Cardiovascular safety and population pharmacokinetic properties of piperaquine in African patients with uncomplicated *falciparum* malaria – a pooled multicentre analysis

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Abstract

Dihydroartemisinin-piperaquine has shown excellent efficacy and tolerability in malaria treatment. However, concerns have been raised of potentially harmful cardiotoxic effects associated with piperaquine. The population pharmacokinetics and cardiac effects of piperaquine were evaluated in 1,000 patients, mostly children enrolled in a multicentre trial from 10 sites in Africa. A linear relationship described the QTc-prolonging effect of piperaquine, estimating a 5.90 ms mean QTc-prolongation per 100 ng/mL increase in piperaquine concentration. The effect of piperaquine on absolute QTc-interval estimated a mean maximum QTc-interval of 456 ms (EC₅₀=209 ng/mL). Simulations from the pharmacokinetic-pharmacodynamic models predicted 1.98-2.46% risk of having QTc-prolongation > 60 ms in all treatment settings. Although piperaquine administration resulted in QTc-prolongation, no cardiovascular adverse events were found in these patients. Thus, the use of dihydroartemisinin-piperaquine should not be limited by this concern.
Introduction

Malaria is a life-threatening disease. The World Health Organization (WHO) reported an estimated 219 million malaria cases and 435,000 malaria-related deaths in 2017. Children aged under 5 years are the most vulnerable group, which accounted for 61% (266,000) of all malaria deaths worldwide (1). Dihydroartemisinin-piperaquine is a WHO-recommended artemisinin-based combination therapy (ACT) that has demonstrated excellent efficacy and tolerability in clinical trials (2-8). This drug is given once-daily for three days, administered according to a weight-based regimen (8-10). Dihydroartemisinin has a rapid but short-lived parasite killing effect (half-life of 1-2 hours), responsible for substantial parasite killing during the first three days of treatment. Piperaquine has a long terminal elimination half-life of 20-30 days, and is responsible for eliminating residual parasites. The long half-life also protects against malaria reinfections for up to 30 days post-treatment. (11-13). Electrocardiographic QT-prolongation has been observed in patients and healthy volunteers following piperaquine administration (6, 7, 14, 15). QT-prolongation is a risk factor for polymorphic ventricular tachycardia (torsades de pointes; TdP), which may lead to ventricular fibrillation and sudden cardiac death. However, reported QT-prolongation after piperaquine administration has not been linked to clinically significant cardiovascular adverse events (16-18). A recent, large systematic meta-analysis reported that the risk of sudden unexplained death associated with dihydroartemisinin-piperaquine was not higher than the baseline rate of sudden cardiac death in the age matched population (19). The cardiovascular safety of dihydroartemisinin-piperaquine has been described previously in patients and volunteers. The majority of studies have been in adults. Evaluation and quantification of the relationship between piperaquine concentration and QT-prolongation in children with uncomplicated falciparum malaria in a large-scale multicentre treatment setting is
very limited. The main aim of this study was to develop a population pharmacokinetic-
pharmacodynamic model to describe and quantify the relationship between piperaquine exposure 
and QT-prolongation in order to assess the cardiovascular safety in patients with uncomplicated 
malaria (n = 1,000) from 10 different sites in Africa.

Results

Patient enrolment and demographics

There were 11,028 uncomplicated malaria patients enrolled in the study (Figure S1). A total of 
10,925 were treated with dihydroartemisinin-piperaquine. Dihydroartemisinin-piperaquine was 
well tolerated and highly effective in the treatment of uncomplicated malaria. The risk of 
recurrent symptomatic malaria was low (0.5%), mostly occurring in children younger than 5 
years of age (76%) (2). Adverse events were reported in only 5% of patients. The most 
frequently reported adverse events were graded as mild, including infections and infestations 
(3.24%), and gastrointestinal disorders (1.37%) (17). A total of 1,305 patients were randomized 
to the nested cohort study. Thirty patients were lost to follow-up, 11 patients had baseline mean 
QTcF > 450 ms, and 262 patients had incomplete study procedures (i.e. incomplete 
electrocardiogram (ECG) measurements and/or incomplete pharmacokinetic samples). Data from 
1,000 patients were included for the current pharmacokinetic-pharmacodynamic analysis. Out of 
1,000 patients, 299 were from Burkina Faso, 442 were from Ghana, 89 were from Mozambique, 
and 170 were from Tanzania. Almost 70% of the patients were children aged < 12 years old. The 
proportion of male and female patients was similar (48.2% vs 51.8%, respectively). ECG 
measurements were recorded in patients in the nested cohort study, and maximum QTc-
prolongations occurred on day 3 after piperaquine administration (i.e. after the last dose of 
treatment) in most of the patients. However, no clinical abnormalities as a result of cardiac
adverse events were reported in this study. The study diagram is shown in Figure S1 and the 
baseline demographics are presented in Table 1.

Population pharmacokinetic modelling of piperaquine
A total of 2,989 piperaquine plasma concentrations from 1,000 patients were included in this 
population pharmacokinetic analysis. A total of 1.44% of observed data were below the limit of 
quantification and were omitted during model development. The pharmacokinetic sampling of 
piperaquine was conducted only over 7 days after the first dose resulting in limited ability to 
describe the disposition and elimination phase accurately. Thus, a frequentist prior approach was 
applied, using information from a pooled pharmacokinetic piperaquine meta-analysis (10). The 
prior model used was a three-compartment disposition model with two-transit absorption 
compartments, allometric scaling of body weight on clearance and volume parameters, age-
related enzyme maturation of elimination clearance, and dose-occasion as a covariate on relative 
bioavailability. Implementing this prior approach resulted in a stable model with reasonable 
parameter estimates close to that previously published for piperaquine (Table 2). No additional 
significant covariate relationships were found for the patients studied here. Goodness-of-fits of 
the final model demonstrated an adequate descriptive performance (Figure S2). Simulation-based 
diagnostics (visual predictive check; n = 2,000 simulations) resulted in satisfactory predictive 
performance of the final model (Figure 1). The numerical predictive check (n = 2,000) resulted in 
2.0% (95% CI: 1.8% to 3.1%) and 3.3% (95% CI: 1.9% to 3.1%) of piperaquine observations 
below and above the simulated 95% prediction interval, respectively. The individually predicted 
piperaquine concentration-time profiles from the final pharmacokinetic model were incorporated 
into the pharmacokinetic-pharmacodynamic model to describe the relationship between 
piperaquine concentration and QT-interval (i.e. sequential modelling approach) (20).
QT-interval correction methods

The QT-interval depends on heart rate. To normalize QT-intervals for comparison, a correction is
applied to the RR-interval. This is usually a power function such as that described by Bazett (0.5)
or Fridericia (0.33). A total of 2,907 pre-treatment (Day 1) QT and RR-interval measurements
were used to estimate the optimal study specific rate correction factor. Ordinary linear regression
analysis estimated the correction factor to be 0.476 (95% CI: 0.468 to 0.484). This estimated
study specific correction factor (SSB) was then applied to all QT-interval measurements
throughout the study (QTcSSB). Additionally, separate correction factors were estimated using the
QT and RR-intervals measured on Day 3 and Day 7. The estimated correction factors for Day 3
and Day 7 were 0.442 (95% CI: 0.433 to 0.450) and 0.435 (95% CI: 0.421 to 0.449),
respectively. The QT-interval measurements were then corrected using the specific correction
factor estimated for each day (QTcDAYS). The traditionally used Fridericia (QTcF) and Bazett
(QTcB) corrections were also applied to all data for completion. The slope of the linear
regression between the corrected QT vs RR-intervals were used to evaluate the performance of
each correction method (21). A slope close to zero represents a complete correction of the QTc
calculations across the heart rate range and a consistent and appropriate performance of the
method. The corrected QT-interval using study specific correction factor (QTcSSB) showed the
least dependence on the heart rate with the estimated slope not significantly different from zero
(slope = 0.00180, p = 0.332). All other correction methods showed varying degrees of bias with
regression slopes of 0.00751, -0.0137, 0.0877, for QTcDAYS, QTcB, and QTcF, respectively (p <
0.0001 for all slopes). Linear regression of QTc-intervals and RR-intervals of each correction
method are presented in Figure S3.
Clinical determinants of QTc-prolongation

Statistical analyses were performed to evaluate clinical determinants associated with QTc-prolongation in each patient strata of QTc-prolongation. Patients were categorized based on the threshold limits suggested in the ICH guideline (22). Both absolute QTc-interval and QTc-interval prolongation (i.e. increase from baseline; ΔQTc-interval) were evaluated. The study specific correction factor (QTcSSB, α=0.476) was used in this analysis. Overall, patients had highest QTcSSB-intervals on day 3 after the third and last dose of piperaquine. The prolongation was transient and the QTcSSB-intervals returned to approximately baseline value on day 7 (Figure 2).

When allocating patients into different ΔQTc-interval strata, 64.2% (638/994), 28.8% (286/994), and 7.0% (70/994) of patients presented a ΔQTcSSB-interval ≤30ms, 31-60ms, and >60ms, respectively. The group of patients with ΔQTcSSB-intervals ≤30ms was used as reference for these comparisons. Patients with higher ΔQTcSSB-intervals had significantly higher body temperature at enrolment (p < 0.0001). The baseline QTcSSB-interval at enrolment was significantly different in each subgroup (p < 0.001), where patients with longer ΔQTcSSB-intervals had shorter baseline values. Additionally, the median piperaquine peak concentration (Cmax) was significantly higher (p < 0.001) in patients with ΔQTcSSB-interval of 31-60ms, but it did not reach significance in patients with QTcSSB-interval >60ms (p = 0.083), most likely due to a much smaller number of patients in this group. No other clinical determinants were significantly different between groups.

