Modeling prevention of malaria and selection of drug resistance with different dosing schedules of dihydroartemisinin-piperaquine preventive therapy during pregnancy in Uganda

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Running title: Drug resistance with DHA-PQ for malaria prevention

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Abstract

Dihydroartemisinin-piperaquine (DHA-PQ) is under study for intermittent preventive treatment during pregnancy (IPTp), but it may accelerate selection for drug resistance. Understanding the relationships between piperaquine concentration, prevention of parasitemia, and selection for decreased drug sensitivity can inform control policies and optimization of DHA-PQ dosing. Piperaquine concentrations, measures of parasitemia, and *Plasmodium falciparum* genotypes associated with decreased aminoquinoline sensitivity in Africa (*pfmdr1* 86Y, *pfcrt* 76T) were obtained from pregnant Ugandan women randomized to IPTp with sulfadoxine-pyrimethamine (SP) or DHA-PQ. Joint pharmacokinetic/pharmacodynamic models described relationships between piperaquine concentration and probability of genotypes of interest using nonlinear mixed effects modeling. Increasing piperaquine plasma concentration was associated with a log-linear decrease in risk of parasitemia. Our models predicted that higher median piperaquine concentrations would be required to provide 99% protection against mutant compared to wild type infections (*pfmdr1* N86: 9.6 ng/mL, 86Y: 19.6 ng/mL; *pfcrt* K76: 6.5 ng/mL, 76T: 19.6 ng/mL). Comparing monthly, weekly, and daily dosing, daily low dose DHA-PQ was predicted to result in the fewest infections and the fewest mutant infections per 1,000 pregnancies (predicted mutant infections for *pfmdr1* 86Y: SP monthly: 607, DHA-PQ monthly: 198, DHA-PQ daily: 1; for *pfcrt 76T*: SP monthly: 1564, DHA-PQ monthly: 283, DHA-PQ daily: 1). Our models predict that higher piperaquine concentrations are needed to prevent infections with *pfmdr1/pfcrt* mutant compared to wild type parasites and that, despite selection for mutants by DHA-PQ, the overall burden of mutant infections is lower for IPTp with DHA-PQ than for IPTp with SP.

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Introduction

Plasmodium falciparum infection during pregnancy, especially during a first pregnancy, places infants at risk for the complications of placental malaria, including intrauterine growth retardation, preterm birth, low birth weight, and death (1). The World Health Organization recommends that pregnant women at risk for malaria in Africa use a long lasting insecticide treated bed net and receive at least three doses of sulfadoxine-pyrimethamine (SP) as intermittent preventive treatment during pregnancy (IPTp) (2). However, in much of Africa, including east Africa, the protective efficacy of SP as chemoprevention for pregnant women and children is inadequate (3-5). Compared to three doses of SP during pregnancy, a monthly course of dihydroartemisinin-piperaquine (DHA-PQ), an artemisinin-based combination therapy administered once daily for three days, dramatically reduced the prevalence of maternal parasitemia and placental malaria in Uganda and Kenya (5, 6). Pharmacokinetic/pharmacodynamic (PK/PD) modeling studies found that plasma piperaquine (PQ) concentrations are excellent predictors of DHA-PQ protective efficacy, and that maintaining higher PQ concentrations in the target population, such as with lower dose weekly or daily DHA-PQ, predicts maximal protective efficacy (7-10).

The long half-life of PQ makes DHA-PQ an ideal choice for malaria chemoprevention, but antimalarials with the longest half-lives may be at the greatest risk for resistance selection (11). Although true resistance to DHA-PQ, as observed in southeast Asia (12, 13), has not been confirmed in Africa (14-16), *P. falciparum* infections that emerge following DHA-PQ treatment have had, compared to parasites not under drug selection, increased prevalence of mutant genotypes in the putative drug transporters *pfmdr1* (86Y) and *pfcrt* (76T) (14, 16, 17). These mutations are associated with decreased sensitivity to chloroquine and amodiaquine, two

aminoquinolines related to piperaquine (14), and these results raise the concern that using DHA-PQ for chemoprevention may provide only a short-term benefit, with eventual loss of efficacy due to accelerated development of resistance.

We are interested in optimizing DHA-PQ dosing during IPTp to maximize protective efficacy, minimize toxicity, and limit selection for less drug sensitive parasites. In this analysis, we used clinical, pharmacokinetic, and molecular data from a trial of pregnant women who were randomized to receive DHA-PQ or SP as IPTp to develop PK/PD models which quantified relationships between PQ exposure, parasitemia, and genetic markers associated with decreased drug sensitivity. We then used the concentration-effect relationships to predict how modifications to DHA-PQ dosing would impact the burden of *P. falciparum* infection, including the risk of infection with parasites with decreased drug sensitivity.

Results

Study cohort and data collection

Data were from a randomized controlled trial, in which 300 pregnant women were randomized to one of three IPTp regimens: SP every 8 weeks beginning at gestational week 20, DHA-PQ every 8 weeks beginning gestational week 20, or DHA-PQ every 4 weeks beginning at gestational weeks 16 or 20 as previously described (Figure 1, Table 1) (5). Clinical characteristics were similar between the three study arms (Table 1). Participants returned monthly for routine visits and for any acute illness. At routine visits or when malaria was suspected, evaluation included a capillary or venous blood draw for plasma PQ concentration and parasite detection. If the woman was parasitemic, the parasite was genotyped at *pfmdr1* 86 and *pfcrt* 76 (16). A subset of 30 women underwent intensive PQ sampling. A total of 652

venous and 558 capillary PQ concentrations were obtained (Table 1, Supplemental Figure 1). Genotyping for the single nucleotide polymorphisms at *pfmdr1* N86Y and *pfcrt* K76T was successful for >84% of episodes of parasitemia in the SP arm, and >93% in the DHA-PQ arms. Prevalences of mutant genotypes were higher in the DHA-PQ arms compared to the SP arm (*pfmdr1* 86Y: SP 27%, DHA-PQ 65%; *pfcrt* 76T: SP 82%, DHA-PQ 87%), as previously reported (Table 1) (16).

