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Efficacy and Safety of Pyronaridine-Artesunate for Treatment of Uncomplicated *Plasmodium falciparum* Malaria in Western Cambodia

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Pyronaridine-artesunate efficacy for the treatment of uncomplicated *Plasmodium falciparum* malaria was assessed in an area of artemisinin resistance in western Cambodia. This nonrandomized, single-arm, observational study was conducted between 2014 and 2015. Eligible patients were adults or children with microscopically confirmed *P. falciparum* infection and fever. Patients received pyronaridine-artesunate once daily for 3 days, dosed according to body weight. The primary outcome was an adequate clinical and parasitological response (ACPR) on day 42, estimated by using Kaplan-Meier analysis, PCR adjusted to exclude reinfection. One hundred twenty-three patients were enrolled. Day 42 PCR-crude ACPRs were 87.2% (95% confidence interval [CI], 79.7 to 92.6%) for the overall study, 89.8% (95% CI, 78.8 to 95.3%) for Pursat, and 82.1% (95% CI, 68.4 to 90.2%) for Pailin. Day 42 PCR-adjusted ACPRs were 87.9% (95% CI, 80.6 to 93.2%) for the overall study, 89.8% (95% CI, 78.8 to 95.3%) for Pursat, and 84.0% (95% CI, 70.6 to 91.7%) for Pailin ($P = 0.353$ by a log rank test). Day 28 PCR-crude and -adjusted ACPRs were 93.2% (95% CI, 82.9 to 97.4%) and 88.1% (95% CI, 75.3 to 94.5%) for Pursat and Pailin, respectively. A significantly lower proportion of patients achieved day 3 parasite clearance in Pailin (56.4% [95% CI, 43.9 to 69.6%]) than in Pursat (86.7% [95% CI, 76.8 to 93.8%]; $P = 0.0019$). Fever clearance was also extended at Pailin versus Pursat ($P < 0.0001$). Most patients (95.9% [116/121]) harbored *P. falciparum kelch13* C580Y mutant parasites. Pyronaridine-artesunate was well tolerated; mild increases in hepatic transaminase levels were consistent with data from previous reports. Pyronaridine-artesunate efficacy was below the World Health Organization-recommended threshold at day 42 for medicines with a long half-life (90%) for first-line treatment of *P. falciparum* malaria in western Cambodia despite high efficacy elsewhere in Asia and Africa. (This study has been registered at ClinicalTrials.gov under registration number NCT02389439.)

The Cambodia-Thailand border is a region with multidrug-resistant *Plasmodium falciparum*. Following the rapid spread of *P. falciparum* resistance to mefloquine monotherapy in the 1990s, artemisinin-based combination therapy (ACT) was introduced to Cambodia in 2000. Initially, the combination of mefloquine plus artesunate had good efficacy, but data suggesting impaired cure rates in the Pailin region in western Cambodia rapidly emerged (1). Molecular investigations showed that parasites with an amplified *P. falciparum* multidrug resistance protein 1 gene (*pfmdr1*) were strongly associated with recrudescence following mefloquine-artesunate therapy (2). Subsequently, the *pfmdr1* copy number was validated as an important surveillance tool for mefloquine-artesunate resistance (3). Alternatives to mefloquine-artesunate were clearly required, and dihydroartemisinin-piperaquine was adopted as the first-line antimalarial agent in Cambodia in 2008 in Pailin and in 2010 in other provinces. However, within 3 years of its introduction, increased treatment failures and parasite clearance times with dihydroartemisinin-piperaquine had undermined its clinical effectiveness (4–6).

