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Significant efficacy of single low dose primaquine compared to stand alone artemisinin combination therapy in reducing gametocyte carriage in Cambodian patients with uncomplicated multidrug resistant *Plasmodium falciparum* malaria

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Running Head (54 characters including spaces): Efficacy of single low dose primaquine in Cambodia

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Abstract (250 words)

Since 2012, single low dose of primaquine (SLDPQ, 0.25mg/kg) has been recommended with artemisinin-based combination therapies, as first-line treatment of acute uncomplicated *Plasmodium falciparum* malaria, to interrupt its transmission, especially in low transmission settings of multidrug, including artemisinin, resistance. Policy makers in Cambodia have been reluctant to implement this recommendation due to primaquine safety concerns and lack of data on its efficacy.

In this randomized controlled trial, 109 Cambodians with acute uncomplicated *P. falciparum* malaria received dihydroartemisinin-piperaquine (DP) alone or combined with SLDPQ on the first treatment day. Transmission-blocking efficacy of SLDPQ was evaluated on Days 0, 1, 2, 3, 7, 14, 21, 28 and recrudescence by reverse transcriptase polymerase chain reaction (RT-PCR) (gametocyte prevalence) and membrane-feeding assays with *Anopheles minimus* mosquitoes (gametocyte infectivity). Without the influence of recrudescence infections, DP+SLDPQ reduced gametocyte carriage 3 fold compared to DP. Of 48 patients tested on Day 0, only three patients were infectious to mosquitoes (~6%). Post-treatment, three patients were infectious: on D14 (3.5%, 1/29), and on the first and seventh day of recrudescence (8.3%, 1/12 for each); this overall low infectivity precluded our ability to assess its transmission blocking efficacy.

Our study confirms effective gametocyte clearance of SLDPQ when combined with DP in multidrug resistant *P. falciparum* and the negative impact of recrudescence infections due to poor DP efficacy. Artesunate-mefloquine (ASMQ) has replaced DP and ASMQ-SLDPQ has been deployed to treat all *P. falciparum* symptomatic patients to further support the elimination of multidrug resistant *P. falciparum* in Cambodia.

Key words: direct-membrane feeding assays, transmission-blocking, malaria, primaquine

Introduction

Malaria remains a major public health challenge with an estimated 228 million cases recorded in 2018 (1). While considerable progress has been made since 2010 when the estimated case burden was 251 million, the recent emergence and spread of both artemisinin and piperaquine resistant *Plasmodium falciparum* lineage (KEL1/PLA1) in the Eastern Greater Mekong subRegion (GMS), threaten this remarkable global achievement (2-7). Eliminating rapidly multidrug resistant *P. falciparum* (MDRPf) is, therefore, the top priority for countries of the Greater Mekong Subregion (GMS). Implementation of novel tools and strategies or repurposing existing tools like primaquine that specifically aim at interrupting malaria transmission are essential to reach this goal.

Although artemisinin derivatives are the more potent compounds in artemisinin-based combination therapies (ACTs) and are active against early gametocyte stages (8-10) and reduce transmission (11, 12), the only currently available drug effective against the transmissible mature stage V gametocytes is primaquine. In 2012, the WHO recommended the addition of single low dose of primaquine (SLDPQ, target dose 0.25mg/kg) to ACTs to interrupt malaria transmission, primarily in low transmission areas of MDRPf like the GMS (13).

To date, several clinical studies conducted in Africa, Colombia and Cambodia, have assessed the safety and efficacy of SLDPQ (14-24), mainly by measuring gametocyte carriage [microscopy or Taqman reverse transcriptase polymerase chain reaction (RT-PCR)] as a surrogate marker of its transmission blocking efficacy. This is due to the challenging logistical requirements of carrying out mosquito infectivity studies. These studies showed that SLDPQ, dosed from 0.20 to 0.75 mg/kg, clearly reduced gametocyte carriage and increased their clearance in a dose dependent

manner (15-17, 19, 21-25). A small number of studies have investigated human-to-mosquito transmission, based on infectivity measures, with variable results due to low pre- and/or post-SLDPQ infectivity (16, 17, 19, 22, 24). Only two studies from Mali demonstrated conclusively the transmission-blocking efficacy of SLDPQ which produced a 92.6% to 100% within-person reduction in infectivity at Day 2 vs. baseline following 0.25 mg/kg of SLDPQ (16, 19).

