

ORIGINAL ARTICLE

Antimalarial Activity of KAF156 in *Falciparum* and *Vivax* Malaria

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ABSTRACT

BACKGROUND

KAF156 belongs to a new class of antimalarial agents (imidazolopiperazines), with activity against asexual and sexual blood stages and the preerythrocytic liver stages of malarial parasites.

METHODS

We conducted a phase 2, open-label, two-part study at five centers in Thailand and Vietnam to assess the antimalarial efficacy, safety, and pharmacokinetic profile of KAF156 in adults with acute *Plasmodium vivax* or *P. falciparum* malaria. Assessment of parasite clearance rates in cohorts of patients with vivax or falciparum malaria who were treated with multiple doses (400 mg once daily for 3 days) was followed by assessment of the cure rate at 28 days in a separate cohort of patients with falciparum malaria who received a single dose (800 mg).

RESULTS

Median parasite clearance times were 45 hours (interquartile range, 42 to 48) in 10 patients with falciparum malaria and 24 hours (interquartile range, 20 to 30) in 10 patients with vivax malaria after treatment with the multiple-dose regimen and 49 hours (interquartile range, 42 to 54) in 21 patients with falciparum malaria after treatment with the single dose. Among the 21 patients who received the single dose and were followed for 28 days, 1 had reinfection and 7 had recrudescence infections (cure rate, 67%; 95% credible interval, 46 to 84). The mean (\pm SD) KAF156 terminal elimination half-life was 44.1 ± 8.9 hours. There were no serious adverse events in this small study. The most common adverse events included sinus bradycardia, thrombocytopenia, hypokalemia, anemia, and hyperbilirubinemia. Vomiting of grade 2 or higher occurred in 2 patients, 1 of whom discontinued treatment because of repeated vomiting after receiving the single 800-mg dose. More adverse events were reported in the single-dose cohort, which had longer follow-up, than in the multiple-dose cohorts.

CONCLUSIONS

KAF156 showed antimalarial activity without evident safety concerns in a small number of adults with uncomplicated *P. vivax* or *P. falciparum* malaria. (Funded by Novartis and others; ClinicalTrials.gov number, NCT01753323.)

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EXPANDING ARTEMISININ RESISTANCE and worsening partner-drug resistance in Southeast Asia threaten the global control of *Plasmodium falciparum* malaria.¹⁻⁵ New drugs are needed. KAF156 represents a new class of antimalarial agents (imidazolopiperazines)⁶ identified by high-throughput phenotypic screening. KAF156 has potent in vitro activity against both asexual and sexual blood stages and the pre-erythrocytic liver stages of the malarial parasite.⁷ The mechanism of antimalarial action is unknown, but drug resistance, mediated by mutations in the *P. falciparum* cyclic amine resistance locus (*PfCARL*) gene, which encodes a protein of unknown function, can be selected.⁷ In a study of 70 healthy adult volunteers,⁶ KAF156 was rapidly absorbed and had an elimination half-life of between 42.5 and 70.7 hours, without evident safety concerns. We assessed KAF156 in a phase 2, open-label study at five centers in Thailand and Vietnam.

METHODS

STUDY DESIGN AND OVERVIEW

We evaluated the efficacy and safety of KAF156 in a two-step process. First, 400 mg of KAF156 was given once daily for 3 days to patients with uncomplicated vivax malaria (cohort 1) or falciparum malaria (cohort 2) in order to assess the safety and initial antimalarial efficacy of the study drug. Next, patients in cohort 3, a separate group of patients with falciparum malaria, were treated with a single 800-mg dose of KAF156 in order to assess the cure rate at 28 days and the potential for a single-dose cure.

The study was sponsored by Novartis and was part of the Wellcome Trust–Mahidol University–Oxford Tropical Medicine Research Programme. The study was approved by the ethics committee at each participating institution and by the Oxford Tropical Research Ethics Committee. All study participants provided written informed consent. Novartis designed the study and collected and analyzed the data. All the authors contributed to the design and execution of the study and the analysis and interpretation of the clinical and laboratory data. The last author wrote the first draft of the manuscript and, along with the coauthors, made the decision to submit it for publication. The authors vouch for the accuracy and completeness of the data and analyses and

for the fidelity of the study to the protocol. For full study details, see the study protocol, available with the full text of this article at NEJM.org.

