Short-Course Regimens of Artesunate-Fosmidomycin in Treatment of Uncomplicated *Plasmodium falciparum* Malaria

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Fosmidomycin is effective against malaria, but it needs to be given for ≥ 4 days when used alone. We conducted a study of 50 children with *Plasmodium falciparum* malaria to evaluate the safety and efficacy of consecutively shortened regimens of artesunate-fosmidomycin (1 to 2 mg/kg of body weight and 30 mg/kg of body weight, respectively; doses given every 12 hours). All dosing regimens were well tolerated. Artesunate-fosmidomycin acted rapidly, resulting in consolidated geometric mean parasite and fever clearance times of 24 h and 15 h, respectively. Treatment regimens of ≥ 2 days led to cure ratios of 100% by day 14 (39/39; 95% confidence interval [95% CI], 91% to 100%). Most importantly, the 3-day regimen achieved 100% cure on day 28 (10/10; 95% CI, 69% to 100%). Treatment with artesunate-fosmidomycin was associated with transient grade I or II neutropenia (absolute neutrophil counts of 750 to 1,200/ μ l and 400 to 749/ μ l, respectively) in six or two patients, respectively. Artesunate-fosmidomycin demonstrates the feasibility and potential value of short-course artemisinin-based combination chemotherapy with rapidly eliminated combination partners.

Fosmidomycin inhibits the isoprenoid biosynthesis of *Plasmodium falciparum* by targeting the 1-deoxy-D-xylulose 5-phosphate (DOXP) reductoisomerase of the DOXP pathway most likely located inside the apicoplast organelle of the parasite (7, 20). Previous clinical studies have demonstrated that fosmidomycin acts rapidly and is well tolerated in the treatment of uncomplicated *Plasmodium falciparum* malaria, but late asexual parasite recrudescences with treatment regimens of less than 4 days preclude the use of fosmidomycin as a monotherapeutic agent (11, 12).

In addition to the combination of fosmidomycin with clindamycin (1, 3), artemisinin, the parental compound of the artemisinin class of antimalarials, was identified as a potential therapeutic partner for fosmidomycin (21). Artesunate, an active semisynthetic artemisinin derivative given orally, acts extremely rapidly and efficiently suppresses posttreatment gametocyte prevalence and density. Artesunate has an excellent safety record (18), and naturally acquired resistance of *P. falciparum* against artesunate or other artemisinins has not yet been reported (19). By analogy to fosmidomycin, artesunate is not sufficiently efficacious on its own in <5 -day regimens (2), and to increase the efficacy of short-course regimens, it needs to be combined with other drugs. With regard to these consid-

erations, artesunate-fosmidomycin was selected for further clinical development, even though a previous in vitro study suggested indifferent, rather than synergistic, interaction of fosmidomycin and artemisinin (21).

Here we report the results of a phase II clinical trial evaluating the safety, tolerability, and efficacy of incrementally shortened regimens of artesunate-fosmidomycin for the treatment of uncomplicated childhood malaria due to infection with *P. falciparum*.

MATERIALS AND METHODS

Study area. The study was conducted at the Albert Schweitzer Hospital in Lambaréné, Gabon. The town of Lambaréné and the surrounding area are characterized by a high year-round transmission of *P. falciparum* and an entomological inoculation rate of around 50 infective bites per person per year (17, 22). The study protocol was approved by the ethics committee of the International Foundation of the Albert Schweitzer Hospital in Lambaréné, Gabon.

Study design. The study was designed as a dose reduction study with consecutive recruitment of eligible patients in cohorts of 10 with no controls. The first cohort was treated with a 5-day regimen of artesunate-fosmidomycin. After the inclusion of 10 patients, the recruitment of further patients was halted. When $\geq 80\%$ of patients within a cohort were clinically and parasitologically cured by day 14, a new cohort of 10 patients received a treatment regimen that was shortened by 1 day (equivalent to a reduction of two treatment doses). Recruitment was discontinued if the day 14 cure ratio of a cohort of 10 patients was $<80\%$.

