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BRIEF DEFINITIVE REPORT

Imatinib augments standard malaria combination therapy without added toxicity

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To egress from its erythrocyte host, the malaria parasite, *Plasmodium falciparum*, must destabilize the erythrocyte membrane by activating an erythrocyte tyrosine kinase. Because imatinib inhibits erythrocyte tyrosine kinases and because imatinib has a good safety profile, we elected to determine whether coadministration of imatinib with standard of care (SOC) might be both well tolerated and therapeutically efficacious in malaria patients. Patients with uncomplicated *P. falciparum* malaria from a region in Vietnam where one third of patients experience delayed parasite clearance (DPC; continued parasitemia after 3 d of therapy) were treated for 3 d with either the region's SOC (40 mg dihydroartemisinin + 320 mg piperaquine/d) or imatinib (400 mg/d) + SOC. Imatinib + SOC–treated participants exhibited no increase in number or severity of adverse events, a significantly accelerated decline in parasite density and pyrexia, and no DPC. Surprisingly, these improvements were most pronounced in patients with the highest parasite density, where serious complications and death are most frequent. Imatinib therefore appears to improve SOC therapy, with no obvious drug-related toxicities.

Introduction

Malaria remains a serious health problem in much of the world today, with ∼228 million new cases and ∼405,000 deaths estimated in 2018 (World Health Organization, 2019). Although artemisinin combination therapies (ACTs) continue to successfully treat most strains of *Plasmodium falciparum* malaria, new drug-resistant mutations leading to delayed parasite clearance (DPC; continued parasitemia after 3 d of standard therapy) have emerged, especially in Southeast Asia (Conrad and Rosenthal, 2019; Ouji et al., 2018; Lu et al., 2017; Pau et al., 2019; Thriemer et al., 2014). The presence of these artemisinin-resistant parasites unfortunately suggests that current treatments may soon prove obsolete. While artemisinin, the cornerstone of ACTs, is fast acting and effective, the duration of its efficacy is short, requiring a companion drug to achieve more prolonged activity (Nsanzabana, 2019; Li and Pybus, 2019). Regrettably, resistance to such companion drugs is also rising (Conrad and Rosenthal, 2019), and while triple-combination therapies are now under investigation for prevention of DPC (Rosenthal, 2020; van der Pluijm et al., 2020; Dini et al., 2018), the third components in most ACTs are antimalarials that have already failed due to a decline in efficacy (Conrad and Rosenthal, 2019). Taken together, these observations suggest that new approaches to treat *P. falciparum* malaria with orthogonal mechanisms of action are critically needed.

We have reported that tyrosine phosphorylation of the erythrocyte membrane protein 3 (band 3, AE1, SLC4A1) induces erythrocyte membrane weakening, leading to membrane vesiculation and fragmentation (Ferru et al., 2011; Puchulu-Campanella et al., 2016; Pantaleo et al., 2011). Because this phosphorylation dramatically increases during *P. falciparum* maturation in infected erythrocytes (Pantaleo et al., 2010; Pantaleo et al., 2012; Bosman et al., 2012), we hypothesized that the resulting membrane weakening might contribute to erythrocyte rupture during merozoite egress from the infected red cell at the end of *P. falciparum*'s life cycle (Pantaleo et al., 2012; Kesely et al., 2016; Pantaleo et al., 2017; Kesely et al., 2020). Indeed, several studies have now demonstrated that blockade of band 3 tyrosine phosphorylation by an inhibitor of spleen tyrosine kinase (SYK) can prevent escape of *P. falciparum* from its erythrocyte host in vitro, thereby terminating the parasitemia (Kesely et al., 2016; Pantaleo et al., 2017, Kesely et al., 2020). The concomitant accretion of denatured hemoglobins within the parasitized erythrocyte is thought to further augment parasite killing by enhancing the redox-mediated activation of artemisinins (Tsamesidis et al., 2020). Because imatinib,

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a Food and Drug Administration–approved tyrosine kinase inhibitor with an excellent safety profile (O'Brien et al., 2003; Hochhaus et al., 2017), has been found to exhibit off-target activity against SYK (Atwell et al., 2004), it seemed reasonable to examine whether a clinically safe dose of imatinib might also exhibit antimalarial activity in infected patients.

We describe here the results of a small clinical trial aimed at examining the safety and efficacy of adding imatinib to the standard of care (SOC) for *P. falciparum* malaria in adult male patients from the Lia Region Huong Hoa district of Vietnam. In this region, patients are administered dihydroartemisinin (DHA) plus piperaquine (PPQ) as the SOC, and they exhibit DPC rates of approximately one in three (Pau et al., 2019; Thriemer et al., 2014). We report that the most commonly used clinical dose of imatinib (400 mg) causes no significant adverse events when administered once daily for 3 d in combination with SOC. We further demonstrate that when administered in combination with SOC, imatinib significantly augments the potency of DHA + PPQ by accelerating elimination of the parasitized cells, thus resulting in a more rapid decline in pyrexia. Because imatinib functions via an orthogonal mechanism from current World Health Organization's recommended treatments, and because its effective dose can be manufactured at minimal cost (due to recent expiration of its patent), we propose that imatinib should be evaluated as a third component of future triple-combination therapies.

Results and discussion

To assess the safety, tolerability, and efficacy of coadministering imatinib mesylate with the SOC therapy for *P. falciparum* malaria (i.e., DHA + PPQ), a small phase 2 clinical trial was designed. For this purpose, a clinical site was required with endemic *P. falciparum* malaria that still responded completely to SOC, yet exhibited DPC (continued parasitemia after 3 d of therapy), a possible precursor of eventual drug resistance. Such a partially resistant population of *P. falciparum* was considered ideal for this initial clinical trial, as it balanced the risk to participants of treatment with an untested combination therapy with the need to demonstrate efficacy against more refractory strains of *P. falciparum* malaria. Therefore, we elected to conduct the clinical trial in the Quang Tri Province of Vietnam, where genetic markers of artemisinin and PPQ resistance (K13 C580Y and PfPM2 multicopies) had been previously identified in only 1.2% of patients, yet DPC had been documented by standard microscopy and PCR in 27.2–39.3% of patients (Pau et al., 2019).

