

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

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

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22 **Abstract**

23 Dihydroartemisinin-piperaquine (DHA-PQ) is under study for intermittent preventive
24 treatment during pregnancy (IPTp), but it may accelerate selection for drug resistance.
25 Understanding the relationships between piperaquine concentration, prevention of parasitemia,
26 and selection for decreased drug sensitivity can inform control policies and optimization of
27 DHA-PQ dosing. Piperaquine concentrations, measures of parasitemia, and *Plasmodium*
28 *falciparum* genotypes associated with decreased aminoquinoline sensitivity in Africa (*pfmdr1*
29 86Y, *pfprt* 76T) were obtained from pregnant Ugandan women randomized to IPTp with
30 sulfadoxine-pyrimethamine (SP) or DHA-PQ. Joint pharmacokinetic/pharmacodynamic models
31 described relationships between piperaquine concentration and probability of genotypes of
32 interest using nonlinear mixed effects modeling. Increasing piperaquine plasma concentration
33 was associated with a log-linear decrease in risk of parasitemia. Our models predicted that higher
34 median piperaquine concentrations would be required to provide 99% protection against mutant
35 compared to wild type infections (*pfmdr1* N86: 9.6 ng/mL, 86Y: 19.6 ng/mL; *pfprt* K76: 6.5
36 ng/mL, 76T: 19.6 ng/mL). Comparing monthly, weekly, and daily dosing, daily low dose DHA-
37 PQ was predicted to result in the fewest infections and the fewest mutant infections per 1,000
38 pregnancies (predicted mutant infections for *pfmdr1* 86Y: SP monthly: 607, DHA-PQ monthly:
39 198, DHA-PQ daily: 1; for *pfprt* 76T: SP monthly: 1564, DHA-PQ monthly: 283, DHA-PQ
40 daily: 1). Our models predict that higher piperaquine concentrations are needed to prevent
41 infections with *pfmdr1/pfprt* mutant compared to wild type parasites and that, despite selection
42 for mutants by DHA-PQ, the overall burden of mutant infections is lower for IPTp with DHA-
43 PQ than for IPTp with SP.

44

45 **Manuscript**46 **Introduction**

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

62 The long half-life of PQ makes DHA-PQ an ideal choice for malaria chemoprevention,
63 but antimalarials with the longest half-lives may be at the greatest risk for resistance selection
64 (11). Although true resistance to DHA-PQ, as observed in southeast Asia (12, 13), has not been
65 confirmed in Africa (14-16), *P. falciparum* infections that emerge following DHA-PQ treatment
66 have had, compared to parasites not under drug selection, increased prevalence of mutant
67 genotypes in the putative drug transporters *pfmdr1* (86Y) and *pfcr1* (76T) (14, 16, 17). These
68 mutations are associated with decreased sensitivity to chloroquine and amodiaquine, two

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

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

80 **Results**

81 **Study cohort and data collection**

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

91 venous and 558 capillary PQ concentrations were obtained (Table 1, Supplemental Figure 1).
92 Genotyping for the single nucleotide polymorphisms at *pfmdr1* N86Y and *pfcr1* K76T was
93 successful for >84% of episodes of parasitemia in the SP arm, and >93% in the DHA-PQ arms.
94 Prevalences of mutant genotypes were higher in the DHA-PQ arms compared to the SP arm
95 (*pfmdr1* 86Y: SP 27%, DHA-PQ 65%; *pfcr1* 76T: SP 82%, DHA-PQ 87%), as previously
96 reported (Table 1) (16).

97 **PK/PD model building**

98 Simultaneous continuous-categorical PK/PD models were developed using a mixed-
99 effects logistic regression approach. Models were evaluated using objective function value
100 (OFV), with a decrease in OFV (Δ OFV) of -3.84 considered a significant improvement if one
101 parameter was added to the model, and by visual predictive check (Supplemental Figure 2). Two
102 types of simultaneous PK/PD models were developed for the analysis. PK/PD-parasitemia
103 models predicted the risk of parasitemia. PK/PD-resistance models predicted the risk of a mutant
104 infection at *pfmdr1* 86 or *pfcr1* 76 when parasitemia was detected.

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

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

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.

136
$$\text{Logit}(P) = B + \theta_{IRS} + \theta_{Season} + \varepsilon + \eta \quad (\text{Eq2})$$

137 Similar to DHA-PQ, being primigravid significantly increased the risk of parasitemia prior to
138 IPTp by 23.3% (ΔOFV -8.0). Receipt of IRS was associated with a reduced risk of parasitemia
139 (32.7%, Table 2, ΔOFV -27). In addition, for the SP arm the dry season was independently
140 associated with a decreased risk of parasitemia (24.4%, ΔOFV -17). After adjusting for
141 significant covariates, the model did not support the addition of an SP effect (added as time
142 varying, treatment arm effect, or binary covariate). In addition, enrollment BMI, change in BMI
143 and trimester were not associated with significant changes in risk of parasitemia.

144 PK/PD-resistance models were then developed to estimate the probability of a mutant
145 infection at *pfmdr1* N86Y or *pfcr1* K76T. A log-linear relationship between PQ concentration and
146 probability of a mutant infection provided the best fit for both *pfmdr1* 86Y (ΔOFV -11) and *pfcr1*
147 76T (ΔOFV -9.6) (Supplemental Figure 2c, 2d). Increasing PQ concentrations were associated
148 with an increased probability of a mutant infection at both loci (Figure 3). As expected, there was
149 no significant relationship between IPTp with SP and detection of a mutant *pfmdr1* 86Y or *pfcr1*
150 76T allele. Compared to the SP group, the odds of detecting *pfmdr1* 86Y increased with
151 increasing PQ concentration, with a maximum median odds of 4.3 occurring at 17.9 ng/mL PQ
152 (Figure 3c). In the setting of a high baseline risk of *pfcr1* 76T, PQ exposure was associated with a
153 slight increase in the odds of detecting a mutant compared to the SP arm, peaking at a maximum
154 median odds of 1.3 at 10.1 ng/mL PQ (Figure 3c).