PK/PD model building

Simultaneous continuous-categorical PK/PD models were developed using a mixed-effects logistic regression approach. Models were evaluated using objective function value (OFV), with a decrease in OFV (ΔOFV) of -3.84 considered a significant improvement if one parameter was added to the model, and by visual predictive check (Supplemental Figure 2). Two types of simultaneous PK/PD models were developed for the analysis. PK/PD-parasitemia models predicted the risk of parasitemia. PK/PD-resistance models predicted the risk of a mutant infection at *pfmdr1* 86 or *pfcrt* 76 when parasitemia was detected.

A two-compartment model for PQ was used to predicted plasma concentrations, as previously described (8). For the PK/PD-parasitemia model for women who received DHA-PQ, a negative log-linear relationship provided an adequate fit for the association between plasma PQ concentration and risk of parasitemia (ΔOFV -230, Supplemental Figure 2b). Being primigravida was associated with a significant 26.6% increased risk of parasitemia prior to IPTp compared to being multigravida participants (ΔOFV -24). However, after initiation of DHA-PQ gravidity was not a significant predictor of parasitemia in the model. Significant covariates after initiation of IPTp included being in the second or third trimester, and household receipt of indoor residual spraying of insecticide (IRS). Compared to the second trimester, the third trimester was

114 associated with a 19.0% reduction in risk of parasitemia while receiving IPTp (ΔOFV -41).
115 Finally, receipt of IRS, which in the clinical trial only occurred after the start of
116 chemoprevention, was associated with complete protection from parasitemia, eliminating the
117 concentration effect of PQ when present (ΔOFV -36) (Table 2). Additional covariates tested
118 included body mass index (BMI) at enrollment, change in BMI compared to enrollment, and
119 presence of dry season, and these were not significantly associated with the risk of parasitemia
120 for women who received DHA-PQ. Gravidity, trimester, and BMI were also tested as covariates
121 on the relationship between PQ and risk of parasitemia; these did not significantly improve the
122 PK/PD-parasitemia model for DHA-PQ. The final model for the probability of parasitemia is
123 described in Equation 1, where P is the probability of parasitemia; B is the baseline risk of
124 parasitemia; sl is the slope of the concentration dependent change in probability; [PQ] is the PQ
125 concentration in ng/mL; θ represents covariates that were estimated in the model; and ε and η
126 indicate residual error.

127 $$Logit\ (P) = B + sl*[\mathrm{PQ}] + \theta_{IRS} + \theta_{Trimester} + \varepsilon + \eta \qquad (Eq1)$$

128 For women who were not exposed to IRS, even low PQ concentrations were associated
129 with a decreased risk of parasitemia as compared to baseline, and PQ was a predictor of
130 parasitemia risk regardless of the trimester (Figure 2).

131 For SP, pharmacokinetic data were not available, and a PD model for parasitemia was
132 developed. In a stepwise manner, binary covariates were added to the baseline probability of
133 parasitemia for the SP PD-parasitemia model as seen in Equation 2, where P is the probability of
134 parasitemia; B is the baseline risk of parasitemia; θ represents covariates that were estimated in
135 the model; and ε and η indicate residual error.

$$Logit\ (P) = B + \theta_{IRS} + \theta_{Season} + \varepsilon + \eta \qquad (Eq2)$$

Similar to DHA-PQ, being primigravid significantly increased the risk of parasitemia prior to IPTp by 23.3% (ΔOFV -8.0). Receipt of IRS was associated with a reduced risk of parasitemia (32.7%, Table 2, ΔOFV -27). In addition, for the SP arm the dry season was independently associated with a decreased risk of parasitemia (24.4%, ΔOFV -17). After adjusting for significant covariates, the model did not support the addition of an SP effect (added as time varying, treatment arm effect, or binary covariate). In addition, enrollment BMI, change in BMI and trimester were not associated with significant changes in risk of parasitemia.

PK/PD-resistance models were then developed to estimate the probability of a mutant infection at *pfmdr1* N86Y or *pfcrt* K76T. A log-linear relationship between PQ concentration and probability of a mutant infection provided the best fit for both *pfmdr1* 86Y (ΔOFV -11) and *pfcrt* 76T (ΔOFV -9.6) (Supplemental Figure 2c, 2d). Increasing PQ concentrations were associated with an increased probability of a mutant infection at both loci (Figure 3). As expected, there was no significant relationship between IPTp with SP and detection of a mutant *pfmdr1* 86Y or *pfcrt* 76T allele. Compared to the SP group, the odds of detecting *pfmdr1* 86Y increased with increasing PQ concentration, with a maximum median odds of 4.3 occurring at 17.9 ng/mL PQ (Figure 3c). In the setting of a high baseline risk of *pfcrt* 76T, PQ exposure was associated with a slight increase in the odds of detecting a mutant compared to the SP arm, peaking at a maximum median odds of 1.3 at 10.1 ng/mL PQ (Figure 3c).

Derivation of PQ concentration targets

The PQ concentrations required to prevent 99% of parasitemia episodes varied by trimester. Women in the second trimester were predicted to require 19.6 ng/mL PQ (95% CI

158 13.2-31.6) to achieve 99% protection from parasitemia, while in the third trimester 12.8 ng/mL
159 (9.2-19.2) was required.

160 The PQ concentrations required to prevent 99% of parasitemia episodes stratified by wild
161 type and mutant genotypes were derived from a joint model of the final PK/PD models for
162 predicting parasitemia and genotype. Since women in the second trimester were predicted to
163 require the highest PQ concentrations for protection (Supplemental Table 1), this population was
164 used to estimate the target protective concentrations, as shown in Figure 4. For *pfmdr1* 86, an
165 increased risk of mutant parasites was predicted compared to baseline at sub-protective plasma
166 concentrations of PQ, peaking at 3.3 ng/mL (Figure 2). PQ concentrations required to prevent
167 99% of parasitemia episodes were predicted to be higher for parasites with mutant *pfmdr1* 86Y
168 (19.6 ng/mL, [95% CI 12.9-32.2]) compared to wild type *pfmdr1* N86 (9.6 ng/mL [7.0-12.4])
169 and for mutant *pfcrt* 76T (19.6 ng/ml [13.1-32.2]) compared to wild type *pfcrt* K76 (6.5 ng/ml
170 [4.1-9.3]) (Figure 4).