Enrollment of patients. The study took place from May 2002 until October 2003. Pediatric patients with uncomplicated *P. falciparum* malaria attending the outpatient pediatric department of the Albert Schweitzer Hospital in Lambaréné, Gabon, were admitted to the study if they met the following inclusion criteria: age of 6 to 12 years; asexual parasite density between 1,000 and 100,000/ μ l; acute manifestation of malaria; body weight between 22 and 62 kg; ability to tolerate oral therapy; informed consent by the legal representative of the subject

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(if possible, the parents); oral agreement of the child if appropriate; and residence in the study area for at least 4 weeks. The exclusion criteria were as follows: adequate antimalarial treatment within the previous 7 days (determined by questioning the caretaker and by consulting the child's health care booklet), antibiotic treatment for a current infection, hemoglobin of <7 g/dl, hematocrit of $<25\%$, leukocyte count of $>15 \times 10^9$ /liter, mixed plasmodial infection, severe malaria, any other severe underlying disease, concomitant disease masking assessment of treatment response, any other disease causing fever, and inflammatory bowel disease.

Study drugs and administration. Fosmidomycin was formulated as capsules containing 150 mg of active substance. Artesunate was supplied as tablets containing 50 mg of active substance. The drugs were coadministered orally under direct supervision using the following dosing schedules: 2 mg of artesunate/kg of body weight every 12 h on days 0 to 2 (1-, 2-, and 3-day regimens) or 2 mg/kg of artesunate every 12 h on days 0 and 1 and then 1 mg/kg of artesunate every 12 h on days 2 to 4 (4- and 5-day regimens) and 30 mg/kg of fosmidomycin every 12 h (targeting mean total doses of 14, 12, 12, 8, and 4 mg/kg of artesunate and 300, 240, 180, 120, and 60 mg/kg of fosmidomycin for the 5-, 4-, 3-, 2-, and 1-day regimens, respectively). Subjects who vomited or rejected the study drug within 30 min received a second full dose. Vomiting or rejecting the second dose led to withdrawal from the study and administration of a rescue treatment. All patients with reappearing asexual parasitemias received oral treatment: a single dose of sulfadoxine-pyrimethamine (25 and 1.25 mg/kg of body weight) in combination with a 7-day course of quinine (10 mg/kg of body weight twice daily).

Study flow and procedures. Patients were seen by a study physician at 12-hour intervals at each drug administration and/or until two consecutive negative blood smears were recorded and again on days 7, 14, 21, and 28 or if otherwise indicated. On each visit during the treatment and follow-up phase, the medical history was taken, vital signs were checked, tympanic temperature was measured, a thick blood smear was prepared from a finger prick, and adverse events were documented. Venipunctures were performed on study days 0, 2, 7, and 28 to monitor hemoglobin, hematocrit, and leukocyte count with differential and platelet counts, as well as liver (alanine aminotransferase) and renal function parameters (creatinine). In addition, urine dipstick tests were done at the same time intervals. To distinguish recrudescences from new infections, EDTA blood samples were stored at -80°C on day 0 and the day of reappearance of asexual parasites for PCR-based genotyping analysis.

End points. The primary efficacy end point of this study was the day 14 cure ratio. Cure was defined as initial and sustained parasite and symptom clearance with no increase in asexual parasite density 48 h after the initiation of treatment and the absence of microscopically detected asexual parasitemia within ≤ 120 h of the start of treatment until day 14. The primary safety end point was the incidence of serious adverse events after the start of treatment. Secondary end points were the incidence of nonserious adverse events, parasite and fever clearance times, and PCR-corrected day 28 cure ratios.

Laboratory. The dried thick blood smears were stained with 20% Giemsa solution at pH 7.2. Parasite species were identified using standard morphological characteristics. Parasite density was counted with the previously described volumetric Lambaréné method and expressed as numbers of parasites/ μl (4, 16). To distinguish recrudescences from new infections, matched pairs of parasite isolates obtained on admission and on the day of reappearing asexual parasite density were compared using PCR-based genotyping analysis of repeat length polymorphisms of the *pfmSP2* gene locus (9, 13). We classified a reappearing asexual parasitemia after initial clearance as reinfection if the sizes of all electrophoretically separated PCR product bands detected on the day of reappearing asexual parasitemia were distinct from those detected on the day of admission.

Data management and statistical analysis. Data were captured using specifically designed concise medical record forms and subsequently entered into an electronic database. Data were then validated by complete manual review of primary and secondary end-point variables. All continuous variables were systematically verified by range checks. Statistical analysis of the data was performed using a commercial software package (Stata v.8 for Mac OS X).