155 **Derivation of PQ concentration targets**

156 The PQ concentrations required to prevent 99% of parasitemia episodes varied by
157 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* 86Y, 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 *pfcr1* 76T (19.6 ng/ml [13.1-32.2]) compared to wild type *pfcr1* 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 *pfcr1* 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%.

181 **Discussion**

182 A monthly treatment course of DHA-PQ markedly reduced the burden of parasitemia
183 during pregnancy in Uganda and Kenya, but there is concern that IPTp with DHA-PQ will
184 accelerate selection for drug resistance. With simultaneous PK/PD modeling, we used PQ
185 concentrations and clinical covariates to predict the probability of detecting malaria parasitemia
186 and the probability of detecting parasites with relevant genotypes associated with drug resistance
187 in women receiving DHA-PQ or SP as IPTp in Uganda. Higher concentrations of PQ were
188 needed to reduce the probability of mutant, compared to wild type infections at *pfmdr1* 86 and
189 *pfcr1* 76, but these concentrations were achievable with practical DHA-PQ dosing regimens,
190 including a novel low dose daily regimen that should minimize toxicity concerns (8, 18). Despite
191 selection for mutants by DHA-PQ, the overall burden of mutant infections was predicted to be
192 lower for IPTp with DHA-PQ than with SP. Thus, a low daily dose of DHA-PQ for
193 chemoprevention during pregnancy is predicted to maximize protective efficacy, with limited
194 burden of mutant parasites with decreased aminoquinoline sensitivity, and decreased risk,
195 compared to monthly dosing, of cardiotoxicity (8, 18).

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

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

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

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

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

231 Our study had some limitations. First, parasitemia was assessed at 28-day intervals. We
232 could not determine the exact time when an individual became parasitemic, and thus the exact
233 concentration required to prevent parasitemia. However, monthly PQ concentrations offered a
234 practical sampling strategy with good predictive power in our models. Second, PK data were not
235 available to assist in detecting a concentration-effect relationship between SP and prevention of
236 malaria. We found that, after controlling for covariates which are associated with reduced risk of
237 malaria infection, a model without an SP effect predicted the data adequately. The absence of a
238 protective benefit for SP was further supported by a placebo-controlled chemoprevention trial in
239 Uganda that did not demonstrate a significant protective effect of SP in children (4). Thirdly,
240 treatment failure due to DHA-PQ resistance and associated genetic markers have not been
241 identified in Africa and thus could not be used in this analysis. The markers associated with
242 DHA-PQ resistance in Southeast Asia (*pfkelch*, *plasmepsin2* copy number, and *exo-E415G* (13,
243 25, 26)) were assessed for this population and were either not present or, in the case of
244 *plasmepsin2* copy number, only present in a minority of isolates (16). *Pfmdr1* 86Y and *pfcr1* 76T
245 have been consistently associated with PQ exposure in Uganda (17, 27, 28), and have recently
246 been associated with a modest increase in ex vivo IC₅₀ for PQ (14). As a result, these markers of
247 antimalarial sensitivity were the most relevant for this population.

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

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

256 **Methods**

257 **Study population**

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

265 **Study design and randomization**

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

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

275 **Pharmacokinetic sampling**

276 Women randomized to receive DHA-PQ underwent sparse venous (gestational weeks:
277 20, 28, and 36) and capillary (gestational weeks: 24, 32, and 40) sampling to determine plasma
278 PQ concentrations (8). Sparse PQ concentrations were determined either 28 days after receiving
279 the drug in the 4 week DHA-PQ arm or every 28 days and every 56 days after receiving the drug
280 in the 8 week DHA-PQ arm (8). Venous or capillary specimens were also collected at the time of
281 any malaria diagnosis. A subset of individuals were enrolled in an intensive PK sub-study. For
282 this study, as previously reported (29), venous plasma samples were obtained pre-dose, and 0.5,
283 1, 2, 3, 4, 6, 8, and 24 hours post dose, and capillary plasma samples were collected at 24 hours
284 and 4, 7, 14 and 21 days post dose. PQ base concentrations were determined using high
285 performance liquid chromatography tandem mass spectrometry (HPLC-MS) (30). Modification
286 and partial-validation of the original method for PQ quantitation was performed, to cover a
287 concentration range of 0.50-1,000 ng/mL, with a coefficient of variation <10% for quality
288 control samples (30).

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

290 A blood spot was collected and stored on filter paper at all routine visits and if malaria
291 was diagnosed at an unscheduled visit. DNA was extracted from dried blood spots using Chelex-
292 100 and tested for the presence of *P. falciparum* DNA by loop-mediated isothermal amplification
293 (LAMP) for all microscopy negative samples, as previously described (5, 31). Genotyping for

294 *pfmdr1* N86Y and *pfert* 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 *pfprt* 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 *pfmdr1* N86Y
323 and *pfprt* 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).