171 **Simulations to predict the optimal DHA-PQ dosing schedule**

172 Simulations were conducted of 1,000 women who received SP every 8 weeks or DHA-
173 PQ monthly, weekly, or daily using the joint PK/PD models to estimate the percentage of time
174 above protective concentrations during pregnancy and the predicted number of mutant *pfmdr1*
175 *86Y* and *pfcrt* 76T infections for each regimen (Table 3, Figure 5). All simulations assumed no
176 exposure to IRS or seasonal variation. Both the number of parasitemia episodes and the number
177 of mutant parasitemia episodes were predicted to be lower with any of the considered DHA-PQ
178 regimens compared to SP. Low dose (320 mg PQ per day) daily DHA-PQ was predicted to result
179 in the lowest median number of infections and mutant infections, with an estimated reduction in
180 mutant infections >99%.

Discussion

A monthly treatment course of DHA-PQ markedly reduced the burden of parasitemia during pregnancy in Uganda and Kenya, but there is concern that IPTp with DHA-PQ will accelerate selection for drug resistance. With simultaneous PK/PD modeling, we used PQ concentrations and clinical covariates to predict the probability of detecting malaria parasitemia and the probability of detecting parasites with relevant genotypes associated with drug resistance in women receiving DHA-PQ or SP as IPTp in Uganda. Higher concentrations of PQ were needed to reduce the probability of mutant, compared to wild type infections at *pfmdr1* 86 and *pfcrt* 76, but these concentrations were achievable with practical DHA-PQ dosing regimens, including a novel low dose daily regimen that should minimize toxicity concerns (8, 18). Despite selection for mutants by DHA-PQ, the overall burden of mutant infections was predicted to be lower for IPTp with DHA-PQ than with SP. Thus, a low daily dose of DHA-PQ for chemoprevention during pregnancy is predicted to maximize protective efficacy, with limited burden of mutant parasites with decreased aminoquinoline sensitivity, and decreased risk, compared to monthly dosing, of cardiotoxicity (8, 18).

In our model, we were unable to predict a malaria protective benefit attributable to IPTp with SP after controlling for covariates. *P. falciparum* polymorphisms associated with antifolate resistance were at high prevalence at the study site (16), and there was a high burden of parasitemia and malarial illness in the SP arm of the study (5). Considering protective efficacy, monthly DHA-PQ was effective for adult males in Thailand (19), and was superior to SP for pregnant women in Uganda and Kenya (5, 6), and for children in Uganda (20). But, as with SP, might regular use of DHA-PQ for IPTp increase the burden of parasites that are no longer inhibited by the regimen? Importantly, in this setting it does not appear to be the case, as the

overall reduction in episodes of parasitemia is predicted to lead to a lower burden of infections with mutant parasites with DHA-PQ as IPTp.

The risk of selecting for *P. falciparum* with decreased susceptibility to antimalarials will be dependent on the prevalence of these mutants in the circulating parasite population, as selection appears to be due primarily to amplification of existing clones, rather than de novo selection of new mutants (11). Since our trial was conducted, there have been significant increases in the prevalence of wild type infections at *pfmdr1* 86 and *pfcrt* 76 in the region, likely selected by use of artemether-lumefantrine (AL) to treat malaria in Uganda (14, 21). An additional wild type polymorphism, *pfmdr1* D1246, also increased in prevalence with AL pressure (14, 21). A haplotype analysis found that mutant *pfmdr1* 1246Y may be required to select for *pfmdr1* 86Y under PQ pressure, further reducing the risk of selecting for *pfmdr1* 86Y under DHA-PQ pressure with current circulating parasites (22). In this setting, a recent Ugandan treatment efficacy study found that, in contrast to results from earlier studies, DHA-PQ did not select for *pfmdr1* and *pfcrt* mutations in recurrent infections (23). Considering our modeling results in this population, it is unlikely that IPTp with DHA-PQ will increase the burden of mutant parasites with decreased sensitivity to the regimen in Uganda. However, risks of resistance selection could change over time based on ACT usage or other factors. Longitudinal surveillance of drug resistance markers and re-evaluation of PK/PD models will remain important as we consider using DHA-PQ for IPTp.

Our analysis identified important covariates which modified the risk of parasitemia among women receiving DHA-PQ chemoprevention, including gravidity in the pre-IPTp period, and trimester and IRS during IPTp. Remarkably, the combination of monthly DHA-PQ and receipt of IRS eliminated the risk of parasitemia. The benefits of IRS were not as large for the SP

arm, likely due to persistent parasitemia despite treatment with SP (3). Recent studies from Uganda found that receipt of IRS is associated with improvements in birth outcomes (24). Taken together, available results suggest enormous potential for the joint use of highly effective intermittent preventive treatment and IRS for the control and potential elimination of malaria.

Our study had some limitations. First, parasitemia was assessed at 28-day intervals. We could not determine the exact time when an individual became parasitemic, and thus the exact concentration required to prevent parasitemia. However, monthly PQ concentrations offered a practical sampling strategy with good predictive power in our models. Second, PK data were not available to assist in detecting a concentration-effect relationship between SP and prevention of malaria. We found that, after controlling for covariates which are associated with reduced risk of malaria infection, a model without an SP effect predicted the data adequately. The absence of a protective benefit for SP was further supported by a placebo-controlled chemoprevention trial in Uganda that did not demonstrate a significant protective effect of SP in children (4). Thirdly, treatment failure due to DHA-PQ resistance and associated genetic markers have not been identified in Africa and thus could not be used in this analysis. The markers associated with DHA-PQ resistance in Southeast Asia (*pfkelch*, *plasmepsin2* copy number, and *exo-E415G* (13, 25, 26)) were assessed for this population and were either not present or, in the case of *plasmepsin2* copy number, only present in a minority of isolates (16). *Pfmdr1* 86Y and *pfcrt* 76T have been consistently associated with PQ exposure in Uganda (17, 27, 28), and have recently been associated with a modest increase in ex vivo IC50 for PQ (14). As a result, these markers of antimalarial sensitivity were the most relevant for this population.

