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Spread of Artemisinin Resistance in *Plasmodium falciparum* Malaria

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Abstract

BACKGROUND—Artemisinin resistance in *Plasmodium falciparum* has emerged in Southeast Asia and now poses a threat to the control and elimination of malaria. Mapping the geographic extent of resistance is essential for planning containment and elimination strategies.

METHODS—Between May 2011 and April 2013, we enrolled 1241 adults and children with acute, uncomplicated falciparum malaria in an open-label trial at 15 sites in 10 countries (7 in Asia and 3 in Africa). Patients received artesunate, administered orally at a daily dose of either 2 mg per kilogram of body weight per day or 4 mg per kilogram, for 3 days, followed by a standard 3-day course of artemisinin-based combination therapy. Parasite counts in peripheral-blood samples were measured every 6 hours, and the parasite clearance half-lives were determined.

RESULTS—The median parasite clearance half-lives ranged from 1.9 hours in the Democratic Republic of Congo to 7.0 hours at the Thailand–Cambodia border. Slowly clearing in fections (parasite clearance half-life >5 hours), strongly associated with single point mutations in the "propeller" region of the *P. falciparum* kelch protein gene on chromosome 13 (*kelch13*), were detected throughout mainland Southeast Asia from southern Vietnam to central Myanmar. The incidence of pretreatment and post-treatment gametocytemia was higher among patients with slow parasite clearance, suggesting greater potential for transmission. In western Cambodia, where artemisinin-based combination therapies are failing, the 6-day course of antimalarial therapy was associated with a cure rate of 97.7% (95% confidence interval, 90.9 to 99.4) at 42 days.

CONCLUSIONS—Artemisinin resistance to *P. falciparum*, which is now prevalent across mainland Southeast Asia, is associated with mutations in *kelch13*. Prolonged courses of artemisinin-based combination therapies are currently efficacious in areas where standard 3-day treatments are failing. (Funded by the U.K. Department of International Development and others; ClinicalTrials.gov number, NCT01350856.)

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Artemisinin derivatives are highly potent, rapidly eliminated antimalarial drugs with a broad stage specificity of action. They clear parasitemia more rapidly than all other currently available antimalarial agents. In the 1990s, resistance to available antimalarial drugs such as chloroquine and sulfadoxine–pyrimethamine worsened across areas of the world where malaria is endemic. As a direct consequence, morbidity and mortality associated with malaria increased, especially among African children, who account for most deaths from malaria.[1] The artemisinin-based combination therapies were introduced in the mid-1990s, when there was an imminent prospect of untreatable malaria in Southeast Asia, where resistance to all available antimalarial drugs had developed. In 2005, the World Health Organization (WHO) recommended that artemisinin-based com bination therapies be used as first-line treatments for falciparum malaria in all countries where malaria was endemic. Recent, marked increases in the availability and use of artemisinin-based combination therapies, together with the increased use of insecticide-treated bed nets, have substantially reduced global morbidity and mortality from malaria.[2] These gains, and the prospects for the elimination of malaria, are now threatened by the emergence of artemisinin resistance in *Plasmodium falciparum*. Artemisinin resistance is characterized by slow parasite clearance,[3,4] which reflects the reduced susceptibility of ring-stage parasites.[5-9] It has recently been linked with point mutations in the “propeller” region of a *P. falciparum* kelch protein.[10]

Artemisinin resistance was reported first in western Cambodia,[3,4] where failure rates for artemisinin-based combination therapies are rapidly increasing[11] and where resistance to previous first-line antimalarial drugs also first emerged. Artemisinin resistance has since spread, emerged independently, or both in other areas of mainland Southeast Asia.[12-15] On the Thailand–Myanmar border, the geometric mean half-life for parasite clearance increased from 2.6 hours in 2001 to 3.7 hours in 2010, as compared with a half-life of 5.5 hours in western Cambodia between 2007 and 2010.[12] Defining the extent and severity of artemisinin resistance is essential for planning containment and elimination strategies.

The Tracking Resistance to Artemisinin Collaboration was created in 2011 to provide evidence and tools to halt or slow the spread of artemisinin resistance. The principal objective of our clinical research was to map the current extent and severity of artemisinin resistance.

METHODS

STUDY DESIGN AND OVERSIGHT

We conducted an open-label, randomized trial at 15 sites in 10 countries (Fig. 1): Cambodia (4 sites), Thailand (3 sites), and Laos, Vietnam, Myanmar, Bangladesh, India, Nigeria, Kenya, and the Democratic Republic of Congo (1 site each). The study was coordinated by the Mahidol–Oxford Tropical Medicine Research Unit (MORU) in Bangkok.

The protocol (available with the full text of this article at NEJM.org) was approved by the Oxford Tropical Research Ethics Committee and the institutional review board, national ethics committee, or both for each site. The trial was monitored by the MORU Clinical Trials Support Group, in collaboration with the Oxford University Clinical Research Unit–

Vietnam and the Kenya Medical Research Institute–Wellcome Trust Clinical Trials Facility. All serious adverse events were reported to an independent data and safety monitoring committee. Quality-assured artesunate manufactured by Guilin Pharmaceutical was provided by the WHO Global Malaria Programme, and artemisinin-based combination therapies were provided free of charge by Beijing Holley-Cotec Pharmaceuticals and Sigma-Tau Pharmaceuticals; these companies did not play a role in the study design, data collection or analysis, or writing of the manuscript.

STUDY SITES AND PATIENTS

Patients were eligible for enrollment in the study if they were 6 months to 65 years of age and had acute, uncomplicated falciparum malaria (including mixed infections with non-falciparum species), parasitemia with a parasite count between 10,000 and 200,000 per cubic millimeter, and fever (a tympanic temperature >37.5°C) or a history of fever. Patients or parents or guardians of minors who were unable to read or write provided oral informed consent in the presence of an impartial witness. Assent was obtained from minors older than 10 years of age. All other patients provided written informed consent. The main exclusion criteria were receipt of an artemisinin antimalarial drug in the previous week, pregnancy or breast-feeding, and a low hematocrit (<25% in Asia and <15% in Africa).