In the Quang Tri province of Vietnam, the SOC consists of administration of DHA (40 mg/d) plus PPQ (320 mg/d). Therefore, a trial was designed in which imatinib (400 mg/d) was coadministered to a cohort of patients concurrently receiving SOC (Im + SOC; n = 20) and compared with a parallel control cohort that received only SOC (n = 21; Fig. 1 and Table 1). Although imatinib had already established a good safety record when administered in perpetuity to chronic myelogenous leukemia cancer patients (O'Brien et al., 2003; Hochhaus et al., 2017), it had not been dosed in malaria patients before this study. Therefore, the primary endpoint for this trial was the safety and tolerability of imatinib in patients with *P. falciparum* malaria, and the secondary endpoint was reduction in parasite density.

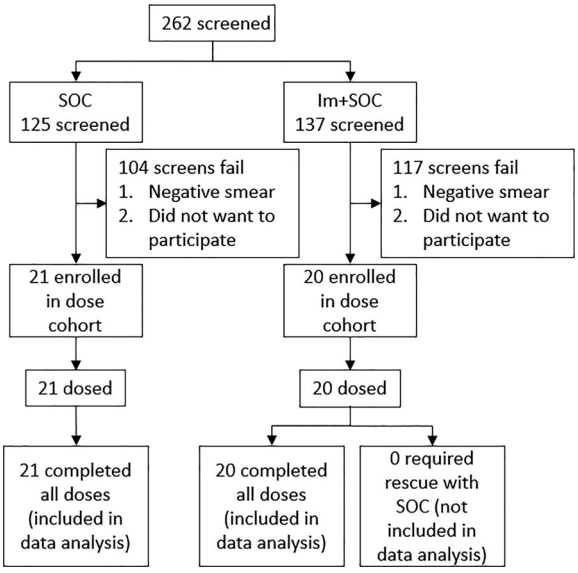

Figure 1. **Overview of triple-combination therapy clinical trial.**

Data from oncology clinical trials reveal that chronic administration of imatinib will cause at least one adverse event in the majority of patients, with the majority of these events being mild to moderate in severity, suggesting that imatinib therapy is generally well tolerated (O'Brien et al., 2003; Hochhaus et al., 2017; Demetri et al., 2002; Druker et al., 2006; Druker et al., 2001a; Druker et al., 2001b; Lyseng-Williamson and Jarvis, 2001). The most common imatinib-induced adverse events are nausea, vomiting, edema, diarrhea, headache, and skin rash (O'Brien et al., 2003; Hochhaus et al., 2017; Demetri et al., 2002; Druker et al., 2006; Druker et al., 2001a; Druker et al., 2001b; Lyseng-Williamson and Jarvis, 2001; Pretel-Irazabal et al., 2014). Therefore, these adverse events, along with fever, body aches/pain, anemia, and jaundice, were monitored in clinical trial participants, and their severity was scored on a scale from 0 to 3 (see Materials and methods; Table S1). As shown in Table 2, the Im+SOC group exhibited the same or similar number/percentage of patients manifesting an adverse event. Moreover, no statistically significant difference was observed in the severity of any adverse event between the two groups (P = 0.30, 0.34, 0.31, 0.96, 0.54, and 0.34 for fever, headache, nausea, diarrhea, anemia, and jaundice, respectively). Additionally, by day 4 and at all subsequent evaluations, participants in both treatment groups reported no adverse events (i.e., severity ranked as 0, normal) in all categories, and all concurrently obtained blood tests (e.g., complete blood count, liver function, and kidney function) were within normal parameters. Most importantly, none of the adverse events in the Im + SOC group were attributed to imatinib (Table 2), suggesting that the triple-combination therapy was as safe as SOC. This benign outcome is perhaps not surprising, since no patient received more than three consecutive doses of Im + SOC, whereas imatinib is

Table 1. **Treatment schedule for trial**

Cohort	0 h	12 h	24 h	48 h
SOC	40 mg DHA + 320 mg PPQ	40 mg DHA + 320 mg PPQ	40 mg DHA + 320 mg PPQ	40 mg DHA + 320 mg PPQ
Im + SOC	40 mg DHA + 320 mg PPQ + 400 mg imatinib	40 mg DHA + 320 mg PPQ	40 mg DHA + 320 mg PPQ + 400 mg imatinib	40 mg DHA + 320 mg PPQ + 400 mg imatinib

commonly administered daily for many years in the treatment of cancer patients.

Although both cohorts entered the trial with similar levels of pyrexia (39.6° ± 0.1°C versus 39.3° ± 0.1°C for SOC versus Im + SOC; P = 0.1425; Fig. 2 A), analysis of participant temperatures before, during, and after the treatments demonstrated that body temperatures (i.e., a good measure of how a patient feels) returned to normal ∼2 d faster (1.55 ± 0.11 versus 3.57 ± 0.26 d; P = 0.00001) in the triple-combination group than in the SOC group (Fig. 2 B). Moreover, while all participants treated with Im + SOC (Fig. 2, C and E) experienced a monotonous decline in body temperature, >30% of participants in the SOC cohort (Fig. 2, C and D) experienced a second increase in temperature during at least 1 d of therapy or follow-up period (P = 0.0191). Taken together, these data suggest that inclusion of imatinib with SOC may facilitate a faster resolution of pyrexia.

