Triple artemisinin-based combination therapies versus artemisinin-based combination therapies for uncomplicated *Plasmodium falciparum* malaria: a multicentre, open-label, randomised clinical trial

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Summary

Background Artemisinin and partner-drug resistance in *Plasmodium falciparum* are major threats to malaria control and elimination. Triple artemisinin-based combination therapies (TACTs), which combine existing co-formulated ACTs with a second partner drug that is slowly eliminated, might provide effective treatment and delay emergence of antimalarial drug resistance.

Methods In this multicentre, open-label, randomised trial, we recruited patients with uncomplicated *P falciparum* malaria at 18 hospitals and health clinics in eight countries. Eligible patients were aged 2–65 years, with acute, uncomplicated *P falciparum* malaria alone or mixed with non-*falciparum* species, and a temperature of 37·5°C or higher, or a history of fever in the past 24 h. Patients were randomly assigned (1:1) to one of two treatments using block randomisation, depending on their location: in Thailand, Cambodia, Vietnam, and Myanmar patients were assigned to either dihydroartemisinin–piperaquine or dihydroartemisinin–piperaquine plus mefloquine; at three sites in Cambodia they were assigned to either artesunate–mefloquine or dihydroartemisinin–piperaquine plus mefloquine; and in Laos, Myanmar, Bangladesh, India, and the Democratic Republic of the Congo they were assigned to either artemether–lumefantrine or artemether–lumefantrine plus amodiaquine. All drugs were administered orally and doses varied by drug combination and site. Patients were followed-up weekly for 42 days. The primary endpoint was efficacy, defined by 42-day PCR-corrected adequate clinical and parasitological response. Primary analysis was by intention to treat. A detailed assessment of safety and tolerability of the study drugs was done in all patients randomly assigned to treatment. This study is registered at ClinicalTrials.gov, NCT02453308, and is complete.

Findings Between Aug 7, 2015, and Feb 8, 2018, 1100 patients were given either dihydroartemisinin–piperaquine (183 [17%]), dihydroartemisinin–piperaquine plus mefloquine (269 [24%]), artesunate–mefloquine (73 [7%]), artemether–lumefantrine (289 [26%]), or artemether–lumefantrine plus amodiaquine (286 [26%]). The median age was 23 years (IQR 13 to 34) and 854 (78%) of 1100 patients were male. In Cambodia, Thailand, and Vietnam the 42-day PCR-corrected efficacy after dihydroartemisinin–piperaquine plus mefloquine was 98% (149 of 152; 95% CI 94 to 100) and after dihydroartemisinin–piperaquine was 48% (67 of 141; 95% CI 39 to 56; risk difference 51%, 95% CI 42 to 59; p<0·0001). Efficacy of dihydroartemisinin–piperaquine plus mefloquine in the three sites in Myanmar was 91% (42 of 46; 95% CI 79 to 98) versus 100% (42 of 42; 95% CI 92 to 100) after dihydroartemisinin–piperaquine (risk difference 9%, 95% CI 1 to 17; p=0·12). The 42-day PCR corrected efficacy of dihydroartemisinin–piperaquine plus mefloquine (96% [68 of 71; 95% CI 88 to 99]) was non-inferior to that of artesunate–mefloquine (95% [69 of 73; 95% CI 87 to 99]) in three sites in Cambodia (risk difference 1%; 95% CI –6 to 8; p=1·00). The overall 42-day PCR-corrected efficacy of artemether–lumefantrine plus amodiaquine (98% [281 of 286; 95% CI 97 to 99]) was similar to that of artemether–lumefantrine (97% [279 of 289; 95% CI 94 to 98]; risk difference 2%, 95% CI –1 to 4; p=0·30). Both TACTs were well tolerated, although early vomiting (within 1 h) was more frequent after dihydroartemisinin–piperaquine plus mefloquine (30 [3·8%] of 794) than after dihydroartemisinin–piperaquine (eight [1·5%] of 543; p=0·012). Vomiting after artemether–lumefantrine plus amodiaquine (22 [1·3%] of 1703) and artemether–lumefantrine (11 [0·6%] of 1721) was infrequent. Adding amodiaquine to artemether–lumefantrine extended the electrocardiogram corrected QT interval (mean increase at 52 h compared with baseline of 8·8 ms [SD 18·6] *vs*

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0·9 ms [16·1]; p<0·01) but adding mefloquine to dihydroartemisinin–piperaquine did not (mean increase of 22·1 ms [SD 19·2] for dihydroartemisinin–piperaquine *vs* 20·8 ms [SD 17·8] for dihydroartemisinin–piperaquine plus mefloquine; p=0·50).

Interpretation **Dihydroartemisinin–piperaquine plus mefloquine and artemether–lumefantrine plus amodiaquine TACTs are efficacious, well tolerated, and safe treatments of uncomplicated *P falciparum* malaria, including in areas with artemisinin and ACT partner-drug resistance.**

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Introduction

Artemisinin-based combination therapies (ACTs) have contributed substantially to the reduction in the global burden of malaria.[1] However, progress is now threatened by the emergence and spread of artemisinin and ACT partner-drug resistance in southeast Asia.[2,3] The combination of resistance first to artemisinins and then to the ACT partner drugs, including piperaquine, mefloquine, amodiaquine, and sulfadoxine–pyrimethamine, has led to unacceptably high rates of treatment failure (ie, recrudescent infections) with artesunate–mefloquine on the Thailand–Myanmar border and dihydroartemisinin-piperaquine in Cambodia, east Thailand, and south Vietnam.[4,5] Molecular epidemiology studies show that the failure of dihydroartemisinin–piperaquine is caused by a single lineage of a multidrug-resistant parasite that has spread across Cambodia, northeast Thailand, southern Laos, and southern Vietnam.[6] The emergence of multidrug resistance has forced a series of treatment policy changes but progressively fewer treatment options for *Plasmodium falciparum* malaria are available in the Greater Mekong subregion. Yet, new compounds will not become generally available within the next few years.[7] A major concern is the potential spread of ACT resistance to the Indian subcontinent and sub-Saharan Africa. In the past, chloroquine resistance and sulfadoxine-pyrimethamine resistance that emerged in southeast Asia spread to sub-Saharan Africa and contributed to millions of childhood deaths.[8–10] Combining drugs with different mechanisms of action to prevent the emergence and

Research in context

Evidence before this study

We searched PubMed for articles published from database inception until Jan 30, 2020, using the terms "resistance" AND "malaria" AND "triple", which resulted in 265 articles. One study in healthy adult volunteers from Thailand reported no difference in the extension of electrocardiogram corrected QT interval after treatment with dihydroartemisinin–piperaquine compared with dihydroartemisinin–piperaquine plus mefloquine. In that study, coadministration of mefloquine with dihydroartemisinin–piperaquine reduced exposure to dihydroartemisinin.
A modelling study published in 2018 modelled the potential for dihydroartemisinin–piperaquine plus mefloquine (with mefloquine given as three doses of 6·7 mg/kg) to achieve parasitological efficacy, even in the scenario in which resistance to dihydroartemisinin and piperaquine had emerged. To date, no studies reporting the safety, tolerability, and efficacy of triple artemisinin-based combination therapies (TACTs) in the clinical setting have been reported.

Added value of this study

To our knowledge, this is the first clinical study to assess the safety, tolerability, and efficacy of two TACTs that combine three existing antimalalarial drugs—dihydroartemisinin–piperaquine plus mefloquine and artemether–lumefantrine plus amodiaquine—compared with currently used ACTs for the treatment of uncomplicated *Plasmodium falciparum* malaria. Incidence of vomiting within the first hour of treatment with both TACTs were low, but adding mefloquine or amodiaquine to the existing ACTs was associated with a slight increase in the incidence of vomiting. Amodiaquine extended the QT interval, but not to the extent associated with cardiac arrhythmias, and overall the two TACTs were safe and well tolerated. Dihydroartemisinin–piperaquine plus mefloquine and artesunate–mefloquine were also very effective in Cambodia, Thailand, and Vietnam, areas where dihydroartemisinin–piperaquine is no longer effective because of high prevalence of both artemisinin and piperaquine resistance. Although adding amodiaquine reduced exposures to lumefantrine, artemether, and its active metabolite dihydroartemisinin, the artemether–lumefantrine-amodiaquine TACT was highly effective.

Implications of all the available evidence

TACTs are a safe, well tolerated, efficacious, and a readily available new option for the treatment of uncomplicated *P falciparum* malaria and could improve treatment outcomes in areas with increasing artemisinin and partner-drug resistance in the Greater Mekong subregion. In areas where such resistance has not yet emerged, deployment of TACTs might delay the emergence and spread of resistance.

spread of antimicrobial resistance is a widely accepted approach, for instance, for the treatment of tuberculosis and infections caused by HIV, *Helicobacter pylori*, and multidrug-resistant bacteria.[11] This principle of combining drugs also underlies ACTs, but the slowly eliminated component is unprotected by the rapidly eliminated artemisinin component after the third day of treatment. Triple artemisinin-based combination therapies (TACTs), which combine a conventional ACT with a second slowly eliminated partner drug, add additional antimalarial activity and provide mutual protection for the partner drugs.[12] Furthermore, combining piperaquine with mefloquine and lumefantrine with amodiaquine potentially exploits counterbalancing resistance mechanisms.[13–18] We did a multicentre randomised controlled trial comparing the efficacy, safety, and tolerability of two TACTs, dihydroartemisinin–piperaquine plus mefloquine and artemether–lumefantrine plus amodiaquine, compared with their corresponding standard ACTs.