DRUG THERAPY AND FOLLOW-UP ASSESSMENTS

At most sites, patients were randomly assigned in blocks of 20 to receive artesunate (Guilin Pharmaceutical), administered orally at a dose of either 2 mg per kilogram of body weight per day or 4 mg per kilogram per day, for 3 days, followed by a standard 3-day course of an artemisinin-based combination therapy (dihydroartemisinin–piperaquine, artemether–lumefantrine, artesunate–sulfadoxine–pyrimethamine, or artesunate-mefloquine, according to local treatment policies). Treatment assignments were concealed in numbered opaque envelopes, which were opened sequentially. In western Cambodia, and Srisaket, Thailand, where higher-grade artemisinin resistance was already established, only the higher dose of artesunate (4 mg per kilogram per day) was evaluated. In response to review by local scientific or ethics committees, the protocol was modified as follows: in India, only the higher dose of artesunate was studied, patients in the Democratic Republic of Congo received either artemether-lumefantrine alone or artesunate at a dose of 4 mg per kilogram per day for 3 days, followed by artemether-lumefantrine, and in Kenya, artesunate was administered at a daily dose of 2 mg per kilogram per day for 7 days.

Patients were admitted to the hospital for supervised treatment. Body temperature and hematocrit were measured and blood smears for parasite counts were obtained at 0, 4, 6, 8, and 12 hours and then every 6 hours thereafter until two consecutive slides were reported as negative.

Follow-up assessments were performed on days 7 and 14 at all study sites; patients were followed weekly for 28 or 42 days at six sites. For recurrent infections, polymerase-chain-reaction (PCR) genotyping was performed with the use of *msp1*, *msp2*, and *glurp* as genetic markers to distinguish a recrudescence from a newly acquired infection.[16] In Kenya, only *msp2* was used for genotyping.[17]

GENETIC MARKERS OF ARTEMISININ RESISTANCE

After PCR amplification, the full sequence of *kelch13* was determined from *P. falciparum* isolates obtained at admission (see the Supplementary Appendix, available at NEJM.org).[10]

MICROSCOPY QUALITY-CONTROL MEASURES

Quality control of microscopy throughout the study was performed by experienced microscopists at four of the study sites. All slides obtained at admission and at 72 hours were rechecked. Complete patient slide sets were rechecked if the standard deviation of residuals for the fit to the linear segment of the parasite-time profile (see below) was greater than 1, the estimated lag phase was longer than 12 hours, the clearance estimate was based on two positive counts, or the last positive parasite count was greater than 1000 per cubic millimeter.

STUDY OUTCOMES

The primary outcome was the parasite clearance half-life, a measure of the parasite clearance rate derived from the linear segment of the log parasitemia–time curve (parasite clearance half-life = $\log_e 2$ divided by the parasite clearance rate).[18-20] Secondary outcomes were the proportions of patients with a parasite clearance half-life of more than 5 hours, parasitemia detected on microscopy[21] on day 3, gametocytemia after treatment, the incidence of anemia, and the efficacy of the 6-day artesunate regimen plus artemisinin-based combination therapy, corrected by means of PCR genotyping.[17]

STATISTICAL ANALYSIS

Sample-size calculations were based on parasite clearance rates among 1952 patients treated with oral artesunate between 2001 and 2010 in northwest Thailand,[12] which had a log-normal distribution, with geometric mean clearance rates between 0.19 and 0.24 per hour and log-scale standard deviations between 0.40 and 0.55 (coefficient of variation on the original scale, 43 to 49%). On the basis of the log-linear component of the parasitemia–time slope, we calculated that a sample of 50 patients per group at each site would provide 12, 15, and 17% precision and 80% power to detect a 20%, 25%, and 27% decrease in the geometric mean of parasite clearance rates for rates between 0.17 and 0.30 per hour, assuming log-transformed standard-deviation values of 0.40, 0.50, and 0.55, respectively. We calculated that we would need to enroll 60 patients per group to allow for a 10% loss to follow-up or incomplete data.

Data were double-entered into a Web-based database (OpenClinica, version 3.0) and cleaned and analyzed with the use of Stata software, version 13 (StataCorp). Parasite clearance was assessed with the use of the WorldWide Antimalarial Resistance Network Parasite Clearance Estimator.[19,20] Half-life values were excluded from numerical comparisons in 14 patients, since these values were based on only two data points. The relationships between *kelch13* mutations and parasite clearance measures were assessed by means of multivariable analysis. A parasite clearance half-life longer than 5.0 hours was considered prospectively to indicate artemisinin resistance on the basis of data from the Thailand–Myanmar border (where this was the 90th centile for parasite clearance half-life in 2004, when resistance had

just emerged).[12] Resolution of fever and treatment efficacy corrected by means of PCR genotyping were estimated with the use of Kaplan–Meier survival analysis.[22,23] A meta-analysis of the differences in mean log-transformed parasite clearance half-life values between treatment groups was performed according to study site with the use of the inverse-variance method and a fixed-effects model. Heterogeneity was evaluated with the use of the I^2 statistic.[24] All comparisons were stratified according to study site with the use of random-effects or fixed-effects models. Anemia was defined as a hemoglobin level below 11 g per deciliter (<10 g per deciliter in children younger than 5 years of age). Full details of the study conduct and analyses are provided in the protocol with the statistical analysis plan.

RESULTS

STUDY PATIENTS

Between May 2011 and April 2013, a total of 18,865 patients were screened and 1241 patients with falciparum malaria were enrolled, of whom 829 (67%) were febrile on admission. The coincident strengthening of efforts to control malaria and the decreasing incidence of malaria prevented 7 of the 15 sites from meeting recruitment targets. The majority of patients were men (74%). The median age was 21 years (interquartile range, 11 to 31) (Table 1).

PARASITE CLEARANCE

There were marked differences in therapeutic responses across the Southeast Asian sites. Median parasite clearance half-life values ranged from 2 hours in Attapeu, Laos, to 7 hours in Srisaket, Thailand (approximately 250 km [155 mi] to the west) (Table 2). There was a gradient of prolonged parasite clearance, with the highest proportion of patients with a prolonged parasite clearance half-life (>5 hours) in western Cambodia and eastern Thailand (49 to 73%), as compared with 14 to 28% in northern Cambodia, Vietnam, and eastern Myanmar, and very low proportions elsewhere. (Fig. 1 and 2 and Table 2). The proportions of patients with parasitemia detectable on microscopy at 72 hours (day 3), a widely used criterion for artemisinin resistance,[21] ranged from 0% in Kenya to 68% in eastern Thailand (Table 2). Among the African sites, median parasite clearance half-life values were similar in Kenya and Nigeria (P = 0.75) but were significantly shorter in the Democratic Republic of Congo than in these two African sites (P<0.001) (Fig. 2 and Table 2).