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346

347 **References**

- 348 1. Desai M, ter Kuile FO, Nosten F, McGready R, Asamo K, Brabin B, Newman RD.
349 2007. Epidemiology and burden of malaria in pregnancy. *Lancet Infect Dis* 7:93-104.
- 350 2. World Health Organization. 2012. Updated WHO policy recommendation: intermittent
351 preventive treatment of malaria in pregnancy using sulfadoxine-pyrimethamine (IPTp-
352 SP). WHO, Geneva, Switzerland.
- 353 3. Desai M, Gutman J, Taylor SM, Wiegand RE, Khairallah C, Kayentao K, Ouma P,
354 Coulibaly SO, Kalilani L, Mace KE, Arinaitwe E, Mathanga DP, Doumbo O, Otieno K,
355 Edgar D, Chaluluka E, Kamuliwo M, Ades V, Skarbinski J, Shi YP, Magnussen P,
356 Meshnick S, Ter Kuile FO. 2016. Impact of Sulfadoxine-Pyrimethamine Resistance on
357 Effectiveness of Intermittent Preventive Therapy for Malaria in Pregnancy at Clearing
358 Infections and Preventing Low Birth Weight. *Clin Infect Dis* 62:323-333.
- 359 4. Bigira V, Kapisi J, Clark TD, Kinara S, Mwangwa F, Muhindo MK, Osterbauer B,
360 Aweeka FT, Huang L, Achan J, Havlir DV, Rosenthal PJ, Kamya MR, Dorsey G. 2014.

- 361 Protective efficacy and safety of three antimalarial regimens for the prevention of malaria
362 in young Ugandan children: a randomized controlled trial. *PLoS Med* 11:e1001689.
- 363 5. Kakuru A, Jagannathan P, Muhindo MK, Natureeba P, Awori P, Nakalembe M, Opira B,
364 Olwoch P, Ategeka J, Nayebare P, Clark TD, Feeney ME, Charlebois ED, Rizzuto G,
365 Muehlenbachs A, Havlir DV, Kamya MR, Dorsey G. 2016. Dihydroartemisinin-
366 Piperaquine for the Prevention of Malaria in Pregnancy. *N Engl J Med* 374:928-39.
- 367 6. Desai M, Gutman J, L'Lanziva A, Otieno K, Juma E, Kariuki S, Ouma P, Were V,
368 Laserson K, Katana A, Williamson J, ter Kuile FO. 2015. Intermittent screening and
369 treatment or intermittent preventive treatment with dihydroartemisinin-piperaquine
370 versus intermittent preventive treatment with sulfadoxine-pyrimethamine for the control
371 of malaria during pregnancy in western Kenya: an open-label, three-group, randomised
372 controlled superiority trial. *Lancet* 386:2507-19.
- 373 7. Permala J, Tarning J, Nosten F, White NJ, Karlsson MO, Bergstrand M. 2017. Prediction
374 of Improved Antimalarial Chemoprevention with Weekly Dosing of Dihydroartemisinin-
375 Piperaquine. *Antimicrob Agents Chemother* 61:e02491-16.
- 376 8. Savic RM, Jagannathan P, Kajubi R, Huang L, Zhang N, Were M, Kakuru A, Muhindo
377 MK, Mwebaza N, Wallender E, Clark TD, Opira B, Kamya M, Havlir DV, Rosenthal PJ,
378 Dorsey G, Aweeka FT. 2018. Intermittent Preventive Treatment for Malaria in
379 Pregnancy: Optimization of Target Concentrations of Dihydroartemisinin-Piperaquine.
380 *Clin Infect Dis* doi:10.1093/cid/ciy218.
- 381 9. Sambol NC, Tappero JW, Arinaitwe E, Parikh S. 2016. Rethinking Dosing Regimen
382 Selection of Piperaquine for Malaria Chemoprevention: A Simulation Study. *PLoS One*
383 11:e0154623.

- 384 10. Bergstrand M, Nosten F, Lwin KM, Karlsson MO, White NJ, Tarning J. 2014.
385 Characterization of an in vivo concentration-effect relationship for piperazine in malaria
386 chemoprevention. *Sci Transl Med* 6:260ra147.
- 387 11. Stepniewska K, White NJ. 2008. Pharmacokinetic determinants of the window of
388 selection for antimalarial drug resistance. *Antimicrob Agents Chemother* 52:1589-96.
- 389 12. Amaratunga C, Lim P, Suon S, Sreng S, Mao S, Sopha C, Sam B, Dek D, Try V, Amato
390 R, Blessborn D, Song L, Tullo GS, Fay MP, Anderson JM, Tarning J, Fairhurst RM.
391 2016. Dihydroartemisinin-piperazine resistance in *Plasmodium falciparum* malaria in
392 Cambodia: a multisite prospective cohort study. *Lancet Infect Dis* 16:357-65.
- 393 13. Spring MD, Lin JT, Manning JE, Vanachayangkul P, Somethy S, Bun R, Se Y, Chann S,
394 Ittiverakul M, Sia-ngam P, Kuntawunginn W, Arsanok M, Buathong N,
395 Chaorattanakawee S, Gosi P, Ta-aksorn W, Chanarat N, Sundrakes S, Kong N, Heng TK,
396 Nou S, Teja-isavadharm P, Pichyangkul S, Phann ST, Balasubramanian S, Juliano JJ,
397 Meshnick SR, Chour CM, Prom S, Lanteri CA, Lon C, Saunders DL. 2015.
398 Dihydroartemisinin-piperazine failure associated with a triple mutant including kelch13
399 C580Y in Cambodia: an observational cohort study. *Lancet Infect Dis* 15:683-91.
- 400 14. Rasmussen SA, Ceja FG, Conrad MD, Tumwebaze PK, Byaruhanga O, Katairo T,
401 Nsohya SL, Rosenthal PJ, Cooper RA. 2017. Changing Antimalarial Drug Sensitivities in
402 Uganda. *Antimicrob Agents Chemother* 61.
- 403 15. Menard D, Khim N, Beghain J, Adegnika AA, Shafiul-Alam M, Amodu O, Rahim-Awab
404 G, Barnadas C, Berry A, Boum Y, Bustos MD, Cao J, Chen JH, Collet L, Cui L, Thakur
405 GD, Dieye A, Djalle D, Dorkenoo MA, Eboumbou-Moukoko CE, Espino FE, Fandeur T,
406 Ferreira-da-Cruz MF, Fola AA, Fuehrer HP, Hassan AM, Herrera S, Hongvanthong B,