By taking a PK/PD modeling approach, we found that higher PQ concentrations are needed to prevent mutant, compared to wild type malaria infections, but that safe and achievable

PQ concentrations can provide >99% protection from parasitemia. In addition, a low dose daily DHA-PQ regimen was predicted to maximally reduce parasitemia. Our findings support the use of DHA-PQ for chemoprevention and the optimization of DHA-PQ dosing to maximize protective efficacy while minimizing toxicity and potential selection of drug resistance. Future clinical trials of DHA-PQ as chemoprevention during pregnancy should consider alternative dosing strategies, including low dose daily DHA-PQ.

Methods

Study population

Pregnant women were enrolled in the clinical trial that provided samples for our analyses in Tororo, Uganda from June through October 2014 (5). Eligible women were ≥16 years of age, HIV-uninfected, and pregnant at 12-20 weeks gestation. Written informed consent was obtained from all study participants. The study protocol was approved by the Makerere University School of Biomedical Sciences Research and Ethics Committee, the Uganda National Council for Science and Technology, and the University of California, San Francisco Committee on Human Research. The clinical trial registration number is NCT02282293.

Study design and randomization

After enrollment, women randomized to SP (1500 mg sulfadoxine/75 mg pyrimethamine) every 8 weeks or DHA-PQ (120 mg DHA/960 mg PQ daily for 3 days) every 8 weeks began chemoprevention at 20 weeks gestational age, and those randomized to DHA-PQ every 4 weeks began chemoprevention at either 16 or 20 weeks gestational age. Administration of the first dose of DHA-PQ was observed in the clinic, and the remaining two doses were taken at home. At enrollment, study participants received a long-lasting insecticide-treated bed net, underwent a

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physical exam, had height and weight determination, and had blood collected. All women attended routine visits at 4 week intervals and were asked to return to the clinic for all of their medical needs. The date of IRS in the household was collected for each subject (24).

Pharmacokinetic sampling

Women randomized to receive DHA-PQ underwent sparse venous (gestational weeks: 20, 28, and 36) and capillary (gestational weeks: 24, 32, and 40) sampling to determine plasma PQ concentrations (8). Sparse PQ concentrations were determined either 28 days after receiving the drug in the 4 week DHA-PQ arm or every 28 days and every 56 days after receiving the drug in the 8 week DHA-PQ arm (8). Venous or capillary specimens were also collected at the time of any malaria diagnosis. A subset of individuals were enrolled in an intensive PK sub-study. For this study, as previously reported (29), venous plasma samples were obtained pre-dose, and 0.5, 1, 2, 3, 4, 6, 8, and 24 hours post dose, and capillary plasma samples were collected at 24 hours and 4, 7, 14 and 21 days post dose. PQ base concentrations were determined using high performance liquid chromatography tandem mass spectrometry (HPLC-MS) (30). Modification and partial-validation of the original method for PQ quantitation was performed, to cover a concentration range of 0.50-1,000 ng/mL, with a coefficient of variation <10% for quality control samples (30).

***P. falciparum* detection and genotyping**

A blood spot was collected and stored on filter paper at all routine visits and if malaria was diagnosed at an unscheduled visit. DNA was extracted from dried blood spots using Chelex-100 and tested for the presence of *P. falciparum* DNA by loop-mediated isothermal amplification (LAMP) for all microscopy negative samples, as previously described (5, 31). Genotyping for

294 *pfmdr1* N86Y and *pfcrt* K76T was conducted using a ligase detection reaction–fluorescent
295 microsphere assay as previously described (28, 32). Isolates were classified as mutant for either
296 pure mutant or mixed mutant and wild type genotypes.

297 **PK/PD models**

298 To estimate the concentration effect relationship between PQ PK and probability of
299 parasitemia, and between PQ PK and the probability of detecting particular alleles at the loci of
300 interest, simultaneous PK/PD models were developed using nonlinear mixed effects modeling
301 and LAPLACE methods (33). All available PQ concentration data above the limit of quantitation
302 were used in the development of a two-compartment PQ PK model, as previously described (8).
303 The population PQ PK model was then used as part of a simultaneous continuous-categorical
304 PK/PD model with logit transformation to determine the probability of parasitemia or mutant
305 genotype. To avoid repeated sampling of persistent circulating parasites, testing for parasitemia
306 was censored after the first episode of parasitemia identified following each administration of
307 study drug. Model appropriateness was evaluated by likelihood ratio test, inspection of the
308 diagnostic plots, and internal model validation techniques, including visual and numerical
309 predictive checks.

310 We first developed a simultaneous continuous-categorical PK/PD-parasitemia model to
311 predict the probability of parasitemia among women who received DHA-PQ. Dose response,
312 linear, and Emax models were tested for the relationship between PQ concentration and
313 probability of parasitemia. Gravidity, trimester (defined as <28 weeks for the second trimester
314 and ≥28 weeks for the third trimester), enrollment BMI, change in BMI compared to enrollment,
315 dry season (defined as December to February), and receipt of IRS were then tested as covariates
316 in the model. We then developed a PD model for the probability of parasitemia for women who

317 received SP. We estimated that SP had a 28 day effect based on prior modeling studies (34). The
318 same covariates were tested for SP as for DHA-PQ.