In Cambodia, glucose-6-phosphate dehydrogenase deficiency (G6PDd) is a common X-linked disorder of the red blood cells with frequencies ranging from 10.8% to 29.6% in males (26) and a prevalence rate in malaria patients seen at health centers estimated at 13.9% (27). This is the main reason why Cambodian policy makers have been reluctant to deploy SLDPQ without local data on the safety and the efficacy of SLDPQ. Therefore, we conducted a trial assessing the tolerability and the safety of SLDPQ (0.25mg/kg) in Cambodia in 2015-2016 (28), which followed on from a safety trial of 0.75 mg/kg/week of PQ in vivax patients (29). Here, we present our investigations on the transmission-blocking efficacy of this treatment through the evaluation of gametocyte prevalence dynamics as well as infectivity measures on a subset of patients using membrane-feeding assays with *Anopheles minimus* mosquitoes, one of the main malaria vectors in Southeast Asia.

Methods

Study design

This open-label randomized control trial assessing the tolerability and the safety of SLDPQ was carried out in Banlung, Rattanakiri province in northeastern Cambodia in 2015-2016

(NCT02434952), as described in Dysoley *et al.* (28). Briefly, 109 non pregnant, non breast feeding patients aged ≥ 1 year with acute uncomplicated falciparum malaria (≥ 1 asexual parasite/500 white blood cells equivalent to 16 asexual parasites/ μ l) and a hemoglobin concentration ≥ 6 g/dL were recruited to receive either dihydroartemisinin piperazine (DP, Duo-Cotecxin®, D40 mg and P320 mg, Zhejiang Holley Nanhu Pharmaceutical Co. Ltd, Jiaxing, Zhejiang, China) alone or combined with SLDPQ (0.25mg/kg given with the first dose of DP, Thai Government Pharmaceutical Organization, 15mg of primaquine base). Clinical and laboratory assessments were performed on Days (D) 0, 1, 2, 3, 7, 14, 21 and 28. *Anopheles minimus* mosquitoes were fed on blood samples collected from a subset of 55 patients and on the days of recurrent (Drec) falciparum parasitaemia (defined by the WHO as late treatment failures) depending on mosquito availability using direct-membrane feeding assays (DMFA).

The sample size calculation was based on the primary outcome of a reduction in the mean D7 hemoglobin (Hb) concentration of 1 g/dL in the G6PDd DP+SLDPQ arm vs. G6PDn DP+SLDPQ arm. Assuming a mean and standard deviation of 11.27 (1.74) g/dL, calculated from a SE Asian database of ~6,800 ACT-treated, falciparum-infected patients of all ages (WRJT, unpublished), a two-sided alpha of 0.05, power of 80%, the sample size was 48/arm, rounded up to 50. G6PD status in the field was diagnosed initially using the fluorescent spot test to allow for the allocation of SLDPQ. The qualitative Carestart® (AcessBio, Solerset, NJ) rapid diagnostic test was assessed in parallel.

Mosquito infection

DMFAs were carried out to assess individual malaria infectivity as described previously (30). Briefly, 3-to-5-day-old *An. minimus* females were fed through membranes on patient's blood.

Mosquitoes were starved for 24h before being provided a blood meal. Venous blood samples were collected in heparinized tubes and 400µl of blood were made available in membrane feeders maintained at 37°C. Females were fed only once on freshly drawn blood. Post-feeding, unfed females were discarded and fed females were kept in cages (20x20x20cm) with constant access to a 10% sucrose solution. Patient infectivity was determined by assessing infection prevalence (i.e. proportion of infected females) and infection intensity (i.e. number of *Plasmodium sp.* oocysts in infected females). Midguts were dissected in a 1% Mercurochrome[®] stain and the presence and the number of oocysts were determined under a microscope (20x magnification). Dissections were performed 7-day post-blood meal. The mean number of dissected females was 49 (range: 15-85, median: 50).

Biological investigations

Parasite RNA was extracted from the Trizol[®] (Life Technologies Holdings Pte Ltd, Singapore) conserved whole blood samples using QIAamp RNA Blood Mini Kit[®] (Qiagen, Germany), following the protocol recommended by the manufacturer. A two-step semi-quantitative real time PCR was performed to detect malaria parasites, as previously described (31). Following PCR amplification, falciparum positive samples were analyzed for the presence of gametocytes by a Taqman RT-PCR, using primers spanning an exon-exon junction and targeting the “*Plasmodium falciparum* meiotic recombination protein DMC1-like protein” gene (AF356553), as described previously (32). Gametocyte dilution series based on *in-vitro* cultured local strain were used to estimate gametocyte blood concentrations.