- 407 Houze S, Ibrahim ML, Jahirul-Karim M, Jiang L, Kano S, Ali-Khan W, Khanthavong M,
408 Kremsner PG, Lacerda M, Leang R, Leelawong M, Li M, Lin K, Mazarati JB, Menard S,
409 Morlais I, Muhindo-Mavoko H, Musset L, Na-Bangchang K, Nambozi M, Niare K,
410 Noedl H, et al. 2016. A Worldwide Map of Plasmodium falciparum K13-Propeller
411 Polymorphisms. *N Engl J Med* 374:2453-64.
- 412 16. Conrad MD, Mota D, Foster M, Tukwasibwe S, Legac J, Tumwebaze P, Whalen M,
413 Kakuru A, Nayebare P, Wallender E, Havlir DV, Jagannathan P, Huang L, Aweeka F,
414 Kanya MR, Dorsey G, Rosenthal PJ. 2017. Impact of intermittent preventive treatment
415 during pregnancy on Plasmodium falciparum drug resistance-mediating polymorphisms
416 in Uganda. *J Infect Dis* doi:10.1093/infdis/jix421.
- 417 17. Tumwebaze P, Conrad MD, Walakira A, LeClair N, Byaruhanga O, Nakazibwe C, Kozak
418 B, Bloome J, Okiring J, Kakuru A, Bigira V, Kapisi J, Legac J, Gut J, Cooper RA,
419 Kanya MR, Havlir DV, Dorsey G, Greenhouse B, Nsobya SL, Rosenthal PJ. 2015.
420 Impact of antimalarial treatment and chemoprevention on the drug sensitivity of malaria
421 parasites isolated from ugandan children. *Antimicrob Agents Chemother* 59:3018-30.
- 422 18. Wallender E, Vucicevic K, Jagannathan P, Huang L, Natureeba P, Kakuru A, Muhindo
423 M, Nakalembe M, Havlir D, Kanya M, Aweeka F, Dorsey G, Rosenthal PJ, Savic RM.
424 2018. Predicting Optimal Dihydroartemisinin-Piperaquine Regimens to Prevent Malaria
425 During Pregnancy for Human Immunodeficiency Virus-Infected Women Receiving
426 Efavirenz. *J Infect Dis* 217:964-972.
- 427 19. Lwin KM, Phyo AP, Tarning J, Hanpithakpong W, Ashley EA, Lee SJ, Cheah P,
428 Singhasivanon P, White NJ, Lindegardh N, Nosten F. 2012. Randomized, double-blind,
429 placebo-controlled trial of monthly versus bimonthly dihydroartemisinin-piperaquine

- 430 chemoprevention in adults at high risk of malaria. *Antimicrob Agents Chemother*
431 56:1571-7.
- 432 20. Nankabirwa JI, Wandera B, Amuge P, Kiwanuka N, Dorsey G, Rosenthal PJ, Brooker SJ,
433 Staedke SG, Kanya MR. 2014. Impact of intermittent preventive treatment with
434 dihydroartemisinin-piperaquine on malaria in Ugandan schoolchildren: a randomized,
435 placebo-controlled trial. *Clin Infect Dis* 58:1404-12.
- 436 21. Tumwebaze P, Tukwasibwe S, Taylor A, Conrad M, Ruhamyankaka E, Asua V,
437 Walakira A, Nankabirwa J, Yeka A, Staedke SG, Greenhouse B, Nsohya SL, Kanya
438 MR, Dorsey G, Rosenthal PJ. 2017. Changing Antimalarial Drug Resistance Patterns
439 Identified by Surveillance at Three Sites in Uganda. *J Infect Dis* 215:631-635.
- 440 22. Taylor AR, Flegg JA, Holmes CC, Guerin PJ, Sibley CH, Conrad MD, Dorsey G,
441 Rosenthal PJ. 2017. Artemether-Lumefantrine and Dihydroartemisinin-Piperaquine Exert
442 Inverse Selective Pressure on *Plasmodium Falciparum* Drug Sensitivity-Associated
443 Haplotypes in Uganda. *Open Forum Infect Dis* 4:ofw229.
- 444 23. Yeka A, Wallender, E, Mulebeke, R, Kibuuka, A, Kigozi, R, Bosco, A, Kyambadde, P,
445 Opigo, J, Kalyesubula, S, Senzoga, J, Vinden, J, Conrad, M, Rosenthal, PJ. 2018.
446 Comparative efficacy of artemether-lumefantrine and dihydroartemisinin-piperaquine for
447 the treatment of uncomplicated malaria in Ugandan children. *JID* in press.
- 448 24. Muhindo MK, Kakuru A, Natureeba P, Awori P, Olwoch P, Ategeka J, Nayebare P,
449 Clark TD, Muehlenbachs A, Roh M, Mpeka B, Greenhouse B, Havlir DV, Kanya MR,
450 Dorsey G, Jagannathan P. 2016. Reductions in malaria in pregnancy and adverse birth
451 outcomes following indoor residual spraying of insecticide in Uganda. *Malar J* 15:437.