Geometric mean parasite clearance half-life values were similar in the group that received artesunate at a dose of 2 mg per kilogram and the group that received artesunate at a dose of 4 mg per kilogram; the overall difference, stratified according to site, was −4.9% (95% confidence interval [CI], −10.7 to 1.4; P = 0.13). In Kin shasa, median parasite clearance half-life values were significantly longer with artemether–lumefantrine than with artesunate at a dose of 4 mg per kilogram (2.2 hours [range, 1.2 to 4.6] vs. 1.9 hours [range, 0.7 to 7.0]; P = 0.003).

GENETIC CORRELATES OF SLOW PARASITE CLEARANCE

Single point mutations in the propeller domain of *kelch13* after position 440 were associated with a mean increase in the parasite clearance half-life of 116% (95% CI, 103 to 131;

P<0.001) (Fig. 2). In a multivariate logistic-regression model, with adjustment for age, baseline parasite count, and dose of artesunate, an infection associated with a *kelch13* propeller mutation was substantially more likely to have a parasite clearance half-life longer than 5 hours (odds ratio, 94.7; 95% CI, 54.6 to 164.0; P<0.001). In this study, *kelch13* propeller mutations had 91.8% sensitivity and 88.4% specificity in identifying a parasite clearance half-life longer than 5 hours. Among infections with wild-type *kelch13* alleles, 3.4% (21 of 611) had a parasite clearance half-life longer than 5 hours. The parasite clearance half-life values for mutant infections were generally similar, except that the values associated with G538V were lower and those associated with I543T and N458Y were higher than the half-life values associated with the other mutations (P = 0.02).

GAMETOCYTE CARRIAGE

Patent gametocytemia (gametocyte densities above the microscopy level of detection) was reported in 125 patients at enrollment, with the highest rates in the Democratic Republic of Congo (31%) and western Cambodia (19% in Pailin and 18% in Pursat). The median duration of patent gametocytemia was 6.7 days (range, <6 hours to 10.5 days); it did not differ significantly between the sites (P = 0.36) and was not correlated with the parasite clearance half-life. Gametocytemia developed in an additional 48 patients within 24 hours and in another 47 patients between day 1 and day 7 (median time to appearance, 42 hours; range, 24 to 169). The prevalence of gametocytemia at enrollment was higher among patients at sites with slow parasite clearance. Excluding the Democratic Republic of Congo, which has substantially higher malaria transmission intensity than the other sites, patent gametocytemia was also more likely to develop in patients with slower parasite clearance after treatment (odds ratio for doubling of the parasite clearance half-life, 2.40; 95% CI, 1.53 to 3.76; P<0.001).

HEMATOLOGIC FINDINGS

More than half the African children enrolled in the study had anemia, as compared with 8 to 37% of Asian patients. The lowest hematocrit occurred on day 3, with partial recovery by day 14. Four patients (one child in the Democratic Republic of Congo and three adults in Thailand) received a blood transfusion during the first week. After adjustment for the baseline hematocrit, baseline parasite count, age, and the total dose of the artemisinin derivative received, delayed recovery from anemia on day 21 was associated with slow parasite clearance (odds ratio for anemia with a doubling of the parasite clearance half-life, 1.53; 95% CI, 1.06 to 2.21; P = 0.02), but there was no association by day 28. Apart from the baseline hematocrit, other covariates associated with anemia on day 21 were age (odds ratio with a doubling of age, 1.53; 95% CI, 1.12 to 2.11; P = 0.008) and initial parasite count (odds ratio with an increase in the count by a factor of 10, 2.09; 95% CI, 1.00 to 4.35; P = 0.05). The development of anemia was not associated with the dose of the artemisinin derivative.

TREATMENT EFFICACY

Patients were followed for 28 days in Kinshasa and for 42 days in Pailin, Attapeu, Binh Phuoc, Shwe Kyin, and Pingilikani. *P. falciparum* parasitemia recurred after day 14 in 39

patients (6 cases of recrudescence). Treatment efficacy corrected by means of PCR genotyping was uniformly high for all regimens at all sites (Table 3).

SAFETY

A total of 12 serious adverse events were reported at seven sites: acute alcohol withdrawal (in 2 patients), anemia requiring blood transfusion (in 3 patients), bacterial infection (in 3 patients), and acute asthma, viral respiratory tract infection, upper gastrointestinal bleeding, and a febrile convulsion (in 1 patient each) (Table S5 in the Supplementary Appendix).

DISCUSSION

Artemisinin-resistant *P. falciparum* is now firmly established in eastern Myanmar, western Cambodia and Thailand, and southern Vietnam, and it is emerging in southern Laos and northeastern Cambodia. Despite the recent spread of resistance, we found that artemisinin-based combination therapies in this region were still highly efficacious, presumably because of increased reliance on the efficacy of the partner drug as the potency of the artemisinin component waned. However, rates of treatment failure with artesunate–mefloquine in Thailand and with dihydroartemisinin–piperaquine in Cambodia have increased by a factor of more than 5.[11,25-29] Mathematical modeling, in vitro experiments, and transcriptomic studies all suggest that reduced susceptibility of ring-stage parasites causes the slow parasite clearance.[5-9] Strong evidence[30,31] of genetic linkage to a region of *P. falciparum* chromosome 13 has now been translated[10] into the discovery of a molecular marker — single-nucleotide polymorphisms (SNPs) in the propeller region of a kelch protein encoded by *kelch13*.