When allocating patients into different absolute QTc-interval strata, 48.4% (481/994), 41.0% (408/994), 7.8% (78/994), and 2.7% (27/994) of patients presented a maximum QTcSSB-interval ≤450ms, 451-480ms, 481-500ms, and >500ms, respectively. The percentage of female/male
patients was similar in all strata (approximately 50%). The baseline QTcSSB-interval at enrolment was significantly different in each strata (p < 0.001), where patients with longer QTcSSB-intervals also had higher baseline values. The median piperaquine $C_{\text{max}}$ was significantly different among the groups (p < 0.0001), with a gradual increase with increasing QTcSSB-interval. No other covariates were significantly different between groups. The results of the statistical analysis of clinical determinants of QTc-prolongation are shown in Table 3.

The possible drug-drug interaction of piperaquine and concomitant medications that prolong QTc-interval was also investigated. There were twelve patients that received at least one medication, listed on www.Crediblemeds.org (accessed: 2019-06-19) as drugs that prolong the QTc-interval, during the study period. The concomitant medications were metronidazole, ketoconazole, fluconazole, ciprofloxacin, furosemide, and metoclopramide. Among these twelve patients, five patients had a $\Delta$QTcSSB-interval ≤30ms, six patients had a $\Delta$QTcSSB-interval of 31-60ms, and one patient had a $\Delta$QTcSSB-interval >60ms. With respect to absolute QTc-interval, seven patients had a QTcSSB-interval ≤450ms, four patients had a QTcSSB-interval of 451-480ms, and one patient had a QTcSSB-interval of 481-500ms.

**Relationship between piperaquine concentration and QTc-interval**

To quantify the magnitude of absolute QTc-interval prolongation resulting from piperaquine administration, a population pharmacokinetic-pharmacodynamic analysis was performed. The absolute QT-intervals were corrected for heart rate using the study specific correction factor (QTcSSB,) and evaluated with nonlinear mixed-effects modelling. There was a 4.87 ms QTc-prolongation per 100 ng/ml increase in piperaquine plasma concentration when described by a linear concentration-response relationship. This model was improved further by implementing an $E_{\text{max}}$ function ($\Delta$OFV = -1886). A stepwise covariate search demonstrated that potassium...
concentration had a significant impact on the estimated QTc-interval at baseline (QTcBaseline) and that age influenced EC50 significantly. This covariate effect estimated that 1 mmol/L increase in potassium level resulted in 1.06 ms (0.25%) decrease in QTcBaseline, but the variation in potassium concentrations over the study period was relatively narrow (IQR: 3.70-4.48). Thus, the effect of potassium level on the QTcBaseline was considered to have negligible clinical relevance and was not included as a covariate in the final model. Age as an effect on EC50 was the only covariate that was retained in the final model (ΔOFV = -53.3). The parameter estimates from the final pharmacokinetic-pharmacodynamic model describing the effect of piperaquine on the absolute QTc-intervals are summarized in Table 4. Goodness-of-fits and visual predictive checks of the final model are shown in Figure 3.

Separate analyses describing the relationship of piperaquine concentration and ΔQTc-interval were conducted using four different correction methods (QTcF, QTcB, QTcSSB, and QTcDAYS). A linear relationship of piperaquine concentrations vs ΔQTcF, ΔQTcB, ΔQTcSSB, and ΔQTcDAYS estimated a QTc-prolongation of 7.97ms, 5.30ms, 5.90ms, and 4.11ms, respectively, per 100 ng/ml increase in piperaquine concentration. Further details of these analyses are provided in the supplementary material.

Population-based simulations of clinical scenarios

The final pharmacokinetic-pharmacodynamic model describing the effects of piperaquine on the absolute QTc-interval was used for large-scale Monte Carlo population simulations of possible clinical scenarios. The simulations included two settings; acute treatment of symptomatic malaria (full 3-day treatment course) and mass drug administration (full 3-day treatment course given once a month for a total of 3 months). Two different dosing recommendations for dihydroartemisinin-piperaquine were evaluated; old recommendation (2nd edition) and new
recommendation (3rd edition) of the WHO guidelines for the treatment of malaria (Table 5).

Overall, the simulated maximum QTc-interval ($QTc_{\text{max}}$) and maximum QTc-prolongation ($\Delta QTc_{\text{max}}$) when using the old and new recommended doses demonstrated similar results when used in both acute treatment and mass drug administration scenarios. For acute malaria treatment, the median predicted $QTc_{\text{max}}$ values were 440ms (95% CI: 401-489ms) and 441ms (95% CI: 401-490ms) for the old and new piperaquine dosing regimens, respectively. In a mass drug administration setting, the median predicted $QTc_{\text{max}}$ values were 440ms (95% CI: 401-490ms) and 441ms (95% CI: 402-491ms) for the old and new piperaquine dosing regimens, respectively. The simulated total probability of having a QTcSSB-interval above 500ms was 1.1% (11 in 1,000 patients) and 1.2% (12 in 1,000 patients) in acute treatment of malaria using the old and new piperaquine dosing regimen, respectively. Similarly, the simulated total probability of having a QTcSSB-interval above 500ms was 1.2% (12 in 1,000 patients) and 1.3% (13 in 1,000 patients) in mass drug administration settings using the old and new piperaquine dosing regimen, respectively. The probability of having $QTc_{\text{max}} > 500$ms, stratified by body weight, is shown in Figure S5. The simulations showed that predicted $\Delta QTc_{\text{max}}$ values of more than 60ms were infrequent (1.98-2.25%). Acute treatment resulted in a predicted median $\Delta QTc_{\text{max}}$ of 16.8ms (95% CI: 2.31-56.9ms) and 18.0ms (95% CI: 2.67-58.6ms) for the old and new piperaquine dosing regimens, respectively. Similarly, the median predicted $\Delta QTc_{\text{max}}$ was 17.6ms (95% CI: 2.58-57.9ms) and 18.5ms (95% CI: 2.90-59.3ms) for the old and new piperaquine dosing regimens given in a mass drug administration scenario. Simulated maximum QTc-intervals stratified by body weight are shown in Figure 4. The distribution of predicted QTc$_{\text{max}}$ and $\Delta QTc_{\text{max}}$ of each clinical scenario is shown in Figure 5. The probability of having QTc$_{\text{max}}$ and $\Delta QTc_{\text{max}}$ at different threshold levels is shown in Table S3.
Discussion

Dihydroartemisinin-piperaquine was well tolerated and highly effective in the treatment of uncomplicated malaria as reported previously in this and earlier studies. (2, 17, 18). This analysis focused on electrocardiographic QT-prolongation, a risk factor for ventricular tachyarrhythmias (TdP). Piperaquine, like several other aminoquinolines and structurally related antimalarial drugs, prolongs the QT-interval. Halofantrine was an antimalarial drug which caused marked QT-prolongation and was associated with sudden death. It has now been discontinued, but concern over a “class effect” remains. This study sought to characterize the relationship between piperaquine plasma concentrations and QT-prolongation, and thereby gauge the potential risk of dangerous tachyarrhythmias. No clinical abnormalities as a result of cardiac adverse events were reported in this post-licensing study. The individual QTc-prolongations recorded in the nested pharmacokinetic and electrocardiographic cohort were not associated with any clinical abnormalities, and 89% of all patients returned to within 20ms of their baseline values on day 7.

Population pharmacokinetic modelling of piperaquine

The use of a frequentist prior model described the population pharmacokinetic properties of piperaquine adequately. Indeed, the application of this technique stabilized the estimation of pharmacokinetic parameters since the study data alone provided insufficient information to generate plausible parameter estimates. Thus, the final pharmacokinetic parameter estimates in this study were generally in agreement with those reported in the prior model (10). The prior model was developed using pooled individual patient data from 11 clinical studies (8,776 samples from 728 individuals) including participants aged 0.56-55 years (68% were children aged <12 years old) with uncomplicated malaria (93%) and healthy volunteers (6.9%) from South East Asia (35%) and Africa (65%). These characteristics of the meta-analysis study were...
considered similar to the current study population that patients were African and 69% of the patients were aged < 12 years old. The inclusion of a wide range of different patients enabled the identification and quantification of several biologically important covariates, including the impact of body weight and age on the elimination of piperaquine. The inclusion of these covariates when fitting the prior model to the data collected in this study provided results that are more generalizable and therefore of higher confidence when using the developed model for simulations. The predicted piperaquine concentrations from the final model were in agreement with the observations, demonstrating that the model was adequate for further pharmacokinetic-pharmacodynamic analysis and translational simulations.

