Pyronaridine-artesunate Shows Promise as an Effective and Well-tolerated Treatment for Artemisinin-resistant *Plasmodium falciparum* Malaria

John Okombo¹ and David A. Fidock^{1,2}

¹Department of Microbiology & Immunology and ²Division of Infectious Diseases, Department of Medicine, Columbia University Irving Medical Center, New York, New York

(See the Major Article by Quang Bui et al on pages 2187–95.)

Keywords. *Plasmodium falciparum* malaria; artemisinin-based combination therapy; drug resistance; clinical trial; K13.

Artemisinin-based combination therapies (ACTs) are the recommended first-line antimalarials for uncomplicated malaria and comprise a short-acting artemisinin-based component for rapid reduction of *Plasmodium* parasite burden partnered with a longer-acting drug to eliminate surviving parasites. The integration of ACT protocols into the national treatment policies of malaria-endemic countries has contributed significantly to the decrease in malaria cases and related deaths observed between 2000 and 2015 [1]. The Greater Mekong sub-region, however, comprising Cambodia, Myanmar, Thailand, Laos, and Vietnam, has seen the emergence of artemisinin resistance in *Plasmodium falciparum*, the cause of most malaria-associated disease and deaths. Emerging resistance is characterized clinically by delayed parasite clearance times and slower parasite clearance half-lives (the number of hours required to halve the numbers of circulating intraerythrocytic asexual blood stage parasites) [2]. Left unprotected,

the ACT partner drugs mefloquine and later piperazine (PPQ) have ultimately succumbed to resistance. Vietnam and Cambodia, which had officially adopted the use of dihydroartemisinin (DHA) plus PPQ, have both reported alarming increases in ACT treatment failure rates, exceeding 35% and 45% respectively [3, 4]. Against this backdrop, the identification of alternative, clinically efficacious ACT combinations with good safety profiles has become urgent. In this issue of *Clinical Infectious Diseases*, Bui Quang et al [5] report the results of a clinical trial of pyronaridine (PND) plus artesunate (AS) (PND-AS; Pyramax; Shin Poong Pharmaceutical), tested against uncomplicated malaria in a region of known artemisinin resistance in Vietnam. PND is a Mannich base antimalarial with potent in vitro activity against *P. falciparum* strains and clinical isolates, including ones resistant to other antimalarials [6]. This drug is also curative for artemisinin-resistant infections in a rodent malaria model [7]. PND has a long terminal elimination half-life of about 11–16 days, compared with AS, whose half-life is about 1 hour [6, 8]. In multiple phase III and phase IIb/IV randomized clinical trials, the oral combination of PND-AS proved efficacious against falciparum and vivax malaria and was well tolerated even after redosing in pediatric and adult populations in Asia and Africa [9].

The prospective, open-label, single-arm observational clinical trial reported by Bui Quang et al [5] was conducted in 5 provinces in central and southern Vietnam, with varying proportions of artemisinin-resistant parasites, between May 2017 and December 2018. Patients with microscopically confirmed *P. falciparum* malaria were administered oral PND-AS once daily for 3 consecutive days, with weekly follow-up visits until day 42. Eligible participants were adults and children ≥ 20 kg in body weight, with microscopically confirmed *P. falciparum* malaria (parasite count, $\geq 5000/\mu\text{L}$ to $<150\,000/\mu\text{L}$) and fever or history of fever within the past 24 hours. Dosing was adjusted to body weight, with a 3:1 fixed ratio of PND-AS.

The primary outcome was polymerase chain reaction (PCR)-adjusted adequate clinical and parasitological response (ACPR) of PND-AS at day 42. This follow-up period was used to identify later recrudescences, and PCR methods were applied to distinguish these from newly acquired infections. In their per-protocol analysis comprising 155 patients, the day 42 PCR-adjusted ACPR was 96.1%, surpassing the efficacy threshold of $>95\%$ ACPR required by the World Health Organization for a new therapy. The relatively modest patient recruitment numbers across the individual provinces (ranging from 9 to 69 patients

Received 20 June 2019; editorial decision 21 June 2019; accepted 26 June 2019; published online June 28, 2019.

Correspondence: D. A. Fidock, Room 1502 Hammer Health Sciences Center, 701 W 168th St, New York, NY 10032 (df2260@cumc.columbia.edu).

Clinical Infectious Diseases® 2020;70(10):2196–8

© The Author(s) 2019. Published by Oxford University Press for the Infectious Diseases Society of America. All rights reserved. For permissions, e-mail: journals.permissions@oup.com. DOI: 10.1093/cid/ciz583

each), however, necessitate cautious interpretation of the degree of therapeutic efficacy. All patients who reported fever at day 0 had fever clearance within 72 hours after treatment, with a mean clearance time of 25 hours. The mean time to parasite clearance was 58 hours.