The cure ratio was calculated from the number of patients with clinical and parasitological cure by days 14 and 28, respectively, divided by the total number of evaluable patients (per protocol population). Patients with genotypically confirmed new infections were considered cured by day 28. Parasite and fever clearance times were calculated from the start of the study until the first of two consecutive negative blood smears (no detectable asexual parasites) and until the first time the tympanic temperature dropped below 37.5°C for at least 24 h, respectively. The recorded times were used to calculate exact parasite and fever clearance times as well as dosing intervals.

Comparisons were made with nonparametric tests (unpaired and paired Wilcoxon or Kruskal-Wallis rank tests as appropriate). Fisher's exact test was used

to assess differences in categorical outcome variables between the cohorts. Other statistical tests are specified in the respective contexts. The safety analysis included laboratory data and adverse events from all subjects who received at least one dose of the study drug (intention-to-treat population). The statistical significance level was set at 5%.

RESULTS

Study cohorts. In total, 50 patients were enrolled in the study and received at least one treatment dose. Enrollment in the study was stopped after the inclusion of 10 patients in the cohort receiving the 1-day regimen. Table 1 details the baseline characteristics of patients receiving treatment regimens with durations between 1 and 5 days. Baseline parameters were similar between cohorts, with the exception of a significant difference in the pulse rate ($P = 0.03$; uncorrected for multiple comparisons). Two minor protocol violations were revealed after study termination: two female patients in the 5-day and 4-day group were included in the study even though their age (13.6 years and 14.9 years, respectively) exceeded the upper limit of age for inclusion (12 years).

Figure 1 details the flow of patients through the trial. A total of 49 subjects were evaluable for the primary and secondary efficacy end points (day 14 and 28 cure ratios, respectively). The first patient of this study, a 9-year old male patient (5-day regimen cohort) was lost to follow-up after day 7.

Efficacy. The results describing the parasitological and clinical responses to treatment with artesunate-fosmidomycin are summarized in Table 2. Treatment with artesunate-fosmidomycin for 5, 4, 3, 2, and 1 days led to rapid asexual parasite reduction with geometric mean asexual parasite clearance times of 22 h (95% confidence interval [95% CI], 18 to 29 h), 35 h (27 to 45 h), 17 h (13 to 22 h), 31 h (22 to 43 h), and 19 h (14 to 25 h), respectively. Artesunate-fosmidomycin also led to prompt fever clearance with a combined median fever clearance time of 15 h (95% CI, 13 to 17 h) and no significant differences between treatments of different durations ($P = 0.50$). By day 7 all evaluable patients in the groups of treatment durations of 5 to 2 days were clinically and parasitologically cured. Two patients in the 1-day treatment group were found to carry asexual parasites on day 7.

By day 14 all evaluable patients who received artesunate-fosmidomycin for 5, 4, 3, and 2 days were clinically and parasitologically cured (10 out of 10 patients in each cohort) (Table 2). In contrast, only 7 out of 10 patients in the 1-day regimen cohort (two doses) remained free of asexual parasites until day 14 (two parasitological failures occurred on day 7 and one on day 12).

Between day 14 and day 28, asexual parasites reemerged in one patient in the 5-day regimen group on day 28; two patients on day 21 in the 4-day regimen group; one patient on day 21 in the 3-day regimen group; one patient, three patients, and one patient on days 17, 21, and 28, respectively, in the 2-day regimen group; and three patients on day 21 in the 1-day regimen group, resulting in crude, PCR-uncorrected parasitological day 28 cure ratios of 8/9 (89%; 95% CI, 52 to 100%), 8/10 (80%; 44 to 97%), 9/10 (90%; 55 to 100%), 5/10 (50%; 19 to 81%), and 4/10 (40%; 12 to 74%) patients, respectively. Samples for parasite genotyping analysis were available for all but three patients (one patient and two patients in the 1-day and 2-day groups, respectively). The results revealed that the two cases of

TABLE 1. Baseline characteristics of pediatric outpatients receiving artesunate-fosmidomycin for the treatment of *P. falciparum* malaria