PATIENTS

We recruited patients at the Hospital for Tropical Diseases, in Bangkok; the Shoklo Malaria Research Unit and Mae Ramat District Hospital, both of which are close to the northwestern border of Thailand; Phusing Hospital, in eastern Thailand; and the National Institute for Malaria, Parasitology, and Entomology, in Hanoi. Eligible patients were 20 to 60 years of age and had a body weight between 40 and 90 kg, with fever or a history of fever and microscopy-confirmed *P. vivax* or *P. falciparum* mono-infection (asexual-stage parasite count, 5000 to 60,000 per cubic millimeter in the multiple-dose cohort and 1000 to 70,000 per cubic millimeter in the single-dose cohort). Women of childbearing potential were excluded. Additional exclusion criteria were severe or complicated malaria,⁵ infection with mixed plasmodium species, a hemoglobin level of less than 9.0 g per deciliter, schizontemia, severe vomiting, receipt of antimalarial treatment within the previous 14 days or treatment with any investigational drug within the previous 30 days, a history of cancer, chronic liver disease, severe malnutrition, or clinically significant electrocardiographic abnormalities (corrected QT interval [calculated with the use of Fridericia's formula], >450 msec for men and >470 msec for women).

TREATMENT AND PROCEDURES

Patients were admitted to an inpatient facility for close monitoring. For both the multiple-dose and single-dose regimens, KAF156 was given orally with water. The dose was repeated if the patient vomited within 45 minutes after the initial administration. In the multiple-dose cohorts, standard antimalarial treatment (artesunate plus mefloquine for *P. falciparum* malaria or chloroquine plus primaquine for *P. vivax* infection) was started on day 5 (or earlier if there was evidence of worsening symptoms) as a precautionary measure because this was the first use of KAF156 in patients with malaria. After confirmation of efficacy with multiple doses, standard antimalarial treatment was not given in the single-dose cohort, unless parasitemia recurred, in order to allow assessment of the cure rate at 28 days.

Clinical observations, vital signs, and symptoms were recorded, and thick and thin blood smears were obtained every 4 hours for 24 hours and then every 6 hours, until two consecutive negative readings had been obtained. Fever clearance was defined as at least two consecutive normal body-temperature measurements. Parasite counts per 1000 red cells were reported for thin-film smears, and parasite counts per 200 white cells (or per 500 white cells if the count was <10 parasites) were reported for thick-film smears.

EFFICACY AND SAFETY ASSESSMENTS

The primary efficacy measure in the multiple-dose cohorts was the parasite clearance time: the interval from the first dose to the first of two consecutive negative blood slides. In the single-dose cohort, efficacy was assessed both as the parasite clearance time and as the cure rate at 28 days, confirmed by means of polymerase-chain-reaction genotyping. Safety was evaluated on the basis of daily assessments of symptoms, as well as clinical examination, predose and postdose electrocardiograms, and hematologic, blood chemical, and urine testing at screening; on days 2, 3, and 5; and at study completion, defined as day 11 in the multiple-dose cohorts and as day 29 in the single-dose cohort. The National Cancer Institute Common Terminology Criteria for Adverse Events,⁸ version 4.0, was used for grading the severity of adverse events.

PHARMACOKINETICS

Blood samples for KAF156 plasma concentration measurements were collected before dose administration (0 hour); at 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours after dose administration on days 1 and 3; and at 36, 48, 96, 144, and 192 hours after the last dose had been administered. Urine was collected and volumes were recorded on days 1 and 3 before drug administration and then between 0 and 24 hours and again between 24 and 48 hours in the single-dose cohort. KAF156 levels in plasma and urine were analyzed with the use of a validated high-performance liquid chromatography–mass spectrometry method with a lower limit of quantification of 1 ng per milliliter in plasma and 1000 ng per milliliter in urine. The pharmacokinetic properties of KAF156 were determined by means of noncompartmental methods with the use of Phoenix WinNonLin, version 6.2 (Certara).

MOLECULAR MARKERS

The *P. falciparum* isolates obtained at admission and during episodes of recrudescence were sequenced fully. The genetic sequences were examined for mutations in the *P. falciparum* gene encoding the propeller region of the kelch protein K13 that are associated with artemisinin resistance,⁹ mutations in *PfCRT* and *PfMDR* that are associated with resistance to aminoquinolines, and mutations in *PfCARL* that are associated with resistance to KAF156.⁷