Methods

Study design and participants

In this multicentre, open-label, randomised controlled trial, we recruited patients from 18 hospitals and health clinics in eight countries: Cambodia (four sites), Thailand (three sites), Myanmar (four sites), India (three sites), Laos, Vietnam, Bangladesh, and the Democratic Republic of the Congo (one site each). Patients presented directly to the study sites with fever or were referred by malaria field workers after pre-screening with a malaria rapid diagnostic test. Eligible patients were aged 2–65 years, with acute, uncomplicated *P falciparum* malaria alone or mixed with non-*falciparum* species, and a temperature of 37·5°C or higher, or a history of fever in the past 24 h. In Democratic Republic of the Congo, only children younger than 12 years were eligible for inclusion. A further inclusion criterion was a parasitaemia of 5000–200 000 parasites per µL of blood, except in the Democratic Republic of the Congo where the range was 10 000–250 000 parasites per µL of blood, and in Cambodia where any parasitaemia of <200 000 parasites per µL of blood was allowed. Exclusion criteria were a contraindication to any study drug, the use of artemisinins in the previous 7 days, a previous splenectomy, pregnancy or breastfeeding, an electrocardiogram (ECG) corrected QT (QTc) interval of more than 450 ms, a documented or self-reported history of cardiac conduction problems, a low haematocrit (<25% in Asian sites and <15% in the Democratic Republic of the Congo), and participation in clinical trials in the previous 3 months.

Written informed consent was obtained from all participants before any study procedures were done. The protocol was approved by the Oxford Tropical Research Ethics Committee and for each site by the relevant institutional review board, national ethics committee, or both. The trial was monitored by the Mahidol-Oxford Tropical Medicine Research Unit Clinical Trials Support Group.

Randomisation and masking

Patients were randomly assigned (1:1) to one of two treatments at all study sites. The randomisation sequence was generated by an independent statistician in blocks of 8, 10, and 12 for all sites. Study number and treatment allocation codes were provided in sequentially numbered opaque envelopes. The comparator treatment was the first-line ACT in that area at the time of the start of the trial. Patients in Thailand, three sites in Cambodia (Pursat, Pailin, and Ratanakiri), Vietnam, and three sites in Myanmar (Thabeikkyin, Pyay, and Ann) were randomly assigned to either dihydroartemisinin–piperaquine or dihydroartemisinin–piperaquine plus mefloquine. In two Cambodian sites (Pursat and Pailin) the comparator treatment was changed from dihydroartemisinin–piperaquine to artesunate–mefloquine after recruitment of 19 patients at the Pursat site and 20 at the Pailin site because of very high failure rates with dihydroartemisinin–piperaquine. In Preah Vihear, the final site in Cambodia, which started later than the other three sites, artesunate–mefloquine was used as the comparator treatment throughout the study. In Laos, Bangladesh, India, Democratic Republic of the Congo, and one site in Myanmar (Pyin Oo Lwin), patients were randomly assigned to either artemether–lumefantrine or artemether–lumefantrine plus amodiaquine. Study staff, patients, and investigators were unmasked to treatment assignment, while laboratory staff were masked.

Procedures

All drugs were administered orally, directly observed by a member of the study team. Artemether–lumefantrine containing study drugs were given with a fatty snack to improve absorption of lumefantrine. The ACTs artemether–lumefantrine and dihydroartemisinin–piperaquine were dosed according to WHO guidelines.[19] All treatments were given as three (for piperaquine and mefloquine containing treatments) or six (for lumefantrine and amodiaquine containing treatments) doses. The target dose of amodiaquine was 10 mg base per kg per day, administered as a split-dose twice daily (together with artemether–lumefantrine). The target dose of mefloquine was 8 mg/kg per day, administered as a once daily dose (together with dihydroartemisinin–piperaquine). Mefloquine doses in the artesunate–mefloquine and dihydroartemisinin–piperaquine plus mefloquine study groups were similar. A single low dose of the gametocytocidal drug primaquine was given after 24 h (0·25 mg base per kg, except for age-based dosing in India, in accordance with national policies). Dosing schedules are detailed in the appendix (pp 26–32).

A full dose of study drug was readministered in case of vomiting within 30 min, or a half dose if vomiting occurred 30–60 min after administration. All patients

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See Online for appendix

were admitted to the study site for at least 3 consecutive nights and followed up on day 7, and thereafter weekly up to day 42. A standardised symptom questionnaire and physical examination, including heart rate, blood pressure, respiratory rate, and tympanic temperature, were done and recorded on each day of admission and each day of follow-up.

In case of the development of signs indicating severe malaria or an increase in parasitaemia 12 h after start of antimalarial therapy, rescue treatment with intravenous artesunate was started.

Adverse events were graded according to the Division of AIDS table for Grading the Severity of Adult and Paediatric Adverse Events (version 2.0).[20] Laboratory abnormalities were graded according to preprepared tables (appendix p 22). A 12-lead ECG was done at screening, baseline, and 4 h, 48 h, and 52 h after drug administration. In patients treated with dihydroartemisinin–piperaquine, ECGs were also done 24 h and 28 h after drug administration, and at the Indian sites also at 60 h and 64 h after drug administration. Recurrent infections were treated with an alternative ACT or a different drug combination; a summary is in the appendix (pp 31–32).

Asexual *P falciparum* densities were counted with microscopy at screening, baseline, and at 4 h, 6 h, 8 h, and 12 h after drug administration, and thereafter every 6 h until two Giemsa-stained consecutive blood films were negative. Asexual parasite clearance half-lives were determined on site with the WorldWide Antimalarial Resistance Network parasite clearance estimator,[21] with a cutoff of 5 h defining an extended parasite clearance half-life. Biochemistry measurements (serum alanine aminotransferase, aspartate aminotransferase, total bilirubin, alkaline phosphatase, and creatinine) and full blood count or haemoglobin measurements, or both, were done at baseline, and at days 3, 7, and 28 after drug administration. Blood samples were taken at baseline, day 7, and at any recurrent infection in all patients for measurement of antimalarial drug concentration (piperaquine, lumefantrine and its active metabolite desbutyl-lumefantrine, and artemether and its active metabolite dihydroartemisinin). Additional dense pharmacokinetic sampling was done in the first 20 patients given ACT and the first 20 patients given TACT at one site using artemether–lumefantrine with and without amodiaquine (Ramu, Bangladesh) and at one site using dihydroartemisinin–piperaquine with and without mefloquine (Binh Phuoc, Vietnam) at 1, 2, 4, 6, 8, 12, 24, 52 h, and day 4, 7, and 28 (and day 42 for patients given dihydroartemisinin–piperaquine plus mefloquine) after administration of the first dose of the study drug. Plasma samples were shipped on dry ice to the Department of Clinical Pharmacology, Mahidol-Oxford Tropical Medicine Unit at Mahidol University (Bangkok, Thailand) for measurement of antimalarial drug concentration. Concentrations of artemether, dihydroartemisinin, piperaquine, lumefantrine, and desbutyl-lumefantrine were measured using validated liquid chromatography-mass spectroscopy methods and standard procedures (details are in the appendix [p 25]).[22–24] Molecular markers of resistance to artemisinins (*P falciparum Kelch 13* mutations [*Pfkelch13*]), piperaquine (*Pfplasmepsin2/3* gene amplification) and mefloquine (*Pfmdr1* gene amplification) were assessed as described previously.[5,25,26] *Pfkelch13* mutations Y493H, R539T, R561H, and C580Y have previously been associated with slow parasite clearance. *Pfkelch13* A578S has been shown not to be associated with slow parasite clearance.[27] Recurrent infections were classified as recrudescent infections if all *msp1*, *msp2*, and *glurp* alleles matched those that were present at baseline.[28]

Outcomes

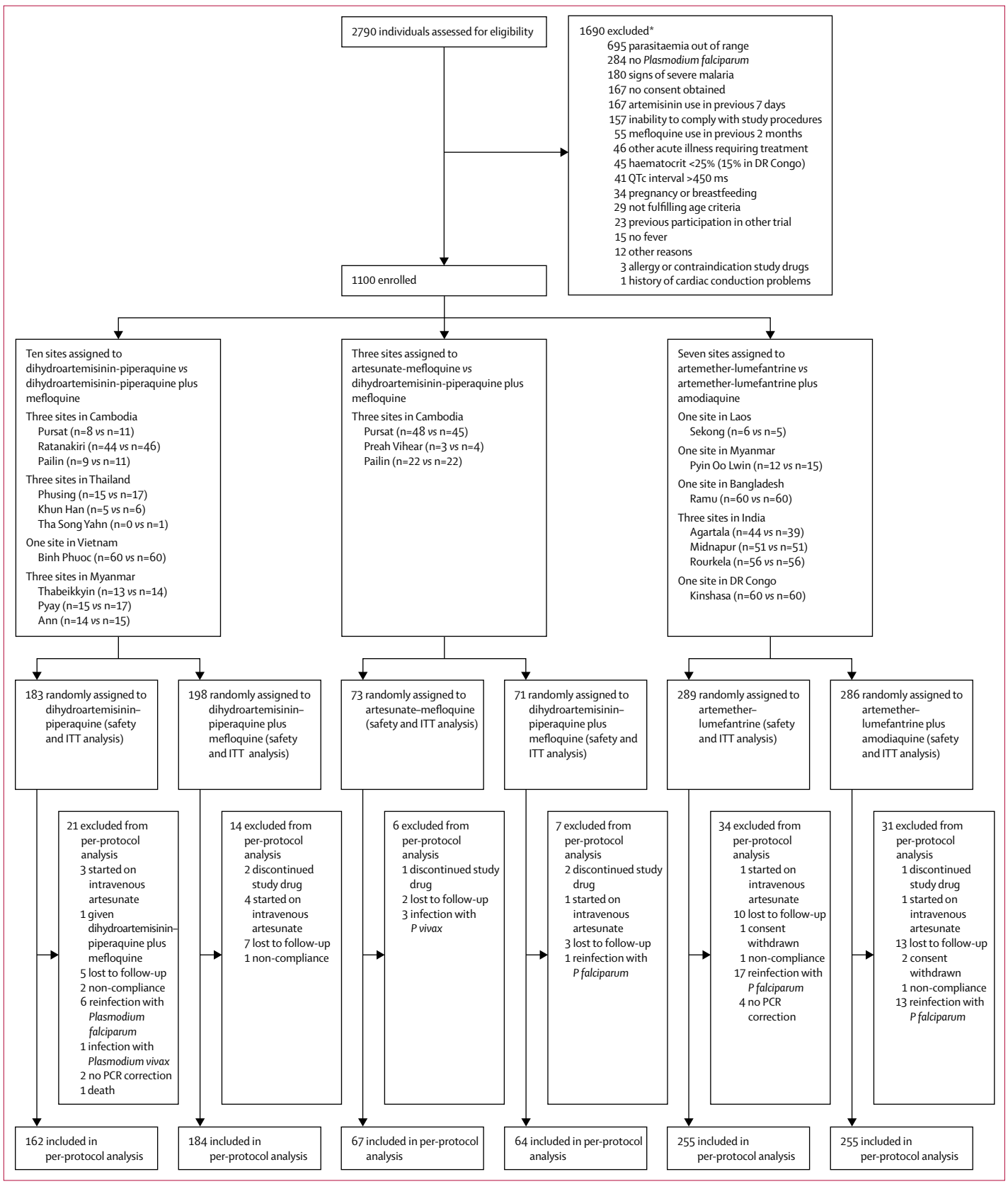
The primary outcome was efficacy, defined by the 42-day PCR-corrected adequate clinical and parasitological response (ACPR) of TACTs and ACTs within each site.[28] Day-42 PCR corrected ACPR was analysed by intention-to-treat (ITT) analysis, and supported by per-protocol and Kaplan-Meier survival analyses.