319 PK/PD-resistance models were developed to estimate the relationship between PQ
320 concentration and parasite genotype at *pfmdr1* N86Y or *pfcrt* K76T, also using simultaneous
321 PK/PD modeling with logit transformation. All PQ PK data and available genotype data from
322 episodes of parasitemia were used to develop models to predict sequences at the *pmfdr1* N86Y
323 and *pfcrt* K76T alleles when parasitemia was detected. Baseline, dose response, linear, and Emax
324 relationships between PQ concentration and genotype were tested for those who received DHA-
325 PQ. Since PK data were not available for SP, a PD-resistance model was used to evaluate a study
326 arm effect of SP chemoprevention on selection for mutant infections compared to the pre-
327 chemoprevention baseline.

328 The final PK/PD-parasitemia models, with epidemiologic covariates, and PK/PD-
329 resistance models for PQ, were utilized sequentially, and concentrations of PQ needed to prevent
330 parasitemia with mutant or wild type infections at each locus were defined as the median value
331 needed to provide 99% protection against parasitemia. One hundred simulations of 1,000
332 pregnancies were conducted using the final PK/PD models to determine the median number of
333 parasitemia episodes and mutant parasitemia episodes with 95% confidence intervals for 1,000
334 pregnancies. Dosing strategies were selected to maximize protective efficacy. Simulated
335 regimens included monthly dosing (2,880 mg PQ and 360 mg DHA divided into three
336 consecutive daily oral doses), once weekly dosing (960 mg PQ and 120 mg DHA), and two once
337 daily dosing options (160 mg PQ with 20 mg DHA and 320 mg PQ with 40 mg DHA). All
338 statistical analyses were conducted in R (version 3.3.2) and STATA (version 14.2).

Acknowledgements. We thank the women who participated in the study, the dedicated study staff, practitioners at the Tororo District Hospital, members of the Infectious Diseases Research Collaboration, and members of the UCSF Drug Research Unit. This work was supported by the National Institutes of Health [R01 AI117001-02, R01 A10750-45, T32 GM007546-41, and P01 HD059454]. The funders had no role in the study design, data collection and interpretation, or decision to submit this work for publication.

Potential conflicts of interest. The authors declare no conflicts of interest.

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499 **Figure Legends**

500 **Figure 1. Trial profile.** Study subjects were tested for *P. falciparum* parasitemia monthly and
501 when they presented for unscheduled visits due to a febrile illness.

502 **Figure 2. (A) Predicted probability of parasitemia with increasing piperaquine**
503 **concentration in the absence of indoor residual spraying of insecticide for women receiving**
504 **DHA-PQ stratified by trimester.** The solid lines (red, second trimester; blue, third trimester)
505 show the median probability and shading encompasses probabilities for 95% of the population.
506 The median probability of parasitemia while receiving SP as IPTp was 39%. **Contributions of**
507 **mutant and wild type genotypes to overall parasitemia probability during the second**
508 **trimester for *pfmdr1* 86 (B) and *pfcrt* 76 (C).** The black line represents the median probability
509 of all parasitemia, and shaded areas indicate the proportion of the probability attributed to wild
510 type (blue) and mutant (red) parasites. Results for the third trimester are shown in Supplemental
511 Figure 3.

512 **Figure 3. Predicted probability of detecting mutant *pfmdr1* 86Y (A) or *pfcrt* 76T (B)**
513 **parasites with increasing piperaquine concentrations for women receiving DHA-PQ with**
514 **parasitemia.** Points are the raw data, showing isolates with mutant (100%) or wild type (0%)
515 genotypes. **(C) Odds of detecting mutant genotypes in the DHA-PQ treatment arms,**
516 **compared to the SP arm.** The solid line is the median probability or increased odds of detecting
517 a mutant parasite during an episode of parasitemia and the shading encompasses the probability
518 or increased odds of detecting a mutant parasite for 95% of the population.

519 **Figure 4. Association between piperaquine concentration and probability of wildtype or**
520 **mutant genotype among women in the second trimester receiving DHA-PQ. Probabilities of**
521 **detecting *pfmdr1* 86 (A) or *pfcrt* 76 (B) genotypes are shown, with closer visualization of the**

curves enclosed in boxes shown for *pfmdr1* 86 (C) and *pfcrt* 76 (D). Arrows indicate the median concentrations (ng/ml) providing 99% protection against parasitemia. Lines indicate the median probabilities, and the shading indicates the probability of detecting mutant parasites for 95% of the population.

Figure 5. (A) Predicted percentage of time above piperaquine concentrations protective against 99% of parasitemia episodes during pregnancy by DHA-PQ regimen. Boxes indicate the interquartile range and error bars represent 95% of the population. **(B) Predicted number of new episodes of parasitemia (gray bars) and episodes of parasitemia with a mutant infection at *pfmdr1* 86 (red) and *pfcrt* 76 (blue) during pregnancy for each chemoprevention regimen.**

542 **Tables**

543 **Table 1. Characteristics of study participants.**

Characteristic	SP every 8 weeks N=106	DP every 8 weeks N=94	DP every 4 weeks N=100
Age in years, mean (SD)	21 (3.6)	22 (4.3)	23 (4.0)
Gravidity (%)			
1	42 (40%)	33 (35%)	36 (36%)
2	32 (30%)	28 (30%)	28 (28%)
≥3	32 (30%)	33 (35%)	36 (36%)
Gestational age at first study drug treatment (%)			
16 weeks	-	-	68
20 weeks	106	94	32
Number of PQ concentration observations			
Venous	-	300	352
Capillary	-	278	280
Visits after participant received indoor residual spraying of insecticide	101	101	153
First episodes of parasitemia after each administration of study drug[a]	140	37	30
Genotypes			
pfmdr1 N86Y genotype available (%)	117 (84%)	37 (100%)	28 (93%)
pfmdr1 86Y (%)	32 (27%)	18 (49%)	24 (86%)
pfcrt K76T genotype available (%)	122 (87%)	37 (100%)	28 (93%)
pfcrt 76T (%)	92 (82%)	31 (84%)	26 (93%)

[a] To avoid consideration of effects of AL or repeat observations of the same parasites, parasitemia detected after treatment with AL and before subsequent receipt of DHA-PQ or parasites detected repeatedly without interval receipt of DHA-PQ were excluded.