STATISTICAL ANALYSIS

We calculated that in the multiple-dose cohorts, 20 patients (10 per cohort) would provide more than 88% power to observe a median parasite clearance time of less than 96 hours, assuming a true value of less than 80 hours with a range of plausible coefficients of variation. The sample size for the single-dose cohort was not formally calculated to provide a specified power but instead was chosen on the basis of a target for the observed cure rate of 50% or higher. We estimated that with a sample of 20 patients, the probability of meeting this target would be 75% or higher, assuming a true underlying cure rate of 55% or higher, and that the probability would be 11% or less, assuming a true cure rate of less than 35%. For the primary efficacy analysis, time-to-event end points were analyzed with the use of the Kaplan–Meier method. Parasite clearance half-lives, estimated with the Worldwide Antimalarial Resistance Network parasite clearance estimator,^{10,11} were compared with those observed after treatment with artemisinin derivatives in studies conducted in Southeast Asia, in which six hourly (or more frequent than hourly) parasite counts were performed. For the cure rates in the single-dose cohort, 95% Bayesian credible intervals are provided on the basis of a noninformative beta prior distribution (meaning that before we recruited any patients, we believed that a cure rate above 50% was just as likely as a cure rate of 50% or lower).

RESULTS

STUDY PATIENTS

From March to August 2013, a total of 21 adults with acute uncomplicated malaria (11 with *P. vivax* malaria and 10 with *P. falciparum* malaria) were enrolled in the multiple-dose cohorts, and 22 patients with uncomplicated *P. falciparum* malaria

were enrolled in the single-dose cohort. Baseline characteristics of the patients are provided in Table S1 in the Supplementary Appendix, available at NEJM.org. Baseline parasitemia ranged from 5077 to 24,160 per cubic millimeter in the patients with *P. vivax* malaria and from 1901 to 59,405 per cubic millimeter in those with *P. falciparum* malaria. Two patients were excluded from the efficacy and pharmacokinetic analyses: 1 patient with *P. vivax* malaria, in whom a mixed infection was detected after receipt of 400 mg of KAF156, and 1 patient with *P. falciparum* malaria, who had repeated vomiting within 45 minutes after receiving 800 mg of KAF156; two further attempts at dose administration were also unsuccessful because of vomiting.

FEVER AND PARASITE CLEARANCE

The median fever clearance times after receipt of KAF156 were 14 hours (range, 4 to 30) in the patients with *P. vivax* malaria and 6 hours (range, 4 to 24) in those with *P. falciparum* malaria in the multiple-dose cohorts and 4 hours (range, 4 to 66) in the single-dose cohort. In one recipient of the single 800-mg dose, parasitemia resolved by 66 hours after dose administration but recurred asymptotically at 84 hours. In all the other patients, parasitemia cleared. In the multiple-dose cohorts, the median parasite clearance time was 24 hours (interquartile range, 20 to 30; range, 16 to 36) in the patients with vivax malaria and 45 hours (interquartile range, 42 to 48; range, 36 to 66) in those with falciparum malaria; in the single-dose cohort, the median clearance time was 49 hours (interquartile range, 42 to 54; range, 16 to 68) (Table 1 and Fig. 1). The mean \log_{10} parasite reduction ratio at 48 hours was 3.49 (range, 3.10 to 3.78) among patients with vivax malaria and 3.18 (range, 1.51 to 3.85) among those with falciparum malaria in the multiple-dose cohorts and 3.17 (range, 2.27 to 4.06) in the single-dose cohort. Corresponding median parasite clearance half-lives in the three cohorts were 1.9 hours (interquartile range, 1.5 to 2.1; range, 0.9 to 2.7), 3.5 hours (interquartile range, 3.4 to 3.8; range, 2.8 to 5.1), and 3.4 hours (interquartile range, 3.2 to 4.1; range, 1.4 to 7.2).

Among the 21 patients with *P. falciparum* malaria who received the single 800-mg dose and were followed for 28 days, 1 had reinfection and 7 had recrudescence infections (cure rate, 67%; 95% credible interval, 46 to 84). Gametocytemia was detected in 2 patients with *P. vivax* malaria at

Table 1. Parasite Clearance in Patients with *Plasmodium vivax* or *P. falciparum* Malaria Treated with Multiple 400-mg Doses or a Single 800-mg Dose of KAF156.*

Variable	Multiple-Dose Regimen		Single-Dose Regimen
	Cohort 1 (N=10)	Cohort 2 (N=10)	Cohort 3 (N=21)
Parasite count, range (per mm ³)	5077–24,160	2350–53,827	1901–59,405
Time to parasite clearance (hr)			
Median	23.6	45.0	48.8
Interquartile range	20.0–30.0	42.0–48.0	42.0–54.2
Range	16.0–36.0	36.3–66.1	15.8–67.6
Parasite clearance half-life (hr)			
Median†	1.9	3.5	3.4
Interquartile range	1.5–2.1	3.4–3.8	3.2–4.1
Range	0.9–2.7	2.8–5.1	1.4–7.2

* The multiple-dose regimen, consisting of three 400-mg doses of KAF156, was administered to patients with *P. vivax* malaria (cohort 1) and those with *P. falciparum* malaria (cohort 2). The single-dose regimen was an 800-mg dose administered to patients with *P. falciparum* malaria (cohort 3). One patient in cohort 1 was excluded owing to a mixed infection, and one patient in cohort 3 was excluded because of vomiting within 45 minutes after dose administration.