Secondary outcomes were the differences in parasite clearance half-lives stratified by *Pfkelch13* mutation status, PCR uncorrected 42-day ACPR (including patients with *P falciparum* reinfection), incidence of vomiting within 1 h after study drug administration, fever clearance time (time until a temperature of <37·5°C), incidence of adverse events and serious adverse events, extension of the Bazett's QTc-interval (QTcB-interval), extension of the QTc-interval of more than 500 ms or of more than 60 ms compared with baseline, and changes in heart rate. A further secondary endpoint was assessment of pharmacokinetic interactions between TACT components. Secondary endpoints not included in this report include the detailed parasite genome-wide and transcriptomic analyses, including gametocyte dynamics, and in-vitro parasite drug sensitivity analyses, which will all be reported separately.

Statistical analysis

We determined sample sizes per site in three different ways. For the sites in Thailand, Cambodia, and Vietnam that were comparing dihydroartemisinin–piperaquine with dihydroartemisinin–piperaquine plus mefloquine, we anticipated a PCR-corrected failure rate of dihydroartemisinin–piperaquine of 30–35%. Recruiting 50 patients in each treatment group would provide 80% power to detect a decrease in PCR-adjusted failure rates at day 42 to 10% or less (α=0·05, two-sided z test). In the sites where the efficacy of dihydroartemisinin–piperaquine plus mefloquine was compared with that of artesunate–mefloquine, the sample size was calculated

Figure 1: **Study profile**
ITT=intention-to-treat. QTc-interval=corrected QT interval. *Reasons are non-exclusive.


2790 individuals assessed for eligibility
1690 excluded*
695 parasitaemia out of range
284 no Plasmodium falciparum
180 signs of severe malaria
167 no consent obtained
167 artemisinin use in previous 7 days
157 inability to comply with study procedures
55 mefloquine use in previous 2 months
46 other acute illness requiring treatment
45 haematocrit <25% (15% in DR Congo)
41 QTc interval >450 ms
34 pregnancy or breastfeeding
29 not fulfilling age criteria
23 previous participation in other trial
15 no fever
12 other reasons
3 allergy or contraindication study drugs
1 history of cardiac conduction problems
1100 enrolled
Ten sites assigned to dihydroartemisinin-piperaquine vs dihydroartemisinin-piperaquine plus mefloquine
Three sites in Cambodia
Pursat (n=8 vs n=11)
Ratanakiri (n=44 vs n=46)
Pailin (n=9 vs n=11)
Three sites in Thailand
Phusing (n=15 vs n=17)
Khun Han (n=5 vs n=6)
Tha Song Yahn (n=0 vs n=1)
One site in Vietnam
Binh Phuoc (n=60 vs n=60)
Three sites in Myanmar
Thabeikkyin (n=13 vs n=14)
Pyay (n=15 vs n=17)
Ann (n=14 vs n=15)
Three sites assigned to artesunate-mefloquine vs dihydroartemisinin-piperaquine plus mefloquine
Three sites in Cambodia
Pursat (n=48 vs n=45)
Preah Vihear (n=3 vs n=4)
Pailin (n=22 vs n=22)
Seven sites assigned to artemether-lumefantrine vs artemether-lumefantrine plus amodiaquine
One site in Laos
Sekong (n=6 vs n=5)
One site in Myanmar
Pyin Oo Lwin (n=12 vs n=15)
One site in Bangladesh
Ramu (n=60 vs n=60)
Three sites in India
Agartala (n=44 vs n=39)
Midnapur (n=51 vs n=51)
Rourkela (n=56 vs n=56)
One site in DR Congo
Kinshasa (n=60 vs n=60)
183 randomly assigned to dihydroartemisinin–piperaquine (safety and ITT analysis)
198 randomly assigned to dihydroartemisinin–piperaquine plus mefloquine (safety and ITT analysis)
73 randomly assigned to artesunate–mefloquine (safety and ITT analysis)
71 randomly assigned to dihydroartemisinin–piperaquine plus mefloquine (safety and ITT analysis)
289 randomly assigned to artemether–lumefantrine (safety and ITT analysis)
286 randomly assigned to artemether–lumefantrine plus amodiaquine (safety and ITT analysis)
21 excluded from per-protocol analysis
3 started on intravenous artesunate
1 given dihydroartemisinin–piperaquine plus mefloquine
5 lost to follow-up
2 non-compliance
6 reinfection with Plasmodium falciparum
1 infection with Plasmodium vivax
2 no PCR correction
1 death
14 excluded from per-protocol analysis
2 discontinued study drug
4 started on intravenous artesunate
7 lost to follow-up
1 non-compliance
6 excluded from per-protocol analysis
1 discontinued study drug
2 lost to follow-up
3 infection with P vivax
7 excluded from per-protocol analysis
2 discontinued study drug
1 started on intravenous artesunate
3 lost to follow-up
1 reinfection with P falciparum
34 excluded from per-protocol analysis
1 started on intravenous artesunate
10 lost to follow-up
1 consent withdrawn
1 non-compliance
17 reinfection with P falciparum
4 no PCR correction
31 excluded from per-protocol analysis
1 discontinued study drug
1 started on intravenous artesunate
13 lost to follow-up
2 consent withdrawn
1 non-compliance
13 reinfection with P falciparum
162 included in per-protocol analysis
184 included in per-protocol analysis
67 included in per-protocol analysis
64 included in per-protocol analysis
255 included in per-protocol analysis
255 included in per-protocol analysis

to assess non-inferiority of dihydroartemisinin–piperaquine plus mefloquine. Assuming an ACT efficacy of 98% and a non-inferiority margin of 8%, a sample size of 49 participants per group was needed to declare non-inferiority with a one-sided α=0·025 and 80% power. Allowing for 20% loss to follow-up, this resulted in a sample size of 60 patients per study group In sites where artemisinin resistance was not established at the start of the study, a sample size of 120 would allow the detection of a prevalence of extended parasite clearance half-lives above 10% compared with a background prevalence of 3·5% with a power of 80%.

We assessed the superiority of the efficacy of ACTs and TACTs at each site using Fisher's exact test. Effect sizes are given as absolute differences with 95% CIs. In the ITT analysis, patients needing intravenous artesunate treatment, patients who discontinued the trial or study drugs before completion, had a PCR result that did not allow the distinction between reinfection and recrudescence, and with *Plasmodium vivax* infection during follow-up were imputed as treatment failures. Patients who re-presented with a PCR-confirmed *P falciparum* reinfection or who were lost to follow-up were imputed as a treatment success. In the per-protocol analysis, patients with any of these events were excluded from the analysis. We obtained day 42 recrudescence-free survival estimates using Kaplan-Meier analysis. We compared changes in heart rate and QTc-interval and parasite clearance half-lives between treatment groups using the unpaired *t* test and incidence of vomiting within the first hour after drug administration using the χ^2 test.

Our analyses for assessing non-inferiority were based on the one-sided CI for the difference in efficacy between treatments. The 42-day PCR-corrected efficacy of dihydroartemisinin–piperaquine plus mefloquine was declared non-inferior to that of artesunate–mefloquine if the lower end of the 95% CI for the difference in efficacy was greater the non-inferiority margin of –8%. We compared PCR uncorrected efficacy using a Fisher's exact test. We normalised the relevant tolerability and safety results as incidence per 100 patients and also calculated incidences using a Poisson distribution and assessed their differences using Fisher's exact test. We also calculated incidence rate ratios comparing treatment groups and the incidences were normalised per 100 patients. Drug exposure (ie, area under the curve [AUC]) was the primary pharmacometric parameter to evaluate drug–drug interactions and we analysed it after the first and last dose separately. Additionally, we calculated C_{max} and T_{max} values, with more details in the appendix [p 25]). Exposure between first dose and second dose (AUC_T) was expressed as AUC_{0-8} for artemether–lumefantrine with or without amodiaquine and as AUC_{0-24} for dihydroartemisinin–piperaquine with or without mefloquine. We report drug concentrations with 90% CIs. Exposure after the last dose ($AUC_{T\_lastdose}$) was defined as the AUC after the last dose (52 h for dihydroartemisinin–piperaquine with or without mefloquine and 96 h for artemether–lumefantrine with or without amodiaquine) to the last collected sample (day 28 for lumefantrine and desbutyl-lumefantrine and day 42 for piperaquine).

All tests were done at a 5% significance level.

A Data and Safety Monitoring Board (DSMB) met before the start of the trial and after recruitment of 20, 100, 200, 500, and 800 patients. We did most analyses using Stata version 15.1. This study was registered on ClinicalTrials.gov, NCT02453308.