Ethical statement

Ethical approvals were obtained from the Cambodian National Ethics Committee for Health Research (0370NECHR) and the trial registered at the clinicaltrials.gov (NCT02434952). The protocols conformed to the Helsinki Declaration on ethical principles for medical research involving human subjects (version 2002) and informed written consent was obtained for all volunteers.

Statistical analyses

Initially, univariate Generalized Linear Mixed Models (GLMMs) with a binomial distribution were fitted to model the PCR measured, gametocyte prevalence rates. In these models, follow-up days (D1, 2, 3, 7, 14, 21 and 28), treatment (DP *vs.* DP+SLDPQ), presence of gametocytes prior to treatment on D0, recrudescence status (recrudescent *vs.* cured patient) and hemoglobin concentration prior to treatment on D0 were included in the model as fixed factors. Patient ID was coded as a random factor to account for repeated measures on the same individual. The univariate analyses were followed by a multivariable GLMM to model the dynamics of gametocyte prevalence post-treatment including all the significant factors from the univariate analysis.

Gametocyte clearance times were not compared in the study for three principal reasons: (i) of the 21 patients with microscopically detected D0 gametocytes, only 7 had complete follow up microscopy data, (ii) Taqman RT-PCR measured gametocyte densities were highly variable during the 28 days follow-up, and, (iii) by D28, 10/34 patients with gametocytes on D0 by Taqman RT-PCR were still gametocytemic (n=1, DP+SLDPQ arm). *P*-values <0.05 were deemed significant. All analyses were performed in R v.3.5.1 (33).

Results

One hundred nine patients with uncomplicated falciparum malaria were enrolled and treated with the standard 3-day DP alone (48.6%, 53/109) or DP+SLDPQ (51.4%, 56/109). Seven patients were lost to follow up (6.4%). Of the remaining 102 patients, 28 (27.4%) had PCR-proven *P. falciparum* recrudescences; 24 occurred within the 28-day follow up on Days 12 (1), 14 (1), 17 (2), 18 (1), 21 (2), 22 (2), 23 (1), 24 (2), 25 (1), 26 (2), 27 (1), 28 (8), and four on D44, 46, 52, and 124. Of the 28 recrudescence patients, 10 were retreated with the standard 3-day DP and 18 with quinine or quinine plus tetracycline; six of the 10 patients retreated with DP experienced a second recrudescence.

At D0, gametocytes were detected in 47/109 patients (43.1%) by Taqman RT-PCR (Table 1; see 30 for further details), with no significant difference between arms ($P=0.3057$).

Gametocyte dynamics

Examining the dynamics of Taqman RT-PCR gametocyte carriage to D28, drug arm, baseline gametocytemia, recrudescence status, baseline hemoglobin and Day of follow up were all significant factors for gametocytemia in the univariate analysis (Table 2).

The DP recipients were more likely to be gametocytemic than DP+SLDPQ recipients during the follow-up, seen more clearly on D7 and D14 (Table 2; Figure 1) as were patients with baseline gametocytemia (Table 2; Figure 2) and recrudescence vs. cured patients (Table 2).

Baseline hemoglobin concentration and Day of follow-up were inversely associated with the probability of being gametocytemic during the follow-up (Table 2).

When the aforementioned factors were tested in a multivariable model, all except drug arm remained significant in the multivariate analysis (Table 3, model 1). However, when removing recrudescence from the multivariable model, drug arm was a significant explanatory factor again (Table 3, model 2).

Post-hoc calculation showed that there was a 99.1% power to detect a difference between the DP and DP-PPQ treatment with the study sample sizes (n=49 and n=56 on D7 for DP and DP-PPQ respectively).