In this large study, multiple *kelch*13 SNPs were highly predictive of slow parasite clearance and were associated with more than double the parasite clearance half-life. Some SNPs (notably C580Y) predominated. With one exception, only one SNP per propeller domain was ever present in “clonal” infections. The majority of SNPs were associated with a similar prolongation of the parasite clearance half-life. The geographic distribution of these SNPs confirms that artemisinin resistance has emerged and spread extensively in mainland Southeast Asia. Widespread availability of artemisinin monotherapies, poor-quality artemisinin–based combination therapies and monotherapies containing subtherapeutic amounts of active ingredients, and unregulated use of antimalarial agents, plus the unusual genetic structure of parasites in this region,[32] may have contributed to the selection of resistant parasites.

Simple surrogate measures for the parasite clearance rate, such as the proportion of patients with detectable parasitemia on day 3, have been useful in ruling out resistance, but they are imprecise and depend on the pretreatment parasite density, accurate timing of samples, and reliable counting.[21] Parasite clearance rates or half-lives are preferable but they are still influenced by many factors, including antimalarial–drug dosing, pharmacokinetics, and pharmacodynamics, as well as host immunity.[4,5,12,13,18,33] Sequencing of *kelch13* provides a potential tool for rapid epidemiologic assessment.

Patients in Bangladesh, Nigeria, and the Democratic Republic of Congo also occasionally had parasite clearance half-life values of more than 5 hours, but these values were not associated with *kelch13* polymorphisms. Conversely, 3 patients in Kinshasa who had *kelch13* propeller polymorphisms had very rapid parasite clearance. However, non-synonymous SNPs before *kelch13* position 441 (not associated with slow parasite clearance) were also more frequent in Kinshasa (30 of 72 patients). The ratio of proximal SNPs to distal SNPs, relative to the 441 cutoff point, may be informative in epidemiologic assessments. Slow parasite clearance after treatment with artemisinin derivatives was observed infrequently soon after they were introduced in Asia.[34] Detection of persistently dormant yet drug-sensitive parasites and splenic hypofunction are possible explanations.[35] Another factor is the initial doses of artemisinin derivatives, which vary considerably among different artemisinin-based combination therapies and range from 1.6 mg of artemether per kilogram in artemether–lumefantrine to 2.5 mg of dihydroartemisinin per kilogram in dihydroartemisinin–piperaquine to 4 mg of artesunate per kilogram in other artemisinin-based combination therapies. In Kinshasa, parasite clearance was faster with artesunate at a dose of 4 mg per kilogram per day than with artemether at a dose of 1.6 mg per kilogram per day, suggesting a submaximal effect with artemether.

Efficacy was good with the 6-day treatment course in areas of established artemisinin resistance. The day 42 failure rate of 2% (95% CI, 1 to 9) after 3 days of artesunate followed by 3 days of dihydroartemisinin–piperaquine in Pailin is more than 10 times lower than the recent failure rate of 25% (95% CI, 10 to 51) with the standard 3-day dihydroartemisinin–piperaquine regimen in the same area.[26] Prolonged courses of treatment are one option for treating artemisinin-resistant malaria.

The higher proportions of pretreatment and post-treatment gametocytemia in patients with slow parasite clearance suggest that artemisinin-resistant *P. falciparum* infections have a transmission advantage that may drive the spread of resistance. This increase in post-treatment gametocytemia may be a harbinger of increased treatment failure rates, as reported previously with sulfadoxine–pyrimethamine.[36] In 2012, the WHO reinforced its recommendation to add a single gametocyto cidal dose of primaquine to artemisinin-based combination therapies in order to limit the spread of artemisinin resistance,[37] but the limited adoption of this policy has been disappointing.

Resistance to artemisinin has not been contained and has now emerged or spread across Southeast Asia. The spread of artemisinin resistance and the consequent emergence of resistance to the increasingly unprotected partner drugs in artemisinin-based combination regimens may well reverse the substantial recent gains in malaria control. New antimalarial drugs are under development but will not be available for several years. Radical measures will be necessary in Southeast Asia to prevent resistance to artemisinins and their partner drugs from spreading to the Indian subcontinent and then to Africa.

Supplementary Material

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REFERENCES

1. Snow RW, Trape JF, Marsh K. The past, present and future of childhood malaria mortality in Africa. Trends Parasitol. 2001; 17:593–7. [PubMed: 11756044]
2. Bhattarai A, Ali AS, Kachur SP, et al. Impact of artemisinin-based combination therapy and insecticide-treated nets on malaria burden in Zanzibar. PLoS Med. 2007; 4(11):e309. [PubMed: 17988171]
3. Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, Fukuda MM. Evidence of artemisinin-resistant malaria in western Cambodia. N Engl J Med. 2008; 359:2619–20. [PubMed: 19064625]
4. Dondorp AM, Nosten F, Yi P, et al. Artemisinin resistance in *Plasmodium falciparum* malaria. N Engl J Med. 2009; 361:455–67. Erratum, N Engl J Med 2009;361:1714. [PubMed: 19641202]
5. Saralamba S, Pan-Ngum W, Maude RJ, et al. Intrahost modeling of artemisinin resistance in *Plasmodium falciparum*. Proc Natl Acad Sci U S A. 2011; 108:397–402. [PubMed: 21173254]
6. Witkowski B, Khim N, Chim P, et al. Reduced artemisinin susceptibility of *Plasmodium falciparum* ring stages in western Cambodia. Antimicrob Agents Chemother. 2013; 57:914–23. [PubMed: 23208708]
7. Mok S, Imwong M, Mackinnon MJ, et al. Artemisinin resistance in *Plasmodium falciparum* is associated with an altered temporal pattern of transcription. BMC Genomics. 2011; 12:391. [PubMed: 21810278]
8. Witkowski B, Amaratunga C, Khim N, et al. Novel phenotypic assays for the detection of artemisinin-resistant *Plasmodium falciparum* malaria in Cambodia: in-vitro and ex-vivo drug-response studies. Lancet Infect Dis. 2013; 13:1043–9. [PubMed: 24035558]
9. Chotivanich K, Tripura R, Das D, et al. Laboratory detection of artemisinin resistant *Plasmodium falciparum*. Antimicrob Agents Chemother. 2014; 58:3157–61. [PubMed: 24663013]
10. Ariey F, Witkowski B, Amaratunga C, et al. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. Nature. 2014; 505:50–5. [PubMed: 24352242]
11. Status report on artemisinin resistance. World Health Organization; Geneva: Jan. 2014 http://www.who.int/malaria/publications/atoz/status_rep_artemisinin_resistance_jan2014.pdf
12. Phyo AP, Nkhoma S, Stepniewska K, et al. Emergence of artemisinin-resistant malaria on the western border of Thailand: a longitudinal study. Lancet. 2012; 379:1960–6. [PubMed: 22484134]

