

## ORIGINAL ARTICLE

# A Trial of Combination Antimalarial Therapies in Children from Papua New Guinea

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## ABSTRACT

**BACKGROUND**

Malaria control is difficult where there is intense year-round transmission of multiple plasmodium species, such as in Papua New Guinea.

**METHODS**

Between April 2005 and July 2007, we conducted an open-label, randomized, parallel-group study of conventional chloroquine–sulfadoxine–pyrimethamine and artesunate–sulfadoxine–pyrimethamine, dihydroartemisinin–piperazine, and artemether–lumefantrine in children in Papua New Guinea 0.5 to 5 years of age who had falciparum or vivax malaria. The primary end point was the rate of adequate clinical and parasitologic response at day 42 after the start of treatment with regard to *Plasmodium falciparum*, after correction for reinfections identified through polymerase-chain-reaction (PCR) genotyping of polymorphic loci in parasite DNA. Secondary end points included the rate of adequate clinical and parasitologic response at day 42 with regard to *P. vivax* without correction through PCR genotyping.

**RESULTS**

Of 2802 febrile children screened, 482 with falciparum malaria and 195 with vivax malaria were included. The highest rate of adequate clinical and parasitologic response for *P. falciparum* was in the artemether–lumefantrine group (95.2%), as compared with 81.5% in the chloroquine–sulfadoxine–pyrimethamine group ( $P=0.003$ ), 85.4% in the artesunate–sulfadoxine–pyrimethamine group ( $P=0.02$ ), and 88.0% in the dihydroartemisinin–piperazine group ( $P=0.06$ ). The rate of adequate clinical and parasitologic response for *P. vivax* in the dihydroartemisinin–piperazine group (69.4%) was more than twice that in each of the other three treatment groups. The in vitro chloroquine and piperazine levels that inhibited growth of local *P. falciparum* isolates by 50% correlated significantly ( $P<0.001$ ). Rash occurred more often with artesunate–sulfadoxine–pyrimethamine and dihydroartemisinin–piperazine than with chloroquine–sulfadoxine–pyrimethamine ( $P=0.004$  for both comparisons).

**CONCLUSIONS**

The most effective regimens were artemether–lumefantrine against *P. falciparum* and dihydroartemisinin–piperazine against *P. vivax*. The relatively high rate of treatment failure with dihydroartemisinin–piperazine against *P. falciparum* may reflect cross-resistance between chloroquine and piperazine. (Australian New Zealand Clinical Trials Registry number, ACTRN12605000550606.)

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**A**NTIMALARIAL THERAPY UNDERPINS strategies to control and eradicate malaria.<sup>1</sup> The progression of resistance of *Plasmodium falciparum* and *P. vivax* to conventional agents including chloroquine and sulfadoxine–pyrimethamine has led the World Health Organization (WHO) to recommend artemisinin-based combination therapy as the first-line treatment for uncomplicated malaria.<sup>2</sup> Because various artemisinin derivatives are similarly effective at initiating parasite clearance, the choice between available artemisinin-based combination therapies should be based on local parasite resistance to the partner drug with the longer half-life.<sup>2</sup>

In parts of Oceania and Asia, such as Papua New Guinea, with hyperendemic or holoendemic transmission of *P. falciparum* similar to that in sub-Saharan Africa, children carry the major disease burden.<sup>3–5</sup> However, unlike in Africa, *P. vivax* transmission can also be substantial<sup>6,7</sup> and may contribute to acute complications, chronic anemia, and death.<sup>8,9</sup> Current first-line treatment for uncomplicated pediatric falciparum or vivax malaria in Papua New Guinea remains chloroquine–sulfadoxine–pyrimethamine.<sup>10</sup> Since this regimen is failing,<sup>11</sup> there is a strong argument for the introduction of artemisinin-based combination therapy.<sup>2</sup> The cost and logistics associated with changes to therapy in poor countries demand firm evidence of efficacy, but local data informing the choice of artemisinin-based combination therapy are usually lacking, and comparative studies of various artemisinin-based combination therapies have often been performed in areas in which falciparum malaria is the only, or the predominant, species or in which transmission is low or variable.<sup>12–20</sup>

We compared the efficacy and safety of chloroquine–sulfadoxine–pyrimethamine and the three commonly used artemisinin-based combination therapies — artesunate–sulfadoxine–pyrimethamine, artemether–lumefantrine, and dihydroartemisinin–piperaquine — in children in Papua New Guinea who had uncomplicated falciparum or vivax malaria. Our primary aim was to establish which of the three combination therapies should replace chloroquine–sulfadoxine–pyrimethamine as treatment for falciparum malaria. Secondary aims were to provide similar relative efficacy data for vivax malaria and to explore host-, parasite-, and drug-specific determinants of outcome.

## METHODS

### STUDY DESIGN AND SITES

This investigator-initiated open-label, randomized, parallel-group trial was conducted at the Alexishafen and Kunjingini Health Centers in Madang and East Sepik Provinces, respectively, in Papua New Guinea. The study started in April 2005, enrollment closed in June 2007, and the last follow-up visit was in July 2007. The primary end point was the reappearance of *P. falciparum* in the blood by day 42 after the start of treatment, after correction for reinfections identified through polymerase-chain-reaction (PCR) genotyping of polymorphic loci in parasite DNA.<sup>21</sup> Secondary end points were the reappearance of *P. falciparum* within 42 days (without correction through PCR genotyping); the reappearance of *P. falciparum* within 28 days (with and without correction through PCR genotyping); the appearance of *P. vivax* within 28 days and 42 days after treatment for vivax malaria and after treatment for falciparum malaria; initial fever clearance and clearance and gametocytogenesis of *P. falciparum* and *P. vivax*; and safety of the study drug. Ethics approval was obtained from the Papua New Guinea Ministry of Health Medical Research Advisory Committee and the University of Western Australia Human Research Ethics Committee. Written informed consent was obtained from a parent or guardian of each patient. The study was conducted in accordance with the Declaration of Helsinki. All authors vouch for the validity and completeness of the data presented.