Artemisinin resistance in 2008 in two patients from Battambang province treated with artesunate monotherapy was defined clinically as prolonged parasite clearance with treatment failure within 28 days of follow-up despite adequate dihydroartemisinin plasma concentrations (7). Subsequently, extended parasite clearance times were described in Pailin province in western Cambodia: the median parasite clearance time was 84 h, versus 48 h in Wang Pha in northwestern Thailand (8). In 2012, extended parasite clearance times were also reported in Pursat province in western Cambodia (9). Artemisinin resistance was not associated with *pfmdr1* copy numbers or mutations in the gene encoding sarcoendoplasmic reticulum calcium ATPase 6 (*pfserca*) (7, 8). Recently, polymorphisms in the *P. falciparum* 3D7_1343700 kelch propeller domain (*K13*), which is normally highly conserved across *Plasmodium* species, have been identified as markers for artemisinin resistance (10–13). Several mutations in *K13* in artemisinin-resistant parasites from western Cambodia have been described (C580Y, R539T, Y493H, and I543T) and are associated with the characteristic delayed parasite clearance *in vivo* and reduced *in vitro* sensitivity (5, 10, 11, 14, 15).

Pyronaridine-artesunate is a novel ACT that received a positive opinion from the European Medicines Agency in 2012 under Ar-

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ticle 58 for the treatment of uncomplicated *P. falciparum* and *Plasmodium vivax* malaria (28). Initial *in vitro* studies performed in the 1990s showed high activity of pyronaridine against multidrug-resistant *P. falciparum* (16). Also, as pyronaridine has not been used as monotherapy in Cambodia, it was hoped that resistance would be uncommon. Across all the Asian and African countries included in phase 2/3 trials, pyronaridine-artesunate had high efficacy against *P. falciparum* malaria: the day 28 PCR-adjusted adequate clinical and parasitological response (ACPR) was 98.5% (per-protocol population), and the efficacy was similar to that of first-line ACTs (17–21). However, data from Pailin obtained in a phase 3 study of *P. falciparum* malaria conducted in 2007 to 2008 reported a day 42 recrudescence rate of 10.2% with pyronaridine-artesunate ($n = 140$), versus 0% for mefloquine-artesunate ($n = 71$; $P = 0.04$) (20). Therefore, reduced susceptibility of *P. falciparum* to the pyronaridine component was present in western Cambodia, although confirmatory studies were necessary. Notably, the parasite clearance rate was significantly increased with both pyronaridine-artesunate and mefloquine-artesunate in Cambodia versus other countries, which is suggestive of artemisinin resistance (20).

Containment of artemisinin resistance in western Cambodia and the Cambodia-Thailand border area is critical for malaria control and elimination efforts both locally and globally. Given the urgent need for effective antimalarial therapies in Cambodia, current efficacy data on pyronaridine-artesunate as an alternative first-line treatment for uncomplicated *P. falciparum* malaria in this region were required. The primary objective of this study was to assess the therapeutic efficacy of pyronaridine-artesunate against uncomplicated *P. falciparum* malaria in an area of artemisinin resistance in western Cambodia.

MATERIALS AND METHODS

Study design and patients. This nonrandomized, single-arm, observational study was conducted between July 2014 and January 2015 at three sites in western Cambodia: a referral hospital in Pailin City (Pailin province) and health centers at Promoy (Pursat province) and Tasanh (Battambang province) (ClinicalTials.gov registration number NCT02389439). For an assumed efficacy of 90%, a sample size of 138 participants was needed for ±5% precision, i.e., 85 to 95%. Allowing for a 5% dropout rate, the planned sample size was 145 patients.

The trial complied with the current version of the Declaration of Helsinki (Seoul 2008) and was performed according to principles of the ICH guidelines for good clinical practice (29). Ethical approval was obtained from the National Ethics Committee for Health Research (NEHCR) of the Ministry of Health of Cambodia; the World Health Organization (WHO) Regional Office, Western Pacific Region; and the Oxford Tropical Research Ethics Committee. All patients or their guardians provided written informed consent prior to participation.

Inclusion criteria. Eligible patients were adults or children with a body weight of ≥20 kg who had microscopically confirmed asexual *P. falciparum* infection (mixed infections were permitted at the Palin site only), with a history of fever within the previous 24 h, and who were able to take oral medication.