Table 2. Pharmacokinetic/pharmacodynamic model parameters.

Model/Parameter	Parameter Estimate	RSE (%)	Between Subject Variability (CV%)	RSE (%)
Sulfadoxine-pyrimethamine pharmacodynamic model				
Baseline logit	-.441	39%	115%	14%
Primigravid baseline logit[a]	.511	78%	-	-
Indoor residual spraying	-.72	60%	-	-
Dry season	-1.13	28%	-	-
Dihydroartemisinin-piperaquine pharmacokinetic/pharmacodynamic model for parasitemia				
Baseline logit	-.508	72%	73%	17%
Primigravid baseline logit[a]	.582	64%	-	-
Slope of concentration dependent effect (mL/ng)	-.204	16%	-	-
Indoor residual spraying	-10 FIXED	-	-	-
Third trimester	-1.45	45%	-	-
Dihydroartemisinin-piperaquine pharmacokinetic/pharmacodynamic model for *pfmdr1 N86Y*				
Baseline logit	-1.16	11%	3.8%	53%
Slope of concentration dependent effect	.317	21%	-	-

Dihydroartemisinin-piperaquine pharmacokinetic/pharmacodynamic model for *pfcrt K76T*					
	Baseline logit	1.06	11%	2.2%	22%
	Slope of concentration dependent effect	.218	22%	-	-

[a] Baseline logit used for all gravidities after start of IPTp as gravidity was not a significant predictor of parasitemia after the start of chemoprevention.

550 **Table 3. Predicted number of mutant infections after starting chemoprevention per 1,000 pregnancies by dosing regimen[a].**

			pfmdr1 86Y				*pfcrt* 76T
Piperaquine dose	Number of infections per 1,000 pregnancies (95% CI)	Mutant Infections (95% CI)	Ratio of mutant infections DP/SP	p-value	Mutant infections (95% CI)	Ratio of mutant infections DP/SP	p-value
0 mg (SP)	2066 (1988-2162)	607 (570-650)	-	-	1564 (1495-1564)	-	-
2,880 mg monthly	317 (280-358)	198 (165-232)	.32	<.001	283 (248-315)	.18	<.001
960 mg weekly	105 (85-122)	87 (71.0-104)	.14	<.001	99 (80.4-115)	.06	<.001
160 mg daily	8.0 (4.0-14.0)	8.0 (3.5-13.5)	.01	<.001	8.0 (4.0-14.0)	.005	<.001
320 mg daily	1.0 (1.0-2.1)	1.0 (.96-2.1)	.002	<.001	1 (1.0-2.1)	.001	<.001

551 [a] Estimated based on monthly surveillance for parasitemia in the absence of indoor residual spraying of pesticide or seasonal variation in

552 transmission

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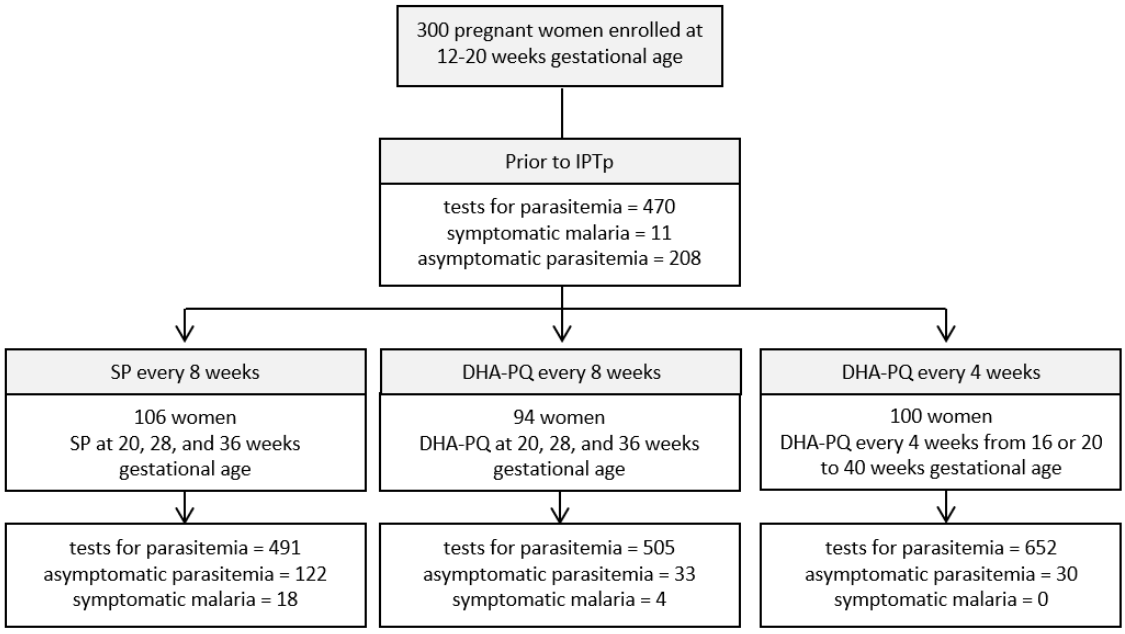

Figure 1. Trial profile. Study subjects were tested for *P. falciparum* parasitemia monthly and when they presented for unscheduled visits due to a febrile illness.