Characteristic	Value for treatment group				
	5 days (10 doses)	4 days (8 doses)	3 days (6 doses)	2 days (4 doses)	1 day (2 doses)
No. of patients	10	10	10	10	10
Dosing					
Median total dose of artesunate (mg/kg) (range)	13 (10–21)	12 (10–14)	12 (10–14)	9 (7–9)	3 (3–5)
Median total dose of fosmidomycin (mg/kg) (range)	310 (280–450)	240 (220–260)	180 (170–200)	130 (120–130)	60 (60–70)
Median dosing interval (h) (range)	12 (10–16)	12 (9–17)	12 (11–13)	12 (10–13)	12 (11–13)
Demography					
Median age (yr) (range)	10.6 (7.5–13.6)	10.3 (8.0–14.9)	9.7 (7.0–12.1)	9.8 (7.5–12.9)	9.5 (7.8–12.1)
Gender (male/female)	6/4	5/5	4/6	4/6	4/6
Median wt (kg) (IQR) ^a	30 (9)	30 (15)	25 (5)	23 (6)	29 (3)
Clinical parameters					
Mean systolic blood pressure (mm Hg) (SD)	90 (11)	94 (7)	100 (9)	93 (11)	97 (13)
Mean diastolic blood pressure (mm Hg) (SD)	54 (9)	56 (12)	60 (8)	62 (8)	63 (11)
Mean pulse (/min) (SD)	93 (15)	94 (16)	109 (17)	88 (12)	103 (16)
Median tympanic temperature (°C) (IQR)	38.0 (2.7)	36.6 (2.3)	37.0 (2.3)	37.2 (1.4)	36.7 (2.4)
Parasitology					
Median density of asexual <i>P. falciparum</i> (/ μ l) (range)	35,400 (2,018–88,200)	34,560 (1,200–98,996)	10,660 (1,200–140,000)	36,800 (5,571–93,000)	39,500 (3,000–81,000)
Gametoocyte carrier rate	3/10	4/10	1/10	0/10	1/10
Hematology					
Mean hemoglobin concentration (g/dl) (SD)	10.7 (0.9)	10.4 (1.6)	9.7 (1.2)	10.0 (1.2)	9.9 (1.5)
Mean hematocrit (%) (SD)	36 (3)	33 (5)	32 (4)	32 (4)	32 (5)
Median leukocyte count (10^9 /liter) (IQR)	6.3 (3.4)	8.0 (4.4)	5.7 (1.1)	6.7 (1.5)	5.4 (3.0)
Median neutrophil count (10^9 /liter) (IQR)	3.0 (5.0)	5.8 (4.4)	2.6 (2.1)	3.5 (1.3)	2.2 (2.2)
Mean thrombocyte count (10^9 /liter) (SD)	180 (57)	165 (70)	171 (81)	186 (62)	177 (79)
Clinical chemistry					
Mean plasma alanine aminotransferase (U/liter) (SD)	22 (7)	23 (13)	24 (15)	14 (15)	26 (13)
Median plasma creatinine (μ mol/liter) (IQR)	59 (27)	50 (34)	62 (18)	64 (8)	66 (34)

^a IQR, interquartile range.

FIG. 1. Trial profile. An asterisk indicates one patient with an asexual-stage parasite reappearance, which was typed as reinfection.

parasite reappearances occurring on day 28 (one each in the 5-day and 2-day groups) and two out of nine cases of parasite reappearances occurring on day 21 (one each in the 3-day and 4-day groups) were due to new infections. The PCR-corrected day 28 cure ratios are given in Table 2. Thus, artesunate-fosmidomycin administered for at least 3 days led to high cure ratios by day 28. Treatment durations of 2 days and 1 day were less efficacious ($P < 0.001$ for the comparison of the combined cure ratios between treatment durations of 5 to 3 days versus 2 to 1 days).

Treatment with artesunate-fosmidomycin cleared sexual parasite stages in 4 out of 9 patients in whom gametocytes were present at baseline, but sexual parasite stages either persisted or newly emerged in 8 out of 50 patients (16%; 95% CI, 7 to 29%) between days 1 and 4 (all in the 4-day and 5-day treatment groups, which also had the highest baseline gametocyte carrier ratios). Treatment with artesunate-fosmidomycin was

associated with a very low, cumulative gametocyte carrier ratio after day 7 until day 28: 2/47 (4%; 95% CI, 0 to 15%).