Exclusion criteria. Subjects were excluded if they had signs or symptoms of severe malaria; parasitemia of >150,000 parasites per μl; alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels >2.5 times the upper limit of normal (ULN); known hypersensitivity to artemisinins or pyronaridine; a history of splenectomy; known active hepatitis A infection (anti-hepatitis A virus [HAV] IgM), hepatitis B virus surface antigen carriage, or hepatitis C virus antibody; or any history or evidence of a clinically significant disorder. Additionally, all women >18 years of age received a pregnancy test, and pregnant or lactating women were excluded; girls 12 to 18 years of age were also excluded.

Treatment. Pyronaridine-artesunate (Pyramax; Shin Poong Pharmaceuticals) was supplied as tablets (180 mg pyronaridine plus 60 mg artesunate) and dosed according to body weight: 1 tablet for subjects weighing 20 to <24 kg, 2 tablets for subjects weighing 24 to <45 kg, 3 tablets for subjects weighing 45 to <65 kg, and 4 tablets for subjects weighing ≥65 kg. Each dose was given orally with water once daily for 3 days (days 0, 1, and 2). Administration of all doses was supervised. Patients were treated as inpatients on days 0 to 3, with follow-up visits as outpatients on days 7, 14, 21, 28, 35, and 42.

Vomiting within 30 min of the first treatment dose on day 0 led to repeat dosing. Any patient who vomited within 30 min of both pyronaridine-artesunate doses was withdrawn from the study and received parenteral therapy according to national guidelines. Any patient who was unable to tolerate pyronaridine-artesunate or who developed severe malaria was treated with parenteral artemether or quinine and tetracycline for 7 days. Recurrent infections were treated with rescue medication according to local clinical guidelines (dihydroartemisinin-piperaquine or quinine and doxycycline).

Assessments. Physical examination was performed, and vital signs were noted at screening and at all patient visits. Biochemical (ALT, AST, total bilirubin, conjugated bilirubin, and alkaline phosphatase) and hematological analyses were conducted at screening; on days 0, 3, 7, 14, and 28; and when clinically indicated. Elevations in levels of liver transaminases have been noted with pyronaridine-artesunate (17). Any case with ALT and/or AST levels >3 times the ULN plus peak total bilirubin levels >2 times the ULN (i.e., potential Hy's law case) or ALT levels 5 times the ULN was recorded as an adverse event of special interest requiring twice-weekly monitoring of liver function test results until resolution.

Blood samples for determination of parasite species and parasitemia were taken at screening, on day 0, daily until day 3, and then at days 7, 14, 21, 28, 35, and 42 and at any unscheduled patient visit. The day 3 sample was taken at 72 h posttreatment. Duplicate Giemsa-stained thick blood smears and one thin smear were examined by microscopists, and parasites were enumerated according to WHO guidelines (22). At each parasite assessment, triplicate blood spots were collected on filter paper (Whatman 3MM) for *P. falciparum* PCR genotyping at the Institut Pasteur in Cambodia, according to established methods (23). Recrudescence was defined as at least one matching allelic band for *P. falciparum* marker genes between baseline samples and samples from post-day 7 recurrences. To further investigate artemisinin resistance markers, the *K13* gene was sequenced by using methods described previously (10).

Study outcomes and statistical analysis. Therapeutic efficacy was evaluated according to WHO methods using ACPRs, i.e., the absence of parasitemia without previous treatment failure (22). The primary efficacy outcome was calculated as the proportion of patients with *P. falciparum* malaria who achieved a PCR-adjusted ACPR at day 42, determined by using Kaplan-Meier analysis, with parasitological recurrence being classified as failure on the day that it occurred; patients lost to follow-up and those who withdrew from the study were censored on the last day of follow-up, and parasitemia with a nonfalciparum species was censored on the day of occurrence. Kaplan-Meier estimates were compared by using the log rank test (a P value of <0.05 was considered significant), and 95% confidence intervals (CIs) were calculated (Greenwood method, log-log).