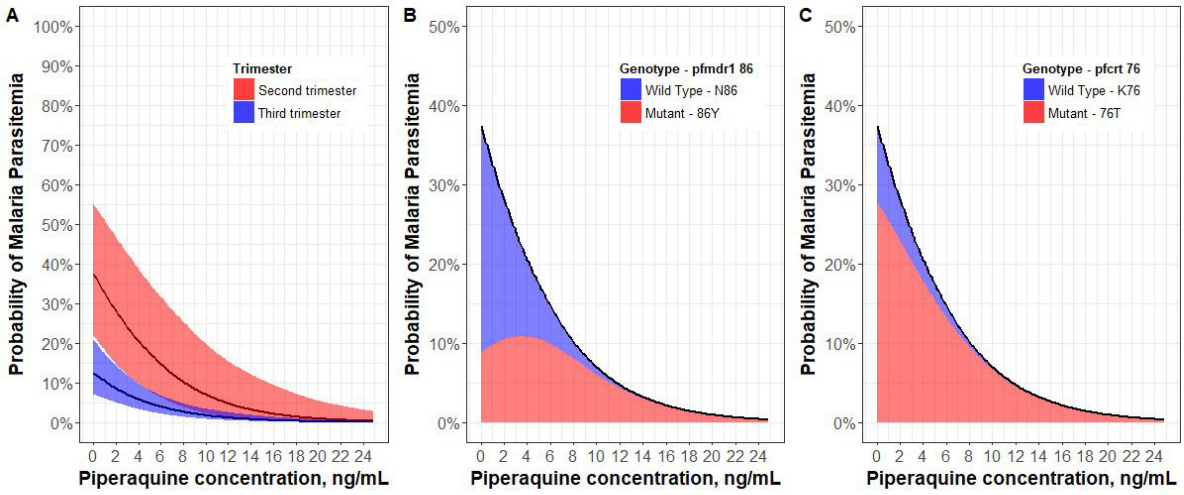

Figure 2. (A) Predicted probability of parasitemia with increasing piperaquine concentration in the absence of indoor residual spraying of insecticide for women receiving DHA-PQ stratified by trimester. The solid lines (red, second trimester; blue, third trimester) show the median probability and shading encompasses probabilities for 95% of the population. The median probability of parasitemia while receiving SP as IPTp was 39%. **Contributions of mutant and wild type genotypes to overall parasitemia probability during the second trimester for *pfmdr1* 86 (B) and *pfcrt* 76 (C).** The black line represents the median probability of all parasitemia, and shaded areas indicate the proportion of the probability attributed to wild type (blue) and mutant (red) parasites. Results for the third trimester are shown in Supplemental Figure 3.

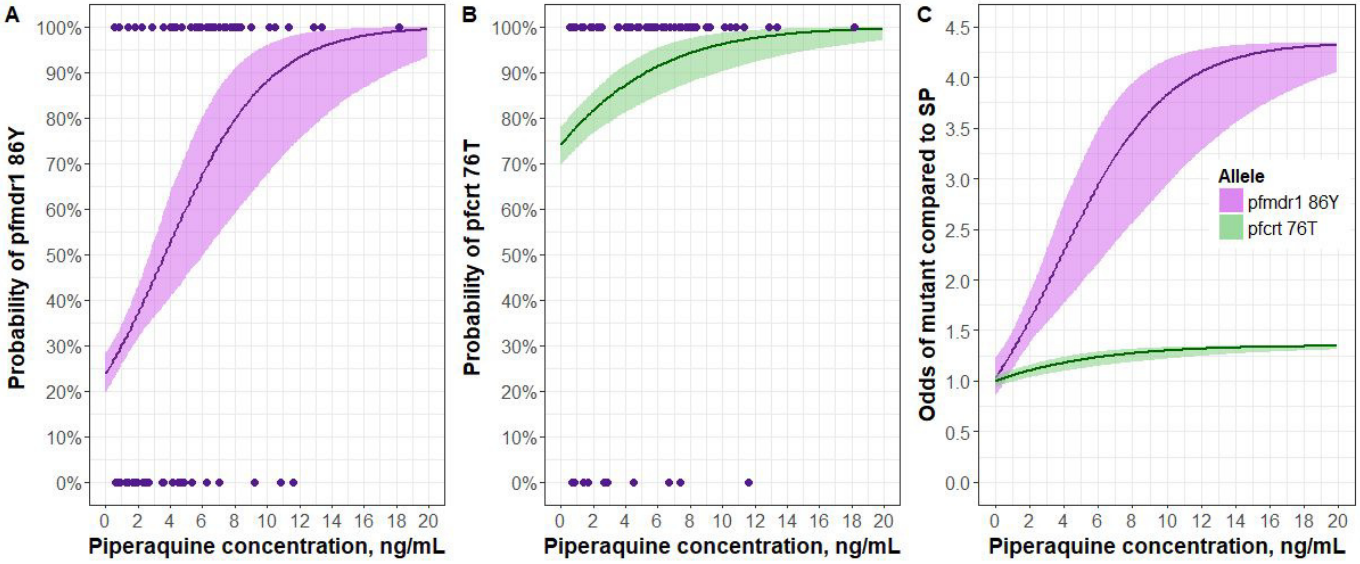

Figure 3. Predicted probability of detecting mutant *pfmdr1* 86Y (A) or *pfcrt* 76T (B) parasites with increasing piperaquine concentrations for women receiving DHA-PQ and parasitemia is detected. Points are the raw data, showing isolates with mutant (100%) or wild type (0%) genotypes. **(C) Odds of detecting mutant genotypes in the DHA-PQ treatment arms, compared to the SP arm.** The solid line is the median probability or increased odds of detecting a mutant parasite during an episode of parasitemia and the shading encompasses the probability or increased odds of detecting a mutant parasite for 95% of the population.