13. Amaratunga C, Sreng S, Suon S, et al. Artemisinin-resistant *Plasmodium falciparum* in Pursat province, western Cambodia: a parasite clearance rate study. Lancet Infect Dis. 2012; 12:851–8. [PubMed: 22940027]
14. Kyaw MP, Nyunt MH, Chit K, et al. Reduced susceptibility of *Plasmodium falciparum* to artesunate in southern Myanmar. PLoS One. 2013; 8(3):e57689. [PubMed: 23520478]
15. Hien TT, Thuy-Nhien NT, Phu NH, et al. In vivo susceptibility of *Plasmodium falciparum* to artesunate in Binh Phuoc Province, Vietnam. Malar J. 2012; 11:355. [PubMed: 23101492]
16. Snounou G, Beck HP. The use of PCR genotyping in the assessment of recrudescence or reinfection after antimalarial drug treatment. Parasitol Today. 1998; 14:462–7. [PubMed: 17040849]
17. Methods and techniques for clinical trials on antimalarial drug efficacy: genotyping to identify parasite populations: informal consultation organized by the Medicines for Malaria Venture and co-sponsored by the World Health Organization, 29-31 May 2007, Amsterdam, the Netherlands. World Health Organization; Geneva: 2008.
18. White NJ. The parasite clearance curve. Malar J. 2011; 10:278. [PubMed: 21939506]
19. Flegg JA, Guerin PJ, White NJ, Stepniewska K. Standardizing the measurement of parasite clearance in falciparum malaria: the parasite clearance estimator. Malar J. 2011; 10:339. [PubMed: 22074219]
20. Worldwide Antimalarial Resistance Network. Parasite Clearance Estimator (PCE). http://www.wwarn.org/toolkit/data-management/parasite-clearance-estimator
21. Stepniewska K, Ashley E, Lee SJ, et al. In vivo parasitological measures of artemisinin susceptibility. J Infect Dis. 2010; 201:570–9. [PubMed: 20085495]
22. Methods for surveillance of antimalarial drug efficacy. World Health Organization; Geneva: 2009.
23. Stepniewska K, White NJ. Some considerations in the design and interpretation of antimalarial drug trials in uncomplicated falciparum malaria. Malar J. 2006; 5:127. [PubMed: 17187673]
24. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002; 21:1539–58. [PubMed: 12111919]
25. Carrara VI, Lwin KM, Phyo AP, et al. Malaria burden and artemisinin resistance in the mobile and migrant population on the Thai-Myanmar border, 1999-2011: an observational study. PLoS Med. 2013; 10(3):e1001398. [PubMed: 23472056]
26. Leang R, Barrette A, Bouth DM, et al. Efficacy of dihydroartemisinin-piperaquine for treatment of uncomplicated *Plasmodium falciparum* and *Plasmodium vivax* in Cambodia, 2008 to 2010. Antimicrob Agents Chemother. 2013; 57:818–26. [PubMed: 23208711]
27. Leang R, Ros S, Duong S, et al. Therapeutic efficacy of fixed dose artesunate-mefloquine for the treatment of acute, uncomplicated *Plasmodium falciparum* malaria in Kampong Speu, Cambodia. Malar J. 2013; 12:343. [PubMed: 24060207]
28. Denis MB, Tsuyuoka R, Lim P, et al. Efficacy of artemether-lumefantrine for the treatment of uncomplicated falciparum malaria in northwest Cambodia. Trop Med Int Health. 2006; 11:1800–7. [PubMed: 17176344]
29. Carrara VI, Zwang J, Ashley EA, et al. Changes in the treatment responses to artesunate-mefloquine on the northwestern border of Thailand during 13 years of continuous deployment. PLoS One. 2009; 4(2):e4551. [PubMed: 19234601]
30. Cheeseman IH, Miller BA, Nair S, et al. A major genome region underlying artemisinin resistance in malaria. Science. 2012; 336:79–82. [PubMed: 22491853]
31. Takala-Harrison S, Clark TG, Jacob CG, et al. Genetic loci associated with delayed clearance of *Plasmodium falciparum* following artemisinin treatment in Southeast Asia. Proc Natl Acad Sci U S A. 2013; 110:240–5. [PubMed: 23248304]
32. Miotto O, Almagro-Garcia J, Manske M, et al. Multiple populations of artemisinin-resistant *Plasmodium falciparum* in Cambodia. Nat Genet. 2013; 45:648–55. [PubMed: 23624527]
33. Lopera-Mesa TM, Doumbia S, Chiang S, et al. *Plasmodium falciparum* clearance rates in response to artesunate in Malian children with malaria: effect of acquired immunity. J Infect Dis. 2013; 207:1655–63. [PubMed: 23448727]
34. Luxemburger C, Brockman A, Silamut K, et al. Two patients with falciparum malaria and poor in vivo responses to artesunate. Trans R Soc Trop Med Hyg. 1998; 92:668–9. [PubMed: 10326118]

35. Teuscher F, Gatton ML, Chen N, Peters J, Kyle DE, Cheng Q. Artemisinin-induced dormancy in *Plasmodium falciparum*: duration, recovery rates, and implications in treatment failure. J Infect Dis. 2010; 202:1362–8. [PubMed: 20863228]

36. Barnes KI, Little F, Mabuza A, et al. Increased gametocytemia after treatment: an early parasitological indicator of emerging sulfadoxine-pyrimethamine resistance in falciparum malaria. J Infect Dis. 2008; 197:1605–13. [PubMed: 18471066]

37. Updated WHO policy recommendation: single dose primaquine as a gametocytocide in *Plasmodium falciparum* malaria. World Health Organization; Geneva: Oct. 2012 http://www.who.int/malaria/publications/atoz/who_pq_policy_recommendation/en