**QT-interval correction methods**

Several different rate correction approaches of the QT-interval have been proposed with no clear consensus. Among the four different correction methods evaluated, unsurprisingly the correction based on the study specific data performed better than the two most commonly used correction methods (Bazett and Fridericia). The correction factor estimated on pre-treatment data alone (QTcSSB) showed an overall better performance than using specific corrections calculated at each day of follow-up (QTcDAYS). The reason for this might be an overfitting of the data when estimating correction factors for each day separately. However, when estimating separate correction factors on day 1, 3 and 7, the estimated correction factor decreased gradually when people were recovering from malaria (α = 0.476, 0.442, 0.435). Thus, this might explain why the Bazett correction (α=0.5) shows an advantage against the more widely used Fridericia correction (α=0.33) in most patient studies, whereas the opposite is true in healthy volunteer studies. The study specific correction factor showed no residual trend when evaluating the relationship between corrected QTc-intervals and RR-intervals. Thus, suggesting this to be the most
appropriate correction factor in the study population. The estimated correction factor of 0.476 (95%CI: 0.468 to 0.484) was higher compared to the estimated correction factor of 0.4, previously reported in Karen patients with uncomplicated malaria on the Thailand-Myanmar border (23). A study in infants and young children (aged 1 month to 5 years) with sensorineural hearing loss who underwent ECG screening for congenital long QT syndrome, demonstrated that the Bazett correction (α=0.5) was the most appropriate correction among the commonly used methods (21). However, the computed slope of the regression between QTc and RR-intervals, using varying correction factors (α) from 0.3 to 0.6, demonstrated that the correction factor α = 0.48 generated a slope equal to zero. This value is very similar to the correction factor estimated in the current study (α =0.476), including mostly children under the age of 5. The estimated correction presented here was based on a large number of patients from 10 different sites at different ages and body weights, and should therefore be representative of the typical malaria patients in Africa. Estimation of individual correction factors, using linear mixed-effect modelling was also attempted using pre-treatment data. However, only one triplicate ECG measurement during the pre-treatment period was available for each patient, which was insufficient to estimate an individual correction factor precisely. Study specific correction factors or individual correction factor should be used when possible, and if data allow these to be estimated reliably. However, the Bazett correction can be used when data or study design do not allow for these study specific or individual correction factors to be estimated.

Clinical determinants of QTc-prolongation

The statistical analysis performed in the current study evaluated and identified biological factors which might influence the QTc-interval, especially in the subpopulations presenting with extreme QTc-prolongation. The analysis revealed that in patients who had ΔQTcSSB-interval
>60ms also had a significantly shorter baseline QTcSSB-interval compared to the other two groups. Moreover, they had the highest median body temperature at enrolment (37.6 °C, IQR: 36.8-38.6). Fever has been identified as a factor associated with QTc-prolongation in patients with congenital long QT syndrome (24, 25). However, in the general population, fever has been reported as a factor associated with QTc-shortening (24, 26, 27). This might partly explain the shorter baseline QTcSSB-interval in this group of patients, and therefore also the apparent large QTcSSB-interval prolongations as patients recover from malaria. The highest QTcSSB-prolongation in most of the patients was observed on day 3 after the last dose of piperaquine administration. This occurred approximately at the same time as patients recovered from fever, and might therefore reflected an additional QTc-prolongation on top of the drug effect. Previous studies report a mean QTc-prolongation of 11-18ms (comparing baseline and day 3 values with varying heart rate correction methods) in patients receiving antimalarial treatments unlikely to increase the QTc-interval (i.e. mefloquine and sulfadoxine-pyrimethamine). The QTc-prolongation reported in these studies was explained preliminary by the resolution of fever associated with the recovery from the malaria disease (23, 28, 29).

The analysis on the absolute QTc-interval found a total of 27 (2.7%) patients with an observed maximum QTcSSB-interval >500ms and 19 patients (70.4%) in this group also had ∆QTcSSB-interval >60ms. These patients also had longer median baseline QTcSSB-interval (435ms, IQR: 425ms-450ms) compared to other groups. The longer baseline QTcSSB-interval might have resulted in a longer absolute QTcSSB-interval at a given piperaquine concentration, compared to patients with lower baseline values. Furthermore, the median piperaquine C_{\text{max}} was significantly higher in patients with a maximum QTcSSB-intervals >500ms compared to those with QTcSSB-intervals ≤450ms, but not different from patients with QTcSSB-intervals 451-480ms. Thus,
piperaquine concentrations alone did not put patients at high risk of QTcSSB-intervals >500ms. This could be partially explained by the non-linear relationship between piperaquine concentration and QTc-prolongation. Other factors associated with QTc-prolongation were not significantly different between the groups described here. One patient received ciprofloxacin for treatment of dysentery, started on the same day as piperaquine was given (no specific stop date recorded). This patient had maximum ΔQTcSSB-interval of 72ms and maximum absolute QTcSSB-interval of 482ms on day 7. Although, there were no clinical cardiovascular events occur in this patient, to minimize the risk of cardiovascular events, the use of medications known to prolong QTc-interval should be avoid or used only when the benefits outweigh the risks during piperaquine administration.

Relationship between piperaquine concentration and QTc-interval

The final pharmacokinetic-pharmacodynamic model of the absolute QTc-interval described the data adequately. The estimated QTcSSB-interval at enrolment in the malaria patients in this study was 421ms with an inter-individual variability of ±17.0ms. This is close to the upper end compared to healthy adults, who commonly have QTc-intervals in the range of 400-423 ms (33). Several factors are known to affect the QT-interval e.g. physical activity, food consumption, genetic factors, age, sex, heart rate, stress, circadian rhythm, and electrolyte imbalances (34, 35). Among these factors, heart rate and circadian rhythm are known to have a large impact and should be taken into consideration when evaluating the effect of drugs on the QT-interval. The correction for heart rate was performed to account for this potential bias. Moreover, the effect of circadian rhythm was evaluated using a cosine function. However, this did not improve the model fit significantly. This is most likely a consequence of ECG measurements being collected only from 8am to 8pm in this study. Thus, it was not possible to
estimate precisely the fluctuation of the QT-interval through the whole 24-hour period. Maximum daily fluctuations of the QT-interval have been reported to be 6.75-7.80 ms in healthy adult subjects (34, 36). However, this value could not be incorporated a priori in the model since the circadian pattern in malaria patients has not been well characterized. A thorough QT study, with measurements over a 24-hour period, in malaria patients receiving antimalarial drugs that do not have an effect on the QT-interval would further our understanding of the circadian rhythm in malaria patients and could benefit cardiotoxicity evaluations of antimalarial drugs in future clinical trials. The $E_{\text{max}}$ relationship of piperaquine exposure and QTc-interval was superior to a linear function in this study, which might be due to a large number of available samples and a wider range of observed piperaquine concentrations, demonstrating a plateau in the QTc-response at high piperaquine concentrations. Age was a significant covariate on EC$_{50}$, resulting in lower EC$_{50}$ values in young children compared to adults, suggesting a relatively greater QT-prolongation in young children compared to adults at equivalent piperaquine concentrations. This should be taken into consideration when evaluating the safety of piperaquine in young children.

From the separate analysis of $\Delta$QTc-interval (in Supplementary material), the estimated QTc-prolongation of 5.90 ms per 100 ng/ml increase in piperaquine concentration was similar to that reported previously in Cambodian malaria patients (5.00 ms QTc-prolongation) and healthy Thai volunteers (4.17 ms QTc-prolongation, using an individual correction method) receiving dihydroartemisinin-piperaquine (30, 31). A study performed in healthy volunteers who received artefenomel (OZ439) in combination with piperaquine reported a similar effect of piperaquine of 4.75 ms QTc-prolongation per 100 ng/ml increase in piperaquine concentration (32).
Population-based simulations of clinical scenarios

Both acute malaria treatment and mass drug administration showed similar patterns of predicted QT-prolongation with the old recommended WHO treatment and the new increased piperaquine dosage in young children. The simulations suggested that the proportion of piperaquine-related maximum QT-prolongation of more than 60ms in all settings were relatively small. However, the main concern for potentially dangerous cardiotoxicity is those patients or subjects with the highest drug levels and the greatest QTc-prolongation (i.e. >500ms). The simulated total probability of having a QTc_{SS50}-interval above 500ms was <2% in all simulated scenarios. No potential risk of piperaquine accumulation that may cause higher risk of QTc-prolongation.

These results were in agreement with a study in healthy volunteers receiving a standard three-day dose of dihydroartemisinin-piperaquine for three consecutive months, which demonstrated that the average increase in QTcF-intervals were comparable between the first-month and third-month of dosing and there was no evidence of cumulative cardiotoxicity reported (37).

Although it is clear that piperaquine increases the QT-intervals when used in acute malaria treatment and mass drug administration efforts, these QTc-prolongations have not resulted in severe cardiac events and sudden death (17, 18). Furthermore, the increases in QTc-intervals observed in this study returned to baseline without any treatment intervention. The results from in vitro animal studies suggested that although piperaquine affect the human ether-à-go-go-related gene (hERG) potassium channel, it demonstrated a low potential to induce TdP, either alone or in combination with dihydroartemisinin (38). In addition, a recent pooled meta-analysis of nearly 200,000 exposed individuals demonstrated that dihydroartemisinin-piperaquine was associated with a low risk of sudden unexplained death, which was not higher than the baseline rate of sudden cardiac death (19).
Limitations

There were some limitations associated with this study. An accurate and unbiased evaluation of drug-induced QT-prolongation can be problematic, especially in patients with malaria since the disease itself affects the electrocardiogram and heart rate. The highest QTc-prolongation observed on day 3 after the last dose of piperaquine, coincide with the recovery of malaria. Moreover, the patients enrolled here had no known predisposing factors for arrhythmias or cardiac conditions. Patients with family history of sudden death, patients taking drugs that prolong the QT-interval, and patients who had baseline QTc-interval >450ms were excluded from the study. Thus, results from this analysis may not be representative of patients who are predisposed of having cardiovascular risks and events.