Mosquito infectivity

A total of 55 patients were included in the DMFAs. Amongst the 292 feeding assays, only 113 of 14,444 (0.78%) dissected mosquitoes were infected (Table 4). Overall, 3 out of 48 ($6.3 \pm 6.8\%$) individuals infected at least one mosquito at baseline, one individual was infectious on D14 (DP) and two individuals with recrudescence infections (Tables 4 & 5) who were repeat DP treatment failures: (i) at presentation on D124 (DP+SLDPQ), and (ii) D35 (7 days post DP started on D28). The three individuals infectious on D0 were all gametocytemic by microscopy, received DP+SLDPQ and were not infectious on D1 or D3 post-treatment (Table 5).

Discussion

In 2012, the WHO recommended adding 0.25 mg/kg SLDPQ to first-line treatments (ACTs) to kill mature gametocytes and block human-to-mosquito transmission (13). As no data were available at that time in Cambodia, we investigated the transmission-blocking efficacy of SLDPQ when given with DP (the recommended first line standard ACT in 2015) by evaluating

gametocyte prevalence dynamics (Taqman RT-PCR) and *Anopheles minimus* infectivity by DMFA.

Our results show that SLDPQ, given on the first day of the 3-day standard ACT treatment, significantly decreased gametocyte carriage over time, starting on D3 and was least on D7 resulting in a 5.59 fold reduced risk, relative to DP alone, over 28 days in the univariate analysis. This is consistent with recent reports on the efficacy of PQ in reducing gametocyte carriage (15-17, 19, 21-25, 34) and earlier reports showing a lag between reduced gametocyte carriage and rapid mosquito infectivity. The multivariable analysis revealed the significant effect of recrudescent (resistant) infections on gametocyte carriage with a 3 fold reduction in gametocyte carriage in the absence of resistance.

Our patients had a high rate of baseline gametocytemia (19% by microscopy, just under 45% by Taqman RT-PCR), which is similar to that reported previously in 2010 in artemisinin resistant western Cambodia (~19%) but is three fold higher than the 6% reported earlier in Rattanakiri province (35). Given the high prevalence of *PfKelch13* mutant *P. falciparum* parasites (63%) in our study and the very high recrudescent rate (~28%), our high gametocyte carriage rate is consistent with the presence of ARPf, which has also led to piperaquine resistance (7). This is consistent with another study in Cambodia which showed that DP treated patients from Oddar Meanchey province (northern Cambodia) also had prolonged gametocyte carriage, irrespective of the slow asexual parasite clearance time, due to ARPf, and in patients with recrudescent infections (22, 35).

However, prolonged gametocyte carriage has also been seen in DP treated African patients without ARPf who, nevertheless, had low human-to-mosquito transmission (16, 35, 36). Moreover, several comparative studies have found that gametocyte carriage is higher in DP compared to artemether lumefantrine (36-39), suggesting reduced gametocytocidal activity of piperaquine compared to lumefantrine.

Overall, we observed a biphasic pattern of gametocyte prevalence with a drop on D7, a rebound on D14, followed by a decrease on D21 and D28. However, this effect was mainly driven by the DP treated patients as their gametocyte carriage rate was similar over the 28-day follow-up whereas it decreased markedly in the DP+SLDPQ treated patients (Figure 1).

We were not able to assess the transmission-blocking efficacy of SLDPQ due to the very low infectivity rate observed in only three patients (~6%) at baseline and three infectious events during follow up. This is a major limitation of DFMA, previously reported by Lin *et al.* (22) at baseline using *Anopheles dirus* mosquitoes and on D7, without assessing baseline, by Okebe *et al* (24). In another study in African children, despite a higher infectivity rate at enrollment after microscopy-based selection of gametocyte positive asymptomatic children (38%), Gonçalves *et al.* (17) were also unable to detect the added value of SLDPQ due to very low infectivity post-treatments in all arms. As our clinical trial aimed primarily at assessing SLDPQ tolerability and safety, we did not particularly select patent or high-density gametocyte carriers and thus would have needed substantially more individuals to assess transmission-blocking efficacy. Only two studies in Mali statistically confirmed high anti-infectivity efficacy (>90%) at 48h of SLDPQ, when dosed at baseline, after selecting gametocyte positive participants by microscopy (16, 19).

249 However, we observed that the three SLDPQ treated infectious patients on D0 did not infect
250 mosquitoes on subsequent days despite the presence of gametocytes. Several reasons may explain
251 this finding. Following primaquine, an anti-infectivity effect is rapid ($\leq 24\text{h}$) and precedes the
252 decline in gametocytaemia (40). Male gametocytes seem to be more sensitive to primaquine than
253 female gametocytes (41) and *P. falciparum* sex ratio is biased towards females, potentially
254 resulting in delayed gametocyte clearance. The relationship between gametocytemia and
255 infectivity is nonlinear and is, thus, an indirect measure of infectivity (42-44).