Secondary efficacy endpoints were Kaplan-Meier estimates of the day 28 PCR-adjusted ACPR and day 28 and day 42 crude ACPRs in *P. falciparum* malaria, the number of patients with parasitemia 72 h following treatment initiation (day 3), and fever clearance time (time for tympanic temperature to reach <37.5°C, maintained for ≥24 h). The proportion of patients with mutations in the *K13* gene was also determined.

Safety outcomes were biochemical and hematological values outside the normal range, adverse events and serious adverse events. All cases of ALT and/or AST levels >3 times the ULN plus peak total bilirubin levels

TABLE 1 Patient baseline characteristics

Characteristic	Value for group		
	Pursat ($n = 60$)	Pailin ($n = 55$)	Battambang ($n = 8$)
No. (%) of female subjects	7 (11.6)	7 (12.7)	1 (12.5)
Mean age (yr) (SD, range)	27.5 (11.4, 9.0–59.0)	33.8 (13.1, 12.0–76.0)	23.3 (13.9, 8.0–45.0)
No. (%) of subjects aged 5–15 yr	8 (13.3)	2 (3.6)	2 (25.0)
No. (%) of subjects aged >15 yr	52 (86.7)	53 (96.4)	6 (75.0)
Mean wt (kg) (SD, range)	51.7 (12.6, 21.0–72.0)	55.5 (8.5, 30.0–76.0)	48.0 (17.0, 21.0–75.0)
Mean temp (°C) (SD, range)	38.7 (0.8, 37.7–40.5)	38.5 (0.8, 37.5–40.0)	38.2 (0.8, 37.5–40.1)
Geometric mean no. of parasites/μl (interquartile range)	9,723 (4,233–22,042)	10,641 (4,302–26,301)	4,009 (1,083–23,292)

>2 times the ULN (i.e., potential Hy's law cases) or ALT levels 5 times the ULN were deemed serious adverse events.

All statistical analyses were done by using R version 3.2.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Patient baseline characteristics. A total of 123 patients were enrolled (60 at Pursat, 55 at Pailin, and 8 at Battambang), and most were adult males. Six patients were lost to follow-up before day 42 (1 at Pursat and 5 at Pailin). Patient baseline characteristics were similar between the study sites, except for the geometric mean parasite count, which was lower for Battambang than for Pursat and Pailin (Table 1). No mixed infections were noted at Pailin. Only five patients had gametocytes detected at baseline, all at Pursat. As there were only eight patients recruited to Battambang, efficacy analysis was restricted to Pursat and Pailin.

Therapeutic efficacy. Efficacy outcomes for all study sites are summarized in Table 2. There were 15 treatment failures reported during the study (9 at Pailin, 6 at Pursat, and 0 at Battambang). PCR genotyping confirmed 14 failures caused by *P. falciparum* recrudescence (8 at Pailin and 6 at Pursat), and there was one reinfection with *P. vivax* (day 35 at Pailin). Most cases of recrudescence were detected on day 28 (4 at Pursat and 5 at Pailin). Recrudescence occurring at the Pailin site was clustered in patients from Phnom Dambang (33.3% [7/21]) versus other villages (2.9% [1/34]). No clustering by location was obvious at the Pursat site. For patients with day 0 parasite counts of >100,000 parasites, 12.5% (1/8) experienced recrudescence, versus 11.3% (13/115) of those with day 0 parasite counts of <100,000 parasites. All cases of recrudescence occurred in adults (age range, 15 to 61 years).