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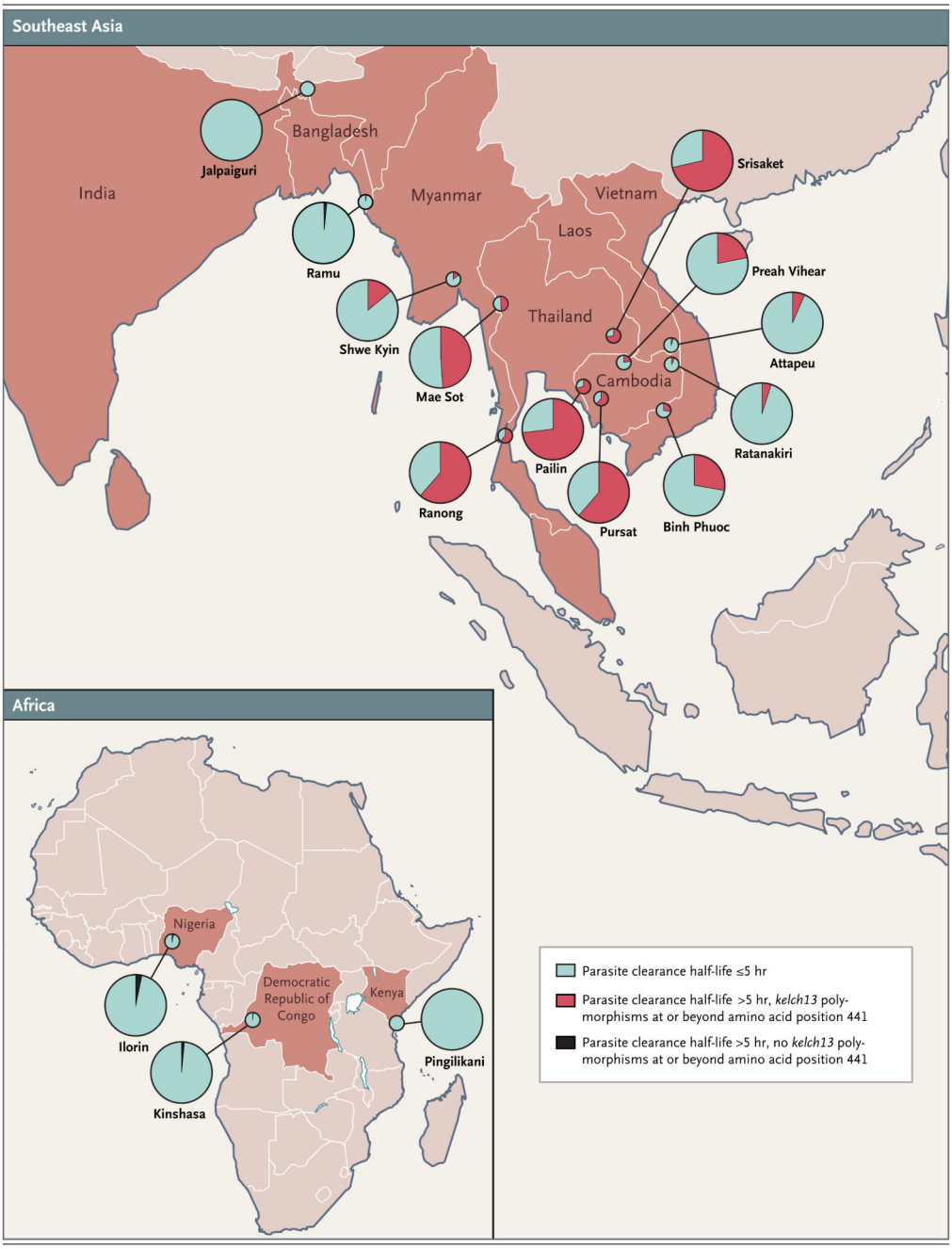

Figure 1. Location of Study Sites and Proportions of Patients with Artemisinin Resistance

Artemisinin resistance was defined by a parasite clearance half-life longer than 5 hours, with some *Plasmodium falciparum* isolates having *kelch13* polymorphisms (at or beyond amino acid position 441).

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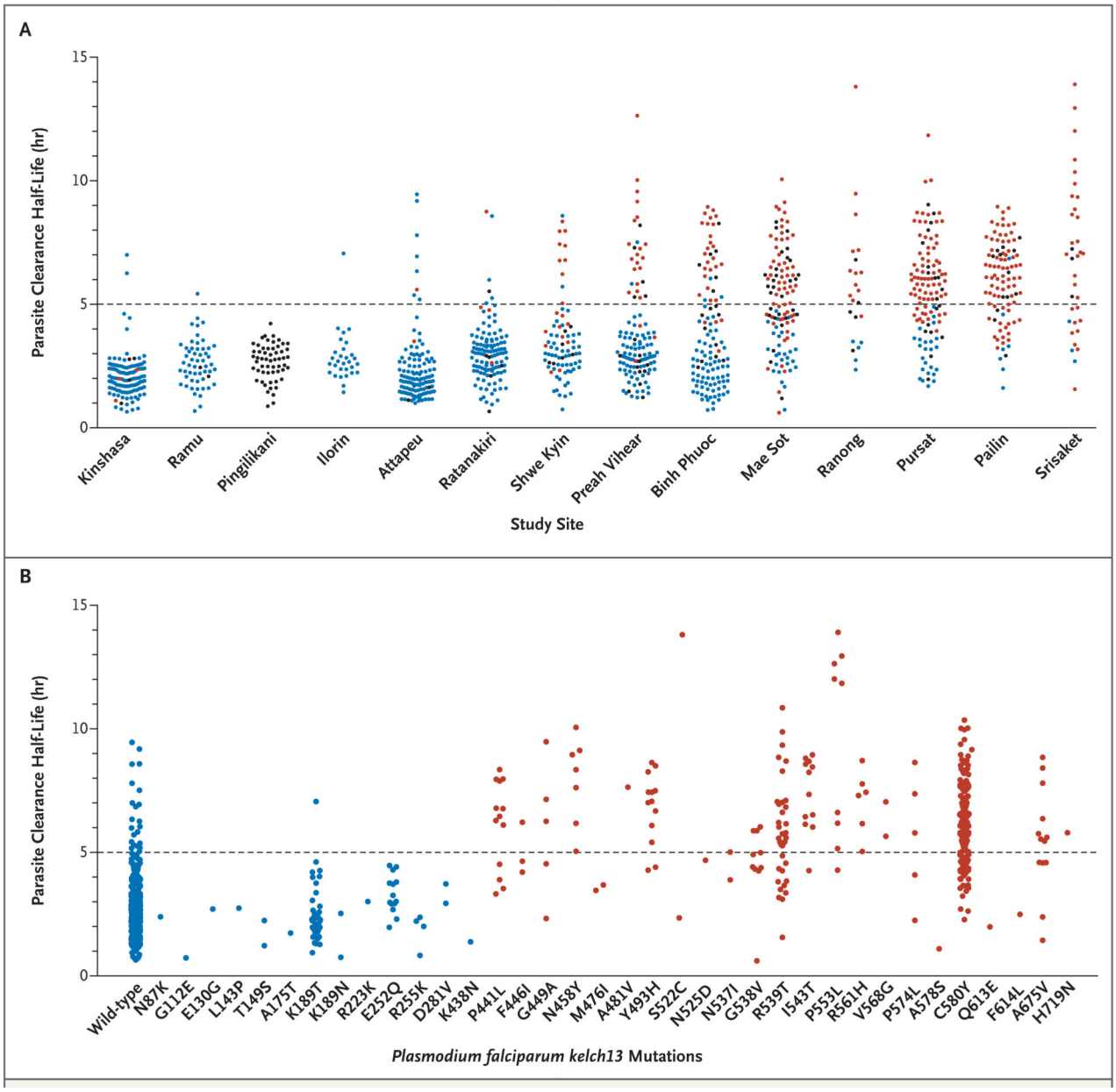