Of note, there were 6 patients with a PCR-corrected recrudescence infection (detected on day 21, 28 or 35), designated as a late treatment failure. PND concentration in blood were not assessed at the time of recrudescence, and treatment failure might have resulted from insufficient drug levels or parasite resistance to this drug. In a separate study in eastern Cambodia, PND-AS was highly effective clinically (98.3% day 42 PCR-adjusted ACPR), including against parasites with an ex vivo half-maximal growth inhibitory concentration 5-fold higher than the median value of 6 nmol/L [10]. These studies highlight the importance of screening for the possible emergence of PND resistance, which would necessitate experimental measures (such as genetic crosses or population genomics and confirmation using gene editing) to define their genetic basis, should resistance arise.

Earlier apprehension regarding the adoption of PND-AS hinged on concerns about incidences of transient increases in liver enzymes, which peaked between days 3 and 7 [9, 11]. Potential Hy's law events (aspartate aminotransferase [AST] or alanine aminotransferase [ALT] levels >3 times the upper limit of normal [ULN] plus peak total bilirubin level more than twice the ULN) had been noted retrospectively in <1% of patients treated with PND-AS in 2 phase III studies in Africa and Asia [9]. Consistent with reversible hepatotoxicity events, restoration of enzyme levels back to baseline typically occurred within a month, and no clinical signs or symptoms of liver injury were observed in any of these patients. In a phase IIb/IV study carried out in West Africa, potential Hy's law cases were reported in 0.2%, 0.3%, 0.1% and 0.2% of patients treated

with PND-AS, artemether-lumefantrine, AS-amodiaquine, and DHA-PPQ respectively [12]. All these cases resolved spontaneously without treatment or sequelae.

Cognizant of the need to focus on hepatic safety, Bui Quang et al [5] tested for AST, ALT, and bilirubin changes from baseline levels. An increase in mean levels of AST and ALT in 5.2% and 7.8% of patients, respectively, was observed at day 7 and resolved by day 28. This included 1 patient with transient 5.1-fold and 3.2-fold increases in the ULN for ALT and bilirubin, respectively. This patient had recrudescence *P. falciparum* at day 28, and the elevated levels of liver enzymes were not thought to be treatment related. Overall, the study reported no clinical adverse events, with observed changes in hematological parameters consistent with recovery from malaria. Nevertheless, the skew toward adult male patients in the overall study recruitment (a reflection of the local epidemiology), warrants further data to profile the risk for hepatotoxicity, particularly in young adult women and children. Of note, a clinical trial to assess the hepatic safety of PND-AS, including in these groups, is underway in several countries in Central and West Africa (NCT03201770).

Emerging artemisinin resistance is associated with mutations within the β -propeller domain of the *P. falciparum* *k13* gene. Genomic mapping has shown that key among these mutations is C580Y, which is strongly associated with longer parasite clearance half-lives [13]. In 2 provinces, K13 C580Y was at near-fixation, with most isolates also harboring *pfpm2* gene amplification, a marker of PPQ resistance. Parasites bearing this K13(C580Y)/multicopy *pfpm2* combination are thought to have swept into Vietnam and neighboring countries from Cambodia, driven by DHA plus PPQ selective pressure [14]. In 4 of the 5 provinces in Vietnam studied herein, the high frequency of artemisinin-resistant K13 mutant parasites aligned with high day 3 positivity rates (ranging from 21% to

56%, contrasting with 0% in the province with almost no K13 mutant parasites). In Binh Phuoc, PND-AS efficacy in 2008 was 99.2%, with 8.7% K13 mutant strains observed in that province the following year [15]. In the current study, the prevalence of K13 mutants there had spiked to 97.7%. It is therefore noteworthy that PND-AS showed an efficacy in Binh Phuoc of 95%.

Recent large-scale molecular surveillance studies have found minimal evidence of K13-propeller sequence polymorphisms associated with artemisinin resistance outside Southeast Asia [16, 17]. Nonetheless, the earlier dissemination of chloroquine resistance from Asia into Africa evokes concern that K13 mutant artemisinin-resistant alleles might also spread, or arise independently. The finding herein that the 6 treatment failures all occurred in areas with a high prevalence of mutant K13 underscores the risk of PND being exposed to substantial selective pressure and highlights the need for vigilance in testing for emerging PND resistance.