	Dihydroartemisinin–piperaquine group (n=183)	Dihydroartemisinin–piperaquine plus mefloquine group (n=269)	Artesunate–mefloquine group (n=73)	Artemether–lumefantrine group (n=289)	Artemether–lumefantrine plus amodiaquine group (n=286)	Total population (n=1100)
Age, years	26·0 (18·0–36·0)	28 (20·0–37·0)	32·0 (24·0–43·0)	18·0 (7·0–28·0)	17·0 (7·0–29·0)	23·0 (13·0–34·0)
Sex						
Male	151 (83%)	231 (86%)	72 (99%)	202 (70%)	198 (69%)	854 (78%)
Female	32 (18%)	38 (14%)	1 (1%)	87 (30%)	88 (31%)	246 (22%)
Number of patients with a baseline tympanic temperature >37·5°C	102 (56%)	158 (59%)	38 (52%)	142 (49%)	134 (47%)	574 (52%)
Weight, kg	51·4 (13·4)	52·5 (11·6)	56·5 (8·2)	38·2 (17·7)	37·6 (17·7)	45·0 (16·9)
Height, cm	157·4 (14·7)	159·6 (11·3)	164·1 (5·7)	140·8 (25·1)	139·7 (24·9)	149·4 (22·1)
QTcB-interval, ms	411·9 (17·2)	412·8 (17·8)	411·5 (16·0)	414·6 (18·8)	415·0 (17·9)	413·6 (17·9)
Haematocrit, %	40·6 (5·3)	40·3 (5·3)	40·1 (5·3)	36·3 (5·6)	37·1 (6·2)	38·5 (5·9)
Parasite count per µL, geometric mean, (range)*	26 035 (160–214 223)	18 776 (48–565 200)	14 179 (96–217 602)	40 278 (384–449 711)	45 396 (1520–379 814)	29 865 (48–565 200)
Mixed infection (*Plasmodium falciparum* and *Plasmodium vivax*) present at baseline	5 (3%)	9 (3%)	7 (10%)	6 (2%)	2 (1%)	29 (3%)

Data are n (%), median (IQR), or mean (SD), unless otherwise stated. QTcB-interval=corrected QT interval using Bazett's formula. *The baseline parasitaemia in some patients was above the screening cutoff as the parasitaemia increased between screening and baseline.

Table 1: **Baseline characteristics of study patients, by randomly assigned treatment**

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Aug 7, 2015, and Feb 8, 2018, of 2790 individuals screened, 1100 patients with acute uncomplicated *P falciparum* malaria were enrolled and randomly assigned to dihydroartemisinin–piperaquine (183 [16·6%]), dihydroartemisinin–piperaquine plus mefloquine (269 [24·5%]), artesunate–mefloquine (73 [6·6%]), artemether–lumefantrine (289 [26·3%]), or artemether–lumefantrine plus amodiaquine (286 [26·0%]; figure 1). Baseline characteristics were similar between study groups (table 1; data for children from Democratic Republic of the Congo are shown in the appendix [p 7]), and 29 (3·0%) patients had mixed *P falciparum* and *P vivax* infections. In 15 sites, the study was stopped before target recruitment was reached because of decreasing malaria transmission based on a DSMB recommendation which was discussed and agreed by the investigators. 113 patients were excluded from the per-protocol analysis, of whom ten patients required intravenous artesunate treatment, as determined by the investigator on site (figure 1). Six patients discontinued the study drug and started standard antimalarial treatment due to abnormal baseline laboratory results or extension of the QTc-interval, as prespecified in the protocol.

Overall, the 42-day PCR corrected efficacy of dihydroartemisinin–piperaquine plus mefloquine (97%; 95% CI 93 to 99) was higher than for dihydroartemisinin–piperaquine (60%; 52 to 67), with a risk difference of 37% (29 to 45; p<0·0001. The 42-day PCR corrected efficacy of dihydroartemisinin–piperaquine plus mefloquine was superior to dihydroartemisinin–piperaquine in Binh Phuoc, Vietnam (p<0·0001); Phusing (p<0·0001) and Khun Han (p=0·015), Thailand; Ratanakiri (p<0·0001) and Pursat (p=0·0002), Cambodia (table 2). Efficacy of

	Dihydroartemisinin–piperaquine	Dihydroartemisinin–piperaquine plus mefloquine	Artesunate–mefloquine	Risk difference	p value
Vietnam					
Binh Phuoc (n=120)	26/60 (43%; 31 to 57)	58/60 (97%; 89 to 100)	..	53% (40 to 67)	<0·0001
Thailand					
Phusing (n=32)	2/15 (13%; 2 to 41)	17/17 (100%; 81 to 100)	..	87% (70 to 100)	<0·0001
Khun Han (n=11)	1/5 (20%; 1 to 72)	6/6 (100%; 54 to 100)	..	80% (45 to 100)	0·015
Tha Song Yang (n=1)	..	1/1 (100%; 3 to 100)	..	NA	NA
Cambodia					
Ratanakiri (n=90)	32/44 (73%; 57 to 85)	46/46 (100%; 92 to 100)	..	27% (14 to 41)	<0·0001
Pursat					
Dihydroartemisinin–piperaquine comparator treatment (n=19)	1/8 (13%; 0 to 53)	11/11 (100%; 72 to 100)	..	88% (65 to 100)	0·0002
Artesunate–mefloquine comparator treatment (n=93)	..	44/45 (98%; 88 to 100)	44/48 (92%; 80 to 98)	6% (–3 to 15)	0·36
Pailin					
Dihydroartemisinin–piperaquine comparator treatment (n=20)	5/9 (56%; 21 to 86)	10/11 (91%; 59 to 100)	..	35% (–1 to 72)	0·13
Artesunate–mefloquine comparator treatment (n=44)	..	20/22 (91%; 71 to 99)	22/22 (100%; 85 to 100)	9% (–3 to 21)	0·50
Preah Vihear (n=7)	..	4/4 (100%; 40 to 100)	3/3 (100%; 29 to 100)	0	NA
Myanmar					
Thabeikkyin (n=27)	13/13 (100%; 75 to 100)	13/14 (93%; 66 to 100)	..	–7% (–21 to 6)	1·00
Pyay (n=32)	15/15 (100%; 78 to 100)	15/17 (88%; 64 to 99)	..	–12% (–27 to 4)	0·50
Ann (n=29)	14/14 (100%; 77 to 100)	14/15 (93%; 68 to 100)	..	7% (–6 to 19)	1·00
Total					
Dihydroartemisinin–piperaquine *vs* dihydroartemisinin–piperaquine plus mefloquine					
Overall (n=381)	109/183 (60%; 52 to 67)	191/198 (97%; 93 to 99)	..	37% (29 to 45)	<0·0001
In Vietnam, Thailand, and Cambodia (n=293)	67/141 (48%; 39 to 56)	149/152 (98%; 94 to 100)	..	51% (42 to 59)	<0·0001
In Myanmar (n=88)	42/42 (100%; 92 to 100)	42/46 (91%; 79 to 98)	..	9% (1 to 17)	0·12
Artesunate–mefloquine *vs* dihydroartemisinin–piperaquine plus mefloquine (n=144)					
Overall (n=144)	..	68/71 (96%; 88 to 99)	69/73 (95%; 87 to 99)	1% (–6 to 8)	1·00

Data are n/N (%; 95% CI) or risk difference. p values are from two-sided Fisher's exact tests. NA=not applicable. ITT=intention-to-treat.

Table 2: **Day-42 PCR-corrected efficacy after dihydroartemisinin–piperaquine, dihydroartemisinin–piperaquine plus mefloquine, and artesunate–mefloquine treatment, by site (ITT analysis)**

	Artemether–lumefantrine	Artemether–lumefantrine plus amodiaquine	Risk difference	p value
Myanmar				
Pyin Oo Lwin (n=27)	12/12 (100%; 74 to 100)	15/15 (100%; 78 to 100)	0 (NA)	NA
Laos				
Sekong (n=11)	5/6 (83%; 36 to 100)	5/5 (100%; 48 to 100)	17% (–13 to 47)	1·00
Bangladesh				
Ramu (n=120)	57/60 (95%; 86 to 99)	58/60 (97%; 89 to 100)	2% (–5 to 9)	1·00
India				
Agartala (n=83)	44/44 (100%; 92 to 100)	38/39 (97%; 87 to 100)	3% (–2 to 8)	0·47
Rourkela (n=112)	52/56 (93%; 83 to 98)	54/56 (96%; 88 to 100)	4% (–5 to 12)	0·68
Midnapur (n=102)	50/51 (98%; 90 to 100)	51/51 (100%; 93 to 100)	2% (–2 to 6)	1·00
Democratic Republic of the Congo				
Kinshasa (n=120)	59/60 (98%; 91 to 100)	60/60 (100%; 94 to 100)	2% (–2 to 5)	1·00
Total				
Artemether–lumefantrine *vs* artemether–lumefantrine plus amodiaquine (n=575)	279/289 (97%; 94 to 98)	281/286 (98%; 96 to 99)	2% (–1 to 4)	0·30

Data are n/N (%; 95% CI) or risk difference. p values are from two-sided Fisher's exact tests. NA=not applicable. ITT=intention-to-treat.

Table 3: **Day-42 PCR corrected efficacy after artemether–lumefantrine and artemether–lumefantrine plus amodiaquine treatment, by site (ITT analysis)**