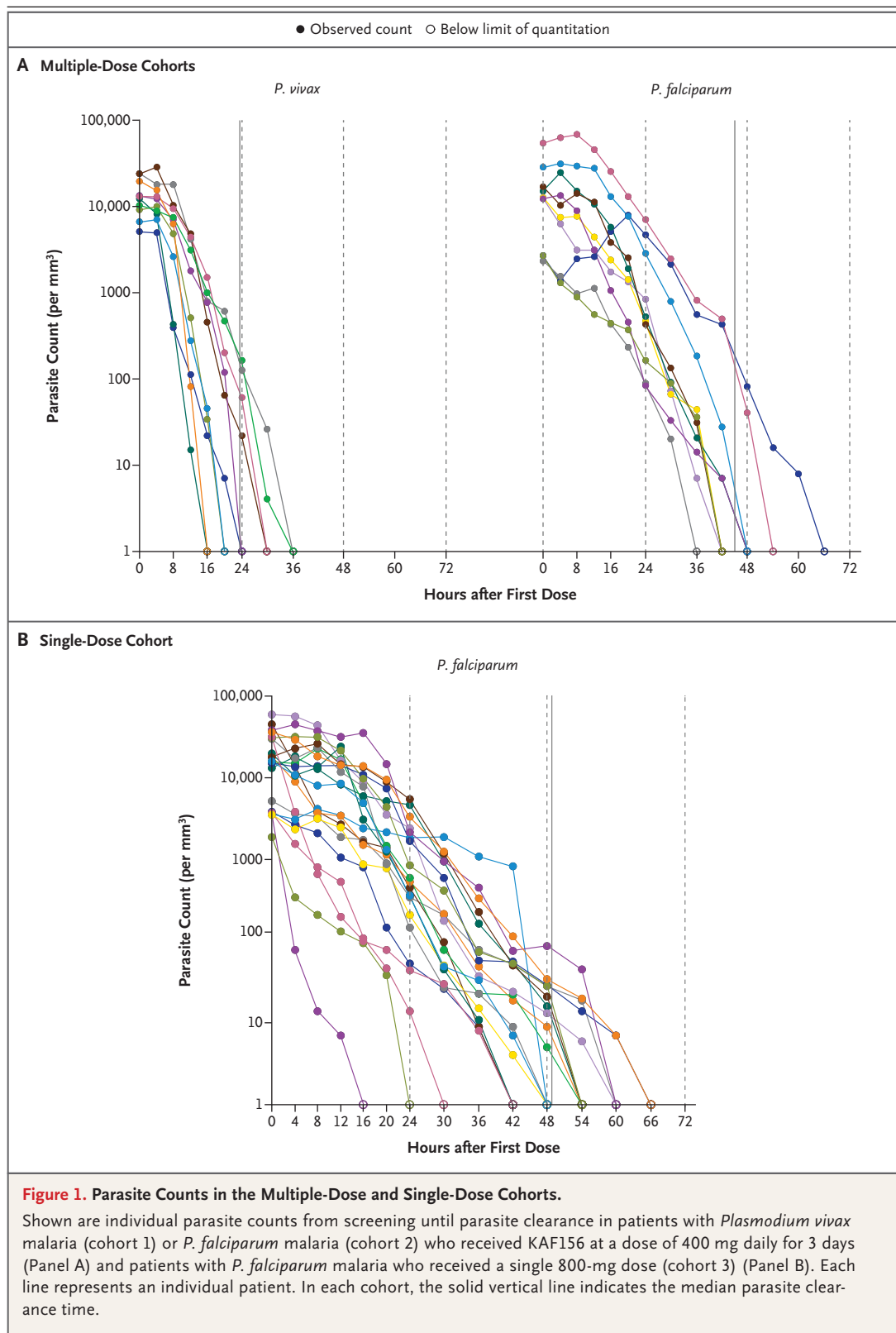
† The median parasite clearance half-life was estimated in a separate analysis with the use of the Worldwide Antimalarial Resistance Network Parasite Clearance Estimator.¹⁰

baseline and cleared in both patients by 16 hours after receipt of KAF156. Among the patients with *P. falciparum* malaria, 1 patient had gametocytemia from baseline to 54 hours after dose administration and 1 had intermittent gametocytemia from baseline until 72 hours after dose administration. In addition, 2 patients had post-treatment gametocytemia: 1 had a single positive reading at 24 hours, and the other had positive readings from 12 to 96 hours (16 gametocytes per cubic millimeter at the last reading), at which time sampling ceased.