Safety and tolerability. Treatment with artesunate-fosmidomycin was safe; no serious adverse events occurred. In total, 29 clinical adverse events were documented: 27 mild and 2 moderate events from which only 3 mild events were judged to be either unlikely or possibly related to the study drugs. All other adverse events were typical symptoms of uncomplicated *P. falciparum* malaria and therefore considered unrelated to the study medication. The most frequent adverse events prior to day 7 were gastrointestinal events (7/12), mostly abdominal pain (5) but not diarrhea or loose stools.

The evolution of laboratory parameters is summarized in Table 3. There was a significant decline in mean hemoglobin concentration and median leukocyte count from day 0 to day 2 (Table 3). Mean hemoglobin concentration and median leukocyte count recovered after days 4 and 7, respectively. Median

TABLE 2. Efficacy of artesunate-fosmidomycin in the treatment of pediatric outpatients with uncomplicated *P. falciparum* malaria

Parasitological or clinical response	Value for treatment group				
	5 days (n = 10)	4 days (n = 10)	3 days (n = 10)	2 days (n = 10)	1 day (n = 10)
Day 14 cure ratio (%; 95% CI)	9/9 (100; 66–100)	10/10 (100; 69–100)	10/10 (100; 69–100)	10/10 (100; 69–100)	7/10 (70; 35–93)
Day 28 cure ratio (%; 95% CI) ^a	9/9 (100; 66–100)	9/10 (90; 55–100)	10/10 (100; 69–100)	6/10 (60; 26–88)	4/10 (40; 12–74)
Geometric mean parasite clearance time (h; 95% CI)	22 (18–29)	35 (27–45)	17 (13–22)	31 (22–43)	19 (14–25)
Geometric mean fever clearance time (h; 95% CI)	15 (12–19)	13 (10–17)	14 (11–19)	14 (11–17)	19 (13–29)
Cumulative gametocyte carrier ratio from day 1 to day 7 (%; 95% CI)	3/10 (30; 7–65)	5/10 (50; 19–81)	0/10 (0–31)	0/10 (0–31)	0/10 (0–31)
Cumulative gametocyte carrier ratio from day 7 to day 28 (%; 95% CI)	0/10 (0–31)	1/10 (10; 0–45)	0/10 (0–31)	1/10 (10; 0–45)	1/10 (10; 0–45)

^a Results are corrected by PCR-based parasite genotyping analysis of paired samples from day 0 and day of recrudescence.

TABLE 3. Changes in laboratory parameters in pediatric outpatients treated with artesunate-fosmidomycin for uncomplicated *P. falciparum* malaria^a

Laboratory parameter	Day 0 to day 2			Day 0 to day 7			Day 0 to day 28		
	No. of pairs	Mean or median difference in parameter	P value	No. of pairs	Mean or median difference in parameter	P value	No. of pairs	Mean or median difference in parameter	P value
Mean hemoglobin concn (g/dl) (SD)	50	-0.6 (0.9)	<0.001	50	-0.4 (1.5)	0.047	45	1.0 (1.6)	<0.001
Median leukocyte count (10 ⁹ /liter) (IQR) ^b	50	-0.8 (2.9)	0.035	50	0.4 (3.6)	0.064	45	0.1 (3.5)	0.72
Median absolute neutrophil count (10 ⁹ /liter) (IQR)	44	-1.5 (3.6)	<0.001	45	-0.8 (3.4)	0.001	39	-0.8 (2.8)	<0.001
Median alanine aminotransferase concn (U/liter) (IQR)	49	-3.0 (22)	0.31	49	-1.0 (22)	0.63	26	-3.5 (26)	0.69
Median plasma creatinine (μmol/liter) (IQR)	48	-3.5 (21)	0.22	48	-5.0 (17)	0.043	34	-3.0 (21)	0.29

^a Data from all patients were pooled.^b IQR, interquartile range.

plasma alanine aminotransferase and creatinine concentrations dropped slightly during the treatment and/or follow-up phase, but these changes were not significant except for the change in plasma creatinine concentrations from day 0 to day 7 ($P = 0.043$ [Table 3]).