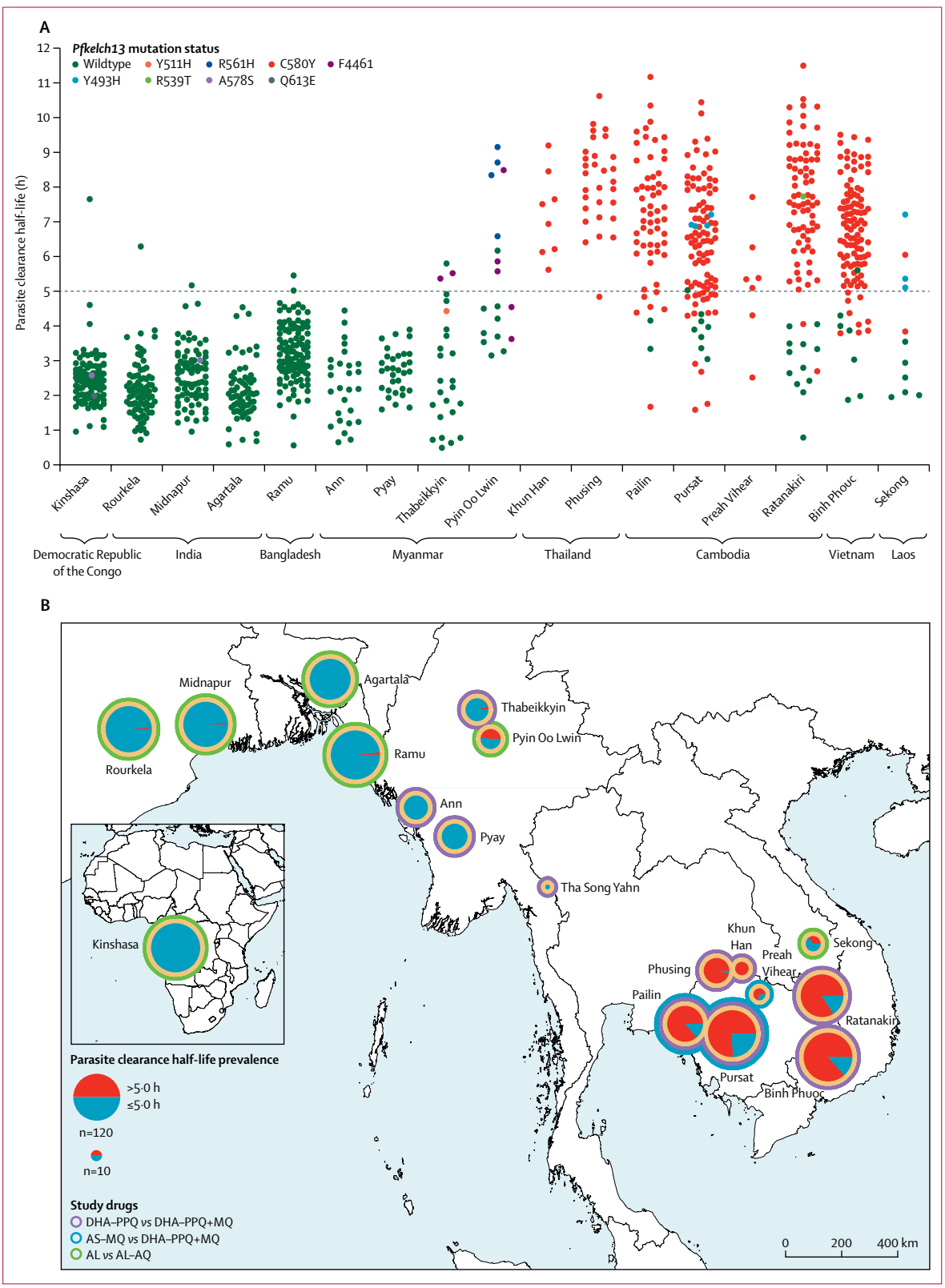
dihydroartemisinin–piperaquine plus mefloquine was not superior to dihydroartemisinin–piperaquine in Pailin, Cambodia (p=0·13; table 2). Because of the high failure rates after dihydroartemisinin–piperaquine and the switch of first-line treatment from dihydroartemisinin–piperaquine to artesunate–mefloquine, the DSMB advised changing the comparator treatment to artesunate–mefloquine in Pailin, Pursat, and Preah Vihear, all in Cambodia. By that timepoint, 19 patients had been recruited in Pursat and 20 patients had been recruited in Pailin. The comparator was not changed in Vietnam and Thailand because national first-line treatment was dihydroartemisinin–piperaquine throughout the duration of the trial. Overall, the 42-day PCR corrected efficacy of dihydroartemisinin–piperaquine plus mefloquine (96%; 88 to 99) was non-inferior to that of artesunate–mefloquine (95%; 87 to 99), risk difference 1% (–6 to 8; p=1·00). The 42-day PCR corrected efficacy of dihydroartemisinin–piperaquine plus mefloquine was non-inferior to that of artesunate–mefloquine in Pursat (p=0·36), Pailin (p=0·50), and Preah Vihear, Cambodia (0 risk difference; table 2; appendix p 2). In Myanmar, 42-day PCR-corrected efficacy of dihydroartemisinin–piperaquine was 100% (42 of 42; 95% CI 92 to 100) in the three sites combined, and efficacy of dihydroartemisinin–piperaquine plus mefloquine in these sites in Myanmar was 91% (42 of 46; 95% CI 79 to 98; p=0·12).

The overall 42-day corrected efficacy of artemether–lumefantrine (97%, 95% CI 94 to 98) was comparable to that of artemether–lumefantrine plus amodiaquine (98% [96 to 99]; risk difference 2%, [–1 to 4, p=0·30]; table 3). The 42-day PCR corrected efficacy of artemether–lumefantrine was above 90% in all seven sites except for Sekong, Laos, where one of six enrolled patients had a recrudescent infection (table 3). The efficacy of artemether–lumefantrine plus amodiaquine ranged between 96% and 100% in all sites where it was tested. No differences in efficacy between artemether–lumefantrine and artemether–lumefantrine plus amodiaquine were observed at any site). The results of the per-protocol analysis and Kaplan-Meier survival analysis showed similar results (appendix pp 3–4, 8–11).

Most patients in Cambodia, Vietnam, and Thailand had extended parasite clearance half-lives of longer than 5 h (figure 2; appendix p 14). Genotyping of the *Pfkelch13* gene, a marker of artemisinin resistance, was possible in 1036 of 1100 patients (appendix pp 15–16). C580Y was the dominant *Pfkelch13* mutation in Cambodia, eastern Thailand, Vietnam, and Laos, but was not observed elsewhere. In Pyin Oo Lwin, Myanmar, ten (37%) of 27 infections were caused by parasites carrying *Pfkelch13* mutations (F446I n=5; R561H n=5; appendix p 15). In the other sites in Myanmar, and in the sites in Bangladesh, India, and the Democratic Republic of the Congo, almost all parasite clearance half-lives were shorter than 5 h. In Midnapur, India, ten (11%) of 89 infections were caused by *Pfkelch13* mutated parasites including mutations at positions 364, 464, 496, 545, 548, 567, 578, 637, 662, and 704. However, nine of these ten *Pfkelch13* mutated infections were mixed strain infections also containing

Figure 2: **Parasite clearance half-lives and the presence of the *Pfkelch13* mutations by study site**

(A) Parasite clearance half-lives for each individual participant by study site, with the dotted line showing the 5 h cutoff point; participants with polyclonal infections were excluded from this graph. (B) Location of the study sites and pie charts show the proportions of participants with a parasite clearance half-life of more than 5 h and less than 5 h and which drugs were trialled at each site. AL=artemether–lumefantrine. AQ=amodiaquine. AS-MQ=artesunate–mefloquine. DHA-PPQ=dihydroartemisinin–piperaquine. MQ=mefloquine.


A
Pfkelch13 mutation status
Wildtype
Y511H
R561H
C580Y
F4461
Y493H
R539T
A578S
Q613E
Parasite clearance half-life (h)
0
1
2
3
4
5
6
7
8
9
10
11
12
Kinshasa
Rourkela
Midnapur
Agartala
Ramu
Ann
Pyay
Thabeikkyin
Pyin Oo Lwin
Khun Han
Phusing
Pailin
Pursat
Preah Vihear
Ratanakiri
Binh Phouc
Sekong
Democratic Republic of the Congo
India
Bangladesh
Myanmar
Thailand
Cambodia
Vietnam
Laos
B
Midnapur
Agartala
Rourkela
Thabeikkyin
Pyin Oo Lwin
Ramu
Ann
Pyay
Tha Song Yahn
Khun Han
Sekong
Phusing
Preah Vihear
Pailin
Ratanakiri
Pursat
Binh Phuoc
Kinshasa
Parasite clearance half-life prevalence
>5·0 h
≤5·0 h
n=120
n=10
Study drugs
DHA–PPQ vs DHA–PPQ+MQ
AS–MQ vs DHA–PPQ+MQ
AL vs AL–AQ
0
200
400 km

Pfkelch13 wildtype parasites (appendix p 16). Similarly, all *Pfkelch13* mutant infections in the other two sites in India, Rourkela (three [3%] of 87) and Agartala (six [9%] of 69) were mixed with wildtype infections. In the sites in Myanmar (other than Pyin Oo Lwin), in Bangladesh, and in the other sites in India and the Democratic Republic of the Congo, *Pfkelch13* mutations were rare. *Pfplasmepsin2/3* gene amplifications, a marker of piperaquine resistance, were present in high frequencies in Cambodia, Thailand, and Vietnam