- 452 25. Amato R, Lim P, Miotto O, Amaratunga C, Dek D, Pearson RD, Almagro-Garcia J, Neal
453 AT, Sreng S, Suon S, Drury E, Jyothi D, Stalker J, Kwiatkowski DP, Fairhurst RM.
454 2017. Genetic markers associated with dihydroartemisinin-piperaquine failure in
455 *Plasmodium falciparum* malaria in Cambodia: a genotype-phenotype association study.
456 *Lancet Infect Dis* 17:164-173.
- 457 26. Witkowski B, Duru V, Khim N, Ross LS, Saintpierre B, Beghain J, Chy S, Kim S, Ke S,
458 Kloeung N, Eam R, Khean C, Ken M, Loch K, Bouillon A, Domergue A, Ma L,
459 Bouchier C, Leang R, Huy R, Nuel G, Barale JC, Legrand E, Ringwald P, Fidock DA,
460 Mercereau-Puijalon O, Arieu F, Menard D. 2017. A surrogate marker of piperaquine-
461 resistant *Plasmodium falciparum* malaria: a phenotype-genotype association study.
462 *Lancet Infect Dis* 17:174-183.
- 463 27. Nankabirwa JI, Conrad MD, Legac J, Tukwasibwe S, Tumwebaze P, Wandera B,
464 Brooker SJ, Staedke SG, Kamya MR, Nsobya SL, Dorsey G, Rosenthal PJ. 2016.
465 Intermittent Preventive Treatment with Dihydroartemisinin-Piperaquine in Ugandan
466 Schoolchildren Selects for *Plasmodium falciparum* Transporter Polymorphisms That
467 Modify Drug Sensitivity. *Antimicrob Agents Chemother* 60:5649-54.
- 468 28. Conrad MD, LeClair N, Arinaitwe E, Wanzira H, Kakuru A, Bigira V, Muhindo M,
469 Kamya MR, Tappero JW, Greenhouse B, Dorsey G, Rosenthal PJ. 2014. Comparative
470 impacts over 5 years of artemisinin-based combination therapies on *Plasmodium*
471 *falciparum* polymorphisms that modulate drug sensitivity in Ugandan children. *J Infect*
472 *Dis* 210:344-53.
- 473 29. Kajubi R, Huang L, Jagannathan P, Chamankhah N, Were M, Ruel T, Koss CA, Kakuru
474 A, Mwebaza N, Kamya M, Havlir D, Dorsey G, Rosenthal PJ, Aweeka FT. 2017.

- 475 Antiretroviral therapy with efavirenz accentuates pregnancy-associated reduction of
476 dihydroartemisinin-piperaquine exposure during malaria chemoprevention. *Clin*
477 *Pharmacol Ther* 102:520-528.
- 478 30. Kjellin LL, Dorsey G, Rosenthal PJ, Aweeka F, Huang L. 2014. Determination of the
479 antimalarial drug piperaquine in small volume pediatric plasma samples by LC-MS/MS.
480 *Bioanalysis* 6:3081-9.
- 481 31. Hopkins H, Gonzalez IJ, Polley SD, Angutoko P, Ategeka J, Asimwe C, Agaba B,
482 Kyabayinze DJ, Sutherland CJ, Perkins MD, Bell D. 2013. Highly sensitive detection of
483 malaria parasitemia in a malaria-endemic setting: performance of a new loop-mediated
484 isothermal amplification kit in a remote clinic in Uganda. *J Infect Dis* 208:645-52.
- 485 32. LeClair NP, Conrad MD, Baliraine FN, Nsanzabana C, Nsohya SL, Rosenthal PJ. 2013.
486 Optimization of a ligase detection reaction-fluorescent microsphere assay for
487 characterization of resistance-mediating polymorphisms in African samples of
488 *Plasmodium falciparum*. *J Clin Microbiol* 51:2564-70.
- 489 33. Bonate PL, Steimer J-L. 2006. Pharmacokinetic-pharmacodynamic modeling and
490 simulation. Springer.
- 491 34. de Kock M, Tarning J, Workman L, Nyunt MM, Adam I, Barnes KI, Denti P. 2017.
492 Pharmacokinetics of Sulfadoxine and Pyrimethamine for Intermittent Preventive
493 Treatment of Malaria During Pregnancy and After Delivery. *CPT Pharmacometrics Syst*
494 *Pharmacol* 6:430-438.
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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 piperazine**
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 *pfcr1* 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 *pfcr1* 76T (B)**
513 **parasites with increasing piperazine 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 piperazine 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 *pfcr1* 76 (B) genotypes are shown, with closer visualization of the**

522 **curves enclosed in boxes shown for *pfmdr1* 86 (C) and *pfprt* 76 (D).** Arrows indicate the
523 median concentrations (ng/ml) providing 99% protection against parasitemia. Lines indicate the
524 median probabilities, and the shading indicates the probability of detecting mutant parasites for
525 95% of the population.