### Patients

For children 0.5 to 5 years of age who had an axillary temperature above 37.5°C or a fever during the previous 24 hours as reported by the family, a blood smear was obtained and examined microscopically on site. Patients with more than 1000 asexual *P. falciparum* or more than 250 asexual *P. vivax*, *P. ovale*, or *P. malariae* per microliter of whole blood were eligible if they also had no features of severity,<sup>22</sup> no intake of a study drug in the previous 14 days, and no clinical evidence of another infection or coexisting condition, including malnutrition.

### Clinical and Laboratory Procedures

An initial clinical assessment was performed, including the measurement of mid-upper-arm cir-

circumference and calculation of the nutrition z score according to weight for age.<sup>23</sup> Blood samples were obtained for measurement of hemoglobin and glucose. Treatment assignments were made on the basis of computer-generated randomized assignment with blocks of 24 for each site.

The four treatment groups were as follows: chloroquine–sulfadoxine–pyrimethamine, with a chloroquine (Aspen Healthcare) dose of 10 mg base per kilogram of body weight daily for 3 days plus sulfadoxine–pyrimethamine (Roche; sulfadoxine, 25 mg per kilogram, and pyrimethamine, 1.25 mg per kilogram) given with the first chloroquine dose; artesunate–sulfadoxine–pyrimethamine, with one dose of sulfadoxine–pyrimethamine (Roche; sulfadoxine, 25 mg per kilogram, and pyrimethamine, 1.25 mg per kilogram) plus artesunate (Sanofi-Aventis) at a dose of 4 mg per kilogram daily for 3 days; dihydroartemisinin–piperaquine (Beijing Holley-Cotec), with a dihydroartemisinin dose of 2.5 mg per kilogram and a piperaquine phosphate dose of 20 mg per kilogram daily for 3 days; and artemether–lumefantrine (Novartis Pharma), with an artemether dose of 1.7 mg per kilogram and a lumefantrine dose of 10 mg per kilogram, twice daily for 3 days.

Combinations of full, half-, or quarter-tablets were swallowed whole or crushed lightly before administration with water or, for artemether–lumefantrine, milk. Only the administration of evening doses of artemether–lumefantrine were unsupervised, given at home by a parent or guardian. Children who vomited within 30 minutes after administration were retreated.

Standardized follow-up, including the measurement of axillary temperature and microscopical examination of a blood smear, was scheduled for days 1, 2, 3, 7, 14, 28, and 42. Children in whom uncomplicated or severe malaria developed during this period were given oral quinine plus sulfadoxine–pyrimethamine or intramuscular quinine, respectively.<sup>10</sup> All blood smears were subsequently reexamined independently by two skilled microscopists who were unaware of the treatment assignments. Parasite density was calculated from the number per 1000 leukocytes and an assumed leukocyte count of 8000 per microliter. Slides with discrepant findings (with a difference of more than a factor of 3) with regard to parasitic positivity or negativity, speciation, or density were adjudicated by a senior microscopist.

Efficacy was assessed using WHO definitions,<sup>22</sup> with a 42-day follow-up period to capture the effect of drugs with a long half-life. Early treatment failure was defined as the development of signs of severity or an inadequate parasitologic response by day 3. Any child in whom parasitemia developed between days 4 and 42 was considered to have had late parasitologic failure or, if febrile, late clinical failure. If none of the three types of failure occurred, an adequate parasitologic and clinical response was recorded. *P. falciparum* reinfection and recrudescence were distinguished with the use of molecular methods.<sup>24,25</sup> Fever and parasite clearance times were defined as the times to the first of two consecutive assessments at which the child was afebrile and had a blood smear negative for malaria, respectively.

The plasma levels on day 7 of chloroquine, its active metabolite monodesethyl chloroquine, piperaquine, and lumefantrine were assayed by means of high-performance liquid chromatography.<sup>26,27</sup> Although the current dihydroartemisinin–piperaquine tablet coformulation has been used in a number of recent efficacy trials,<sup>12,16,18,20</sup> it was not produced according to Good Manufacturing Practice standards. Tablets from each batch were therefore assayed for dihydroartemisinin and piperaquine,<sup>26</sup> and both drugs were consistently within 90 to 110% of the stated content<sup>28</sup> up to the expiration date. The levels of chloroquine, piperaquine, and lumefantrine that inhibited by 50% the growth of field isolates of parasites from the Madang area were determined with the use of a plasmodium lactate dehydrogenase colorimetric assay.<sup>29</sup>