	Dihydroartemisinin–piperaquine group (n=183)	Dihydroartemisinin–piperaquine plus mefloquine group (n=269)	Artesunate–mefloquine group (n=73)	Artemether–lumefantrine group (n=289)	Artemether–lumefantrine plus amodiaquine group (n=286)
Vomiting within 1 h after treatment/number of treatments	8/543 (1·5%)	30/794 (3·8%)	3/219 (1·4%)	11/1721 (0·6%)	22/1703 (1·3%)
Serious adverse events	6/183 (3·3%)	10/269 (3·7%)	2/73 (2·7%)	4/289 (1·4%)	2/286 (0·7%)
Drug-related serious adverse events	4/183 (2·2%)	4/269 (1·5%)	1/73 (1·4%)	0/289 (0%)	1/286 (0·3%)
QTcB >60 ms above baseline	5/183 (2·7%)	6/269 (2·2%)	0/73 (0·0%)	1/289 (0·3%)	1/286 (0·3%)
QTcB >500 ms	0/183 (0·0%)	1/269 (0·4%)	0/73 (0·0%)	0/289 (0·0%)	0/286 (0·0%)
Bradycardia	24/183 (13·1%)	44/269 (16·4%)	9/73 (12·3%)	18/289 (6·2%)	52/286 (18·2%)
Symptoms					
Headache					
Grade 1–2	43 (23·5%)	40 (14·9%)	7 (9·6%)	25 (8·7%)	13 (4·5%)
Grade 3–4	0 (0·0%)	1 (0·4%)	0 (0·0%)	1 (0·3%)	0 (0·0%)
Fatigue					
Grade 1–2	26 (14·2%)	29 (10·8%)	3 (4·1%)	14 (4·8%)	21 (7·3%)
Grade 3–4	0 (0·0%)	1 (0·4%)	1 (1·4%)	0 (0·0%)	0 (0·0%)
Abdominal pain					
Grade 1–2	9 (4·9%)	17 (6·3%)	6 (8·2%)	9 (3·1%)	13 (4·5%)
Grade 3–4	0 (0·0%)	0 (0·0%)	0 (0·0%)	0 (0·0%)	0 (0·0%)
Loss of appetite					
Grade 1–2	19 (10·4%)	19 (7·1%)	8 (11·0%)	25 (8·7%)	31 (10·8%)
Grade 3–4	0 (0·0%)	0 (0·0%)	0 (0·0%)	0 (0·0%)	0 (0·0%)
Nausea					
Grade 1–2	17 (9·3%)	39 (14·5%)	5 (6·8%)	3 (1·0%)	14 (4·9%)
Grade 3–4	0 (0·0%)	0 (0·0%)	0 (0·0%)	0 (0·0%)	0 (0·0%)
Vomiting*					
Grade 1–2	15 (8·2%)	28 (10·4%)	6 (8·2%)	10 (3·5%)	22 (7·7%)
Grade 3–4	0 (0·0%)	0 (0·0%)	0 (0·0%)	0 (0·0%)	0 (0·0%)
Diarrhoea					
Grade 1–2	9 (4·9%)	25 (9·3%)	8 (11·0%)	7 (2·4%)	5 (1·7%)
Grade 3–4	0 (0·0%)	0 (0·0%)	0 (0·0%)	0 (0·0%)	0 (0·0%)
Itching					
Grade 1–2	3 (1·6%)	3 (1·1%)	2 (2·7%)	4 (1·4%)	4 (1·4%)
Grade 3–4	0 (0·0%)	0 (0·0%)	0 (0·0%)	0 (0·0%)	0 (0·0%)
Dizziness					
Grade 1–2	21 (11·5%)	38 (14·1%)	16 (21·9%)	18 (6·2%)	25 (8·7%)
Grade 3–4	1 (0·5%)	2 (0·7%)	2 (2·7%)	0 (0·0%)	0 (0·0%)
Blurred vision					
Grade 1–2	1 (0·5%)	9 (3·3%)	11 (15·1%)	3 (1·0%)	2 (0·7%)
Grade 3–4	0 (0·0%)	0 (0·0%)	0 (0·0%)	0 (0·0%)	0 (0·0%)
Sleep disturbance					
Grade 1–2	8 (4·4%)	25 (9·3%)	16 (21·9%)	2 (0·7%)	3 (1·0%)
Grade 3–4	0 (0·0%)	1 (0·4%)	3 (4·1%)	0 (0·0%)	0 (0·0%)
Total					
Grade 1–2	171/183 (93·4%)	272/269 (101·1%)	88/73 (120·5%)	120/289 (41·5%)	153/286 (53·5%)
Grade 3–4	1/183 (0·5%)	5/269 (1·9%)	6/73 (8·2%)	1/289 (0·3%)	0/286 (0·0%)

(Table 4 continues on next page)

	Dihydroartemisinin–piperaquine group (n=183)	Dihydroartemisinin–piperaquine plus mefloquine group (n=269)	Artesunate–mefloquine group (n=73)	Artemether–lumefantrine group (n=289)	Artemether–lumefantrine plus amodiaquine group (n=286)
(Continued from previous page)					
Laboratory abnormalities					
Creatinine†					
Grade 1–2	17/148 (11·5%)	17/229 (7·4%)	2/73 (2·7%)	23/232 (9·9%)	38/230 (16·5%)
Grade 3–4	0/148 (0·0%)	1/229 (0·4%)	0/73 (0·0%)	2/232 (0·8%)	6/230 (2·6%)
Total bilirubin					
Grade 1–2	22/183 (12·0%)	21/269 (7·8%)	2/73 (2·7%)	11/289 (3·8%)	9/286 (3·1%)
Grade 3–4	2/183 (1·1%)	3/269 (1·1%)	0/73 (0·0%)	1/289 (0·3%)	0/286 (0·0%)
Alkaline phosphatase					
Grade 1–2	5/183 (2·7%)	7/269 (2·6%)	3/73 (4·1%)	17/289 (5·9%)	21/286 (7·3%)
Grade 3–4	0/183 (0·0%)	0/269 (0·0%)	0/73 (0·0%)	0/289 (0·0%)	0/286 (0·0%)
Alanine aminotransferase‡					
Grade 1–2	31/163 (19·0%)	43/246 (17·5%)	23/73 (31·5%)	43/289 (14·9%)	32/286 (11·1%)
Grade 3–4	3/163 (1·8%)	2/246 (0·8%)	2/73 (2·7%)	1/289 (0·3%)	0/286 (0·0%)
Aspartate aminotransferase§					
Grade 1–2	35/183 (19·1%)	33/269 (12·2%)	18/73 (24·7%)	63/283 (22·2%)	47/281 (16·7%)
Grade 3–4	1/183 (0·5%)	1/269 (0·4%)	1/73 (1·3%)	2/283 (0·7%)	1/281 (0·3%)
Anaemia (haemoglobin)					
Grade 1–2	40/159 (25·2%)	39/244 (16·0%)	11/73 (15·1%)	66/235 (28·1%)	71/227 (31·3%)
Grade 3–4	5/159 (1·4%)	3/244 (1·2%)	1/73 (1·4%)	25/235 (10·6%)	16/227(7·0%)
Leucocytopenia					
Grade 1–2	0/114 (0·0%)	0/149 (0·0%)	1/22 (4·5%)	0/165 (0·0%)	1/160 (0·6%)
Grade 3–4	0/114 (0·0%)	1/149 (0·7%)	1/22 (4·5%)	1/165 (0·6%)	0/160 (0·6%)
Neutropenia					
Grade 1–2	2/113 (1·8%)	4/149 (2·7%)	1/22 (4·5%)	6/161 (3·7%)	6/156 (3·8%)
Grade 3–4	0/113 (0·0%)	0/149 0·0%)	0/22 (0·0%)	6/161 (3·7%)	1/156 (0·6%)
Thrombocytopenia					
Grade 1–2	9/115 (7·8%)	16/149 (10·7%)	2/22 (9·1%)	26/162 (16·0%)	23/157 (14·6%)
Grade 3–4	2/115 (1·7%)	2/149 (1·3%)	0/22 (0·0%)	2/162 (1·2%)	1/157 (0·6%)

Data are n/N, where n is number of events and N is number of patients, with a normalised incidence per 100 patients in parentheses, unless otherwise indicated. Incidence of QTcB increases of >60 ms above baseline or bradycardia (defined as ≤54 heartbeats per min) are defined as a patient encountering these abnormalities at one or more timepoints at 4 h, 48 h, or 52 h after treatment. QTcB=Bazett's corrected QT-interval. *Any worsening of self-reported vomiting was recorded as an adverse event. †Results of creatinine measurements from sites in Midnapur (India), Pyay (Myanmar), Phusing and Khun Han (Thailand), and Sekong (Laos) were not available, and the denominator number of patients is amended to reflect this fact. ‡Results from Phusing and Khun Han (Thailand) were not available, and the denominator number of patients is amended to reflect this fact. §Results from Sekong, Laos were not available, and the denominator number of patients is amended to reflect this fact.

Table 4: **Incidence of adverse events and other indicators of study drug toxicity, by study treatment group**

(appendix p 15), but were absent in all the other countries. *Pfmdr1* gene amplifications, a marker for mefloquine resistance, were not observed anywhere. Parasite half-lives in *Pfkelch13* C580Y mutated infections were shorter in patients treated with dihydroartemisinin–piperaquine plus mefloquine (mean 6·93 h [SD 1·77]) than among those treated with dihydroartemisinin–piperaquine (7·39 h [1·46]; p=0·019) and were similar to the half-lives in those treated with artesunate–mefloquine (7·02 h [1·81]; p=0·752; appendix p 5). In patients with a *Pfkelch13* wildtype infection, parasite clearance half-lives were longer with dihydroartemisinin–piperaquine plus mefloquine (2·90 h [1·15]) than with dihydroartemisinin–piperaquine alone (2·40 h [SD 0·98]; p=0·020). We found no difference in parasite clearance half-lives in *Pfkelch13* wildtype infections after treatment with artemether–lumefantrine (2·65 h [1·39]) compared with after artemether–lumefantrine plus amodiaquine (2·69 h [1·17]; p=0·702).

PCR-uncorrected day-42 ACPR were similar to the PCR-corrected ACPR outcome data, except for the high-transmission site in the Democratic Republic of the Congo, where reinfection is common and the uncorrected ACPR was 75% (45 of 60; 95% CI 62–85) with artemether-lumefantrine and 78% (47 of 60; 66–88) with artemether-lumefantrine plus amodiaquine (appendix p 13). Fever clearance times were not different between the ACTs and corresponding TACTs (data not shown).

Overall all drug regimens were well tolerated and most reported adverse clinical symptoms were mild