To assess whether the accelerated decline in body temperature might correlate with a more rapid reduction in parasite density, we next compared the number of parasites/µl of peripheral blood in the two treatment groups. Although the average initial parasite concentration in peripheral blood did not differ significantly between SOC and Im + SOC cohorts (16,601 ± 2,099 versus 31,380 ± 13,351 parasites/µl, respectively; P = 0.267), parasite density decreased more rapidly in the triple-combination than SOC cohort (Fig. 3, A–D). Thus, 30% of participants receiving Im + SOC displayed no residual parasites at 24 h after ingestion of the initial dose of therapy (i.e., before receipt of the second dose), whereas none of the participants on SOC alone displayed an absence of parasites at the same 24-h time point (Fig. 3 B). Moreover, 90% of participants in the Im + SOC cohort were devoid of blood parasites by 48 h after initiation of therapy, with most of the remaining patients becoming parasite free by day 3. In contrast, only 14% of participants in the SOC group were devoid of parasites at the end of day 2, and one third still retained measurable parasites after the full 3-d course of therapy (Fig. 3 B), confirming the established DPC in this region of Vietnam (Pau et al., 2019). While the presence of a large number of participants with K13 resistance mutations was deemed unlikely due to the low prevalence of K13 mutants in the region (Pau et al., 2019), nonetheless, we sequenced participants' pathogens and did identify a single participant (in the Im + SOC cohort) that harbored parasites with the common K13 resistance mutation (C580Y). Importantly, this participant responded similarly to the other Im + SOC participants. Collectively, the secondary endpoint of faster parasite clearance by the Im + SOC cohort was met.

Upon further scrutiny of the individual participant data in Fig. 3, we noted that responses to the triple-combination therapy were bimodal, with individuals initially diagnosed with high concentrations of parasites (Fig. 3 H) responding more rapidly than those initially diagnosed with low parasite densities (Fig. 3 F). Indeed, all 10 participants presenting with >10,000 parasites/µl blood in the Im + SOC treatment group experienced a rapid decline in parasite density (∼80% in 24 h; Fig. 3 H). In

Table 2. **Number and severity of adverse events**

	SOC (*n* = 21)			Im + SOC (*n* = 20)		
Adverse event	**Patients numbers with severity ≥1**	**Average severity score**	**Imatinib related**	**Patients numbers with severity ≥1**	**Average severity score**	**Imatinib related**
Itching/skin reaction/rash	0 (0%)	0 (0; 0–0)	NA	0 (0%)	0 (0; 0–0)	NA
Edema	0 (0%)	0 (0; 0–0)	NA	0 (0%)	0 (0; 0–0)	NA
Fever	21 (100%)	2.4 (0.74; 1–3)	NA	20 (100%)	2.2 (0.67; 1–3)	0 (0; 0–0)
Headache	4 (19.0%)	0.2 (0.40; 0–1)	NA	3 (15.0%)	0.2 (0.37; 0–1)	0 (0; 0–0)
Body aches/pain	0 (0%)	0 (0; 0–0)	NA	0 (0%)	0 (0; 0–0)	NA
Nausea	0 (0%)	0 (0; 0–0)	NA	1 (5.0%)	0.05 (0.22; 0–1)	0 (0; 0–0)
Vomiting	0 (0%)	0 (0; 0–0)	NA	0 (0%)	0 (0; 0–0)	NA
Diarrhea	2 (9.5%)	0.10 (0.30; 0–1)	NA	2 (10.0%)	0.10 (0.31; 0–1)	0 (0; 0–0)
Anemia	2 (9.5%)	0.10 (0.30; 0–1)	NA	1 (10.0%)	0.05 (0.22; 0–1)	0 (0; 0–0)
Jaundice	1 (4.8%)	0.05 (0.22; 0–1)	NA	0 (0%)	0 (0; 0–0)	NA

Data are presented as *n* (%) or mean (SD; range). Severity score of 0, 1, 2, and 3 represent normal, mild, moderate, and severe, respectively (see Table S3 for additional information). NA, not applicable.

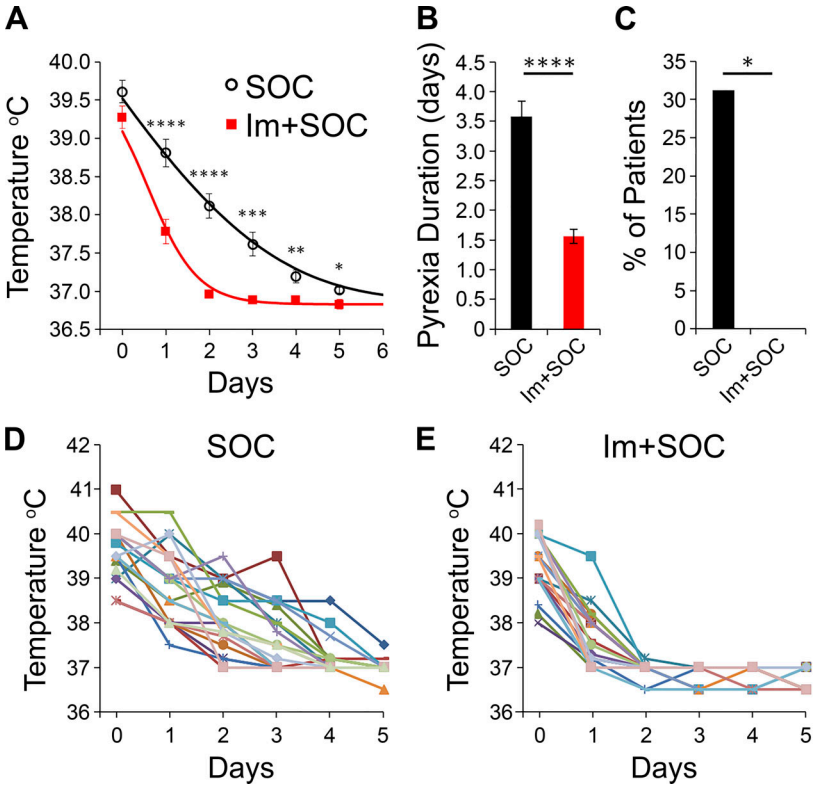

Figure 2. **Changes in body temperature as a function of time on therapy. (A)** Participants were randomly assigned to receive either 40 mg DHA + 320 mg PPQ (SOC; black circles; *n* = 21) or 400 mg Im + SOC (red squares; *n* = 20). Participant temperatures were measured daily, plotted, and analyzed using mixed ANOVA followed by post hoc multi-comparison testing (P = 0.1425, 0.00001, 0.00001, 0.0002, 0.0094, and 0.016 for days 0–5, respectively). **(B)** Average duration of pyrexia in the two cohorts (*n* = 21 and *n* = 20 for SOC and Im + SOC, respectively) were plotted, and the means were compared using *t* test (P = 0.00001). **(C)** The percentage of participants who exhibited a second fever spike (*n* = 21 and *n* = 20 for SOC and Im + SOC, respectively) was plotted and compared using *t* test (P = 0.0191). **(D)** Plots of individual body temperatures versus time in the SOC cohort (*n* = 21). **(E)** Plots of individual body temperatures in the Im + SOC cohort (*n* = 20). Error bars express SEM; *, P < 0.05; **, P < 0.01; ***, P < 0.001, ****, P < 0.0001.