Figure 2. Distribution of Parasite Clearance Half-Lives

Panel A shows the parasite clearance half-life according to study site (treatment groups have been pooled in all sites), and Panel B shows the location of *P. falciparum kelch13* nonsynonymous polymorphisms. One circle represents one patient. Infections with mutations in the *P. falciparum kelch13* are indicated by blue circles (wild-type or mutation before amino acid position 441), red circles (mutation after position 440), or black circles (at least part of *kelch13* sequence is missing or heterozygous). Two patients had double mutations (n87k and k92n, and p441l and n725y); the site of the first mutation is shown.

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Table 1

Baseline Characteristics of the Patients, According to Study Site*

Study Site	No. of Patients	Artesunate	Artemisinin-Based Combination Therapy	Male Sex	Weight	Median Age (range)	Median Duration of Fever (range)	Parasite Count (range)	Gametocytemia on Day 0	Mixed Infection	Hematocrit
		mg/kg		*no. of patients (%)*	*kg*	*yr*	*days*	*no./mm^3*	*no. of patients/ total no. (%)*	*no. of patients (%)*	*%*
All sites	1241			920 (74)	42±17	21 (11–31)	3 (2–4)	52,250 (2560–605,329)	125/1225 (10)	67 (5)	37±6.5
Jalpaiguri, India	1	4	AS-SP	1	49	38	—	93,600	0/1	0	30
Ramu, Bangladesh	56	2 or 4	AL	48 (86)	46±10	26 (19–30)	4 (3–5)	27,130 (10,048–224,196)	0/56 (0)	0	41±4.3
Shwe Kyin, Myanmar	80	2 or 4	AL	66 (82)	52±7.6	24 (19–31.5)	3 (2–4)	66,066 (10,640–420,006)	9/80 (11)	4(5)	39±5.7
Thailand											
Mae Sot	120	2 or 4	AS-MQ	94 (78)	52±6.8	29 (22.5–37)	3 (2–4)	38,685 (2560–327,062)	11/118 (9)	5 (4)	38±6
Srisaket	41	4	AS-MQ	41 (100)	58±7.5	29 (22–38)	3 (2–4)	31,902 (4346–192,997)	1/38 (3)	3 (7)	41±5.4
Ranong	24	2 or 4	AS-MQ	16 (67)	59±8.4	33 (26–39)	3 (2–4)	38,999 (5903–94,451)	0/23	0	39±4.5
Attapeu, Laos	120	2 or 4	AL	85 (71)	43±14	20.5 (13.5–28)	3 (3–4)	46,346 (10,048–198,574)	6/120 (5)	4(3)	38±4.1
Cambodia											
Pailin	100	4	DP	87 (87)	52±9.6	25 (19–37.5)	2.5 (1.5–3.5)	45,216 (3712–389,988)	19/100 (19)	16 (16)	39±5.1
Preah Vihear	120	2 or 4	DP	82 (68)	42±14	20 (14–29)	2.8 (2.0–3.0)	56,583 (13,942–311,237)	6/120 (5)	2 (2)	37±5.8
Ratanakiri	120	2 or 4	DP	78 (65)	35±15.2	14 (9–19.5)	2 (2–3)	62,109 (5024–310,860)	7/120 (6)	19 (16)	37±5.7
Pursat	120	4	DP	109 (91)	50±12.3	25 (19–33.5)	3 (3–4)	56,583 (9797–284,861)	22/120 (18)	4 (3)	39±5.7
Binh Phuoc, Vietnam	120	2 or 4	DP	92 (77)	51±11.3	26 (18.5–38.5)	2 (2–3)	49,738 (9797–205,230)	7/119 (6)	9 (8)	38±4.5
ilorin, Nigeria	40	2 or 4	AL	29 (72)	16±7.1	3 (2–6)	2 (1–3)	52,250 (3391–219,549)	1/35 (3)	1 (2)	32±4.7
Kinshasa, Democratic Republic of Congo	120	4 or 0	AL	63 (52)	15±5	5 (3–6)	3 (2–4)	60,037 (11,555–60,5329)	36/117 (31)	0	31±4.7
Pingilikani, Kenya	59	2	—	28 (47)	15±4.7	5 (3–8)	2 (1–3)	91,562 (16,579–488,333)	0/58	0	30±4.8

*Plus–minus values are means±SD. AL denotes artemether–lumefantrine, AS-MQ artesunate–mefloquine, AS-SP artesunate–sulfadoxine–pyrimethamine, and DP dihydroartemisinin–piperaquine.