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

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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			
<i>pfmdr1</i> N86Y genotype available (%)	117 (84%)	37 (100%)	28 (93%)
<i>pfmdr1</i> 86Y (%)	32 (27%)	18 (49%)	24 (86%)
<i>pfert</i> K76T genotype available (%)	122 (87%)	37 (100%)	28 (93%)
<i>pfert</i> 76T (%)	92 (82%)	31 (84%)	26 (93%)

544 ^aTo avoid consideration of effects of AL or repeat observations of the same parasites, parasitemia
 545 detected after treatment with AL and before subsequent receipt of DHA-PQ or parasites detected
 546 repeatedly without interval receipt of DHA-PQ were excluded.

547 **Table 2. Pharmacokinetic/pharmacodynamic model parameters.**

Model/Parameter	Parameter		Between Subject	
	Estimate	RSE (%)	Variability (CV%)	RSE (%)
Sulfadoxine-pyrimethamine pharmacodynamic model				
Baseline logit	-.441	39%	115%	14%
Primigravid baseline	.511	78%	-	-
logit ^a				
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	.582	64%	-	-
logit ^a				
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 <i>pfmdr1</i> N86Y				
Baseline logit	-1.16	11%	3.8%	53%
Slope of concentration dependent effect	.317	21%	-	-

Dihydroartemisinin-piperaquine pharmacokinetic/pharmacodynamic model for <i>pfprt</i> K76T				
Baseline logit	1.06	11%	2.2%	22%
Slope of concentration dependent effect	.218	22%	-	-

548 ^a Baseline logit used for all gravidities after start of IPTp as gravidity was not a significant predictor of

549 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.**

Piperaquine dose	<i>pfmdr1</i> 86Y				<i>pfert</i> 76T		
	Number of	Mutant	Ratio of	p-value	Mutant infections	Ratio of	p-value
	infections per	Infections	mutant		infections	mutant	
	1,000 pregnancies	(95% CI)	infections	DP/SP	(95% CI)	DP/SP	
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

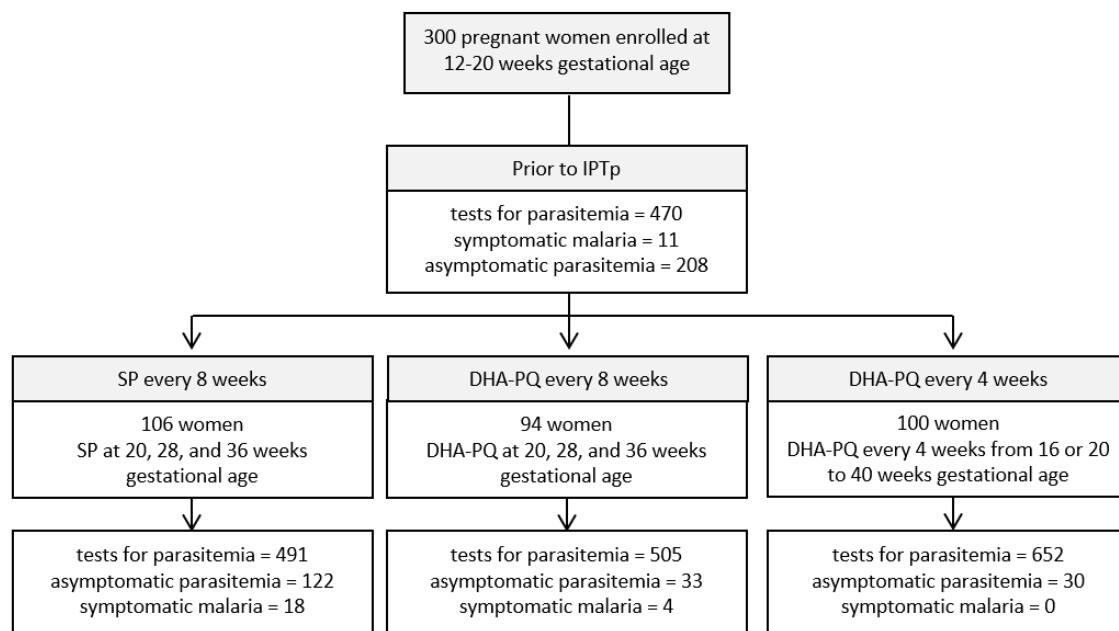


Figure 1. Trial profile. Study subjects were tested for *P. falciparum* parasitemia monthly and when they presented for unscheduled visits due to a febrile illness.

