppositories in this situation, ArTiMist appears to be a valuable alternative.

ACKNOWLEDGMENTS

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