256
257 To conclude, our study shows that the addition of SLDPQ to ACT treatment in symptomatic
258 falciparum malaria decreases substantially gametocyte carriage in patients with MDRPf.
259 Although unable to reconfirm the elegant studies of Dicko *et al.* (16, 19), SLDPQ, dosed on the
260 first day of treatment, is likely to further decrease the transmission potential of *P. falciparum*
261 patients in our setting when combined with an effective ACT.

262 Owing to the high failure rate of DP, Cambodia has switched to artesunate-mefloquine treatment
263 as first line treatment for uncomplicated *P. falciparum* and in January 2018 deployed SLDPQ in
264 the whole country, based partly on the results of these findings and the good tolerability of
265 SLDPQ in G6PDd patients (28). An age based dosing regimen of SLDPQ, designed for
266 Cambodia, will aid elimination efforts where weighing scales are unavailable (45).

267
268 **Conflicts of interest.** All authors declare no conflicts of interest.

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References

1. World Health Organization. 2019. World malaria report 2019. Geneva, Switzerland: 232p.
2. Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, Fukuda MM, Consortium ARCS. 2008. Evidence of Artemisinin-Resistant Malaria in Western Cambodia. N Engl J Med 359:2619-2620.
3. Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J. 2009. Artemisinin resistance in *Plasmodium falciparum* malaria. N Engl J Med 361.

- 292 4. Saunders DL, Vanachayangkul P, Lon C. 2014. Dihydroartemisinin-piperaquine failure in
293 Cambodia. *N Engl J Med* 371:484-5.
- 294 5. Leang R, Taylor WR, Bouth DM, Song L, Tarning J, Char MC, Kim S, Witkowski B, Duru V,
295 Domergue A, Khim N, Ringwald P, Menard D. 2015. Evidence of *Plasmodium falciparum*
296 malaria multidrug resistance to artemisinin and piperaquine in Western Cambodia:
297 dihydroartemisinin-piperaquine open-label multicenter clinical assessment. *Antimicrob Agents*
298 *Chemother* 59:4719-26.
- 299 6. Imwong M, Hien TT, Thuy-Nhien NT, Dondorp AM, White NJ. 2017. Spread of a single
300 multidrug resistant malaria parasite lineage (*PfPailin*) to Vietnam. *Lancet Infect Dis* 17:1022-
301 1023.
- 302 7. van der Pluijm RW, Imwong M, Chau NH, Hoa NT, Thuy-Nhien NT, Thanh NV, Jittamala P,
303 Hanboonkunupakarn B, Chutasmit K, Saelow C, Runjarern R, Kaewmok W, Tripura R, Peto
304 TJ, Yok S, Suon S, Sreng S, Mao S, Oun S, Yen S, Amaratunga C, Lek D, Huy R, Dhorda M,
305 Chotivanich K, Ashley EA, Mukaka M, Waithira N, Cheah PY, Maude RJ, Amato R, Pearson
306 RD, Goncalves S, Jacob CG, Hamilton WL, Fairhurst RM, Tarning J, Winterberg M,
307 Kwiatkowski DP, Pukrittayakamee S, Hien TT, Day NP, Miotto O, White NJ, Dondorp AM.
308 2019. Determinants of dihydroartemisinin-piperaquine treatment failure in *Plasmodium*
309 *falciparum* malaria in Cambodia, Thailand, and Vietnam: a prospective clinical,
310 pharmacological, and genetic study. *Lancet Infect Dis* 19:952-961.
- 311 8. Adjui M, Babiker A, Garner P, Olliaro P, Taylor W, White N. 2004. Artesunate combinations
312 for treatment of malaria: meta-analysis. *Lancet* 363:9-17.