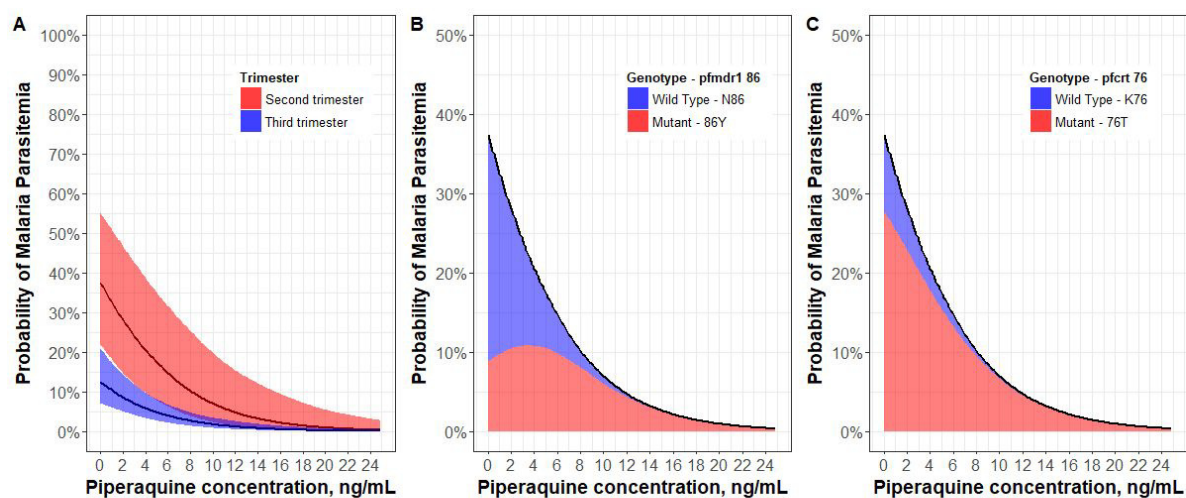


Figure 2. (A) Predicted probability of parasitemia with increasing piperazine concentration in the absence of indoor residual spraying of insecticide for women receiving DHA-PQ stratified by trimester. The solid lines (red, second trimester; blue, third trimester) show the median probability and shading 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 *pfcr7* 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.

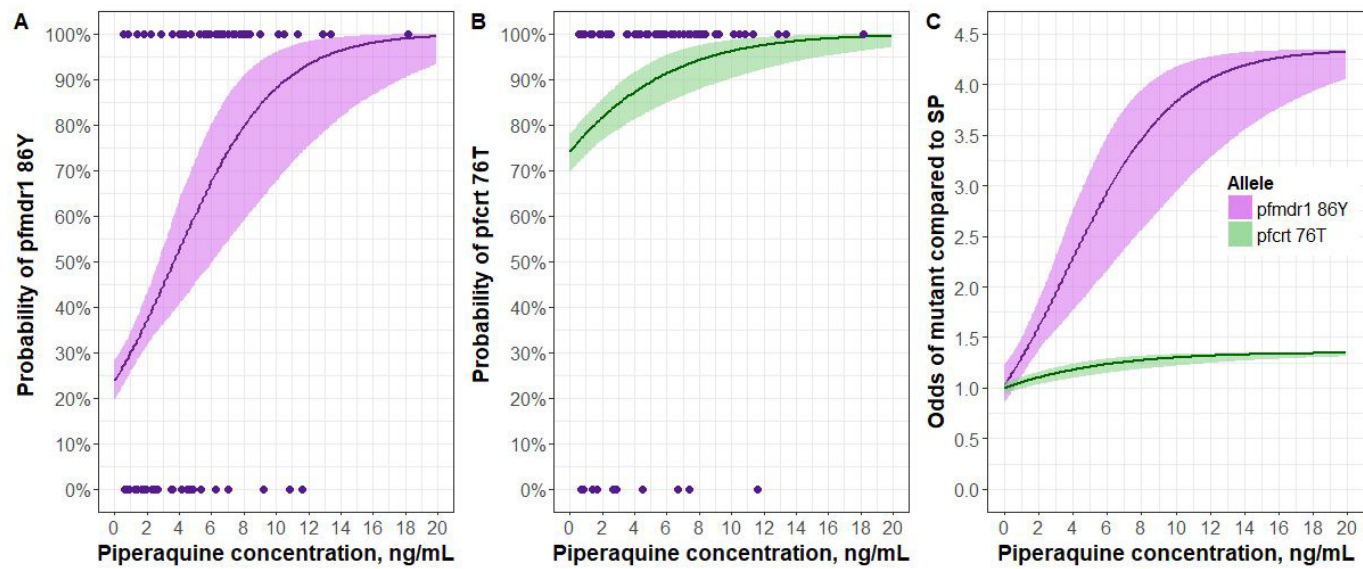


Figure 3. Predicted probability of detecting mutant *pfmdr1* 86Y (A) or *pfcr1* 76T (B) parasites with increasing piperazine concentrations for women receiving DHA-PQ and parasitemia is detected. Points are the raw data, showing isolates with mutant (100%) or wild type (0%) genotypes. (C) Odds of detecting 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.