#### STATISTICAL ANALYSIS

We calculated the number of patients who needed to be enrolled on the basis of the assumption that there would be an adequate clinical and parasitologic response with regard to *P. falciparum* in 95% of patients or more in each of the three artemisinin-based combination treatment groups at day 42, after correction for reinfections identified through PCR genotyping, a rate that falls within the WHO-recommended range for adoption of new antimalarial therapy.<sup>2</sup> Because there were no robust local chloroquine–sulfadoxine–pyrimethamine efficacy data when the study was designed, we aimed to enroll 100 children in each treatment group to detect a rate of treatment fail-

ure of 5% or more in any of the four groups with 5% precision and 95% confidence after allowing for 20% loss to follow-up.<sup>21</sup> Early in 2006, data became available from Madang and East Sepik Provinces from a 2004 efficacy study that revealed that, 28 days after treatment with chloroquine-sulfadoxine-pyrimethamine, 76.7% of patients had an adequate clinical and parasitologic response with regard to *P. falciparum* after correction for reinfections identified on PCR genotyping of polymorphic parasite loci.<sup>11</sup> Under the conservative assumption that this adequate clinical and parasitologic response persisted to day 42 and allowing for 20% attrition, 452 children (113 in each of the four treatment groups) would be required to detect a significant difference in the primary end point between each of the three artemisinin-based combination therapy groups and the chloroquine-sulfadoxine-pyrimethamine group, with a statistical power of 80% and a two-tailed type I error rate of 1%.<sup>30</sup>

The statistical analysis was prespecified. Per-protocol analyses included data for children with complete follow-up or confirmed treatment failure and excluded data for those who had been treated for malaria but whose parasitemia had not been confirmed through microscopy or who had been lost to follow-up despite repeated attempts to contact them. Data from these excluded patients were included in modified intention-to-treat analyses of two types: a worst-case approach in which patients excluded by day 3 were assumed to have had early treatment failure and those excluded after day 3 assumed to have had late parasitologic failure or late clinical failure, and a best-case approach in which all missing blood smears during the follow-up period were assumed to be parasite-negative.

Kaplan-Meier estimates were computed for each end point defined by parasite species. The treatment groups were compared by means of the log-rank test, including post hoc comparisons between the three artemisinin-based combination therapies. No interim efficacy analyses were performed. Cox regression involving backward-stepwise modeling was used to determine predictors of treatment failure among the prespecified variables of age, sex, measures of growth or nutrition, and baseline parasite density and the exploratory variable of drug levels at day 7. Safety and tolerability were assessed on the basis of the incidence of symptoms or signs through day 7 with

the use of Poisson regression (for frequent events) or Fisher's exact test (for infrequent events). All P values are two-tailed and were not adjusted for multiple comparisons.

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## RESULTS

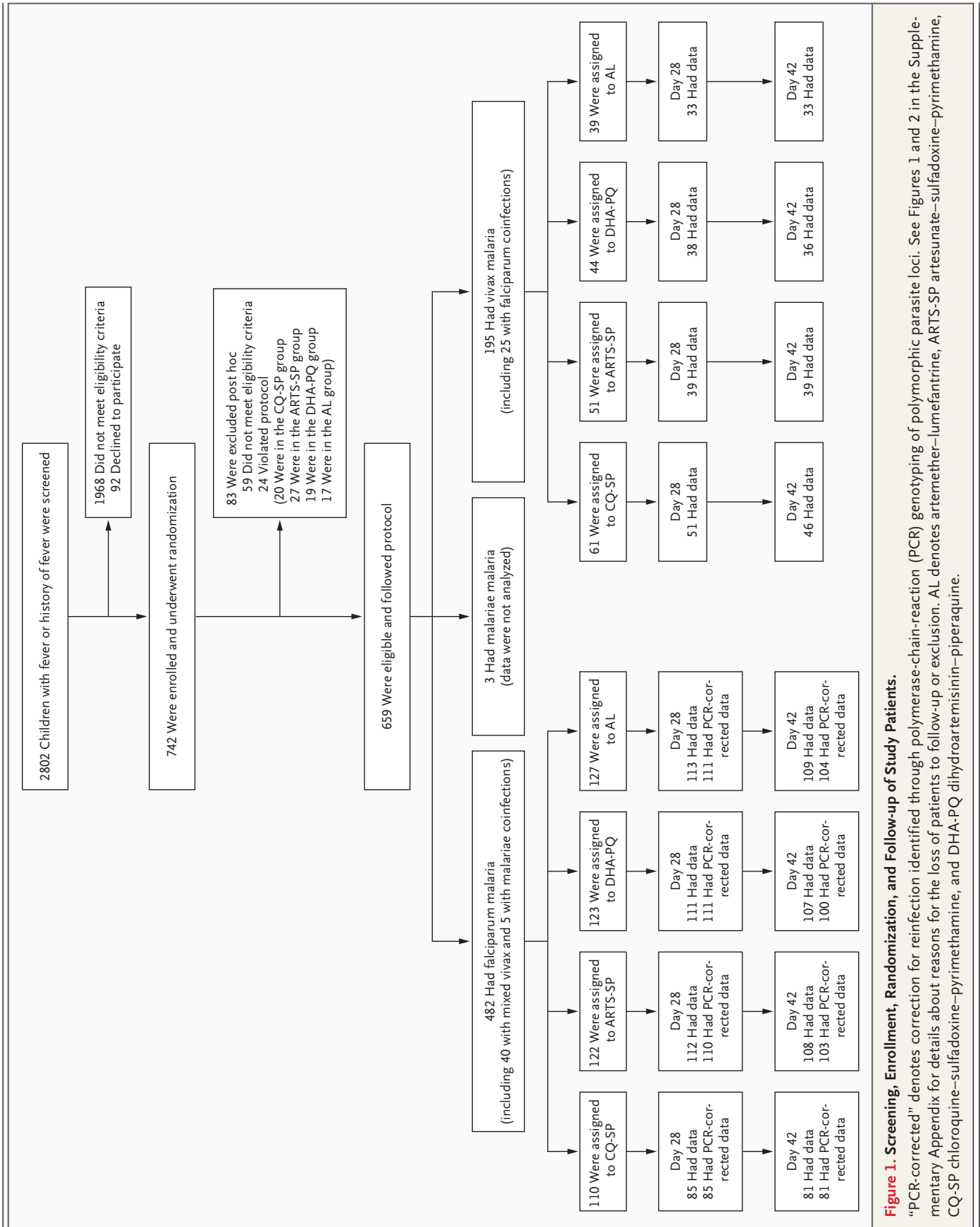
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### PATIENTS