In the current study, dihydroartemisinin-piperaquine was given in fasted patients, and food effects could not be evaluated. A recent study demonstrated that concomitant food, especially high-fat and high-caloric food, increase piperaquine exposure, resulting in an increased QTc-prolongation, suggesting that dihydroartemisinin-piperaquine should be given in the fasting state (39). Although high-fat meals should be avoided, normal meals do not substantially alter the absorption of piperaquine (8). The simulations in the mass drug administration setting were extrapolated from acute malaria treatment in the current study. Several clinical factors could be different between the populations receiving mass drug administration compared to acute malaria patients. The QTc-prolongation may be more pronounced in acute malaria treatment compared to a healthy population or individuals with asymptomatic infections. However, we have shown that there was no accumulation in the risk of QTc-prolongation associated with repeated piperaquine administration for prophylactic or elimination treatment.

In conclusion, no clinical abnormalities related to cardiovascular adverse event were reported in the 1,000 patients receiving dihydroartemisinin-piperaquine in this study. The developed
population pharmacokinetic-pharmacodynamic model described the relationship between QTc-prolongation and piperaquine concentrations, resulting in increasing QTc-intervals with increasing concentrations. Simulations from the developed model suggested that the risk of QTc-prolongation was similar in the previously and newly recommended dose regimen of dihydroartemisinin-piperaquine, and there were no accumulation of cardiotoxicity associated with mass drug administration over several months. Although piperaquine increases the QTc-interval, clinical studies have demonstrated that piperaquine had a low potential to induce TdP and have a low risk of sudden unexplained death. Therefore, in the life-saving setting and the limited availability of effective treatment in the area, the concern about cardiotoxicity should not limit the current use of dihydroartemisinin-piperaquine. However, symptomatic screening of cardiac conditions and factors associated with QTc-prolongation is recommended to diminish the risk of undesirable adverse events.
Methods

Study design and patient enrolment

This was a post-licensing pharmacovigilance study, evaluating the safety of dihydroartemisinin-piperaquine (Eurartesim®) conducted in patients with uncomplicated malaria from four African countries, including Ghana, Tanzania, Burkina Faso, and Mozambique. The study was designed as a prospective, observational, open-label, non-comparative, multicentre study. The protocol was approved by the institutional and national ethics committees in all four countries before patients were enrolled into the study. The study was registered on Clinical trials.gov on May 1, 2013 (NCT02199951). Written informed consent was obtained from all patients or from their parents or guardians if they aged <18 years. Patients were enrolled into two groups, the main study, and the nested cohort study with identical inclusion and exclusion criteria. In the main study, it was planned to collect data from 10,000 patients who received dihydroartemisinin-piperaquine treatment to assess the treatment outcome and adverse events. In the nested cohort, collection of data was planned in 1,000 patients to assess the effect of dihydroartemisinin-piperaquine on electrocardiography. The study flow-chart can be seen in Figure S1 Patients with confirmed uncomplicated malaria infection were recruited from the outpatient departments of the public health centres in each study site. The inclusion criteria were (1) age ≥6 months, (2) weight ≥5 kg, (3) ability to tolerate oral medication, and (4) willingness to participate in the study based on written informed consent. The exclusion criteria were (1) allergy to artemisinin or piperaquine, (2) history of taking dihydroartemisinin-piperaquine in the previous 4 weeks, (3) pregnant women, (4) lactating women, (5) severe malaria, (6) history of taking medicinal products that are known to prolong the QT-interval (i.e. anti-arrhythmic, neuroleptic and certain antimicrobial agents), (7) family history of sudden unexplained death, (8) personal or family
history of predisposing cardiac conditions for arrhythmia/QT-prolongation (including congenital long-QT syndrome, arrhythmia, and any known QTcF or QTcB of more than 450ms).

Laboratory and ECG measurements

Detailed laboratory assessments were performed at each visit including white blood cell and red blood cell counts, haemoglobin level, platelet count, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, blood urea nitrogen (BUN), serum potassium, and serum chloride. Parasite count was performed under microscopy using thick blood smears. The parasite density was calculated based on 200 white cell counted, assuming 8000 white cells/µL. Blood samples for pharmacokinetic analysis were collected randomly for each patient at approximately 0, 48, 52, 120, 144, 168 h after the first dose of dihydroartemisinin-piperaquine administration (1-5 samples/patient). ECG measurements were performed using the 12-leads digitalized ELI 150 Cardiograph® provided by CardiaBase (40). ECG measurements were collected in all patients pre-dose on Day 1 (baseline), pre-dose on Day 3, post-dose on Day 3, and on Day 7. All measurements were recorded once per occasion except on Day 1 (baseline) and post-dose on Day 3, which were recorded in triplicate with 1-2 minute intervals between each reading. The positioning of the 12 leads was standardized for all ECG measurements. The ECGs were recorded at least three hours apart from food intake and were taken from patients in a relaxed supine position in a quiet room. The ECGs were read by trained and ECG-certified study clinicians. The ECG readers were blinded for the time and the day of the ECG recording. A computer-assisted, semi-automatic, on-screen measurement of the digital ECG waveform was used for the reading (ECG Manager®). The ECG reading of each particular patient was performed by the same cardiologist. The QTcF was calculated automatically and the QT interval was verified by the study clinician. Participants with an average QTcF of ≥ 450 ms
were excluded from the study and prescribed alternative anti-malarial medicines. The complete
details of ECG measurement method have been described in previous studies (17, 18).

Drug administration

Dihydroartemisinin-piperaquine was administered based on body weight as a once daily dosing
for 3 days (Table 5). Paediatric (20/160 mg) and adult (40/320 mg) fixed-dose formulations of
dihydroartemisinin/piperaquine were used in children and adults, respectively.

Dihydroartemisinin-piperaquine administration was directly observed by the research team for
all 3 days of dosing in all patients. The drug was given with water and patients were encouraged
to avoid food intake for 3 hours before and after dosing. In small children, the tablets were
crushed and given on a spoon with water. The dissolution profiles for the crushed and whole
tablets of dihydroartemisinin-piperaquine were superimposed, indicating that the absorption
should be the same in children taking either a crushed or a whole tablet (41). A full dose was re-
administered in patients who vomited within 30 minutes after drug administration. For patients
vomiting between 30 and 60 minutes after drug administration, half dose was re-administered.

Re-dosing was performed once and rescue treatment was given for unsuccessful re-dosing.

Piperaquine quantification

Piperaquine plasma samples were shipped on dry ice to the Department of Clinical
Pharmacology, Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand, for drug
quantification and population pharmacokinetic-pharmacodynamic analyses. Piperaquine plasma
concentrations were determined using solid-phase extraction followed by liquid chromatography
coupled with tandem mass spectrometry as per published method (42). The lower limit of
quantification (LLOQ) was set at 1.50 ng/ml. Three replicates of quality control samples at low,
middle, and high concentration (4.50, 20.0, and 400 ng/ml) were analysed within each batch of
clinical samples to ensure accuracy and precision of the drug assay. The relative standard deviations (%CV) were less than 6% for all quality control samples.

**Population pharmacokinetic modelling of piperaquine**

The population pharmacokinetic analysis was performed using nonlinear mixed-effects modelling in NONMEM software, version 7.3 (Icon Development Solution, Ellicott City, MD). The first-order conditional estimation method with interaction (FOCE-I) was used throughout the model development. Piperaquine has a long terminal elimination half-life of approximately 18-28 days, however, the plasma samples were collected only for up to 7 days. A frequentist prior approach (43) was implemented to stabilize the estimation and avoid structural model misspecification due to this limited sampling design. Regarding to this, the SPRIOR record was implemented to allow a Bayesian penalty function to be added to the NONMEM objective function. This constrains the parameter estimates (i.e. fixed and random effects) in the model, thus stabilizing the estimates obtained from insufficient and/or uninformative data. The prior pharmacokinetic parameters of piperaquine were adopted from an individual participant data meta-analysis (8,776 samples from 728 individuals) which included children aged < 12 years (68%) and adults (32%) with uncomplicated malaria (93%) and healthy volunteers (6.9%) from South East Asia (35%) and Africa (65%) (10). The prior model consisted of a three-compartment disposition model with two-transit absorption compartments. The identified and quantified covariates were body weight (fixed allometric function), enzyme maturation (maturation half-time of 7 months), and dose occasion (24% increased relative bioavailability between each consecutive dose of piperaquine). The model structure, parameter estimates, and covariate effects of this model were applied using a frequentist prior methodology as described above.
Pharmacokinetic parameters were assumed to be log-normally distributed and the inter-individual variability were implemented as an exponential function (Equation 1).

\[ \theta_i = \theta \times e^{\eta_i} \]  

where \( \theta_i \) represents individual i’s parameter estimate, \( \theta \) represents the typical parameter estimate in the population, and \( \eta_i \) represents the inter-individual variability for individual i, which is normally distributed with a zero mean and variance \( \omega^2 \). Inter-occasion variability between dose occasions was investigated on absorption parameters (Equation 2).

\[ \theta_{ij} = \theta \times e^{\eta_{ij} + \kappa_{ij}} \]  

where \( \kappa_{ij} \) represents the inter-occasion variability of the pharmacokinetic parameter \( \theta \) at the \( j \)'th occasion. The residual unexplained variability was assumed to be additive on a logarithmic scale.