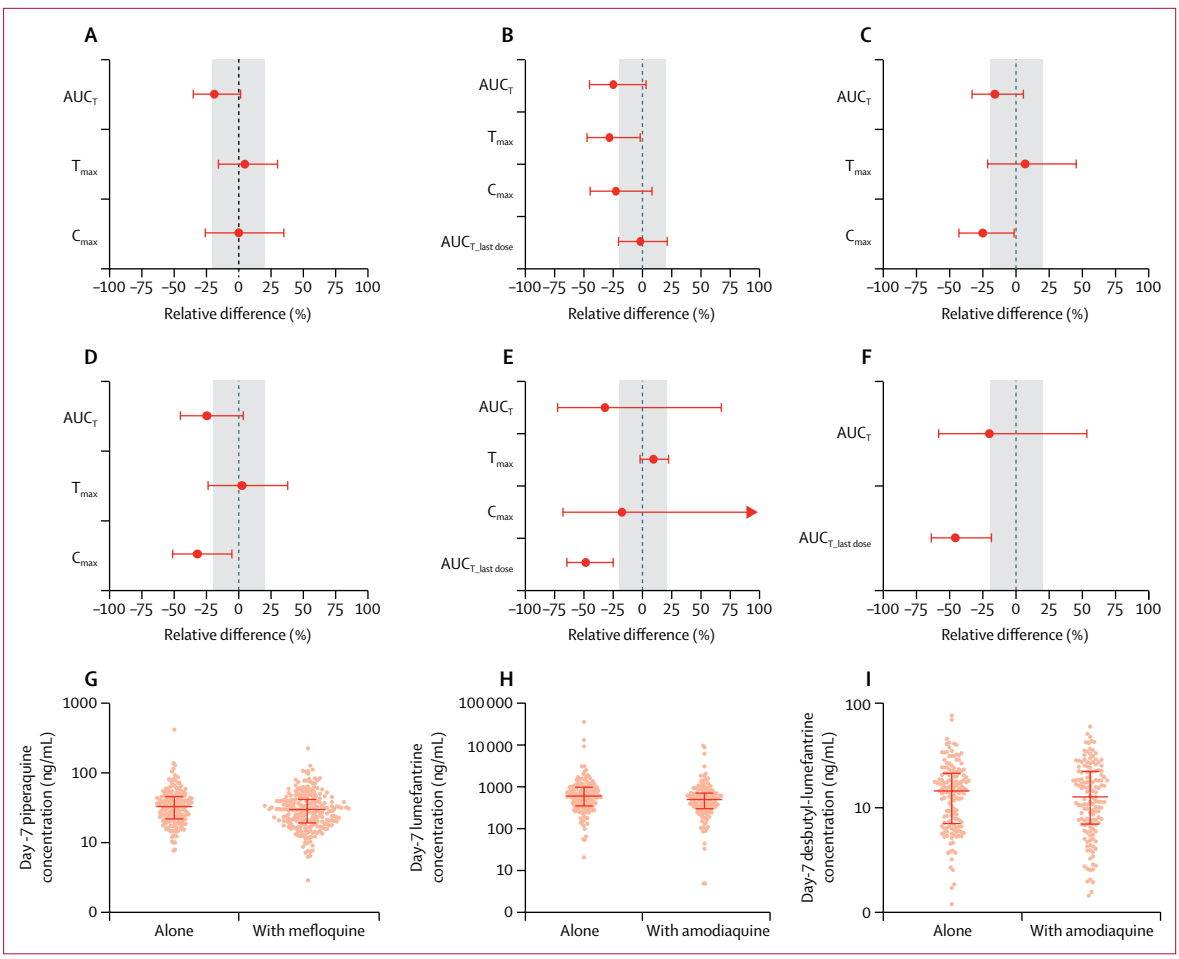


Figure 3: **Pharmacokinetic drug–drug interactions**

Effect of mefloquine on dihydroartemisinin (A) and on piperaquine (B) when treatment is dihydroartemisinin–piperaquine with or without mefloquine. Effect of amodiaquine on artemether (C), active metabolite dihydroartemisinin (D), lumefantrine (E), and desbutyl-lumefantrine when treatment is artemether–lumefantrine with or without amodiaquine. (G) Effect of mefloquine on day-7 piperaquine plasma concentrations when the treatment is dihydroartemisinin–piperaquine with or without mefloquine. Effect of amodiaquine on day 7 lumefantrine (H) and desbutyl-lumefantrine (I) plasma concentrations when the treatment is artemether–lumefantrine with or without amodiaquine. The plots in panels A–F show the geometric mean ratios and 90% CIs of drug–drug interactions related to the specific pharmacokinetic parameters. The dashed line represents zero effect, and the dotted lines show plus or minus 20% effect. In the scatter plots in panels G–I, the red bars show the median and IQR of day 7 plasma concentrations. C_{max}=maximum plasma concentration divided with mg/kg dose. T_{max}=time to reach maximum concentration. AUC_T=area under the concentration-time curve to time T after administration of the first dose, divided by the mg/kg dose. $AUC_{T\_lastdose}$=area under the concentration-time curve to time T after administration of the last dose, divided by the mg/kg dose.

or moderate in severity (table 4). Most clinical adverse events occurred in the first week after enrolment (appendix p 18). The incidence of clinical adverse events in patients treated with dihydroartemisinin–piperaquine plus mefloquine (277 adverse events in 269 patients) were not different from dihydroartemisinin–piperaquine (171 adverse events in 183 patients; incidence rate ratio 1·1, 95% CI 0·9–1·3; p=0·32), whereas patients treated with artesunate–mefloquine (94 adverse events in 73 patients) had more clinical adverse events than did those treated with dihydroartemisinin–piperaquine (incidence rate ratio 1·4, 95% CI 1·1–1·8; p=0·014), including more abdominal complaints, dizziness, blurred vision, and sleeping disturbances. The incidence of clinical adverse events was also higher with artemether–lumefantrine plus amodiaquine (153 adverse events in 286 patients) than with artemether–lumefantrine (121 adverse events in 289 patients; incidence rate ratio 1·3, 95% CI 1·0–1·6; p=0·0436), including more abdominal symptoms—eg, loss of appetite, nausea, and vomiting. Vomiting within the first hour after administration of study drug was infrequent but occurred more after dihydroartemisinin–piperaquine plus mefloquine (30 [3·8%] of 794) than after dihydroartemisinin–piperaquine (eight [1·5%] of 543; p=0·012; table 4; appendix p 19). A similar proportion of patients had vomiting within the first hour after artemether–lumefantrine plus amodiaquine (22 [1·3%] of 1703) versus artemether–lumefantrine (11 [0·6%] of 1721; p=0·055).

No difference in extension of the ECG QTcB-interval was seen at 52 h compared with baseline after treatment with

dihydroartemisinin–piperaquine (mean increase in QTcB 22·1 ms [SD 19·2]) compared with dihydroartemisinin–piperaquine plus mefloquine (20·8 ms [SD 17·8]; p=0·50; appendix p 6). Frequency of QTcB-interval extensions of more than 60 ms compared with baseline was similar between dihydroartemisinin–piperaquine (five [2·7%] of 183), dihydroartemisinin–piperaquine plus mefloquine (six [2·2%] of 269), and artesunate–mefloquine (0 of 73). One patient developed a QTcB-interval of more than 500 ms at 52 h after dihydroartemisinin–piperaquine. The decrease in heart rate after dihydroartemisinin–piperaquine plus mefloquine (mean decrease of 21·8 beats per min [bpm; SD 13·7]) and artesunate–mefloquine (14·5 bpm [13·7]) was less than that after dihydroartemisinin–piperaquine alone (25·8 bpm [SD 15·0]; appendix pp 6, 21). The incidence of bradycardia (ie, ≤54 bpm) at 4 h, 48 h, or 52 h after treatment was similar with dihydroartemisinin–piperaquine and with dihydroartemisinin–piperaquine plus mefloquine (p=0·42; table 4). The QTcB-interval was more extended at 52 h compared with baseline with artemether–lumefantrine plus amodiaquine than with artemether–lumefantrine alone (mean increase of 8·8 ms [SD 18·6] *vs* 0·9 ms [16·1]; p=0·01), and the decrease in heart rate was more pronounced with artemether–lumefantrine plus amodiaquine than with artemether–lumefantrine alone (mean decrease of 29·6 bpm [SD 16·3] *vs* 20·9 bpm [SD 16·9]; p=0·01; appendix pp 6, 21). Overall, bradycardia was more frequent in patients treated with artemether–lumefantrine plus amodiaquine than with artemether–lumefantrine alone (p=0·01).

We saw no haematological differences between any of the treatment groups (table 4). The incidence of mild-to-moderate increases in liver enzymes was similar with all treatments. 20 patients developed a hepatotoxic adverse event that was graded as severe or higher (defined as an alanine aminotransferase, aspartate aminotransferase, or alkaline phosphatase concentration of >5·0×the upper limit of normal [ULN] or total bilirubin >2·5×ULN), with no difference between treatment groups. None of the patients fulfilled Hy's law criteria for liver toxicity (alanine aminotransferase or aspartate aminotransferase >3×ULN and total bilirubin >2×ULN). We found no evidence of nephrotoxicity of the two TACTs, although small increases in serum creatinine were more frequent after amodiaquine-containing TACTs than with the other treatments (table 4).

24 serious adverse events were reported in 1100 patients, of which 11 were judged to be possibly (n=10) or probably (n=1) drug related (appendix pp 23–24). The incidence of serious adverse events was similar after treatment with ACTs or TACTs. In northeast Thailand (Khun Han), one patient died of severe malaria after treatment with dihydroartemisinin–piperaquine; this incident has been reported in detail elsewhere.[5] Two male patients in Myanmar treated with dihydroartemisinin-piparaquine plus mefloquine, progressed to severe malaria in the first 12 h of treatment, and fortunately intravenous artesunate resulted in a rapid clinical recovery. In two patients (one in Myanmar given dihydroartemisinin–piperaquine plus mefloquine and one in Vietnam given dihydroartemisinin–piperaquine) an initial decrease in parasitaemia in the first 12 h after treatment was followed by an increase in parasitaemia, after which intravenous artesunate was started, resulting in rapid parasite clearance. None of these four patients had early vomiting after the study drug. Two young previously healthy males (aged 14 and 17 years), one treated with dihydroartemisinin–piperaquine and one with dihydroartemisinin–piperaquine plus mefloquine, developed sinus bradycardia (<54 beats per min) on the first day of treatment, both interpreted as physiological or possibly related to study drug. One male patient, aged 23 years, who was treated with dihydroartemisinin–piperaquine plus mefloquine developed convulsions at day 2. This event was interpreted as a post-malaria neurological syndrome, which can be associated with use of mefloquine, but generally occurs later in the course after severe malaria.[29] One male child aged 11 years who was given dihydroartemisinin–piperaquine plus mefloquine developed a QTcB interval extension to 503 ms at 52 h, the time of the expected peak level of piperaquine. One male child aged 5 years who was given artemether–lumefantrine plus amodiaquine developed general weakness and a relative bradycardia (45–55 beats per min) at day 2 of enrolment; investigators deemed this event to probably be due to a pre-existing hypokalaemia and malnourished state. The patient recovered after intravenous replacements of electrolytes and fluids.