A total of 742 children were randomly assigned to a study treatment, but 83 (11.2%) were excluded because of protocol violations, including 41 with subthreshold parasite densities on expert microscopy and 24 who received incorrect or nonstudy treatment (Fig. 1). Of the remaining 659, 21 (3.2%) were infected with both *P. falciparum* and *P. vivax* at confirmed densities above each species-specific threshold; they were considered to have both types of malaria and were included in both malaria groups. An additional 24 of 482 children with *P. falciparum* (5.0%) and 4 of 195 with *P. vivax* (2.1%) had low-level coinfections with another plasmodium species. A higher percentage of children with falciparum malaria in the chloroquine-sulfadoxine-pyrimethamine group were lost to follow-up than from the other groups combined (22.7% vs. 9.7% at day 28,  $P < 0.001$ ), but there were no significant between-treatment differences in attrition for vivax malaria (Fig. 1, and Fig. 2 in the Supplementary Appendix, available with the full text of this article at [www.nejm.org](http://www.nejm.org)). There were no significant differences in demographic, clinical, or parasitologic variables, according to parasite species or treatment (Table 1).

### EFFICACY AGAINST *P. FALCIPARUM*

Over one third of all children who had falciparum malaria at enrollment had reinfection or recrudescence by day 42 (Table 1 in the Supplementary Appendix), with three (0.6%) having early treatment failure (two in the artesunate-sulfadoxine-pyrimethamine group and one in the chloroquine-sulfadoxine-pyrimethamine group) and more than 80% having late parasitologic failure. There were no significant between-treatment differences in outcomes at day 42 (without correction for reinfection through PCR genotyping): the rate of adequate clinical and parasitologic response was 67.9% for chloroquine-sulfadoxine-pyrimethamine, 63.9% for artesunate-sulfadoxine-pyrimethamine, 62.6% for dihydroartemisinin-piperazine, and 64.2% for artemether-lumefantrine



**Figure 1. Screening, Enrollment, Randomization, and Follow-up of Study Patients.**

“PCR-corrected” denotes correction for reinfection identified through polymerase-chain-reaction (PCR) genotyping of polymorphic parasite loci. See Figures 1 and 2 in the Supplementary Appendix for details about reasons for the loss of patients to follow-up or exclusion. AL denotes artemether–lumefantrine, ARTS-SP artesunate–sulfadoxine–pyrimethamine, CQ-SP chloroquine–sulfadoxine–pyrimethamine, and DHA-PQ dihydroartemisinin–piperaquine.

**Table 1. Baseline Characteristics of the Study Patients, According to Type of Malaria and Treatment Group.\***

Characteristic	Falciparum Malaria (N=482)				Vivax Malaria (N=195)				P Value
	CQ-SP	ARTS-SP	DHA-PQ	AL	CQ-SP	ARTS-SP	DHA-PQ	AL	
Female sex (%)	52	39	49	43	54	45	59	51	0.58
Mean age (mo)	36	35	37	38	27	24	24	25	0.49
Mean weight (kg)	11.4	11.3	11.2	11.7	10.0	9.6	9.5	9.4	0.60
Mean WAZ	-1.68	-1.75	-1.79	-1.74	-1.66	-1.66	-1.75	-1.87	0.78
Mean MUAC (cm)	14.1	14.0	14.5	14.0	13.5	13.9	13.5	14.0	0.73
Body-mass index†	14.5	14.6	14.6	14.6	15.5	15.2	15.4	15.2	0.91
Parasite density (no. of parasites/ $\mu$ l)									
Median	43,869	50,986	56,009	48,507	4,068	5,716	5,890	4,192	0.43
Range	1160-467,160	1600-366,280	1320-609,960	2280-450,440	320-55,680	320-86,840	320-93,760	280-223,040	
Mean axillary temperature (°C)	38.0	38.0	37.9	38.0	37.3	37.7	37.2	37.1	0.18
Enlarged spleen (%)	52	51	52	59	40	37	36	44	0.88
Hemoglobin (g/dl)	8.5	8.6	8.3	8.4	8.7	9.0	8.9	9.0	0.71
Blood glucose (mmol/liter)	7.2	7.0	7.0	7.0	7.2	6.9	7.1	7.2	0.86
Heart rate (beats/min)	119	120	125	118	119	113	125	111	0.17
Respiratory rate (breaths/min)	33	33	33	35	34	37	35	36	0.81

\* AL denotes artemether-lumefantrine, ARTS-SP artesunate-sulfadoxine-pyrimethamine, CQ-SP chloroquine-sulfadoxine-pyrimethamine, DHA-PQ dihydroartemisinin-piperaquine, MUAC mid-upper-arm circumference, and WAZ nutrition z score according to weight for age. To convert values for blood glucose to milligrams per deciliter, divide by 0.05551.  
 † The body-mass index is the weight in kilograms divided by the square of the height in meters.

( $P=0.99$ ). However, after correction for reinfection through PCR genotyping, the artemether-lumefantrine group had a higher rate of adequate clinical and parasitologic response at days 28 and 42 than the other treatment groups (Table 2 and Fig. 2). There was a similar result in the best-case intention-to-treat analysis, whereas in the worst-case model, the rate of treatment failure was highest with chloroquine-sulfadoxine-pyrimethamine (Table 2 in the Supplementary Appendix).