The drop in the neutrophil counts from day 0 to 2 was associated with transient neutropenia in a total of eight patients from all but the 4-day treatment group as defined by an absolute neutrophil count of less than 1,200/ μ l (six cases of grade I [750 to 1,200/ μ l] and two cases of grade II [400 to 749/ μ l] [14]). Neutrophil counts returned to normal values by day 7 in all eight patients without further intervention. However, the median time to fever clearance was prolonged in these patients compared to patients without such an episode (23 h versus 12 h, respectively; $P = 0.047$). There were two cases of transient posttreatment plasma alanine aminotransferase elevations in the 1-day treatment group: in an 8-year-old female patient (up to 96 U/liter on day 2 from 24 U/liter on admission) and in a 10-year-old male patient (up to 157 U/liter from 26 U/liter and 13 U/liter on admission and day 2, respectively). In addition, temporarily elevated plasma creatinine concentrations were detected in four patients (on either day 2 or day 7). No abnormal urine parameters were detected during the treatment and/or follow-up phase.

DISCUSSION

In this study the novel combination of artesunate-fosmidomycin was shown to be well tolerated and highly efficacious in the treatment of uncomplicated *P. falciparum* malaria. Treatment regimens of 5, 4, 3, and 2 days duration led to 100% parasitological and clinical cure ratios by day 14, and even 1 day of artesunate-fosmidomycin cured 7/10 patients by day 14. Most importantly, the 3-day regimen of artesunate-fosmidomycin achieved 100% cure by day 28, which is a highly sensitive marker for the efficacy of an antimalarial in a high transmission area, especially considering the short half-life of artesunate and fosmidomycin (less than 1 h and 2 h, respectively [8, 15]). A study design-related limitation of this phase II proof-of-principle study was the small sample size, which resulted in point estimates with wide confidence intervals. In addition, a higher degree of parasite-specific immunity in the study population (aged 6 to 12 years) compared to younger children might have contributed to an initial complete asexual parasite

clearance and/or successful suppression of recrudescence asexual infection, thus resulting in an overestimate of the true efficacy of artesunate-fosmidomycin in nonimmune patients. The encouraging results of the 3-day regimen require confirmation in a large randomized controlled trial including non-immune patients. In addition, the potential for simplifying the twice-daily dosing regimen could be explored in an extended phase II program.

Artesunate-fosmidomycin was associated with extremely low cumulative sexual-stage parasite carrier ratios during the follow-up period, and no sexual-stage parasites were detected in patients with recrudescence infections between day 7 and day 28. This is reassuring with regard to the resistance-conferring potential of sexual-stage parasites. However, artesunate-fosmidomycin was insufficiently active against mature sexual-stage parasites (more than half of the initially detected sexual-stage parasites persisted for more than 1 day), suggesting that the low cumulative sexual-stage carrier ratios during follow-up are a result of an inhibitory effect of artesunate-fosmidomycin on immature, and metabolically active, sexual-stage parasites.

Treatment with artesunate-fosmidomycin was associated with transient episodes of grade I and II neutropenia in 8 out of 50 patients (16%) on day 2. This was also related to a slightly prolonged median time to fever clearance in these patients compared to patients not experiencing such an episode. However, all cases resolved without further intervention until day 7. In the absence of a control group in this phase II study, it is difficult to attribute these episodes to the study medication in light of strong evidence for both immunosuppression in patients with uncomplicated *P. falciparum* malaria (6) and direct suppressive effects of antimalarials on neutrophil functions (10). Clearly, this issue requires further study.

A potential disadvantage of the evaluated twice-daily regimen of artesunate-fosmidomycin might be its reduced effectiveness under operational circumstances, although a recent study reported high compliance with an at least equally complex six-dose regimen of artemether-lumefantrine (5). It remains to be seen whether this optimistic conclusion can be substantiated in further studies.

Artemisinin-based combination chemotherapy has been widely advocated to delay the spread of parasite resistance to the combination partner (19). Artesunate-fosmidomycin appeared to be at least as efficacious as fosmidomycin-clindamycin in previous studies (20). The combination of artesunate

with fosmidomycin, however, might represent distinct advantages due to its inhibitory effect on sexual-stage parasites and, possibly, improved tolerability. Artesunate-fosmidomycin, unlike other artemisinin-based combination chemotherapies, such as artesunate-amodiaquine and artemether-lumefantrine, is characterized by the approximately matching short half-life of its components in plasma (8, 15), which eliminates the risk of selecting resistant parasite alleles through prolonged subtherapeutic concentrations in plasma. This might be an important advantage in the high transmission areas of sub-Saharan Africa.

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