contrast, participants in the SOC treatment cohort showed no such trend, with participants presenting at diagnosis with high and low parasite densities responding similarly (Fig. 3, D, E, and G). Importantly, no participant in either the SOC or Im + SOC treatment group experienced recrudescence after completion of therapy (data collected for 42 d after therapy initiation).

While the number of participants in this pilot clinical trial was relatively small, the data presented support the conclusion that the safety and efficacy of Im + SOC combination therapy is superior to that of the current SOC for treatment of *P. falciparum* malaria. Thus, not only were no serious adverse events detected in participants treated with Im + SOC, but both parasite density and pyrexia subsided more rapidly in the Im + SOC than SOC cohort. Because malaria-associated deaths occur most frequently in patients with high levels of parasite density (World Health Organization, 2015), the unexpectedly rapid rate of parasite clearance in the Im + SOC arm may have the potential to save lives.

Imatinib has already established a good safety record in the treatment of chronic myelogenous leukemia and gastrointestinal stromal tumors, allowing many patients to take imatinib chronically without serious adverse events (O'Brien et al., 2003; Hochhaus et al., 2017; Demetri et al., 2002). While some chronic myelogenous leukemia and gastrointestinal stromal tumor patients indeed report limiting side effects such as abdominal pain, edema, nausea, muscle cramps, rashes, diarrhea, musculoskeletal pain, fatigue, joint pain, or headaches (Druker et al., 2006), participants in the Im + SOC arm reported no more adverse events than those in the SOC arm, and in fact, they commonly reported feeling better sooner than those in the SOC cohort, probably due to the more rapid decline in pyrexia. Because clinical exams also revealed no obvious drug-related toxicities, we suspect that the cumulative toxicities that can arise from chronic dosing of imatinib do not normally emerge after only three doses of the drug. However, due to the small number of participants in this pilot trial, larger studies will still need to be conducted before the safety of imatinib in malaria patients can be more confidently determined. Nonetheless, it is conceivable that imatinib could be used to enhance SOC potency without contributing significantly to toxicity.

The ability of imatinib to improve the potency of DHA + PPQ supports the hypothesis that strengthening the erythrocyte membrane can inhibit propagation of the malaria parasite (Kesely et al., 2016; Pantaleo et al., 2017; Kesely et al., 2020). Mechanistically, by blocking tyrosine phosphorylation of erythrocyte membrane band 3, imatinib prevents the prominent conformational change in band 3 (Ferru et al., 2011) that leads to dissociation of the membrane cytoskeleton from the lipid bilayer (Ferru et al., 2011; Puchulu-Campanella et al., 2016). Blockade of this disjunction of the RBC membrane from its spectrin-actin cytoskeleton then inhibits the membrane weakening that induces both blebbing and fragmentation of the membrane. Because toxic hemichromes and oxidizing hemes are normally discharged within the blebbing membrane vesicles, the imatinib-induced retention of these oxidizing components within the parasitized cell must render the intra-erythrocytic environment increasingly toxic to the parasite (Tsamesidis et al., 2020). Together with the fact that the parasite's egress from the erythrocyte is blocked, the parasite becomes trapped in its RBC host where the available food (i.e., hemoglobin) is largely consumed and toxic waste products (such as heme iron) are accumulating. Whether the resulting incarcerated parasite dies from starvation, toxification, or