Body weight was implemented as an allometric function on all clearance (exponent of 0.75) and volume of distribution (exponent of 1) parameters. Additionally, the effect of the enzyme maturation process of clearance in young children was also applied (Equation 3).

\[ CL_i = TVCL \times \frac{AGC_{Hill}}{MF_{50} + AGC_{Hill}} \times e^{\eta_{i,CL}} \]  

where \( CL_i \) represents the individual clearance parameter, \( TVCL \) represents the typical population mean value of the elimination clearance, \( AGC \) represents individual age, \( MF_{50} \) represents the age corresponding to 50% enzyme maturation, and \( Hill \) represents the slope of the maturation function. Physiologically relevant demographic covariates, including body temperature, malaria parasite count, hemoglobin, total bilirubin, AST, ALT, serum creatinine, BUN, potassium, and chloride were investigated with a stepwise covariate approach. A stepwise forward inclusion and
backward deletion approach (p < 0.05 and < 0.001 for forward and backward step, respectively) were implemented in PsN.

**QT-interval correction methods**

All QT-intervals were corrected for heart rate before further analyses were conducted. Four different correction methods were used to calculate QTc-intervals. The most commonly used formulas are the Fridericia correction (QTcF, Equation 4) and Bazett correction (QTcB, Equation 5). The QTcF- and QTcB-intervals were used as a reference in this study.

$$\text{QTcF} = \frac{QT}{\sqrt{RR}} = QT \times RR^{-0.33}$$  \hspace{1cm} (4)

$$\text{QTcB} = \frac{QT}{RR} = QT \times RR^{-0.5}$$ \hspace{1cm} (5)

where QT represents the measured QT-interval in ms and RR represents the RR-interval in second. Study specific correction factors were investigated using the observed data generated in this study. Pre-treatment QT and RR measurements were transformed into their natural logarithms and used to estimate a study specific correction factor by applying a simple linear regression analysis (Equation 6).

$$\ln(QT) = \beta + \alpha \times \ln(RR)$$ \hspace{1cm} (6)

where $\beta$ represents the intercept and $\alpha$ represents the slope of the regression. The estimated slope of the regression model was implemented as the correction factor and applied to all QT-interval measurements in the study (Equation 7):

$$\text{QTc} = QT \times RR^{-\alpha}$$ \hspace{1cm} (7)

Furthermore, QT-interval measurements were also stratified on the day of measurement (i.e. Day 1, Day 3, and Day 7) and a specific correction factor was estimated for each day and applied as a
correction for data collected on that day (\(\text{QTc}_{\text{DAYS}}\)). QT-intervals, corrected by the four methods (\(\text{QTc}_F\), \(\text{QTc}_B\), \(\text{QTc}_{\text{SSB}}\), and \(\text{QTc}_{\text{DAYS}}\)), were used to investigate the effect of piperaquine on QTc-prolongation. The slope of the linear regression between QTc vs RR-intervals were used to evaluate the performance of each correction method (21). A slope close to zero represents a complete correction of the QTc calculations across the heart rate range and a consistent and appropriate performance of the method.

Clinical determinants of QTc-prolongation

According to the ICH-E14, the guidance for clinical evaluation of QT/QTc Interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs (44), the following categories of QTc-interval prolongation should be used as a reference limits for clinical analysis; absolute QTc-interval \(\leq 450\text{ms}\), \(451-480\text{ms}\), >\(481-500\text{ms}\), and >\(500\text{ms}\), and \(\Delta\text{QTc}\)-interval \(\leq 30\text{ms}\), 31-60ms, and >60ms. These categories were used as a reference in this study for the categorical analysis. Factors associated with the risk of QTc-interval prolongation were evaluated in each of the above category of QTc-interval prolongation. The list of the non-drug factors associated with QTc-interval prolongation (moderate to high quality of evidence) and the list of drugs that prolong QTc-interval stated at www.Crediblemeds.org as known, possible, and conditional risk of TdP were used as references (45). Non-drug factors associated with QTc-prolongation included in the analysis were female, age, potassium level, hypokalaemia (<3.5 mmol/L), body temperature, and QTc-interval at enrolment. Potential differences between groups were compared with Kruskal-Wallis test or Dunn’s test for continuous and Chi-square test or Fisher’s exact test for categorical variables.
Relationship between piperaquine concentration and QTc-interval

Individually predicted piperaquine concentration-time profiles were constructed based on the Empirical Bayes (“post hoc”) estimates (EBEs) from the final population pharmacokinetic model. The relationship between piperaquine concentrations and the absolute QTc-interval was evaluated using the best performing QT-interval correction method from previous stage. The linear and $E_{\text{max}}$ functions were evaluated as shown in Equation 8 and 9, respectively.

$$QTc(t) = (QTc_{\text{Baseline}} + \eta) + (\text{Slope} \times Cp) + \epsilon_i$$  \hspace{1cm} (8)

$$QTc(t) = (QTc_{\text{Baseline}} + \eta) + \left(E_{\text{max}} \times \frac{C_p^\gamma}{C_p^\gamma + EC_{50}^\gamma}\right) + \epsilon_i$$  \hspace{1cm} (9)

where $QTc_{\text{Baseline}}$ represents baseline value of the QTc-interval (ms) at enrolment. Slope represents the slope of the linear relationship of piperaquine and QTc-interval (QTc-prolongation in ms per 100 ng/ml), $C_p$ represents the piperaquine concentration (ng/ml), $E_{\text{max}}$ represents the maximum QTc-interval (ms) achieved at infinite drug concentration, $EC_{50}$ represents the piperaquine concentration (ng/ml) generating half of maximum drug effect, $\gamma$ represents the hill factor, $\eta$ represents the inter-individual variability and $\epsilon_i$ represents the residual error. The influence of patient characteristics on pharmacodynamic parameters was investigated using a stepwise covariate approach as described in the pharmacokinetic model building process.

Model diagnostics and evaluation

Model diagnostics and automation were performed using Xpose version 4.0, Pirana, and Pearl-speaks-NONMEM (PsN; version 3.6.0). Goodness-of-fits and simulation-based diagnostics were used to evaluate the descriptive and predictive performances of the model. The robustness of parameter estimates from the final model was performed using a 1,000 bootstrap runs. Numerical and visual predictive checks ($n=2,000$) were used to evaluate the predictive performance of the model.
final model. The individual parameter estimates from final pharmacokinetic model were used further to generate the concentration-time profiles of piperaquine for the pharmacokinetic-pharmacodynamic analysis.

**Population-based simulations of clinical scenarios**

The updated dosing recommendation for dihydroartemisinin-piperaquine in the latest edition of the WHO Guidelines for the Treatment of Malaria (2015) suggested an increased dosage in young children (8). This adjustment was a strong recommendation based on pharmacokinetic modelling (9, 10). The final pharmacokinetic-pharmacodynamic model describing the effect of piperaquine on the absolute QTc-interval was used to simulate the impact of the newly recommended dose as well as the previous recommendation. The impact on absolute QTc-interval was assessed both in acute treatment and in mass drug administration settings.

Once daily dosing of dihydroartemisinin-piperaquine for three days was simulated to evaluate the cardiac safety of the different dosing regimens in the acute malaria treatment scenario. For mass drug administration, a full 3-day treatment regimen of dihydroartemisinin-piperaquine, once a month for a total of 3 months was simulated to evaluate the cardiac safety of the different dosing regimens. The detailed dosing regimens used are summarized in Table 5. The relationship between age and body weight in the study population was used to assign an age to each simulated patient at different body weights. A total of 480,000 patients were simulated, including 5,000 patients at each body weight (5 to 100 kg) for each dosing scenario.
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Transparency declarations

All authors declare that they have no competing interests.

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References

1. World Health Organization. 2018. World Malaria Report 2018. World Health Organization, Geneva, Switzerland.

2. Adjei A, Narh-Bana S, Amu A, Kukula V, Nagai RA, Owusu-Agyei S, Oduro A, Macete E, Abdulla S, Halidou T, Sie A, Osei I, Severe E, Asante KP, Mulokozii A, Compaore G, Valea I, Adjuk M, Baiden R, Ogutu B, Binka F, Gyapong M. 2016. Treatment outcomes in a safety observational study of dihydroartemisinin/piperaquine (Eurartesim®(R)) in the treatment of uncomplicated malaria at public health facilities in four African countries. Malar J 15:43.

3. Tran TH, Dolecek C, Pham PM, Nguyen TD, Nguyen TT, Le HT, Dong TH, Tran TT, Stepniewska K, White NJ, Farrar J. 2004. Dihydroartemisinin-piperaquine against multidrug-resistant Plasmodium falciparum malaria in Vietnam: randomised clinical trial. Lancet 363:18-22.

4. Denis MB, Davis TM, Hewitt S, Incardona S, Nimol K, Fandeur T, Poravuth Y, Lim C, Socheat D. 2002. Efficacy and safety of dihydroartemisinin-piperaquine (Artekin) in Cambodian children and adults with uncomplicated falciparum malaria. Clin Infect Dis 35:1469-76.