The mean parasite clearance time was longer in the chloroquine-sulfadoxine-pyrimethamine group (4.2 days) than in any of the three artemisinin-based combination therapy groups ( $\leq 3.1$  days,  $P \leq 0.001$ ), but there were no significant between-group differences in the fever clearance time (Table 3 in the Supplementary Appendix). The prevalence of post-treatment gametocytemia was greatest for chloroquine-sulfadoxine-pyrimethamine (maximum on day 7 of 83%, vs.  $\leq 22\%$  for the three artemisinin-based combination therapies; Fig. 3 in the Supplementary Appendix). In the artesunate-sulfadoxine-pyrimethamine group, treatment failure (after correction for reinfection through PCR genotyping) was significantly associated with age (hazard ratio for each 1-year increase in age, 1.12; 95% confidence interval [CI], 1.05 to 1.20;  $P=0.001$ ) and the body-mass index (the weight in kilograms divided by the square of the height in meters) (hazard ratio for each increase of 1.0, 1.43; 95% CI, 1.16 to 1.76;  $P=0.001$ ). In the dihydroartemisinin-piperaquine group, independent predictors of treatment failure were baseline parasite density (hazard ratio for each increase of 10,000 per microliter, 1.07; 95% CI, 1.03 to 1.11;  $P=0.001$ ) and nutrition z score according to weight for age (hazard ratio for each increase of 1.0, 1.22; 95% CI, 1.09 to 1.38;  $P=0.001$ ). There were no baseline variables significantly associated with treatment failure in the chloroquine-sulfadoxine-pyrimethamine group or the artemether-lumefantrine group.

#### EFFICACY AGAINST *P. VIVAX*

Almost two thirds of patients who had vivax malaria at enrollment had redevelopment of *P. vivax* parasitemia by day 42, and most had late parasitologic failure (Table 2). The highest rates of adequate clinical and parasitologic response and lowest rates of late clinical failure were found for children receiving dihydroartemisinin-piperaquine (Table 2 and Fig. 2). The least efficacious treat-

ment was chloroquine-sulfadoxine-pyrimethamine, with only 13.0% of children without parasitemia at day 42. Intention-to-treat analyses were consistent with per-protocol results at days 28 and 42 (Table 4 in the Supplementary Appendix). Age, sex, baseline parasite density, body-mass index, and nutrition z score according to weight for age were not significant independent predictors of treatment failure. The mean parasite clearance time was longer with chloroquine-sulfadoxine-pyrimethamine (3.1 days) than with any of the three artemisinin-based combination therapies ( $\leq 1.4$  days,  $P=0.05$ ), but fever clearance times were similar (Table 3 in the Supplementary Appendix). Approximately half the 371 children who had *P. falciparum* mono-infections also had *P. vivax* parasitemia by day 42 (Table 2), with the lowest incidence occurring in the dihydroartemisinin-piperaquine group.

#### DRUG LEVELS AND OUTCOME AT DAY 7

Among children who had falciparum malaria, in univariate analyses, there was a trend toward a lower risk of any treatment failure (not corrected through PCR genotyping) at day 7 with a higher plasma piperaquine level in the dihydroartemisinin-piperaquine group (hazard ratio for each increase of 10  $\mu\text{g}$  per liter, 0.86; 95% CI, 0.73 to 1.01;  $P=0.06$ ) and with a higher plasma lumefantrine level in the artemether-lumefantrine group (hazard ratio for each increase of 100  $\mu\text{g}$  per liter, 0.87; 95% CI, 0.74 to 1.02;  $P=0.09$ ). According to the Cox model of treatment failure in the dihydroartemisinin-piperaquine group, after correction through PCR genotyping, there was a trend toward association in plasma piperaquine levels at day 7 ( $P=0.08$ ), but the nutrition z score according to weight for age was no longer significantly associated ( $P=0.25$ ).

In the chloroquine-sulfadoxine-pyrimethamine group, there was no association between the plasma chloroquine and monodesethyl chloroquine levels at day 7 and treatment failure with regard to *P. falciparum* ( $P>0.60$  for each comparison), but treatment failure with regard to *P. vivax* was negatively associated with both the plasma chloroquine level (hazard ratio, 0.97; 95% CI, 0.95 to 1.00;  $P=0.04$ ) and the plasma level of the metabolite monodesethyl chloroquine (hazard ratio, 0.97; 95% CI, 0.94 to 0.99;  $P=0.01$ ). There was an increased risk of development of *P. vivax* parasitemia among children treated for *P. falciparum* infec-

**Table 2. Per-Protocol Analysis of Responses among Children with Falciparum Malaria or Vivax Malaria, after Correction for Reinfection, According to Treatment Group.\***