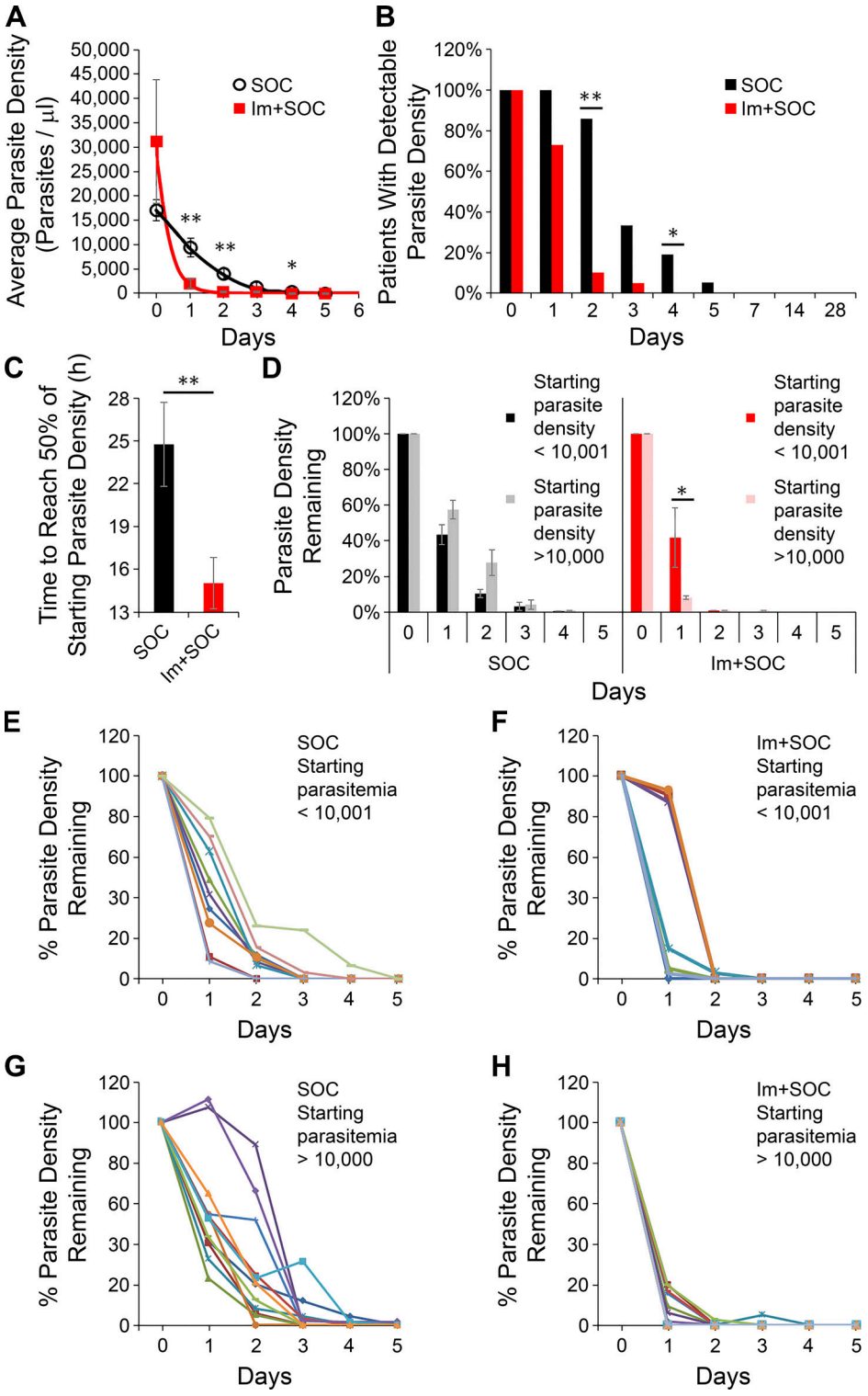

Figure 3. **Comparison between SOC and Im + SOC treatment on the reduction of parasite density. (A)** Participants were randomly assigned to receive either 40 mg DHA + 320 mg PPQ (SOC; black circles; *n* = 21) or 400 mg Im + SOC (red squares; *n* = 20). The level of parasitemia was determined daily, and averages were plotted and analyzed using mixed ANOVA followed by post hoc multicomparison testing (P = 0.2670, 0.0010, 0.0016, 0.1638, 0.0432, and 0.3354 for days 0–5, respectively). **(B)** The percentage of patients with detectable parasites on different days after initiation of therapy (*n* = 21 and *n* = 20 for SOC and Im + SOC, respectively) was plotted and compared using *t* test (P = 0.6762, 0.7163, 0.0012, 0.1035, 0.0432, and 0.3354 for days 0–5, respectively). **(C)** The average time for parasite density to decline to 50% of its starting level (*n* = 21 and *n* = 20 for SOC and Im + SOC, respectively) was plotted and compared using *t* test (P = 0.0091). **(D–H)** For analysis, treatment cohorts were split into two groups: participants who presented with an initial parasite density <10,001 parasites/µl blood and those who presented with >10,000. The decrease in parasite density as a function of time was plotted separately for patients with <10,001 parasites/µl blood in the SOC (*n* = 9; E) and Im + SOC (*n* = 7; F) arms and for patients with >10,000 parasites/µl blood in the SOC (*n* = 12; G) and Im + SOC (*n* = 13; H) arms. Error bars express SEM; *, P < 0.05; **, P < 0.011.

phagocytosis has not been determined, but the absence of recrudescence in any patients treated with triple-combination therapy indicates that all viable parasites have been eliminated. Although this absence of recrudescence and DPC argue that the coadministration of imatinib with its orthogonal mechanism of action may have overcome the drug resistance common to this region of Southeast Asia, larger clinical trials will have to be conducted to confirm this hypothesis.

A major objective of any future malaria therapy will most likely involve reducing the number of days a patient must be treated to achieve a cure. Thus, drug-resistant strains have been hypothesized to emerge in part because many patients feel better after intake of their first or second malaria pill and then decide to save the remaining pills for a subsequent infection. Although a much larger clinical study will have to be performed before a conclusion can be proposed, the data in Figs. 2 and 3 suggest that addition of imatinib to DHA + PPQ could conceivably reduce the required days on therapy from three to two. Indeed, if the residual parasites detected in the 10% of participants at the end of day 2 were to prove to be inviable, it might be possible to optimize the Im + SOC therapy to eliminate all parasites after only two doses. Such a treatment would not only reduce the probability of patients saving pills for future infections, it would also decrease the cost of the therapy, rendering it more affordable in indigent countries.

As noted by others (Dini et al., 2018), a triple combination will generally outperform a dual-combination therapy, because multiple mechanisms of action are more difficult to evade than a single or dual mechanism of action. While this principle alone would argue that Im + SOC should prove more mutation resistant than SOC, we foresee still another mechanism by which the triple-combination therapy should suppress mutation-induced drug resistance. Thus, the target of imatinib is a tyrosine kinase found in the erythrocyte (SYK; Atwell et al., 2004) that phosphorylates protein band 3 in the human RBC (Brunati et al., 2000). Because the parasite's genome does not encode SYK (Solyakov et al., 2011), it cannot mutate SYK to become imatinib resistant. While it is conceivable that the parasite could still mutate one of its own tyrosine kinases to replace the function of erythrocyte SYK, this mutational strategy is also unlikely to succeed, since the *P. falciparum* genome contains no obvious tyrosine kinase (Solyakov et al., 2011). Thus, evolution of mutations that could evade an Im + SOC combination therapy should be probabilistically unlikely.

Finally, there are still important issues that must be addressed before an imatinib + DHA + PPQ combination therapy can be considered a candidate for potential clinical use. These include an expanded evaluation of its safety and efficacy in larger patient populations that include women and children, an assessment of its potency against other species and strains of malaria, an appraisal of its efficacy in other countries and patient populations, an analysis of its compatibility with other ACTs, and an evaluation of strategies to render it affordable (now off patent) to patients in indigent countries. Assuming that imatinib + DHA + PPQ passes these hurdles, it is hoped that the data obtained here will facilitate development of an improved therapy for treatment of this devastating disease.