Overall, the study by Bui Quang et al [5] shows that PND-AS is efficacious and well tolerated in populations where DHA-PPQ efficacy has fallen rapidly. Other efforts to address multidrug resistance have involved trials of triple ACTs with 2 partner drugs (NCT02612545 and NCT02453308). The findings in the current report, supplemented by data on PND-AS clinical trials from East and West Africa [9], provide reassurance that this combination could be an effective addition to the antimalarial arsenal in Asia and Africa.

Note

Potential conflicts of interest. Both authors report no potential conflicts. Both authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Gething PW, Casey DC, Weiss DJ, et al. Mapping *Plasmodium falciparum* mortality in Africa between 1990 and 2015. *N Engl J Med* 2016; 375:2435–45.

2. Dondorp AM, Nosten F, Yi P, et al. Artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med* **2009**; 361:455–67.
3. Phuc BQ, Rasmussen C, Duong TT, et al. Treatment failure of dihydroartemisinin/piperaquine for *Plasmodium falciparum* malaria, Vietnam. *Emerg Infect Dis* **2017**; 23:715–7.
4. Spring MD, Lin JT, Manning JE, et al. Dihydroartemisinin-piperaquine failure associated with a triple mutant including kelch13 C580Y in Cambodia: an observational cohort study. *Lancet Infect Dis* **2015**; 15:683–91.
5. Bui Quang P, Huynh Hong Q, Tran Thanh D, et al. Pyronaridine-artesunate efficacy and safety in uncomplicated *Plasmodium falciparum* malaria in areas of artemisinin-resistant falciparum in Viet Nam (2017–2018). *Clin Infect Dis* **2019**; pii: ciz580.
6. Croft SL, Duparc S, Arbe-Barnes SJ, et al. Review of pyronaridine anti-malarial properties and product characteristics. *Malar J* **2012**; 11:270.
7. Henrich PP, O'Brien C, Sáenz FE, Cremers S, Kyle DE, Fidock DA. Evidence for pyronaridine as a highly effective partner drug for treatment of artemisinin-resistant malaria in a rodent model. *Antimicrob Agents Chemother* **2014**; 58:183–95.
8. Jittamala P, Pukrittayakamee S, Ashley EA, et al. Pharmacokinetic interactions between primaquine and pyronaridine-artesunate in healthy adult Thai subjects. *Antimicrob Agents Chemother* **2015**; 59:505–13.
9. Pryce J, Hine P. Pyronaridine-artesunate for treating uncomplicated *Plasmodium falciparum* malaria. *Cochrane Database Syst Rev* **2019**; 1:CD006404.
10. Leang R, Mairet-Khedim M, Chea H, et al. Efficacy and safety of pyronaridine-artesunate plus single-dose primaquine for treatment of uncomplicated *Plasmodium falciparum* malaria in Eastern Cambodia. *Antimicrob Agents Chemother* **2019**; 63:e02242-18.
11. Duparc S, Borghini-Fuhrer I, Craft CJ, et al. Safety and efficacy of pyronaridine-artesunate in uncomplicated acute malaria: an integrated analysis of individual patient data from six randomized clinical trials. *Malar J* **2013**; 12:70.
12. West African Network for Clinical Trials of Antimalarial Drugs (WANECAM). Pyronaridine-artesunate or dihydroartemisinin-piperaquine versus current first-line therapies for repeated treatment of uncomplicated malaria: a randomised, multicentre, open-label, longitudinal, controlled, phase 3b/4 trial. *Lancet* **2018**; 391:1378–90.
13. Arieu F, Witkowski B, Amaratunga C, et al. A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. *Nature* **2014**; 505:50–5.
14. Imwong M, Suwannasin K, Kunasol C, et al. The spread of artemisinin-resistant *Plasmodium falciparum* in the Greater Mekong subregion: a molecular epidemiology observational study. *Lancet Infect Dis* **2017**; 17:491–7.
15. Thuy-Nhien N, Tuyen NK, Tong NT, et al. K13 propeller mutations in *Plasmodium falciparum* populations in regions of malaria endemicity in Vietnam from 2009 to 2016. *Antimicrob Agents Chemother* **2017**; 61:e01578-16.
16. MalariaGEN *Plasmodium falciparum* Community Project. Genomic epidemiology of artemisinin resistant malaria. *Elife* **2016**; 5:e08714.
17. Ménard D, Khim N, Beghain J, et al; KARMA Consortium. A worldwide map of *Plasmodium falciparum* K13-propeller polymorphisms. *N Engl J Med* **2016**; 374:2453–64.