5. Ashley EA, Krudsood S, Phaiphun L, Srivilairit S, McGready R, Leowattana W, Hutagalung R, Wilairatana P, Brockman A, Looareesuwan S, Nosten F, White NJ. 2004. Randomized, controlled dose-optimization studies of dihydroartemisinin-piperaquine for the treatment of uncomplicated multidrug-resistant falciparum malaria in Thailand. J Infect Dis 190:1773-82.

6. Valecha N, Phyto AP, Mayxay M, Newton PN, Krudsood S, Keomany S, Khanthavong M, Pongvongsa T, Ruangveerayuth R, Uthaisil D, Ubven D, Duparc S, Bacchieri A, Corsi M, Rao BH, Bhattacharya PC, Dubhashii N, Ghosh SK, Dev V, Kumar A, Pukrittayakamee S. 2010. An open-label, randomised study of dihydroartemisinin-piperaquine versus artesunate-mefloquine for falciparum malaria in Asia. PLoS One 5:e11880.

7. Bassat Q, Mulenga M, Tinto H, Piola P, Borrmann S, Menendez C, Nambozi M, Valea I, Nsanzabana C, Sasi P, Bacchieri A, Corsi M, Ubven D, Talisuna A, D’Alessandro U. 2009. Dihydroartemisinin-piperaquine and artemether-lumefantrine for treating uncomplicated malaria in African children: a randomised, non-inferiority trial. PLoS One 4:e7871.

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

9. Tarning J, Zongo I, Some FA, Rouamba N, Parikh S, Rosenthal PJ, Hanpithakpong W, Jongrak N, Day NP, White NJ, Nosten F, Ouedraogo JB, Lindegardh N. 2012. Population pharmacokinetics and pharmacodynamics of piperaquine in children with uncomplicated falciparum malaria. Clin Pharmacol Ther 91:497-505.

10. Hoglund RM, Workman L, Edstein MD, Thanh NX, Quang NN, Zongo I, Ouedraogo JB, Borrmann S, Mwai L, Nsanzabana C, Price RN, Dahal P, Sambol NC, Parikh S, Nosten F, Ashley EA, Phyto AP, Lwin KM, McGready R, Day NP, Guerin PJ, White NJ, Barnes KI.
Tarning J. 2017. Population Pharmacokinetic Properties of Piperaquine in Falciparum Malaria: An Individual Participant Data Meta-Analysis. PLoS Med 14:e1002212.

11. Zongo I, Dorsey G, Rouamba N, Dokomajilar C, Sere Y, Rosenthal PJ, Ouedraogo JB. 2007. Randomized comparison of amodiaquine plus sulfadoxine-pyrimethamine, artemether-lumefantrine, and dihydroartemisinin-piperaquine for the treatment of uncomplicated Plasmodium falciparum malaria in Burkina Faso. Clin Infect Dis 45:1453-61.

12. Zwang J, Ashley EA, Karema C, D’Alessandro U, Smithuis F, Dorsey G, Janssens B, Mayxay M, Newton P, Singhasivanon P, Stepniewska K, White NJ, Nosten F. 2009. Safety and efficacy of dihydroartemisinin-piperaquine in falciparum malaria: a prospective multi-centre individual patient data analysis. PLoS One 4:e6358.

13. Nankabirwa J, Cundill B, Clarke S, Kabateriene N, Rosenthal PJ, Dorsey G, Brooker S, Staedke SG. 2010. Efficacy, safety, and tolerability of three regimens for prevention of malaria: a randomized, placebo-controlled trial in Ugandan schoolchildren. PLoS One 5:e13438.

14. Mytton OT, Ashley EA, Peto L, Price RN, La Y, Hae R, Singhasivanon P, White NJ, Nosten F. 2007. Electrocardiographic safety evaluation of dihydroartemisinin piperaquine in the treatment of uncomplicated falciparum malaria. Am J Trop Med Hyg 77:447-50.

15. Karunajeewa H, Lim C, Hung TY, Ilett KF, Denis MB, Socheat D, Davis TM. 2004. Safety evaluation of fixed combination piperaquine plus dihydroartemisinin (Artekin) in Cambodian children and adults with malaria. Br J Clin Pharmacol 57:93-9.

16. Keating GM. 2012. Dihydroartemisinin/Piperaquine: a review of its use in the treatment of uncomplicated Plasmodium falciparum malaria. Drugs 72:937-61.

17. Baiden R, Oduro A, Halidou T, Gyapong M, Sie A, Macete E, Abdulla S, Owusu-Agyei S, Mulokozzi A, Adjei A, Severe E, Compaore G, Valea I, Osei I, Yawson A, Adjui M, Akparibo R, Ogutu B, Upunda GL, Smith P, Binka F. 2015. Prospective observational study to evaluate the clinical safety of the fixed-dose artemisinin-based combination Eurartesim(R) (dihydroartemisinin/piperaquine), in public health facilities in Burkina Faso, Mozambique, Ghana, and Tanzania. Malar J 14:160.

18. Kabanywanyi AM, Baiden R, Ali AM, Mahende MK, Ogutu BR, Oduro A, Tinto H, Gyapong M, Sie A, Severe E, Macete E, Owusu-Agyei S, Adjei A, Compaore G, Valea I, Osei I, Yawson A, Adjui M, Akparibo R, Kakolwa MA, Abdulla S, Binka F. 2016. Multi-Country Evaluation of Safety of Dihydroartemisinin/Piperaquine Post-Licensure in African Public Hospitals with Electrocardiograms. PLoS One 11:e0164851.

19. Chan XHS, Win YN, Mawer LJ, Tan JY, Brugada J, White NJ. 2018. Risk of sudden unexplained death after use of dihydroartemisinin-piperaquine for malaria: a systematic review and Bayesian meta-analysis. Lancet Infect Dis 18:913-923.

20. Zhang L, Beal SL, Sheiner LB. 2003. Simultaneous vs. sequential analysis for population PK/PD data I: best-case performance. J Pharmacokinet Pharmacodyn 30:387-404.
21. Phan DQ, Silka MJ, Lan YT, Chang RK. 2015. Comparison of formulas for calculation of the corrected QT interval in infants and young children. J Pediatr 166:960-4.e1-2.

22. Committee for Medicinal Products for Human Use (CHMP). 2005. ICH E14 Note for Guidance on The Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs (CHMP/ICH/2/04) European Medicines Agency, London.

23. Price R, Nosten F, White N. 1998. Prolongation of the QTc interval in African children treated for falciparum malaria. The American journal of tropical medicine and hygiene 59:503-503.

24. Amin AS, Herfst LJ, Delisle BP, Klemens CA, Rook MB, Bezzina CR, Underkofler HA, Holzem KM, Ruijter JM, Tan HL, January CT, Wilde AA. 2008. Fever-induced QTc prolongation and ventricular arrhythmias in individuals with type 2 congenital long QT syndrome. J Clin Invest 118:2552-61.

25. Burashnikov A, Shimizu W, Antzelevitch C. 2008. Fever accentuates transmural dispersion of repolarization and facilitates development of early afterdepolarizations and torsade de pointes under long-QT Conditions. Circ Arrhythm Electrophysiol 1:202-8.

26. Drew D, Baranchuk A, Hopman W, Brison RJ. 2017. The impact of fever on corrected QT interval. J Electrocardiol 50:570-575.

27. Amin AS, Meregalli PG, Bardai A, Wilde AA, Tan HL. 2008. Fever increases the risk for cardiac arrest in the Brugada syndrome. Ann Intern Med 149:216-8.

28. vn Seidlein L, Jaffar S, Greenwood B. 1997. Prolongation of the QTc interval in African children treated for falciparum malaria. Am J Trop Med Hyg 56:494-7.

29. Krudsood S, Looareesuwan S, Wilairatama P, Leowattana W, Tangpukdee N, Chalermrut K, Ramanathan S, Navaratnam V, Olliero P, Vaillant M, Kiechel JR, Taylor WR. 2011. Effect of artesunate and mefloquine in combination on the Fridericia corrected QT intervals in Plasmodium falciparum infected adults from Thailand. Trop Med Int Health 16:458-65.

30. Vanachayangkul P, Lon C, Spring M, Sok S, Ta-Aksorn W, Kodchakorn C, Pann ST, Chann S, Ittiverakul M, Sriansrithai S, Buathong N, Kuntawungwinn W, So M, Youdaline T, Milner E, Wojnarski M, Lanteri C, Manning J, Prom S, Haigney M, Cantilena L, Saunders D. 2017. Piperaquine Population Pharmacokinetics and Cardiac Safety in Cambodia. Antimicrob Agents Chemother.

31. Chotsiri P, Wattanakul T, Hoglund R, Hanboonkunupakarn B, Pukrittayakamee S, Blessborn D, Jittamala P, White NJ, Day NPJ, Tarning J. 2017. Population pharmacokinetics and electrocardiographic effects of dihydroartemisinin-piperaquine in healthy volunteers. Br J Clin Pharmacol doi:10.1111/bcp.13372.
32. Darpo B, Ferber G, Siegl P, Laurijssens B, Macintyre F, Toovey S, Duparc S. 2015. Evaluation of the QT effect of a combination of piperaquine and a novel anti-malarial drug candidate OZ439, for the treatment of uncomplicated malaria. Br J Clin Pharmacol 80:706-15.

33. Palhares DMF, Marcelino MS, Santos TMM, da Silva JLP, Gomes PR, Ribeiro LB, Macfarlane PW, Ribeiro ALP. 2017. Normal limits of the electrocardiogram derived from a large database of Brazilian primary care patients. BMC Cardiovasc Disord 17:152.