Kaplan-Meier estimates for PCR-adjusted ACPRs at day 42 were 87.9% (95% CI, 80.6 to 93.2%) for the overall study, 89.8% (95% CI, 78.8 to 95.3%) for Pursat, and 84.0% (95% CI, 70.6 to 91.7%) for Pailin ($P = 0.353$ by a log rank test) (Fig. 1). The proportion of patients achieving parasite clearance by day 3 was significantly lower in Pailin than in Pursat (Fig. 2A): Kaplan-Meier estimates for parasite clearance at day 3 were 86.7% (95% CI, 76.8 to 93.8%) for Pursat and 56.4% (95% CI, 43.9 to 69.6%) for Pailin ($P = 0.002$). Fever clearance time was also extended at Pailin versus Pursat ($P < 0.0001$) (Fig. 2B).

For patients who had *P. falciparum* recrudescence, 5/8 patients at Pailin and 1/6 at Pursat had not achieved parasite clearance at day 3. At Pailin, the Kaplan-Meier estimate of the risk of recrudescence was 23.6% (95% CI, 10.6 to 47.8%) in patients who were

TABLE 2 Kaplan-Meier estimates for efficacy outcomes following pyronaridine-artesunate treatment of *P. falciparum* malaria at three study sites in western Cambodia

Outcome	Value for group[b]		
	Pursat ($n = 60$)	Pailin ($n = 55$)	Battambang ($n = 8$)
Day 42 PCR-adjusted ACPR (%) (95% CI)	89.8 (78.8, 95.3)	84.0 (70.6, 91.7)	100 (100, 100)
Day 42 crude ACPR (%) (95% CI)	89.8 (78.8, 95.3)	82.1 (68.4, 90.2)	100 (100, 100)
Day 28 PCR-adjusted ACPR (%) (95% CI)[a]	93.2 (82.9, 97.4)	88.1 (75.3, 94.5)	100 (100, 100)
% of patients with parasite clearance (95% CI) on day:			
1	10.0 (4.6, 20.9)	5.5 (1.8, 16.0)	12.5 (1.9, 61.3)
2	40.0 (28.9, 53.5)	29.1 (18.9, 43.0)	37.5 (13.9, 77.1)
3	86.7 (76.8, 93.8)	56.4 (43.9, 69.6)	75.0 (44.2, 96.3)
Median parasite clearance time (days) (95% CI)	3.0 (2.0, 3.0)	3.0 (3.0, NA)	3.0 (1.0, NA)
% of patients with fever clearance (95% CI) on day:			
1	85.0 (74.9, 92.6)	36.4 (25.2, 50.5)	50.0 (22.5, 84.8)
2	100 (100, 100)	74.5 (62.6, 85.1)	100 (100, 100)
3	100 (100, 100)	98.2 (91.5, 99.9)	100 (100, 100)
Median fever clearance time (days) (95% CI)	1.0 (1.0, 1.0)	2.0 (1.0, 2.0)	1.5 (1.0, NA)

[a] Results for the day 28 crude ACPR were the same as those for the day 28 PCR-adjusted ACPR.
[b] NA, not applicable.

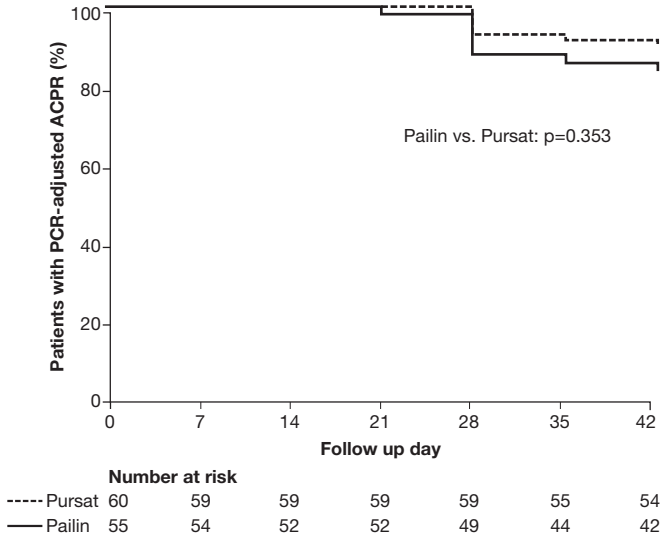

FIG 1 Kaplan-Meier probability of PCR-adjusted adequate clinical and parasitological responses (ACPRs) following pyronaridine-artesunate treatment of *P. falciparum* malaria in western Cambodia.