Value	CQ-SP	ARTS-SP	DHA-PQ	AL	All	ARTS-SP vs. DHA-PQ	ARTS-SP vs. AL	DHA-PQ vs. AL
<i>Plasmodium falciparum</i> assessed at day 28 — no.	85	110	111	111	417			
Adequate clinical and parasitologic response								
No.	72	99	100	108	379			
Percent (95% CI)	84.7 (75.3–91.6)	90.0 (82.8–94.9)	90.1 (83.0–94.9)	97.3 (92.3–99.4)	90.9 (87.7–93.5)			
P value, vs. CQ-SP		0.26	0.26	0.001				
P values between ACT-based therapies						0.98	0.03	0.03
Early treatment failure — %	3.5	1.8	0	0	1.2			
Late clinical failure — %	1.2	0	0.9	0.9	0.7			
Late parasitologic failure — %	10.6	8.2	9.0	1.8	7.2			
<i>P. falciparum</i> assessed at day 42 — no.	81	103	100	104	388			
Adequate clinical and parasitologic response								
No.	66	88	88	99	341			
Percent (95% CI)	81.5 (71.3–89.2)	85.4 (77.1–91.6)	88.0 (80.0–93.6)	95.2 (89.1–98.4)	87.9 (83.9–90.7)			
P value, vs. CQ-SP		0.47	0.22	0.003				
P values between ACT-based therapies						0.59	0.02	0.06
Early treatment failure — %	3.7	1.9	0	0	1.3			
Late clinical failure — %	1.1	0	1.0	1.9	1.0			
Late parasitologic failure — %	13.6	12.6	11.0	2.9	10.1			
<i>P. vivax</i> assessed at day 28 — no.	51	39	38	33	161			
Adequate clinical and parasitologic response								
No.	26	20	32	16	94			
Percent (95% CI)	51.0 (36.6–65.2)	51.3 (34.8–67.6)	84.2 (68.7–94.0)	48.5 (30.8–66.5)	58.4 (50.4–66.1)			
P value, vs. CQ-SP		0.98	0.001	0.82				
P values between ACT-based therapies						0.002	0.81	0.001
Early treatment failure — %	0	0	0	0	0			
Late clinical failure — %	7.8	2.6	0	6.1	4.3			
Late parasitologic failure — %	41.2	46.2	15.8	45.5	37.3			

<i>P. vivax</i> assessed at day 42 — no.						
Adequate clinical and parasitologic response						
No.	6	13	25	36	33	154
Percent (95% CI)	13.0 (4.9–26.3)	33.3 (19.1–50.2)	69.4 (51.9–83.7)	<0.001	0.06	35.1 (27.6–43.2)
P value, vs. CQ-SP	0.03					
P values between ACT-based therapies						
Early treatment failure — %	0	0	0	0	0	0
Late clinical failure — %	21.7	17.9	2.8	15.2	14.9	14.9
Late parasitologic failure — %	65.3	48.7	27.8	54.5	50.0	50.0
<i>P. vivax</i> parasitemia after treatment for <i>P. falciparum</i> , assessed at day 42 — no.						
Adequate clinical and parasitologic response						
No.	30	38	73	36	177	177
Percent (95% CI)	40.0 (28.9–52.0)	40.4 (30.4–51.0)	73.0 (63.2–81.4)	35.3 (26.1–45.4)	47.7 (42.5–52.9)	47.7 (42.5–52.9)
P value, vs. CQ-SP	1.0		<0.001	0.52	<0.001	<0.001
P values between ACT-based therapies						
Early treatment failure — %	0	0	0	0	0	0
Late clinical failure — %	2.7	1.1	1.0	3.9	2.2	2.2
Late parasitologic failure — %	57.3	58.5	26.0	60.8	50.1	50.1

\* Data are reported after correction for reinfection through polymerase-chain-reaction genotyping. ACT denotes artemisinin-based combination therapy, AL artemether–lumefantrine, ARTS-SP artesunate–sulfadoxine–pyrimethamine, CQ-SP chloroquine–sulfadoxine–pyrimethamine, and DHA-PQ dihydroartemisinin–piperazine.

**SAFETY MONITORING**

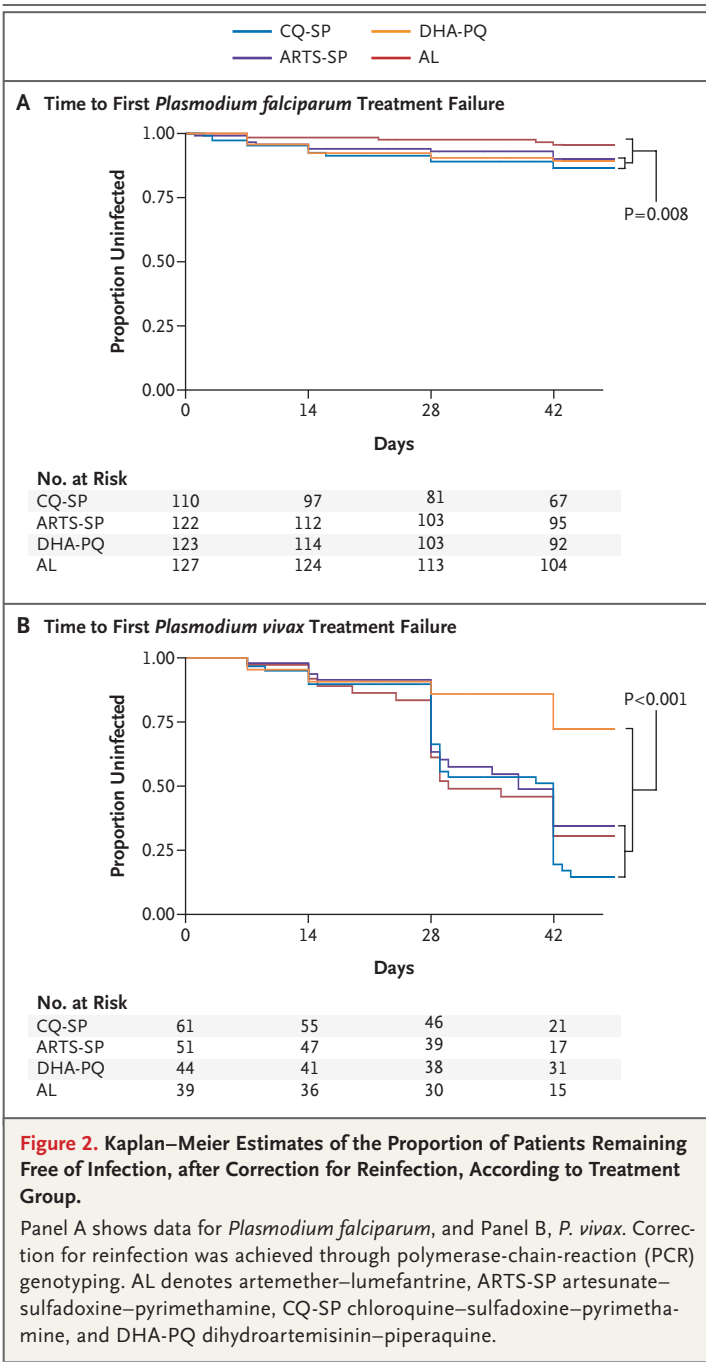
No treatment withdrawals were attributable to adverse effects related to a study drug. There was a lower incidence rate ratio for fever and vomiting between days 0 and 7 with the artemisinin-based combination therapy regimens than with chloroquine–sulfadoxine–pyrimethamine ( $P < 0.04$  for all three comparisons; Table 5 in the Supplementary Appendix) but no significant between-treatment difference for other symptoms. There was a higher incidence rate ratio for rash with artesunate–sulfadoxine–pyrimethamine and dihydroartemisinin–piperazine than with chloroquine–sulfadoxine–pyrimethamine ( $P = 0.004$ ). A palpable spleen was found least often in the dihydroartemisinin–piperazine group ( $P = 0.006$ ). Hemoglobin levels remained similar across the treatment groups, with a mean increase in the level at day 42 of 1.7 g per deciliter as compared with the baseline value. Clinically significant hypoglycemia did not develop in any child.