Materials and methods

Study agents

Generic imatinib mesylate was purchased from TEVA Pharmaceuticals and provided to participants in two 200-mg tablets per treatment to conform with the typical dosage of imatinib indicated for treatment of chronic myelogenous leukemia. CV Artecan, the SOC for treatment of *P. falciparum* malaria in Vietnam, was purchased from OPC Pharmaceutical and administered to participants as a tablet containing 40 mg DHA plus 320 mg PPQ.

Study participants

P. falciparum–infected males age 16–55 yr with no complicating comorbidities as confirmed by blood testing (Table S2), who also had received no antimalarial drug within the previous 4 wk, were eligible for the study. Those individuals who met eligibility criteria and provided informed written consent were enrolled in the trial (Fig. 1 and Table S3). Women were not enrolled because of imatinib's unknown effects on pregnancy.

Study protocols

The study was an open-label trial aimed at determining the safety, tolerability, and efficacy of Im + SOC in adult male patients with uncomplicated *P. falciparum* malaria. Uncomplicated malaria was defined as a positive, microscopy-confirmed *P. falciparum* infection in symptomatic patients with no complicating comorbidities and a malaria count <150,000 parasites/µl blood. Participants were randomly assigned to receive either SOC (n = 21), in which patients were administered 40 mg DHA plus 320 mg PPQ orally twice (12 h apart) on the first day and then once a day on the following 2 d, or one dose of imatinib/day for three days plus SOC arm (n = 20), where they received SOC exactly as above with the addition of 400 mg imatinib mesylate orally with a meal and a full glass of water once a day for 3 d (Table 1).

Participant temperatures and peripheral blood parasite levels were monitored before, during, and after the trial on days 0, 1, 2, 3, 4, 5, 7, 28, and 42. Participants were also examined for the usual symptoms of *P. falciparum* malaria, including fever, chills, headache, fatigue, anorexia, and mild diarrhea, along with symptoms of potential adverse events related to administration of imatinib. If any participant in the Im + SOC cohort exhibited either an increase in parasite density >150,000 parasites/µl or adverse symptoms exceeding those normally associated with uncomplicated malaria, the participant was transferred immediately to SOC alone.

Both trials were approved by the Vietnam Ministry of Health, the Vietnamese Ministry of Health's Ethical Assessment Committee for Biomedical Research, the Institutional Review Board at the Hue University of Medicine and Pharmacy, and the Institutional Review Board at Purdue University. All participants consented to being included in this study. The trial was conducted in the six communes of the Lia Region Huong Hoa district, Quang Tri province, owing to the high levels of DPC present in this region of Vietnam (Thriemer et al., 2014; Pau et al., 2019). This study was registered with ClinicalTrials.gov (NCT03697668).

Randomization and masking

The trial was open label, so no blinding of the attending physicians was performed. Participants were randomly assigned to a

cohort by alternating assignments based on the date and time of hospital admission, and all participants and microscopists who quantitated parasite density were blinded to the treatment regimens.

Outcomes

The primary endpoint for the study was safety and tolerability. Secondary endpoints were reduction in parasite density and decline in pyrexia. Safety and tolerability were assessed at protocol-specified time points, and adverse events were classified as toxicities not normally associated with uncomplicated *P. falciparum* malaria, including edema, rash, and severe diarrhea. The severity of any adverse event was classified as normal, event not present or values within normal levels (score = 0); mild, events requiring minimal or no treatment that did not interfere with the participant's daily activities (score = 1); moderate, events resulting in a low level of inconvenience or concern that may have caused some interference with the participant's daily functioning (score = 2); and severe, events interrupting a participant's daily activity and that could also be incapacitating or require medical intervention (score = 3). More specific details of this scoring system can be found in Table S3. Attribution of imatinib to adverse events was assessed using a 5-point scale: not (0), unlikely (1), possibly (2), probably (3), and definitely (4) related. Primary endpoints were met if the imatinib treatment group exhibited an absence of severe adverse events and an insignificant increase or actual decrease in moderate to mild adverse events.

Analyses and statistics

All authors had access to the primary clinical trial data. Data from all participants who received the full three doses of treatment were included in the analysis. For parasite density and pyrexia, cohort values from all participants were averaged, and unless stated otherwise, all error bars represent SEM. To estimate the time required for clearance of ∼50% of the parasites, the number of parasites at each time point for each patient was fitted using an asymmetric sigmoidal function. The time at which the parasite number equaled half of the initial value on the fitted line was designated as the 50% (halfway) time. Determination of K13 gene mutations were performed exactly as described previously (Pau et al., 2019). A mixed ANOVA was conducted to determine the effect of drug treatment on parasite density and pyrexia. Post hoc multicomparison testing was used to determine which time points were statistically different. Two-tailed *t* test was used to determine statistical differences between means of independent groups. Significance was assumed for P values <0.05.

Data sharing

Data collected for this study will be made available to others upon publication until 36 mo after article publication. De-identified datasets containing the variables analyzed for the primary and secondary endpoints, as well as other supporting documents such as the study protocol and informed consent, will be made available. Investigators who seek access to individual-level data will need to contact the corresponding author to receive instructions on the formal request process. A data usage agreement will need to be signed by the respective institutions/individuals before data are transferred.

Online supplemental material

Table S1 details the criteria for how each of the various adverse events were scored from normal to severe. Table S2 describes the results of blood tests for complete blood counts, liver function, and kidney function on admission, which were performed to ensure that all participants had uncomplicated malaria. Table S3 lists the baseline characteristics of trial participants.

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Disclosures: K.S. Putt reported "other" from Erythrocure, Inc. outside the submitted work. K.S. Putt, P.S. Low, and F.M. Turrini are members of the Board of Directors for Erythrocure, Inc. P.S. Low reported a patent to the US Patent Office pending. No other disclosures were reported.