Assessing the pharmacokinetics of the addition of mefloquine to dihydroartemisinin–piperaquine, the only observed significant drug–drug interaction was a shorter absorption time for piperaquine (T_{max} –28·4%, 90% CI –47·6 to –2·07) when administered with mefloquine (figure 3; appendix p 17). We found a non-significant decrease in the exposure to dihydroartemisinin (–18·8%, –35·1 to 1·53) and piperaquine (–25·1%, –45·5 to 2·93) after adding mefloquine to the first dose of dihydroartemisinin–piperaquine. Exposure to piperaquine after the last dose ($AUC_{T\_lastdose}$) or the piperaquine day 7 concentrations were unaffected by adding mefloquine (figure 3). Adding amodiaquine to artemether–lumefantrine resulted in lower peak concentrations of both artemether (C_{max} –24·9%, 90% CI –42·9 to –1·31) and its active metabolite dihydroartemisinin (C_{max} –32·0%, –51·1 to –5·39), and a non-significant decrease in the exposure to artemether (AUC_T –15·9%, 90% CI –33·1 to 5·77) and dihydroartemisinin (–24·6%, –45·0 to 3·38). We also saw a non-significant decrease in exposure to both lumefantrine (AUC_T –32·0%, 90% CI –72·3 to 67·5) and desbutyl-lumefantrine (–20·0%, –58·3 to –53·4) after the first dose. After the last dose, exposure to both lumefantrine ($AUC_{T\_lastdose}$ –48·4%, 90% CI –64·5 to –25·0%) and desbutyl-lumefantrine (–45·7%, –63·8 to –18·5) were decreased and day-7 plasma lumefantrine concentrations were lower after

artemether–lumefantrine plus amodiaquine (n=148; median 508·5 ng/mL [IQR 305·8–727·8]) than after artemether–lumefantrine (n=152; median 614·5 ng/mL [355·3–1008]; figure 3). A more detailed description on the pharmacokinetic profiles of the study drugs will be reported separately.

Discussion

To our knowledge, this is the first clinical study of the two TACTs, dihydroartemisinin–piperaquine plus mefloquine and artemether–lumefantrine plus amodiaquine. We found that both combinations were highly efficacious in the treatment of uncomplicated falciparum malaria, and were safe and well tolerated. Except for a slight increase in incidence of vomiting within 1 h of treatment, neither combination was associated with more adverse effects than those known for the individual components. The addition of mefloquine to dihydroartemisinin–piperaquine did not further extend the QTc-interval[30] and the addition of amodiaquine to artemether–lumefantrine resulted in small increases in QTc-interval and decreases in heart rate, which do not have clinical importance.

Dihydroartemisinin–piperaquine plus mefloquine was highly efficacious even in areas in Cambodia, Thailand, and Vietnam where dihydroartemisinin–piperaquine alone gave unacceptably high rates of recrudescent infections. Artesunate–mefloquine was also an effective treatment in Cambodia, but this combination is known to be vulnerable to the emergence of mefloquine resistance in artemisinin-resistant parasite populations.[4] In Cambodia, *P falciparum* isolates did not show *Pfmdr1* amplification, the molecular marker of mefloquine resistance, presumably as a consequence of the cessation of drug pressure 5–8 years previously when increasing rates of treatment failure led to artesunate–mefloquine to being abandoned as first-line therapy (unpublished, Dondorp AM). On the Thailand–Myanmar border, artesunate–mefloquine was highly efficacious for over a decade, but mefloquine resistance was rapidly acquired after the arrival of artemisinin-resistant *P falciparum*, a scenario likely to repeat in Cambodia and southern Vietnam.[4] However, the current high efficacy of dihydroartemisinin–piperaquine plus mefloquine in areas with high rates of dihydroartemisinin–piperaquine failure is threatened by worsening piperaquine resistance. Although initial observations suggested that concomitant amplification of *Pfmdr1* and *Pfplasmepsin2/3* was rare, implying the presence of counter-balancing resistance mechanisms,[14,31] in recent years parasites carrying both amplifications have been observed more frequently in Cambodia.[32] In a previous study in healthy volunteers, dihydroartemisinin exposure was reduced by 23% with the addition of mefloquine to dihydroartemisinin–piperaquine.[33] This finding is of concern because of the relatively low dose of dihydroartemisinin in the fixed dose dihydroartemisinin–piperaquine regimen. The reduction in exposure to dihydroartemisinin was not observed in the current study, although parasite clearance half-life was extended in wild-type parasite infections treated with dihydroartemisinin–piperaquine plus mefloquine compared with such infections treated with dihydroartemisinin–piperaquine. In artemisinin-resistant infections, parasite clearance was more rapid with dihydroartemisinin–piperaquine plus mefloquine.

Artemether–lumefantrine plus amodiaquine was well tolerated, with only 1% of patients given this treatment vomiting within 1 h. This rate is lower than the approximately 5% reported in previous studies in which artesunate-amodiaquine was given as a once daily dose.[34] This improved tolerability might be explained by lower peak concentrations of amodiaquine and its active metabolite desethyl-amodiaquine, resulting from splitting the daily dose, which will not affect overall drug exposure.

Amodiaquine and mefloquine both have bitter tastes, which could compromise acceptability in young children. In future pharmaceutical development, masking the taste of both amodiaquine and mefloquine in paediatric formulations might be necessary to optimise treatments in this important age group. Adding amodiaquine resulted in reduced exposures to artemether and the active metabolite dihydroartemisinin and almost 50% lower exposure to lumefantrine after the last dose. The mechanism underlying these interactions is unknown. Nevertheless, clinical efficacy of this TACT was excellent and observed drug concentrations in plasma remained adequate for parasite clearance. Whether higher doses of artemether–lumefantrine should be used is currently uncertain. In Myanmar, Bangladesh, India, and the Democratic Republic of the Congo both artemether–lumefantrine and artemether–lumefantrine plus amodiaquine were highly efficacious with cure rates over 98%, which is in accordance with the observed low prevalence of *Pfkelch13* mutations in these study locations. A trial comparing artemether–lumefantrine with artemether–lumefantrine plus amodiaquine in areas with high levels of multidrug-resistant falciparum malaria in Cambodia and Vietnam is ongoing (NCT03355664). This TACT might be the preferred choice for countries in the eastern Greater Mekong subregion where ACTs are increasingly unsuccessful, and where deployment of artesunate–mefloquine plus piperaquine is suboptimal because of potential resistance to all three components. In the Indian study sites, including in Midnapur, west Bengal, a variety of *Pfkelch13* mutant *P falciparum* strains were observed. These mutations are not in the current list of *Pfkelch13* mutations associated with delayed parasite clearance and were observed at very low frequencies.[27] Parasite clearance half-lives were not extended in these infections, but nearly all were multiclonal admixed with wildtype genotypes, confounding the parasite clearance assessment. The mutations were also different from the *Pfkelch13* mutations reported previously from west Bengal,[35] and might represent low frequencies of background *Pfkelch*

mutations that are not under selection, as also observed in African parasite populations in higher transmission settings.[36]

Our study had several limitations. For instance, it was unblinded. Although this factor could have affected assessment of subjective outcomes, such as symptom severity and attribution of causality of adverse events to study drugs, it is unlikely to have affected objective endpoints, such as treatment efficacy, and measures of cardiac, renal, and hepatic toxicity. The study might have been underpowered to declare non-inferiority between study groups in sites without ACT failure, because the sample size in those areas was based on the detection of changes in parasite clearance half-lives. Another limitation is that children, who carry most of the malaria burden in sub-Saharan Africa, were under-represented with only the site in the Democratic Republic of the Congo explicitly studying this patient group. Our focus initially was on areas where ACT resistance is established, or that are threatened by such resistance due to geographical proximity. A large follow-up project testing artesunate–mefloquine plus piperaquine and artemether–lumefantrine plus amodiaquine with greater focus on sub-Saharan Africa is currently in preparation (NCT03923725 and NCT03939104).

With the increasing failure of conventional ACTs, use of TACTs might become essential for treatment of uncomplicated *falciparum* malaria in the Greater Mekong subregion in the near future. This region is aiming for accelerated malaria elimination before increasing antimalarial drug resistance renders *P falciparum* malaria close to untreatable. The TACTs we studied here could prevent a resurgence of malaria that often accompanies spreading antimalarial drug resistance. Because two well-matched partner drugs provide mutual protection against resistance, deployment of TACTs is expected to extend the useful life of the few effective available and affordable antimalarial drugs. This approach would break the well known repeated historical inefficient sequence in malaria chemotherapy—waiting for resistance to emerge and spread before changing therapy. Fortunately, to date, artemisinin resistance-related delayed parasite clearance has not worsened in southeast Asia and has not spread to or emerged in sub-Saharan Africa, so this class of drugs still provides useful antimalarial activity in combinations. The presented TACTs, combine existing antimalaria drugs and could be made available in the near future and might buy important time before new antimalarial compounds become available. In areas not yet affected by antimalarial resistance, TACTs might have the potential to delay the emergence and spread of antimalarial resistance and could help prevent importation of drug resistance from the Greater Mekong subregion. This study shows that artemether–lumefantrine plus amodiaquine and dihydroartemisinin–piperaquine plus mefloquine are well tolerated, safe, and efficacious TACTs.

Contributors

RWvdP, RMH, DL, ARA, CA, MMu, NW, CJW, MPG, MvV, RMF, PYC, LvS, MD, RJM, DPK, MI, PJ, KL, TMH, KC, RH, CF, EA, MMa, PNN, TTH, NV, FS, SP, AF, OM, JT, NPJD, NJW, and AMD designed the study or were involved in the organisation of the trial, or both, and training of the study teams or data management or both. RT, APP, AuI, PS, SSa, PKB, AT, SB, MO, NHC, YS, SSu, SSr, SM, SO, SY, KC, CS, RR, WK, NTH, NVT, BH, JJC, AKM, JH, MT, KG, TG, TJP, and NTT-N recruited the study participants, collected samples, or took part in laboratory work at the study site or in the central laboratories. RWvdP, RMH, MW, JT, NJW, and AMD generated or analysed the pharmacological data, or both. RA, RDP, CGJ, SG, OM, and MI generated and analysed the parasite genetic data. RWvdP, MMu, NJW, and AMD wrote the manuscript.

Declaration of interests

We declare no competing interests.

Data sharing

De-identified, individual participant data that underlie this Article, along with a data dictionary describing variables in the dataset, are available to researchers whose proposed purpose of use is approved by the Mahidol-University Oxford Tropical Medicine Research Unit data access committee. Related documents such as the study protocol and informed consent form will be made available on request. To request the dataset, please send a signed data request form to datasharing@tropmedres.ac. The data request form can be found online.

For the **data request form** see https://www.tropmedres.ac/files/moru-bangkok-files/2-dataapplicationformv3-16nov2018.docx

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