**PARASITE SENSITIVITY IN VITRO**

There was a significant correlation between the in vitro levels of chloroquine and piperazine that inhibited growth of local *P. falciparum* isolates by 50% ( $r = 0.54$  for 57 samples,  $P < 0.001$ ) (Fig. 3). The correlation between chloroquine and lumefantrine was not significant ( $r = 0.15$  for 16 samples,  $P = 0.58$ ).

**DISCUSSION**

This study shows that artemether–lumefantrine is an effective treatment for uncomplicated falciparum malaria in children from Papua New Guinea. However, artemether–lumefantrine is less efficacious than dihydroartemisinin–piperazine against *P. vivax*, both for primary infection and in suppressing its emergence after treatment for *P. falciparum*. Although dihydroartemisinin–piperazine has been used rarely, if at all, in Papua New Guinea, the relatively high failure rate of the combination treatment against *P. falciparum* is in contrast to that reported in Asia, Africa, and South America, where efficacies have exceeded 95% over periods of up to 63 days.<sup>31</sup> Artesunate–sulfadoxine–pyrimethamine was inferior to artemether–lumefantrine for treatment against *P. falciparum* and inferior to dihydroartemisinin–piperazine against *P. vivax*, whereas chloroquine–sulfadoxine–



**Figure 2.** Kaplan–Meier Estimates of the Proportion of Patients Remaining Free of Infection, after Correction for Reinfection, According to Treatment Group.

Panel A shows data for *Plasmodium falciparum*, and Panel B, *P. vivax*. Correction for reinfection was achieved through polymerase-chain-reaction (PCR) genotyping. AL denotes artemether–lumefantrine, ARTS-SP artesunate–sulfadoxine–pyrimethamine, CQ-SP chloroquine–sulfadoxine–pyrimethamine, and DHA-PQ dihydroartemisinin–piperazine.

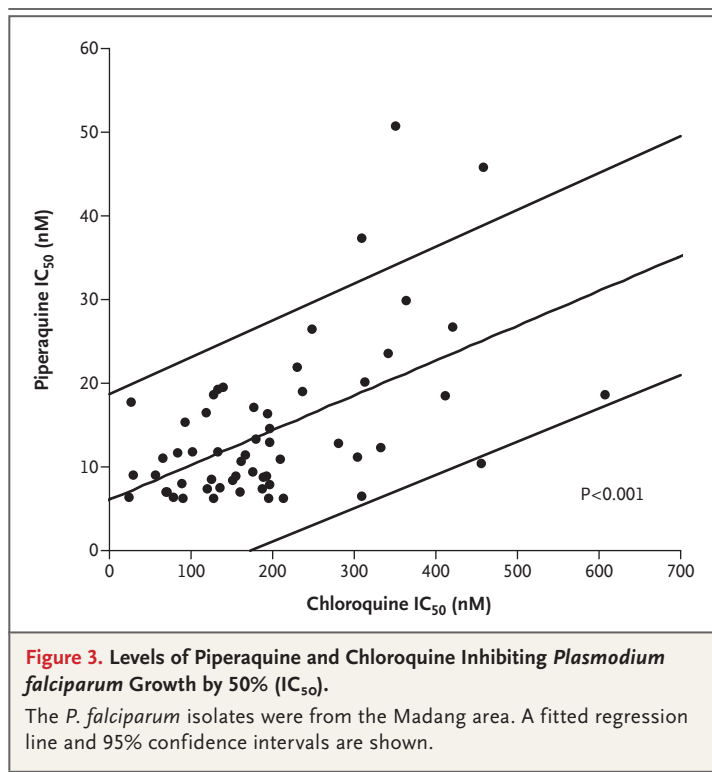
tion, for those in the artemether–lumefantrine group who had plasma lumefantrine levels of less than 175  $\mu\text{g}$  per liter (hazard ratio, 2.65; 95% CI, 1.43 to 4.91;  $P = 0.003$ ) and for those in the dihydroartemisinin–piperazine group with plasma piperazine levels of less than 20  $\mu\text{g}$  per liter (hazard ratio, 2.42; 95% CI, 1.03 to 5.70;  $P = 0.04$ ).

pyrimethamine was the least efficacious regimen against both plasmodium species.