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References

Atwell, S., J.M. Adams, J. Badger, M.D. Buchanan, I.K. Feil, K.J. Froning, X. Gao, J. Hendle, K. Keegan, B.C. Leon, et al. 2004. A novel mode of Gleevec binding is revealed by the structure of spleen tyrosine kinase. *J. Biol. Chem.* 279: 55827–55832. https://doi.org/10.1074/jbc.M409792200

Bosman, G.J.C.G.M., E. Lasonder, Y.A.M. Groenen-Döpp, F.L.A. Willekens, and J.M. Werre. 2012. The proteome of erythrocyte-derived microparticles from plasma: new clues for erythrocyte aging and vesiculation. *J. Proteomics.* 76:203–210. https://doi.org/10.1016/j.jprot.2012.05.031

Brunati, A.M., L. Bordin, G. Clari, P. James, M. Quadroni, E. Baritono, L.A. Pinna, and A. Donella-Deana. 2000. Sequential phosphorylation of protein band 3 by Syk and Lyn tyrosine kinases in intact human erythrocytes: identification of primary and secondary phosphorylation sites. *Blood.* 96:1550–1557. https://doi.org/10.1182/blood.V96.4.1550

Conrad, M.D., and P.J. Rosenthal. 2019. Antimalarial drug resistance in Africa: the calm before the storm? *Lancet Infect. Dis.* 19:e338–e351. https://doi.org/10.1016/S1473-3099(19)30261-0

Demetri, G.D., M. von Mehren, C.D. Blanke, A.D. Van den Abbeele, B. Eisenberg, P.J. Roberts, M.C. Heinrich, D.A. Tuveson, S. Singer, M. Janicek, et al. 2002. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N. Engl. J. Med.* 347:472–480. https://doi.org/10.1056/NEJMoa020461

Dini, S., S. Zaloumis, P. Cao, R.N. Price, F.J.I. Fowkes, R.W. van der Pluijm, J.M. McCaw, and J.A. Simpson. 2018. Investigating the Efficacy of Triple Artemisinin-Based Combination Therapies for Treating *Plasmodium falciparum* Malaria Patients Using Mathematical Modeling. *Antimicrob. Agents Chemother.* 62:e01068-18. https://doi.org/10.1128/AAC.01068-18

Druker, B.J., M. Talpaz, D.J. Resta, B. Peng, E. Buchdunger, J.M. Ford, N.B. Lydon, H. Kantarjian, R. Capdeville, S. Ohno-Jones, and C.L. Sawyers. 2001a. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N. Engl. J. Med.* 344:1031–1037. https://doi.org/10.1056/NEJM200104053441401

Druker, B.J., C.L. Sawyers, H. Kantarjian, D.J. Resta, S.F. Reese, J.M. Ford, R. Capdeville, and M. Talpaz. 2001b. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *N. Engl. J. Med.* 344:1038–1042. https://doi.org/10.1056/NEJM200104053441402

Druker, B.J.F., F. Guilhot, S.G. O'Brien, I. Gathmann, H. Kantarjian, N. Gattermann, M.W. Deininger, R.T. Silver, J.M. Goldman, R.M. Stone, et al. IRIS Investigators. 2006. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. *N. Engl. J. Med.* 355:2408–2417. https://doi.org/10.1056/NEJMoa062867

Ferru, E., K. Giger, A. Pantaleo, E. Campanella, J. Grey, K. Ritchie, R. Vono, F. Turrini, and P.S. Low. 2011. Regulation of membrane-cytoskeletal interactions by tyrosine phosphorylation of erythrocyte band 3. *Blood.* 117: 5998–6006. https://doi.org/10.1182/blood-2010-11-317024

Hochhaus, A., R.A. Larson, F. Guilhot, J.P. Radich, S. Branford, T.P. Hughes, M. Baccarani, M.W. Deininger, F. Cervantes, S. Fujihara, et al. IRIS Investigators. 2017. Long-Term Outcomes of Imatinib Treatment for Chronic Myeloid Leukemia. *N. Engl. J. Med.* 376:917–927. https://doi.org/10.1056/NEJMoa1609324

Kesely, K.R., A. Pantaleo, F.M. Turrini, P. Olupot-Olupot, and P.S. Low. 2016. Inhibition of an Erythrocyte Tyrosine Kinase with Imatinib Prevents *Plasmodium falciparum* Egress and Terminates Parasitemia. *PLoS One.* 11: e0164895. https://doi.org/10.1371/journal.pone.0164895

Kesely, K., P. Noomuna, M. Vieth, P. Hipskind, K. Haldar, A. Pantaleo, F. Turrini, and P.S. Low. 2020. Identification of tyrosine kinase inhibitors that halt *Plasmodium falciparum* parasitemia. *PLoS One.* 15:e0242372. https://doi.org/10.1371/journal.pone.0242372

Li, Q., and B. Pybus. 2019. Pharmacokinetic and Pharmacodynamic Profiles of Rapid- and Slow-Acting Antimalarial Drugs. In Malaria. Kasenga, F.H., editor. IntechOpen, Rijeka, Croatia. https://doi.org/10.5772/intechopen.83505

Lu, F., R. Culleton, M. Zhang, A. Ramaprasad, L. von Seidlein, H. Zhou, G. Zhu, J. Tang, Y. Liu, W. Wang, et al. 2017. Emergence of Indigenous Artemisinin-Resistant *Plasmodium falciparum* in Africa. *N. Engl. J. Med.* 376:991–993. https://doi.org/10.1056/NEJMc1612765

Lyseng-Williamson, K., and B. Jarvis. 2001. Imatinib. *Drugs.* 61:1765–1774. https://doi.org/10.2165/00003495-200161120-00007

Nsanzabana, C. 2019. Resistance to Artemisinin Combination Therapies (ACTs): Do Not Forget the Partner Drug! *Trop. Med. Infect. Dis.* 4:26. https://doi.org/10.3390/tropicalmed4010026