34. Piotrovsky V. 2005. Pharmacokinetic-pharmacodynamic modeling in the data analysis and interpretation of drug-induced QT/QTc prolongation. AAPS J 7:E609-24.

35. White NJ. 2007. Cardiotoxicity of antimalarial drugs. Lancet Infect Dis 7:549-58.

36. Kervezee L, Gotta V, Stevens J, Birkhoff W, Kamerling I, Danhof M, Meijer JH, Burggraaf J. 2016. Levofloxacin-Induced QTc Prolongation Depends on the Time of Drug Administration. CPT Pharmacometrics Syst Pharmacol 5:466-74.

37. Millat-Martinez P, Ila R, Laman M, Robinson L, Karunajeewa H, Abel H, Pulai K, Sanz S, Manning L, Moore B, Bassat Q, Mitja O. 2018. Electrocardiographic Safety of Repeated Monthly Dihydroartemisinin-Piperaquine as a Candidate for Mass Drug Administration. Antimicrob Agents Chemother 62.

38. Borsini F, Crumb W, Pace S, Ubben D, Wible B, Yan GX, Funck-Brentano C. 2012. In vitro cardiovascular effects of dihydroartemisin-piperaquine combination compared with other antimalarials. Antimicrob Agents Chemother 56:3261-70.

39. Funck-Brentano C, Bacchieri A, Valentini G, Pace S, Tommasini S, Voiriot P, Ubben D, Duparc S, Evene E, Felices M, Corsi M. 2019. Effects of Dihydroartemisinin-Piperaquine Phosphate and Artemether-Lumefantrine on QTc Interval Prolongation. Sci Rep 9:777.

40. Banook Group. 2015. Solutions: Cardiac safety. http://www.banookgroup.com/for-all/solutions/cardiac-safety/. Accessed 28 January

41. European Medicines Agency. 2011. EMA/739355/2011 - Assessment report of Eurartesim. https://www.ema.europa.eu/en/documents/assessment-report/eurartesim-epar-public-assessment-report_en.pdf.

42. Lindegardh N, Annerberg A, White NJ, Day NP. 2008. Development and validation of a liquid chromatographic-tandem mass spectrometric method for determination of piperaquine in plasma stable isotope labeled internal standard does not always compensate for matrix effects. J Chromatogr B Analyst Technol Biomed Life Sci 862:227-36.

43. Gisleskog PO, Karlsson MO, Beal SL. 2002. Use of prior information to stabilize a population data analysis. J Pharmacokinet Pharmacodyn 29:473-505.

44. U.S. Department of Health and Human Services. 2005. Guidance for Industry: E14 Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs. Food and Drug Administration, Maryland.
45. Woosley RL HC, Romero KA. www.Crediblemeds.org, QTdrugs List.
### Table 1. Baseline patient characteristics.

|                  | Burkina Faso (n = 299) | Ghana (n = 442) | Mozambique (n = 89) | Tanzania (n = 170) | Total (n = 1,000) |
|------------------|------------------------|-----------------|---------------------|--------------------|------------------|
| **Patient enrollment** |                        |                 |                     |                    |                  |
| Age group [n (%)]  |                        |                 |                     |                    |                  |
| < 1 yr            | 5 (0.5)                | 1 (0.1)         | 0                   | 0                  | 6 (0.6)          |
| 1 yr to < 5 yrs   | 93 (3.1)               | 116 (11.6)      | 2 (0.2)             | 27 (2.7)           | 238 (23.8)       |
| 5 yrs to < 12 yrs | 143 (14.3)             | 184 (18.4)      | 49 (4.9)            | 74 (7.4)           | 450 (45.0)       |
| 12 yrs to < 18 yrs| 18 (1.8)               | 64 (4.4)        | 22 (2.2)            | 23 (2.3)           | 127 (12.7)       |
| ≥ 18 yrs          | 40 (4.0)               | 77 (7.7)        | 16 (1.6)            | 46 (4.6)           | 179 (17.9)       |
| **Sex [n (%)]**   |                        |                 |                     |                    |                  |
| Male              | 142 (48.2)             | 203 (20.3)      | 47 (4.7)            | 90 (9.0)           | 482 (48.2)       |
| Female            | 157 (51.8)             | 239 (23.9)      | 42 (4.2)            | 80 (8.0)           | 518 (51.8)       |
| **Patient characteristics** |           |                 |                     |                    |                  |
| Median (IQR)      |                        |                 |                     |                    |                  |
| Age (yrs)         | 6 (4-9)                | 7.5 (4-13)      | 11 (9-14)           | 10 (6-18)          | 7.5 (5-12)       |
| Body weight (kg)  | 17 (13-23)             | 22 (16-42)      | 31 (26-43)          | 22 (18-45)         | 21 (15-38)       |
| Body temperature (°C) | 37.4 (36.8-37.7) | 37.0 (36.5-38.0) | 37.2 (36.5-38.2) | 37.7 (37.0-38.9) | 37.2 (36.7-38.0) |
| Pulse rate (beats/minute) | 100 (87-111) | 110 (90-126)   | 91 (76-111)         | 99 (84-112)        | 54 (87-120)      |
| Parasite density (parasites/µl) | 960 (440-7,605) | 13,466 (1,199-46,210) | 23,951 (7,981-74,824) | 140 (33-787) | 2,537 (297-25,418) |
| Haemoglobin (g/dl) | 10.6 (9.5-11.5)       | 10.9 (9.8-11.7) | 10.8 (9.4-11.9)    | 10.7 (9.4-11.9)   | 10.7 (9.6-11.7)  |
| Total bilirubin (µmol/l) | 9.8 (6.6-15.4) | 14.0 (9.1-23.6) | 17.0 (12.0-25.0) | 18.1 (8.3-26.7) | 13.4 (8.0-22.9)  |
| ALT (U/l)         | 24.3 (18.2-31.1)       | 20.9 (15.3-31.5) | 26.0 (22.0-34.0)   | 29.1 (21.8-35.6)  | 24.0 (17.4-33.0) |
| AST (U/l)         | 25.2 (19.1-32.5)       | 28.4 (22.1-37.9) | 33.0 (28.0-44.0)   | 35.5 (29.6-41.7)  | 23.9 (18.8-34.9) |
| BUN (mmol/l)      | 2.9 (2.2-3.7)          | 3.4 (2.4-4.6)   | 13.0 (11.0-16.0)   | 6.3 (4.4-10.0)    | 3.6 (2.6-5.6)    |
| Serum creatinine (µmol/l) | 30.5 (25.0-38.6) | 47.8 (38.3-65.3) | 44.6 (38.5-53.9) | 77.0 (54.0-99.5) | 43.9 (32.0-64.0) |
| Potassium (mmol/l) | 4.2 (4.0-4.4)         | 3.8 (3.5-4.2)   | 3.8 (3.9-4.5)      | 3.8 (3.7-5.7)     | 4.05 (3.7-4.4)   |
| Chloride (mmol/l) | 102.8 (95.8-105.3)     | 101.2 (97.0-107.4) | 100.0 (99.0-103.0) | 108.5 (98.0-114.0) | 102.2 (97.0-106.9) |
Table 2. Pharmacokinetic parameters from the final population pharmacokinetic model for piperaquine.

| Parameter         | Population estimate<sup>a</sup> (%) | 95% Cl<sup>b</sup> (%) | IIV/IOV<sup>†</sup> (%) | 95% Cl<sup>b</sup> |
|-------------------|-------------------------------------|-------------------------|--------------------------|---------------------|
| F                 | 1 fixed                             | -                       | 38.2 (3.90)              | 36.0-42.3          |
|                   |                                     |                         | †42.8 (2.07)             | 43.5-47.8          |
| MTT (h)           | 2.13 (1.11)                         | 2.09-2.18               | 37.5 (9.83)              | 36.1-52.8          |
|                   |                                     |                         | †44.7 (1.20)             | 46.2-48.7          |
| CL/F (L/h)        | 53.1 (2.77)                         | 50.2-56.1               | -                        | -                   |
| V<sub>C</sub>/F(L) | 1730 (8.04)                         | 1,441-1,991             | 90.5 (19.8)              | 31.4-165           |
| Q<sub>1</sub>/F(L/h) | 282 (5.60)                         | 249-310                 | -                        | -                   |
| V<sub>P</sub>/F(L) | 3,290 (5.10)                        | 2,949-3,595             | 23.4 (24.1)              | 17.2-39.9          |
| Q<sub>2</sub>/F(L/h) | 82.9 (2.42)                         | 78.9-86.6               | 27.1 (11.9)              | 24.0-36.7          |
| V<sub>P</sub>/F(L) | 25,100 (1.77)                       | 24,170-25,925           | 31.8 (1.01)              | 32.0-33.3          |
| Dose occasion effect on F | 0.237 fixed                      | -                       | -                        | -                   |
| AGE<sub>50</sub> (years) | 0.575 fixed                | -                       | -                        | -                   |
| Hill              | 5.51 fixed                          | -                       | -                        | -                   |
| σ                 | 0.198 (4.13)                        | 0.167-0.232             | -                        | -                   |

Abbreviations: F, relative bioavailability; MTT, mean transit time; CL/F, apparent oral clearance; V<sub>C</sub>/F, apparent central volume of distribution; Q/F, inter-compartmental clearance; V<sub>P</sub>/F, apparent peripheral volume of distribution; AGE<sub>50</sub>, the age to reach 50% of the full maturation of the elimination clearance; Hill, the shape function in the maturation equation; σ,
residual unexplained error of drug measurements (variance); IIV, inter-individual variability; IOV, inter-occasion variability.