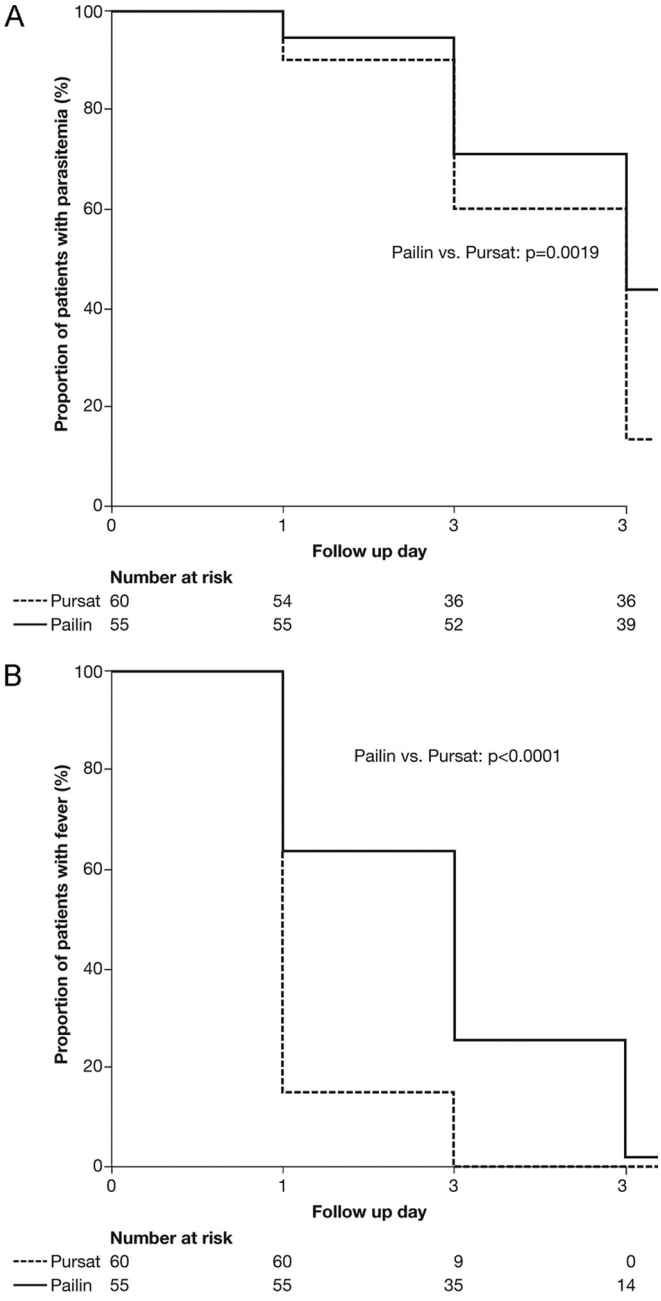

FIG 2 Kaplan-Meier probability of patients having parasitemia (A) and fever (B) following pyronaridine-artesunate treatment of *P. falciparum* malaria in western Cambodia.

parasitemic at day 3, versus 10.5% (95% CI, 3.5 to 29.1%) in those with day 3 parasite clearance ($P = 0.184$). At Pursat, the risk of recrudescence in patients who were parasitemic at day 3 was 12.5% (95% CI, 1.9 to 61.3%), versus 9.8% (95% CI, 4.2 to 22.0%) in those with day 3 parasite clearance ($P = 0.84$).