O'Brien, S.G., F. Guilhot, R.A. Larson, I. Gathmann, M. Baccarani, F. Cervantes, J.J. Cornelissen, T. Fischer, A. Hochhaus, T. Hughes, et al. IRIS Investigators. 2003. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N. Engl. J. Med.* 348:994–1004. https://doi.org/10.1056/NEJMoa022457

Ouji, M., J.-M. Augereau, L. Paloque, and F. Benoit-Vical. 2018. *Plasmodium falciparum* resistance to artemisinin-based combination therapies: A sword of Damocles in the path toward malaria elimination. *Parasite.* 25: 24. https://doi.org/10.1051/parasite/2018021

Pantaleo, A., E. Ferru, F. Carta, F. Mannu, G. Giribaldi, R. Vono, A.J. Lepedda, P. Pippia, and F. Turrini. 2010. Analysis of changes in tyrosine and serine phosphorylation of red cell membrane proteins induced by *P. falciparum* growth. *Proteomics.* 10:3469–3479. https://doi.org/10.1002/pmic.201000269

Pantaleo, A., E. Ferru, F. Carta, F. Mannu, L.F. Simula, A. Khadjavi, P. Pippia, and F. Turrini. 2011. Irreversible AE1 tyrosine phosphorylation leads to membrane vesiculation in G6PD deficient red cells. *PLoS One.* 6:e15847. https://doi.org/10.1371/journal.pone.0015847

Pantaleo, A., E. Ferru, R. Vono, G. Giribaldi, O. Lobina, F. Nepveu, H. Ibrahim, J.P. Nallet, F. Carta, F. Mannu, et al. 2012. New antimalarial indolone-N-oxides, generating radical species, destabilize the host cell membrane at early stages of *Plasmodium falciparum* growth: role of band 3 tyrosine phosphorylation. *Free Radic. Biol. Med.* 52:527–536. https://doi.org/10.1016/j.freeradbiomed.2011.11.008

Pantaleo, A., K.R. Kesely, M.C. Pau, I. Tsamesidis, E. Schwarzer, O.A. Skorokhod, H.D. Chien, M. Ponzi, L. Bertuccini, P.S. Low, and F.M. Turrini. 2017. Syk inhibitors interfere with erythrocyte membrane modification during *P falciparum* growth and suppress parasite egress. *Blood.* 130: 1031–1040. https://doi.org/10.1182/blood-2016-11-748053

Pau, M.C., A. Pantaleo, I. Tsamesidis, H. Hoang, A. Tuan Tran, T.L. Hanh Nguyen, T.H. Giang Phan, P.A. Ton Nu, T.M. Chau Ngo, G. Marchetti, et al. 2019. Clinical impact of the two ART resistance markers, K13 gene mutations and DPC3 in Vietnam. *PLoS One.* 14:e0214667. https://doi.org/10.1371/journal.pone.0214667

Pretel-Irazabal, M., A. Tuneu-Valls, and N. Ormaechea-Pérez. 2014. Adverse skin effects of imatinib, a tyrosine kinase inhibitor. *Actas Dermosifiliogr.* 105:655–662. https://doi.org/10.1016/j.ad.2013.01.009

Puchulu-Campanella, E., F.M. Turrini, Y.-H. Li, and P.S. Low. 2016. Global transformation of erythrocyte properties via engagement of an SH2-like sequence in band 3. *Proc. Natl. Acad. Sci. USA.* 113:13732–13737. https://doi.org/10.1073/pnas.1611904113

Rosenthal, P.J. 2020. Are three drugs for malaria better than two? *Lancet.* 395: 1316–1317. https://doi.org/10.1016/S0140-6736(20)30560-2

Solyakov, L., J. Halbert, M.M. Alam, J.P. Semblat, D. Dorin-Semblat, L. Reininger, A.R. Bottrill, S. Mistry, A. Abdi, C. Fennell, et al. 2011. Global kinomic and phospho-proteomic analyses of the human malaria parasite *Plasmodium falciparum*. *Nat. Commun.* 2:565. https://doi.org/10.1038/ncomms1558

Thriemer, K., N.V. Hong, A. Rosanas-Urgell, B.Q. Phuc, M. Ha, E. Pockele, P. Guetens, N.V. Van, T.T. Duong, A. Amambua-Ngwa, et al. 2014. Delayed parasite clearance after treatment with dihydroartemisinin-piperaquine in *Plasmodium falciparum* malaria patients in central Vietnam. *Antimicrob. Agents Chemother.* 58:7049–7055. https://doi.org/10.1128/AAC.02746-14

Tsamesidis, I., K. Reybier, G. Marchetti, M.C. Pau, P. Virdis, C. Fozza, F. Nepveu, P.S. Low, F.M. Turrini, and A. Pantaleo. 2020. Syk Kinase Inhibitors Synergize with Artemisinins by Enhancing Oxidative Stress in *Plasmodium falciparum*-Parasitized Erythrocytes. *Antioxidants.* 9:753. https://doi.org/10.3390/antiox9080753

van der Pluijm, R.W., R. Tripura, R.M. Hoglund, A. Pyae Phyo, D. Lek, A. Ul Islam, A.R. Anvikar, P. Satpathi, S. Satpathi, P.K. Behera, et al. Tracking Resistance to Artemisinin Collaboration. 2020. Triple artemisinin-based combination therapies versus artemisinin-based combination therapies for uncomplicated *Plasmodium falciparum* malaria: a multicentre, open-label, randomised clinical trial. *Lancet.* 395:1345–1360. https://doi.org/10.1016/S0140-6736(20)30552-3

World Health Organization. 2015. Guidelines for the treatment of malaria, 3rd edition. World Health Organization, Geneva, Switzerland. 313 pp.

World Health Organization. 2019. World Malaria Report 2019. World Health Organization, Geneva, Switzerland. 232 pp.

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Supplemental material

Provided online are three tables. Table S1 characterizes adverse events. Table S2 shows participant blood counts and blood tests for liver and kidney function upon admission. Table S3 shows baseline characteristics of participants.