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Table 2

Time to Resolution of Fever and Parasite Clearance*

Study Site	Median Time to Resolution of Fever (IQR)	Median Parasite Clearance Half-Life (range)	Median Time to 50% Parasite Clearance (range)	Median Time to 90% Parasite Clearance (range)	Parasite Clearance Half-Life >5 Hr	Positive for Parasitemia on Day 3	Nonsynonymous SNPs *kelch13* after Position 440†
	hours					*no. of patients/total no. (%)*	
Jalpaiguri, India	12	2.8	8.2	11.9	0	0/1	—
Ramu, Bangladesh	12 (4–18)	2.5 (0.7–5.4)	5 (2.1–12.1)	11 (3.7–22.4)	1/55 (2)	1/53 (2)	0/55
Shwe Kyin, Myanmar	6 (4–18)	3.1 (1.3–8.6)	7.4 (2.3–22.1)	15.7 (4.6–39.7)	11/79 (14)	7/80 (9)	19/73 (26)
Thailand							
Mae Sot	24 (8–36)	4.9 (0.6–10.1)	7.9 (2.0–27.5)	20.8 (3.7–50.4)	57/117 (49)	41/118 (35)	60/89 (67)
Srisaket	36 (12–36)	7.0 (1.6–13.9)	7.7 (2.1–27.3)	28 (3.9–60.6)	23/35 (66)	23/34 (68)	30/33 (91)
Ranong	36 (30–36)	5.4 (2.4–13.8)	8 (2.9–18.9)	29.7 (11.6–44.2)	14/23 (61)	11/22 (50)	13/18 (72)
Attapeu, Laos	18 (7–31)	2 (1.1–9.5)	7.9 (2.4–23.1)	13.7 (5.4–28.8)	7/108 (6)	6/115 (5)	2/116 (2)
Cambodia							
Pailin	36 (24–60)	6.1 (2.4–9.0)	9.5 (2.1–22.3)	26.4 (3.7–47.4)	71/97 (73)	65/98 (66)	78/85 (92)
Preah Vihear	18 (6–24)	3.0 (1.2–12.6)	7.7 (2.0–42.6)	16.2 (3.6–59.5)	26/120 (22)	21/120 (18)	22/104 (21)
Ratanakiri	12 (6–18)	3 (0.7–8.8)	6.9 (2.0–26.0)	15.5 (3.6–36.3)	6/119 (5)	8/119 (7)	4/114 (4)
Pursat	36 (24–42)	5.6 (1.7–11.8)	9.6 (2.3–32.1)	25.7 (6.4–66.5)	73/119 (61)	71/118 (60)	76/100 (76)
Binh Phuoc, Vietnam	18 (8–30)	3.1 (0.7–8.9)	8.5 (2.0–33.3)	16.4 (3.6–53.3)	33/118 (28)	32/118 (27)	28/102 (27)
ilorin, Nigeria	8 (4–18)	2.6 (1.4–7.1)	6.8 (2.7–22.0)	13.8 (5.7–33.3)	1/29 (3)	1/27 (4)	0/30
Democratic Republic of Congo							
Kinshasa‡	4 (4–12)	1.9 (0.7–7.0)	6.0 (2.0–16.0)	11.3 (3.6–20.0)	2/59 (3)	1/59 (2)	1/58 (2)
Kinshasa§	12 (4–24)	2.2 (1.2–4.6)	8.9 (2.3–18.8)	16.7 (4.6–23.6)	0/57	0/57	2/56 (4)
Pingilikani, Kenya	18 (4–24)	2.8 (0.9–4.2)	5.3 (1.3–13)	11.6 (3.4–27.5)	0/56	0/46	—

*The time to the resolution of fever is the time until the temperature decreased to 37.5°C or lower and remained at that level for 24 hours. The time to the resolution of fever was estimated with the use of the Kaplan–Meier method. IQR denotes interquartile range, and SNP single–nucleotide polymorphism.

†The denominator excludes missing and heterozygous genotypes.

‡Patients in this group received artesunate at a dose of 4 mg per kilogram of body weight.

§Patients in this group received artemether–lumefantrine.

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Table 3

Efficacy of Various Treatment Regimens at Sites that Followed Patients for More than 14 Days*

Study Site	Treatment	Duration of Treatment	Duration of Follow-up	Total No. of Patients	No. of Patients with Recurrence of Infection	Efficacy	
						Without PCR Correction	With PCR Correction†
		days				*% (95% CI)*	
Pailin, Cambodia	AS4-DP	6	42	100	2	97.7 (90.9–99.4)	97.7 (90.9–99.4)
Vietnam							
Binh Phuoc	AS2-DP	6	42	60	0	100 (93.2–100)	100 (93.2–100)
Binh Phuoc	AS4-DP	6	42	60	0	100 (93.0–100)	100 (93.0–100)
Laos							
Attapeu	AS4-AL	6	42	60	1	98.2 (88.0–99.8)	100 (93.5–100)
Attapeu‡	AS2-AL	6	42	60	2	96.6 (86.9–99.1)	100 (93.6–100)
Myanmar							
Shwe Kyin	AS4-AL	6	42	40	0	100 (87.9–100)	100 (87.9–100)
Shwe Kyin	AS2-AL	6	42	40	0	100 (89.0–100)	100 (89.0–100)
Pingilikani, Kenya§	AS2	7	42	59	19	65.5 (51.3–76.4)	93.4 (80.8–97.9)
Democratic Republic of Congo							
Kinshasa	AS4-AL	6	28	60	3	94.6 (84.3–98.2)	100 (93.6–100)
Kinshasa	AL	3	28	60	12	77.4 (64.3–88.2)	98.3 (88.4–99.8)

*AL denotes artemether–lumefantrine, AS4 artesunate at a dose of 4 mg per kilogram of body weight, AS2 artesunate at a dose of 2 mg per kilogram, and DP dihydroartemisinin–piperaquine.

†For recurrent infections, polymerase-chain-reaction (PCR) genotyping was performed with the use *mspl*, *msp2*, and *glurp* as genetic markers for *Plasmodium falciparum* to distinguish a recrudescence from a newly acquired infection.[16] In Kenya, only *msp2* was used for genotyping.[17]

‡Genotypes were missing for two patients.

§DNA failed to amplify in three patients.