The adequate clinical and parasitologic response against *P. falciparum* in the artemether-lumefantrine group was more than 95% at day 42 (after correction for reinfection through PCR genotyping), which is within the WHO-recommended range for adoption of a new therapy.<sup>2</sup> However, most patients receiving artemether-lumefantrine had *P. vivax* parasitemia by day 28; this was true for many fewer patients receiving dihydroartemisinin-piperazine, probably reflecting faster elimination of lumefantrine than piperazine.<sup>32</sup> However, the 28-day *P. falciparum* failure rate for dihydroartemisinin-piperazine, after correction through PCR genotyping, was well below the WHO-recommended threshold of 95% for adequate clinical and parasitologic response.<sup>2</sup> The association between in vitro chloroquine and piperazine levels that inhibited growth of local *P. falciparum* isolates by 50%, which parallels a weaker positive correlation in isolates from Cameroon,<sup>33</sup> suggests that cross-resistance of local *P. falciparum* strains may have contributed.

Consistent with previous studies,<sup>20,34</sup> we found that children receiving dihydroartemisinin-piperazine in whom treatment failed tended to have low plasma piperazine levels at day 7. Although piperazine bioavailability is improved by coadministration of fat,<sup>35</sup> this is not part of current dosing recommendations (unlike for artemether-lumefantrine) and was not required in several dihydroartemisinin-piperazine efficacy studies with high cure rates.<sup>20,36</sup> In a study in which dihydroartemisinin-piperazine was administered with fat,<sup>16</sup> 38% of children under 15 years of age had a plasma piperazine level of less than 30 ng per milliliter,<sup>34</sup> as compared with 52% in our patients, who were generally younger. Although this difference might reflect reduced absorption in our patients, it could also result from age-specific differences in the pharmacokinetic properties of piperazine<sup>37</sup> that have led to a call for increased dihydroartemisinin-piperazine doses in children.<sup>34</sup> The few treatment failures occurring with artemether-lumefantrine in our study were associated with low plasma lumefantrine levels, consistent with the results of an African study<sup>38</sup> and highlighting the importance of adherence to complex dosing regimens.

We found that the *P. falciparum* density at base-



line predicted whether dihydroartemisinin-piperazine treatment would fail. This finding has not been reported in other studies of dihydroartemisinin-piperazine with high cure rates,<sup>16,20,36</sup> perhaps because the study children had limited malarial immunity and a consequently lower pyrogenic parasite burden (geometric mean, <10,000 per microliter, vs. approximately 50,000 per microliter in our patients). Piperazine has the longest half-life of the drugs used in our study,<sup>39</sup> and exposure to therapeutic drug levels over many parasite life cycles is an important determinant of response.<sup>40</sup> However, pharmacokinetic data from children in Papua New Guinea suggest that piperazine is distributed extensively, with a substantial post-treatment fall in plasma levels.<sup>26</sup> This phenomenon, together with relatively high densities of piperazine-resistant *P. falciparum*, could result in early subtherapeutic plasma piperazine levels in children treated with dihydroartemisinin-piperazine.

The differences between the rates of adequate clinical and parasitologic response with and without correction for reinfection through PCR genotyping suggest that one quarter of patients were reinfected with *P. falciparum* by day 42, confirm-

ing intense transmission. The emergence of *P. vivax* parasitemia after treatment for *P. falciparum* mono-infection is well recognized, even with artemisinin-based combination therapy,<sup>19</sup> but not to the extent observed in the current study. Most of our patients who had falciparum malaria at enrollment became positive for *P. vivax* during the follow-up period. Although the vivax infection was largely asymptomatic, it could still contribute to adverse outcomes.<sup>8,9</sup> For example, although hemoglobin levels increased in each treatment group, the levels at day 42 remained lower than those reported in a study from an area with less intense transmission that also showed a greater improvement with dihydroartemisinin-piper-quine than artemether-lumefantrine.<sup>16</sup>

Our study had limitations. There was an unexpectedly large attrition rate among the children with falciparum malaria receiving chloroquine-sulfadoxine-pyrimethamine. This potential source of bias may have reflected a relatively slow initial symptomatic response or disappointment of parents or guardians that their children did not receive one of the new treatments. However, even if all these children had completed the study with adequate clinical and parasitologic response, the failure rate of chloroquine-sulfadoxine-pyrimethamine would have remained relatively high. The relatively small numbers of children in the *P. vivax* treatment groups and the fact that PCR correction for reinfection is not currently feasible for this plasmodium species mean that these data should be viewed as preliminary. Nevertheless,

clear between-treatment differences emerged for both falciparum and vivax malaria.

This study highlights key issues complicating choice of antimalarial therapy in areas with intense transmission of multiple plasmodium species. Although the similar rates of reappearance of any *P. falciparum* parasitemia across treatments suggest that chloroquine-sulfadoxine-pyrimethamine might still be useful, the need to ensure continuing high-level efficacy against this potentially life-threatening infection favors artemether-lumefantrine as a replacement for chloroquine-sulfadoxine-pyrimethamine in Papua New Guinea at this time. Dihydroartemisinin-piper-quine could be used where *P. vivax* is especially problematic. However, neither regimen is optimal for this epidemiologic circumstance, suggesting the need for more broadly efficacious and affordable treatment that could include other artemisinin-based combination therapies, including new partner drugs.

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