ADVERSE EVENTS

Most patients had at least one adverse event. A total of 31 of the 43 patients (72%) had grade 1 events; 35% had grade 2 events, and 14% had grade 3 events (Table 2). No grade 4 or serious adverse events were noted. One patient discontinued treatment (because of repeated vomiting after receipt of the 800-mg single dose).

In the two cohorts that received multiple lower doses of KAF156, nausea and vomiting occurred once each among the patients with *P. falciparum* malaria, whereas in the cohort that received a single higher dose, 4 patients (18%) had nausea



and 6 (27%) had vomiting. Overall, there were more adverse events after the single 800-mg dose than after multiple 400-mg doses. Asymptomatic sinus bradycardia (heart rate, <60 beats per minute) was the most common adverse event and was reported in a total of 13 patients (62%) in cohorts 1 and 2 and in 14 patients (64%) in cohort 3. Overall, plasma potassium values ranged from 2.8 to 4.7 mmol per liter. Hypokalemia was recorded as an adverse event in 11 patients (50%) in cohort 3 but was not considered to be drug-related.

Post-treatment elevation of the alanine aminotransferase levels, ranging from 41 to 136 U per liter, occurred in seven patients (33%) in the multiple-dose cohorts (grade 1 in six patients and grade 2 in one); in two of the patients (one in each cohort), the elevation was reported as an adverse event. At the end of the study (day 11), six of the patients continued to have elevated levels. In the single-dose cohort, six patients (27%) had post-treatment elevations in alanine aminotransferase levels, ranging from 46 to 126 U per liter. In five of the patients (23%), the elevation was considered to be a drug-related adverse event (grade 1 in four patients and grade 2 in one). At the end of the study, three of the patients still had elevated levels.

Post-treatment elevations in aspartate aminotransferase levels (all grade 1) occurred in seven patients (33%) in the multiple-dose cohorts (range, 41 to 115 U per liter) and in six patients (27%) in the single-dose cohort (range, 38 to 49 U per liter). One elevation (cohort 2) was reported as an adverse event. At the end of the study, two patients in cohort 2 and four patients in cohort 3 still had elevated aspartate aminotransferase levels.

In 1 patient with falciparum malaria who received multiple doses of KAF156, the hemoglobin level fell from 9.1 g per deciliter to 6.8 g per deciliter on day 2 (grade 3 event), fluctuated during the study, was 6.6 g per deciliter on day 11, but was normal 2 weeks later. In 2 patients in the single-dose cohort who had anemia at baseline (hemoglobin level, 10.6 g per deciliter and 10.5 g per deciliter), the hemoglobin level fell further after receipt of the study drug (hemoglobin level, 8.4 g per deciliter and 7.7 g per deciliter, respectively), constituting grade 2 events, but improved by day 11 (11.6 g per deciliter and

Table 2. Adverse Events.

Event	Cohort 1 (N=11)	Cohort 2 (N=10)	Cohort 3 (N=22)
	number of patients (percent)		
Any adverse event	5 (45)	8 (80)	22 (100)
Drug-related adverse event*	1 (9)	6 (60)	22 (100)
Discontinuation due to adverse event	0	0	1 (5)†
Serious adverse event	0	0	0
Grade 1 adverse event	5 (45)	6 (60)	20 (91)
Adverse event of grade 2 or 3‡	0	5 (50)	16 (73)
Anemia	0	1 (10)	2 (9)
Lymphopenia	0	0	1 (5)
Thrombocytopenia	0	0	5 (23)
Vomiting	0	0	2 (9)
Hyperbilirubinemia	0	1 (10)	3 (14)
Increased alanine aminotransferase	0	1 (10)	1 (5)
Prolonged QT interval	0	0	1 (5)
Hypokalemia	0	1 (10)	4 (18)
Hyponatremia	0	0	1 (5)
Allergic dermatitis	0	1 (10)	0
Hypotension	0	0	2 (9)

* In the multiple-dose cohorts, 14 of 31 adverse events (45%), reported in 7 patients (1 with *P. vivax* malaria and 6 with *P. falciparum* malaria), were considered by the investigator to be drug-related. All 22 patients with *P. falciparum* malaria who were treated with a single dose had at least 1 adverse event that was considered to be related to the study medication. Overall, 60% of adverse events were considered to be drug-related; the proportion was higher in the single-dose cohort (65%) than in the multiple-dose cohorts (45%).