Nearly all patients (95.9% [116/121]) had *P. falciparum* parasites with the C580Y mutation; two patients from Battambang had missing data. All cases of recrudescence harbored *P. falciparum* C580Y mutant parasites. At Pailin, there were three cases who had wild-type parasites and one who had parasites with the R539T mutation, and at Pursat, there was one case who had wild-type parasites; all five cases had parasite clearance by day 2.

Safety. Across the three study sites, adverse events of any cause occurred in 91.1% (112/123) of patients. Most adverse events occurred on day 0 and were consistent with the symptoms of malaria (Table 3). There were no adverse events after day 3 and no serious or severe adverse events. There were no deaths during the study.

Mean ALT levels increased from 27.5 IU/liter (standard deviation [SD], 20.0 IU/liter) at baseline to 50.5 IU/liter (SD, 64.3 IU/liter) on day 7, returning to normal by day 14 (see Table S1 in the supplemental material). Six patients had eight instances of postbaseline AST levels >3 times the ULN (range, 114 to 298 IU/liter). Three patients had ALT levels >3 times the ULN (204, 254, and 598 IU/liter, all on day 7). One patient had postbaseline total bilirubin levels >2 times the ULN (2.9 IU/liter on day 3), with AST/ALT levels within the normal range. There were no potential Hy's law cases, no clinical sequelae associated with increased levels in liver function tests, and no events classified as a serious adverse event or an adverse event of special interest. Hematological findings were consistent with recovery from malaria (see Table S1 in the supplemental material).

DISCUSSION

PCR-adjusted recrudescence rates for pyronaridine-artesunate in this study at day 42 were 16.0% (95% CI, 8.3 to 29.4%) for Pailin and 10.2% (95% CI, 4.7 to 21.2%) for Pursat. Most of the patients included in this study were adults with low-level parasitemia (<100,000 parasites/μl); thus, failures were not associated with these known risk factors, i.e., young age and high parasite burden. The high day 42 recrudescence rate reported for Pailin is consistent with that noted in 2007 to 2008 for pyronaridine-artesunate at this site (10.2%) (20). In contrast, across other regions of Asia and Africa, Kaplan-Meier estimates of day 42 PCR-adjusted recrudescence rates in three phase 3 trials of pyronaridine-artesunate for treatment of falciparum malaria were 1.2%, 4.5%, and 5.0% (18, 21). Pyronaridine-artesunate was well tolerated, with a safety profile consistent with those reported in previous studies (17, 18, 20, 21, 24).

TABLE 3 Adverse events of any cause and any severity occurring with pyronaridine-artesunate treatment of *P. falciparum* malaria across three study sites in western Cambodia[a]

Adverse event	No. (%) of patients with adverse event ($n = 123$) on day:	
	0+	1+
Headache	100 (81.3)	2 (1.6)
Vertigo	85 (69.1)	0
Insomnia	64 (52.0)	0
Abdominal pain	55 (44.7)	6 (4.9)
Tinnitus	55 (44.7)	2 (1.6)
Nausea	33 (26.8)	2 (1.6)
Palpitation	22 (17.9)	0
Diarrhea	16 (13.0)	2 (1.6)
Vomiting	10 (8.1)	1 (0.8)
Itching	10 (8.1)	5 (4.1)
Deafness	7 (5.7)	1 (0.8)
Tachycardia	6 (4.9)	0
Rash	3 (2.4)	1 (0.8)
Dark urine	3 (2.4)	0
Abdominal bleeding	2 (1.6)	1 (0.8)
Confusion	1 (0.8)	0
Other	1 (0.8)	0

[a] 0+ includes all adverse events recorded at day 0, and 1+ includes those recorded at day 1.