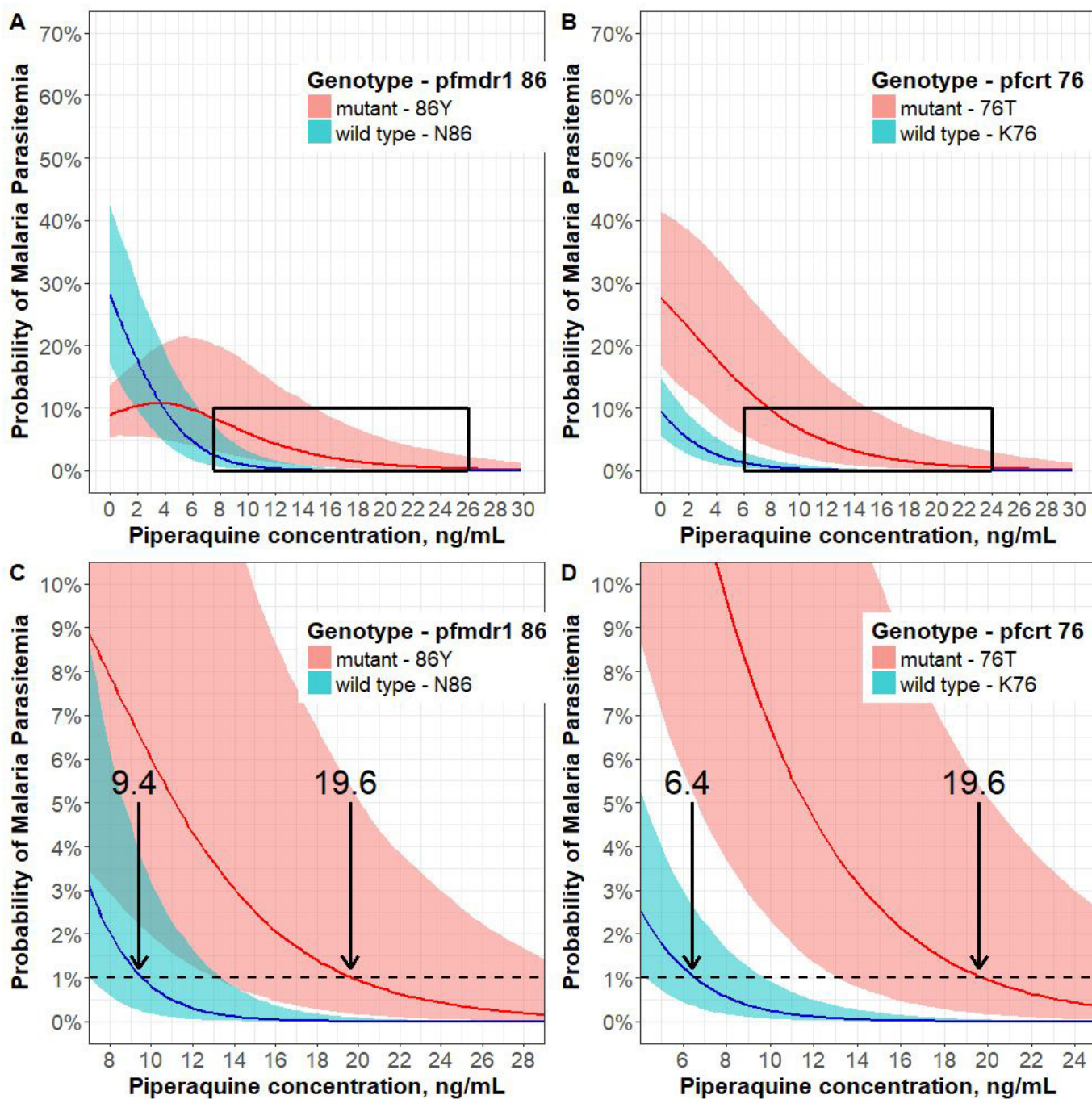


Figure 4. Association between piperazine concentration and probability of mutant genotype among women in the second trimester receiving DHA-PQ. Probabilities of detecting *pfmdr1* 86 (A) or *pfcr1* 76 (B) genotypes are shown, with closer visualization of the curves enclosed in boxes shown for *pfmdr1* 86 (C) and *pfcr1* 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.

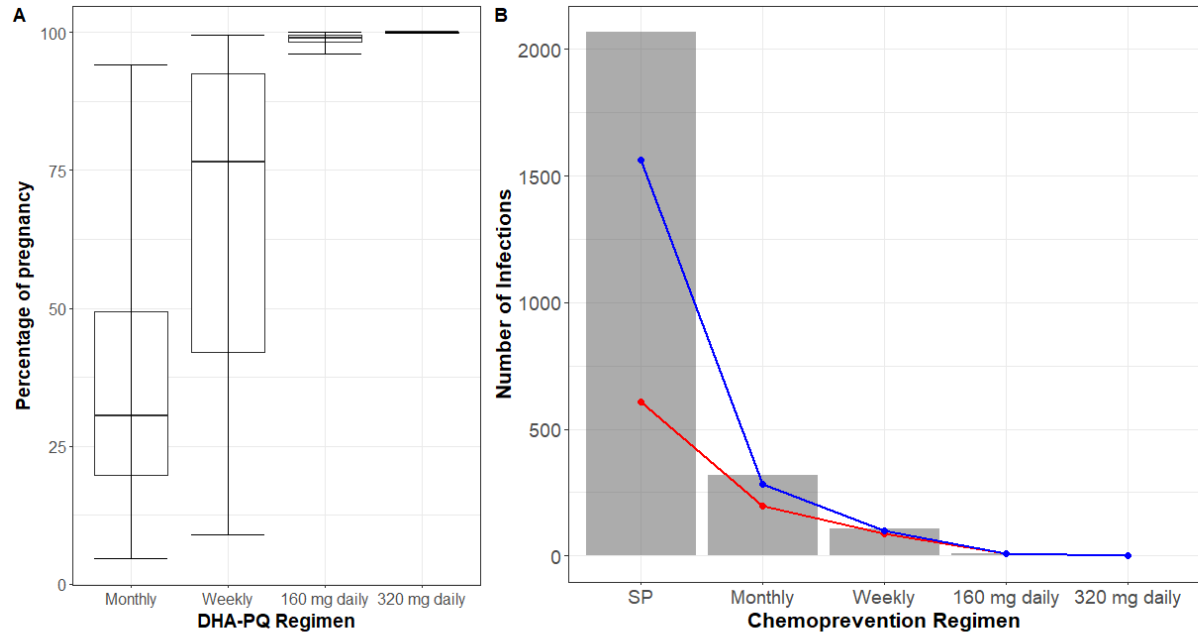


Figure 5. (A) Predicted percentage of time above piperaquine concentrations protective against 99% of parasitemia episodes during pregnancy by DHA-PQ regimen. Boxes indicate the interquartile range and error bars represent 95% of the population. (B) Predicted number of new episodes of parasitemia (gray bars) and episodes of parasitemia with a mutant infection at *pfndr1* 86 (red) and *pfert* 76 (blue) during pregnancy for each chemoprevention regimen.