† The patient vomited repeatedly during the 45-minute postdose period and was withdrawn from the study after two further attempts at dose administration were also unsuccessful because of vomiting.

‡ Adverse events are listed according to the preferred terms in the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. The most frequently reported grade 2 or 3 adverse events were thrombocytopenia, hypokalemia, hyperbilirubinemia, and anemia. Patients with multiple occurrences of an adverse event were counted only once in the adverse-event category. No grade 4 or serious adverse events were noted.

10.8 g per deciliter, respectively). The 2 patients declined further follow-up. Overall, 12 of 21 patients (57%) in the multiple-dose cohorts and 5 of 22 (23%) in the single-dose cohort had anemia.

In all, 60% of adverse events were considered to be drug-related. The percentage was higher in the single-dose cohort (65%) than in the multiple-dose cohorts (45%).

PHARMACOKINETICS

The median time from the administration of KAF156 to the maximum plasma concentration (C_{max}) was approximately 3 hours on both day 1

Table 3. Pharmacokinetic Variables on Day 1 and Day 3 after the Administration of KAF156 at a Dose of 400 mg Daily for 3 Days or a Single Dose of 800 mg.*

Variable	Cohort 1 (N=10)	Cohort 2 (N=10)	Cohort 3 (N=18)
Day 1			
AUC (CV) — hr·ng/ml			
AUC ₀₋₂₄	9,470±2140 (22.6)	10,100±2440 (24.1)	21,700±5680 (26.2)
AUC _{0-last}			54,900±16,600 (30.2)
AUC _{0-inf}			58,300±18,600 (31.9)
C _{max} (CV) — ng/ml	795±182 (22.8)	856±158 (18.4)	1,800±404 (22.5)
Median T _{max} (range) — hr	3.00 (2.00–6.00)	3.00 (1.00–4.03)	3.52 (2.00–11.60)
Day 3			
AUC ₀₋₂₄ (CV) — hr·ng/ml	20,200±3730 (18.4)	21,700±6630 (30.5)	
C _{max} (CV) — ng/ml	1,440±299 (20.7)	1,620±384 (23.7)	
Median T _{max} (range) — hr	3.00 (2.00–5.98)	2.52 (2.00–3.02)	
CL/F (CV) — liters/hr			15.1±4.8 (32.0)
V _z /F (CV) — liters			1,030±264 (25.6)
Terminal elimination half-life (CV) — hr	39.0±7.4 (19.0)	40.8±8.4 (20.5)	48.7±7.9 (16.1)
Accumulation ratio (CV)†	2.16±0.26 (11.9)	2.15±0.38 (17.8)	

* Plus-minus values are means ±SD. Pharmacokinetic data were not available for one patient in cohort 1. Four patients in the single-dose cohort were excluded from the analysis of pharmacokinetic properties because of vomiting within 3 hours after dose administration. AUC₀₋₂₄ denotes the area under the plasma concentration–time curve from time 0 to 24 hours, AUC_{0-inf} the area under the plasma concentration–time curve from time 0 to infinity, AUC_{0-last} the area under the plasma concentration–time curve from time 0 to the time of the last quantifiable concentration, CL/F the apparent clearance of the drug after oral dosing, C_{max} the observed maximum plasma concentration after drug administration, CV the coefficient of variation, T_{max} the time from drug administration to C_{max}, and V_z/F the apparent volume of distribution after oral dosing.

† The accumulation ratio is the AUC₀₋₂₄ on day 3 divided by the AUC₀₋₂₄ on day 1.

and day 3, with an overall mean (±SD) terminal elimination half-life of 44.1±8.9 hours (Table 3 and Fig. 2, and Fig. S1 in the Supplementary Appendix). The interindividual variation in pharmacokinetic profiles was relatively low for an antimalarial drug, with coefficients of variation of 20 to 30% for C_{max} and the area under the curve during a 24-hour period (AUC₀₋₂₄) in all three cohorts. The mean accumulation ratios (calculated as the AUC₀₋₂₄ on day 3 divided by the AUC₀₋₂₄ on day 1) were 2.16 and 2.15 after daily dose administration for 3 days in cohorts 1 and 2, respectively; these values were consistent with those predicted from the elimination half-life. The pharmacokinetic exposure (C_{max} and AUC₀₋₂₄) on day 3 after the administration of multiple doses was similar in the two multiple-dose cohorts and was also similar to the pharmacokinetic exposure after the administration of a single 800-mg dose. Less than 10% of the administered dose was excreted unchanged in urine during the collection intervals.