Pyronaridine-artesunate efficacy was numerically higher in Pursat than in Pailin, although this difference did not reach statistical significance. Most of the failures at Pailin were clustered in patients from Phnom Dambang. It is possible either that parasites from Phnom Dambang are resistant to pyronaridine-artesunate or that recrudescence could have been overestimated by using PCR methods because of a clonal parasite population in this area; i.e., reinfection with the clone would not be easily distinguishable from recrudescence. However, given the supporting evidence of extended parasite and fever clearance times for Pailin versus Pursat, it would be expected that the recrudescence rate would also be higher at Pailin. Also, rescue treatment with dihydroartemisinin-piperaquine resulted in 3/8 patients at Pailin and 2/4 patients at Pursat failing therapy within 42 days. These consecutive failures suggest possible cross-resistance between piperaquine and pyronaridine, as was reported in 2003 for African *P. falciparum* strains *in vitro* (25). If piperaquine resistance also confers resistance to pyronaridine, this might explain why pyronaridine-artesunate has insufficient efficacy at specific locations in Cambodia, despite its very limited use in this region.

At Pailin, the risk of recrudescence was approximately doubled in patients with detectable parasites at day 3 versus those who had parasite clearance. However, at Pursat, the risk of recrudescence was similar irrespective of the presence of parasites at day 3. This finding is inconsistent with data from a previous large study that indicated that the parasite clearance rate at 72 h (day 3) predicts subsequent treatment failure (26). However, numbers in this study were small.

The C580Y mutation in the *K13* gene, which is associated with extended parasite clearance times following ACT therapy, was virtually ubiquitous in this study. In previous studies of pyronaridine-artesunate treatment of *P. falciparum* malaria, >96% of non-Cambodian patients had parasite clearance by day 3, versus 62.9% in Pailin (17, 18, 20, 21). Similarly, the median parasite clearance times were 23.9 h in African countries, 23.8 h in India, 24.0 to 32.0 h in Vietnam, and 31.3 to 31.8 h in Thailand, versus 64.1 h in Pailin (17, 18, 20, 21). In this study, only 86.7% of patients at Pursat and 56.4% at Pailin were aparasitic at 72 h, and the median parasite clearance time was 72 h at both sites, which is suggestive of artemisinin resistance. Although the parasite clearance time was extended at both Pailin and Pursat, the difference in the day 3 parasite clearance rates between the two sites suggests that factors other than the presence of mutations in the *K13* gene may be involved.

There are several limitations to this study. Pyronaridine-artesunate plasma concentrations were not measured, so the possibility that treatment failure resulted from subtherapeutic dosing cannot be excluded. *In vitro* susceptibility of parasites to pyronaridine, artesunate, or other antimalarial drugs was not investigated. Also, despite the study being conducted in the rainy season, the required number of patients could not be reached. The overall trend in the number of malaria cases is decreasing in Cambodia, and it is becoming more difficult to include adequate sample sizes in clinical studies. The number of patients recruited was sufficient to draw conclusions because pyronaridine-artesunate efficacy was lower than expected, compared to previously reported data from the study region.

Despite high efficacy in other countries in Asia and Africa, pyronaridine-artesunate did not meet World Health Organization efficacy criteria for first-line treatment of *P. falciparum* malaria in western Cambodia, i.e., a >90% PCR-adjusted ACPR at day 42 (27). Further work is warranted on *in vitro* susceptibility and molecular markers for pyronaridine resistance in *P. falciparum* and potential cross-resistance with piperaquine. This study confirms the ongoing challenge in maintaining effective antimalarial therapy in western Cambodia against parasites that are resistant to artemisinin and available partner drugs. Pyronaridine-artesunate has important clinical utility in other countries in Asia and Africa and could possibly be used in other parts of Cambodia or in combination with other ACTs to prevent the emergence of multidrug resistance. However, this study highlights the potential consequences for this drug and other first-line ACTs should multidrug-resistant, artemisinin-resistant *P. falciparum* fail to be contained and ultimately eliminated in the Cambodia-Thailand border area.

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