*a* Computed population mean parameter estimates from NONMEM. Parameter estimates are based on the typical individual in the prior population with a body weight of 54 kg. IIV and IOV were implemented as an exponential function and are presented as the coefficient of variation (CV), calculated as $100 \times \sqrt{\text{exp(estimate)}-1}$.

*b* Based on nonparametric bootstrap diagnostics (n = 1,000). Parameter precision is presented as relative standard deviation (%RSE), calculated as $100 \times \frac{\text{Standard deviation}}{\text{Mean value}}$. 
Table 3. Clinical determinants associated with QTc-prolongation.

| Clinical determinants associated with ∆QTcSSB-interval prolongation | Factor | ≤30ms | 31-60ms | >60ms | P ≤30ms vs 31-60ms | P ≤30ms vs >60ms | P All groups |
|---|---|---|---|---|---|---|---|
| No. of patients | 658 (64.2) | 286 (28.8) | 70 (7.0) | | | | |
| Female | 322 (48.3) | 184 (64.3) | 37 (53.1) | 0.157 | 0.617 | 0.186 | |
| Hypokalaemia (< 3.5 mmol/l) | 87 (13.6) | 52 (18.2) | 7 (10.0) | 0.000 | 0.463 | 0.101 | |
| Body temperature (°C) | 37.1 (36.6-37.8) | 37.5 (36.8-38.4) | 37.6 (36.8-38.6) | < 0.001 * | 0.617 | < 0.001 * | |
| Age (years) | 7 (5-14) | 8 (5-12) | 7 (5-10) | > 0.999 | >0.999 | 0.873 | |
| Potassium (mmol/l) | 4.05 (3.70-4.47) | 4.01 (3.70-4.40) | 4.16 (3.80-4.49) | 0.045 | 0.383 | 0.111 | |
| Baseline QTcSSB-interval (ms) | 425 (413-435) | 422 (406-434) | 415 (391-433) | < 0.0001 * | 0.0004 * | < 0.0001 * | |
| Piperaquine Cmax (ng/mL) | 694 (442-1030) | 814 (546-1110) | 830 (550-1120) | 0.0004 * | 0.083 | 0.0003 * | |

| Clinical determinants associated with absolute QTc-interval prolongation | Factor | ≤450ms | 451-600ms | >600ms | P ≤450ms vs 451-600ms | P ≤450ms vs >600ms | P All groups |
|---|---|---|---|---|---|---|---|
| No. of patients | 481 (48.4) | 408 (41.0) | 78  (7.8) | 27 (2.7) | | | |
| Female | 229 (47.6) | 234 (57.4) | 39 (50.0) | 14 (50.0) | 0.045 | 0.715 | 0.696 |
| Hypokalaemia (< 3.5 mmol/l) | 65 (13.5) | 63 (15.4) | 11 (14.1) | 14 (18.7) | 0.125 | 0.696 | 0.345 |
| Body temperature (°C) | 37.1 (36.6-38.6) | 37.5 (36.8-38.4) | 37.6 (36.8-38.6) | > 0.999 | >0.999 | 0.545 | 0.437 |
| Age (years) | 8 (5-15) | 7 (4-13) | 9 (6-12) | 0.675 | >0.999 | 0.999 | 0.493 |
| Potassium (mmol/l) | 4.05 (3.70-4.47) | 4.01 (3.70-4.40) | 4.16 (3.80-4.49) | 0.351 | 0.833 | 0.164 | 0.126 |
| Baseline QTc-interval (ms) | 425 (405-427) | 428 (419-439) | 436 (424-450) | < 0.001 * | <0.0001 * | < 0.0001 * | |
| Piperaquine Cmax (ng/mL) | 631 (421-960) | 783 (517-1191) | 962 (658-1320) | < 0.001 * | <0.0001 * | < 0.0001 * | |

*Reference group, *Statistically significant
Table 4. Parameter estimates from the final pharmacokinetic-pharmacodynamic model for the piperaquine effect on absolute QTc-interval.

| Parameter | Population estimate<sup>a</sup> (% RSE)<sup>b</sup> | 95% CI | IIV %CV (% RSE)<sup>b</sup> | 95% CI<sup>b</sup> |
|-----------|------------------|--------|-------------------|--------|
| Piperaquine effect on absolute QTc-interval | | | | |
| QTc<sub>Baseline</sub> (ms) | 421 (0.15) | 420-423 | 17.0<sup>c</sup> (3.12) | 16.0-18.0 |
| E<sub>max</sub> (ms) | 35 (11.0) | 29.0-44.2 | 49.1<sup>d</sup> (13.0) | 34.1-62.4 |
| EC<sub>50</sub> (ng/mL) | 209 (16.7) | 155-296 | 119.3<sup>d</sup> (9.28) | 88.7-152 |
| γ | 1.69 (11.6) | 1.36-2.17 | - | - |
| Effect of age on EC<sub>50</sub> (%) | 4.10 (19.5) | 2.68-5.88 | - | - |
| σ (ms) | 11.6 (5.74) | 10.5-13.2 | - | - |

Abbreviations: QTc<sub>Baseline</sub>, baseline value of the QTc-interval at enrolment; E<sub>max</sub>, maximum QTc-interval associated with drug effect; EC<sub>50</sub>, piperaquine concentration needed to achieve 50% of the maximum drug effect; γ, shape function of the E<sub>max</sub> model; σ, additive residual error (variance) of QTc-interval measurements; IIV, inter-individual variability.

<sup>a</sup>Computed population mean parameter estimates from NONMEM.

<sup>b</sup>Based on nonparametric bootstrap diagnostics (n = 1,000). Parameter precision is presented as relative standard deviation (%RSE), calculated as 100× Standard deviation/Mean value.

<sup>c</sup>Additive inter-individual variability, presented as absolute variability on an arithmetic scale.

<sup>d</sup>Exponential inter-individual variability, presented as the coefficient of variation (%CV), calculated as 100×√exp(estimate)-1.
Table 5. Old and new dihydroartemisinin-piperaquine dosing regimen recommended by WHO for the treatment of uncomplicated malaria.

| Body weight (kg) | Old piperaquine dosing regimen | New piperaquine dosing regimen |
|------------------|--------------------------------|--------------------------------|
|                  | DHA/PQP (mg)                   | DHA/PQP (mg)                   |
| 5-12             | 20/160                         | 5 to < 8                       |
| 13-23            | 40/320                         | 8 to < 11                      |
| 24-35            | 80/640                         | 11 to < 17                     |
| 36-74            | 120/960                        | 17 to < 25                     |
| >74              | 160/1280                       | 25 to < 36                     |
|                  |                                | 36 to < 60                     |
|                  |                                | 60 to < 80                     |
|                  |                                | >80                            |
|                  |                                | 200/1600                       |
Figure 1. Visual predictive check of the final piperaquine pharmacokinetic model.

The open circles represent the observed piperaquine concentrations. Solid red lines represent the 50th percentiles of the observations, and dashed red lines represent the 5th and 95th percentiles of the observations. The shaded areas represent the 95% confidence intervals of each simulated percentile (n=2,000).
Figure 2. Observed QTc-intervals, stratified by ECG measurement schedule.

The solid lines and error bars represent the median and interquartile range of QTcSSB-interval recorded at each ECG measurement occasion, stratified by QTc-interval threshold categories.
Figure 3. Diagnostics of the final pharmacokinetic-pharmacodynamic model.

Goodness-of-fit plots showing (A) observed QTc-interval vs individually predicted QTc-interval, and (B) conditionally weighted residual vs time after dose. The solid black lines represent the line of identity and the dashed red lines represent a local polynomial regression fitting of all observations (i.e. trend line). Visual predictive check (C) of the model describing the relationship between piperaquine concentrations and absolute QTc-intervals using an E_{max} function (n=2,000). The open circles represent the observations. Solid red line represents the 50th percentile of the observations, and dashed red lines represent the 5th and 95th percentiles of the observations. The shaded areas represent the 95% confidence intervals of each simulated percentile.
Figure 4. Predicted maximum QTc-intervals after different dosing regimens, simulated from the final pharmacokinetic-pharmacodynamic model.

The box plots represent the simulated maximum QTc-interval, stratified by body weight, in children weighting 5 to 25 kg (data on adults are presented in the supplementary material; Figure S5) after receiving the old and new dosing regimen for (A) acute malaria treatment (3-day regimen) and (B) mass drug administration (monthly 3-day regimen). The dashed red lines represent an absolute QTc-interval regulatory safety cut-off of 500 ms.
Figure 5. Probability density of maximum QTc-intervals and ΔQTc-intervals after different dosing regimens, simulated from the final pharmacokinetic-pharmacodynamic model.

The graphs show the probability density distribution of maximum QTc-intervals and maximum ΔQTc-intervals based on a total 480,000 simulated patients after receiving the old (grey solid lines) and new (red solid lines) dosing regimen for (A, B) acute malaria treatment (3-day regimen) and for (C, D) mass drug administration (monthly 3-day regimen).