MOLECULAR MARKERS OF RESISTANCE

In the baseline and recrudescence samples, 14 of 31 *P. falciparum* infections had nonsynonymous single-nucleotide polymorphisms (SNPs) in the K13 gene, 10 of which were the C580Y mutation strongly associated with artemisinin resistance (Table S2 in the Supplementary Appendix). Parasite clearance times and rates were similar in infections with and those without these molecular markers (Table S2 in the Supplementary Appendix). For 28 of the 31 isolates from the patients with *P. falciparum* malaria, there was sufficient coverage of the PfCARL gene. For each of the 28 isolates, there was either one of eight distinct, nonsynonymous SNPs or one of three insertions or deletions in PfCARL, as compared with the 3D7 reference genome (Table S3 and Fig. S2 in the Supplementary Appendix). None of those polymorphisms overlapped with any of the previously identified mutations conferring drug resistance to KAF156,⁷ and parasite clearance rates did not differ according to the PfCARL poly-

Figure 2. Mean Plasma Concentration–Time Profiles of KAF156.

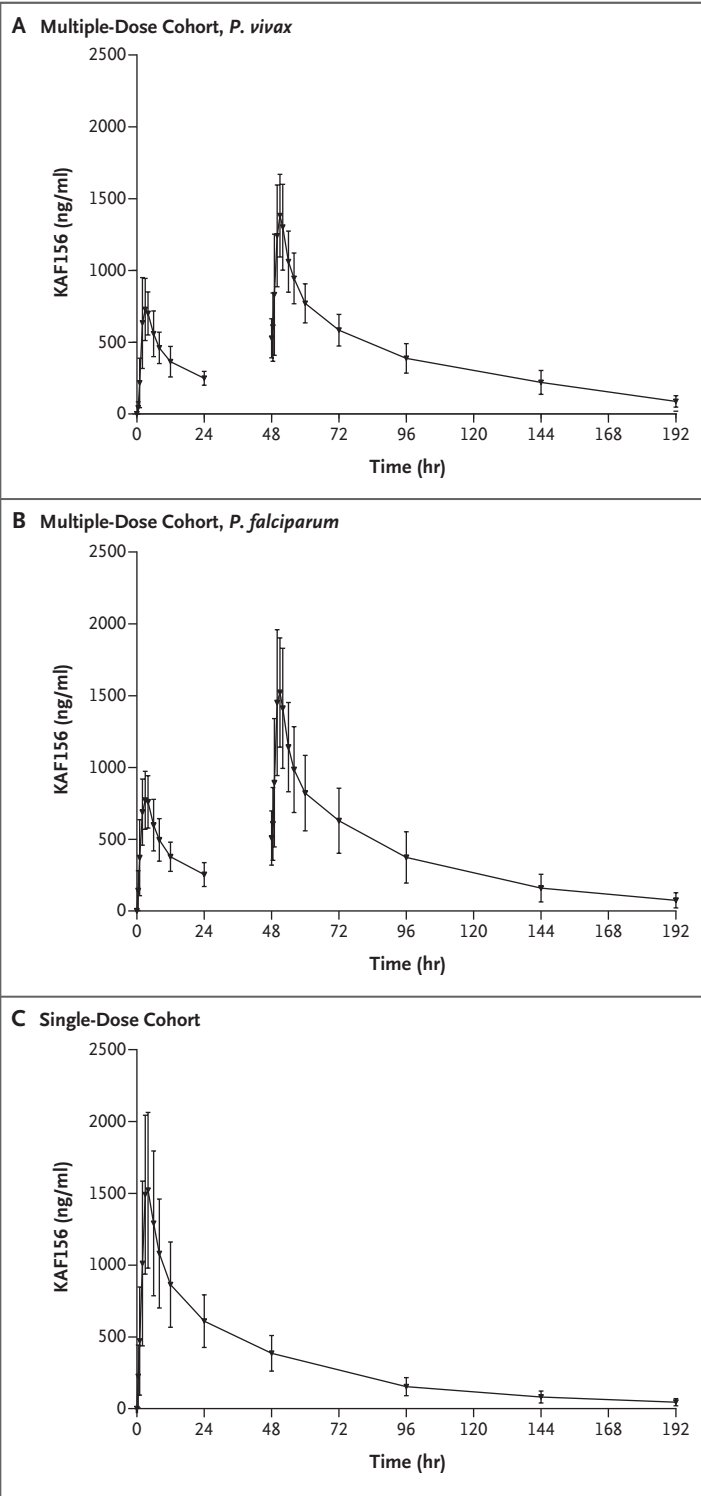
The data for patients in the multiple-dose cohorts on day 1 and day 3 after oral administration of 400 mg once daily (day 1 to day 3) are shown in patients with *P. vivax* infection (Panel A) and in those with *P. falciparum* infection (Panel B). Panel C shows the data for patients with *P. falciparum* infection in the single-dose cohort. I bars indicate standard deviation.