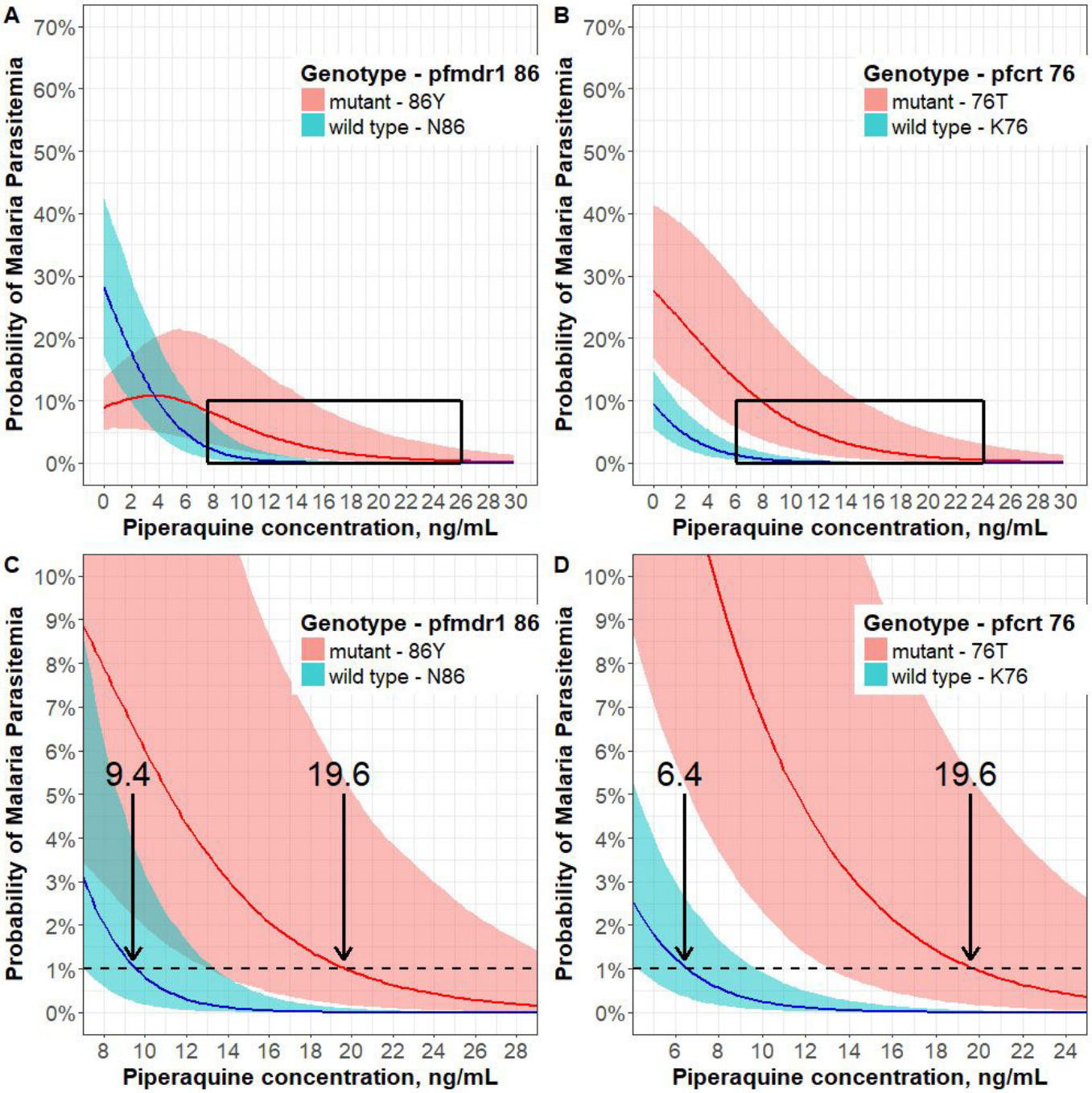

Figure 4. Association between piperaquine concentration and probability of mutant genotype among women in the second trimester receiving DHA-PQ. Probabilities of detecting *pfmdr1* 86 (A) or *pfcrt* 76 (B) genotypes are shown, with closer visualization of the curves enclosed in boxes shown for *pfmdr1* 86 (C) and *pfcrt* 76 (D). Arrows indicate the median concentrations (ng/ml) providing 99% protection against parasitemia. Lines indicate the median probabilities, and the shading indicates the probability of detecting mutant parasites for 95% of the population.

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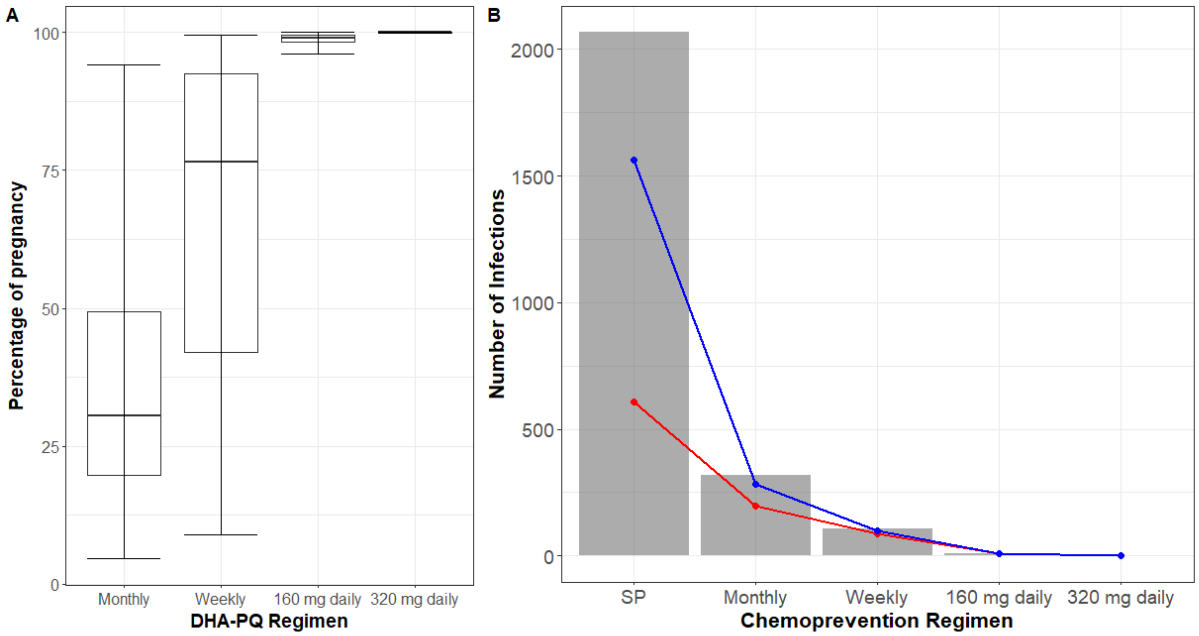

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