morphisms identified (Fig. S3 in the Supplementary Appendix).

DISCUSSION

New antimalarial drugs are needed as artemisinin resistance spreads in Southeast Asia and partner-drug resistance follows.^{12–14} Untreatable falciparum malaria would reverse many of the substantial gains in malaria control and elimination that have been made in recent years. The imidazolopiperazine KAF156 represents a new class of antimalarial agent with both preerythrocytic and blood-stage activity. In this study of the antimalarial activity of KAF156 in humans, signs and symptoms of illness resolved and parasitemia cleared rapidly in patients with vivax malaria and in those with falciparum malaria, including infections with artemisinin-resistant parasites. The rates of parasite clearance after treatment with KAF156 were slightly slower than those associated with artemisinin treatment in sensitive infections and were substantially slower than the rates associated with spiroindolone KAE609 (cipargamin) treatment, which provides the fastest parasite clearance recorded so far,¹⁵ but they were more rapid than the rates associated with treatment with sulfadoxine–pyrimethamine, atovaquone–proguanil, quinine, or mefloquine in susceptible infections.

Antimalarial drugs that are eliminated rapidly (terminal elimination half-life, <3 days) usually cannot cure falciparum malaria in a single dose,¹ so an overall 28-day cure rate, adjusted for the parasite clearance rate, of 67% after a single dose of KAF156 suggests clinically significant in vivo potency. By comparison, cure rates after short courses of artemisinin derivatives (<5 days) are typically less than 80%.^{16–18} However, as with all antimalarial agents, it is expected that KAF156 would become part of a combination regimen for the treatment of malaria. Therapeutic responses to KAF156 (assessed as the para-



site clearance time and 28-day cure rate) were similar in patients with the *PfMDR*, *PfCRT*, and *K13* mutations associated with either aminoquinoline or artemisinin resistance. These data suggest that KAF156 should be effective against

infections resistant to all currently available antimalarial drugs. None of the patients in our study, including those with recrudescence infections, had parasite isolates bearing the *PfCARL* mutations that are associated with the selection of KAF156 resistance in experimental studies.

Thirteen of the 21 patients who received multiple 400-mg doses of KAF156 and all 22 patients who received a single 800-mg dose had at least one adverse event. One patient who received the single 800-mg dose had repeated vomiting and was withdrawn from the study. Five other patients vomited after receiving the single 800-mg dose, as compared with 1 patient with *P. falciparum* malaria in the multiple-dose cohort. Four patients who received the single 800-mg dose reported nausea, as compared with 1 patient with *P. falciparum* malaria in the multiple-dose cohort. Between 27% and 33% of all the patients in all three cohorts had mild post-treatment elevations in aminotransferase levels (maximum elevation, 136 U per liter). Overall, the 3-day regimen of multiple 400-mg doses was associated with fewer adverse events than the single 800-mg dose. Anemia is common in patients with malaria. In our study, anemia that worsened from baseline or that developed dur-

ing treatment had resolved or improved by the end of the study.

The pharmacokinetic properties of KAF156 varied relatively little between patients and were similar to the values reported previously in healthy volunteers.⁶ The therapeutic responses were uniformly rapid. No clear relationship between drug exposure (measured as C_{max} or AUC_{0-24}) and the initial parasitologic response (measured as the parasite clearance time or parasite clearance half-life) was established for either the multiple-dose or single-dose regimen. Of the 7 patients with recrudescence *P. falciparum* infections after receipt of a single dose, only 2 had total KAF156 plasma concentrations above 58 ng per milliliter (more than twice the 99% inhibitory concentration) for 6 days (three asexual cycles), as compared with 10 of the 13 patients who did not have a recrudescence.

In conclusion, our study showed that KAF156, a new antimalarial drug, has activity against vivax and falciparum malaria, including artemisinin-resistant parasites.

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