9. Sinclair D, Zani B, Donegan S, Olliaro P, Garner P. 2009. Artemisinin-based combination therapy for treating uncomplicated malaria. Cochrane Database Syst Rev doi:10.1002/14651858.CD007483.pub2:CD007483.
10. Dechy-Cabaret O, Benoit-Vical F. 2012. Effects of antimalarial molecules on the gametocyte stage of *Plasmodium falciparum*: the debate. J Med Chem 55:10328-10344.
11. Targett G, Drakeley C, Jawara M, von Seidlein L, Coleman R, Deen J, Pinder M, Doherty T, Sutherland C, Walraven G, Milligan P. 2001. Artesunate reduces but does not prevent posttreatment transmission of *Plasmodium falciparum* to *Anopheles gambiae*. J Infect Dis 183.
12. Drakeley CJ, Jawara M, Targett GA, Walraven G, Obisike U, Coleman R, Pinder M, Sutherland CJ. 2004. Addition of artesunate to chloroquine for treatment of *Plasmodium falciparum* malaria in Gambian children causes a significant but short-lived reduction in infectiousness for mosquitoes. Trop Med Int Health 9:53-61.
13. World Health Organization. 2012. Updated WHO policy recommendation (October 2012). Single dose primaquine as gametocytocide in *Plasmodium falciparum* malaria. Geneva WHO, 2012.
14. Mwaiswelo R, Ngasala BE, Jovel I, Gosling R, Premji Z, Poirot E, Mmbando BP, Björkman A, Mårtensson A. 2016. Safety of a single low-dose of primaquine in addition to standard artemether-lumefantrine regimen for treatment of acute uncomplicated *Plasmodium falciparum* malaria in Tanzania. Malar J 15:1-11.
15. Eziefula AC, Bousema T, Yeung S, Kamya M, Owaraganise A, Gabagaya G, Bradley J, Grignard L, Lanke KHW, Wanzira H, Mpimbaza A, Nsobya S, White NJ, Webb EL, Staedke SG, Drakeley C. 2014. Single dose primaquine for clearance of *Plasmodium falciparum*

gametocytes in children with uncomplicated malaria in Uganda: a randomised, controlled, double-blind, dose-ranging trial. *Lancet Infect Dis* 14:130-139.

16. Dicko A, Brown JM, Diawara H, Baber I, Mahamar A, Soumare HM, Sanogo K, Koita F, Keita S, Traore SF, Chen I, Poirrot E, Hwang J, McCulloch C, Lanke K, Pett H, Niemi M, Nosten F, Bousema T, Gosling R. 2016. Primaquine to reduce transmission of *Plasmodium falciparum* malaria in Mali: a single-blind, dose-ranging, adaptive randomised phase 2 trial. *Lancet Infect Dis* 16:674-84.

17. Goncalves BP, Tiono AB, Ouedraogo A, Guelbeogo WM, Bradley J, Nebie I, Siaka D, Lanke K, Eziefula AC, Diarra A, Pett H, Bougouma EC, Sirima SB, Drakeley C, Bousema T. 2016. Single low dose primaquine to reduce gametocyte carriage and *Plasmodium falciparum* transmission after artemether-lumefantrine in children with asymptomatic infection: a randomised, double-blind, placebo-controlled trial. *BMC Med* 14:40.

18. Chen I, Diawara H, Mahamar A, Sanogo K, Keita S, Kone D, Diarra K, Djimde M, Keita M, Brown J, Roh ME, Hwang J, Pett H, Murphy M, Niemi M, Greenhouse B, Bousema T, Gosling R, Dicko A. 2018. Safety of Single-Dose Primaquine in G6PD-Deficient and G6PD-Normal Males in Mali Without Malaria: An Open-Label, Phase 1, Dose-Adjustment Trial. *J Infect Dis* 217:1298-1308.

19. Dicko A, Roh ME, Diawara H, Mahamar A, Soumare HM, Lanke K, Bradley J, Sanogo K, Kone DT, Diarra K, Keita S, Issiaka D, Traore SF, McCulloch C, Stone WJR, Hwang J, Muller O, Brown JM, Srinivasan V, Drakeley C, Gosling R, Chen I, Bousema T. 2018. Efficacy and safety of primaquine and methylene blue for prevention of *Plasmodium falciparum* transmission in Mali: a phase 2, single-blind, randomised controlled trial. *Lancet Infect Dis* 18:627-639.

20. Bastiaens GJH, Tiono AB, Okebe J, Pett HE, Coulibaly SA, Goncalves BP, Affara M, Ouedraogo A, Bougouma EC, Sanou GS, Nebie I, Bradley J, Lanke KHW, Niemi M, Sirima SB, d'Alessandro U, Bousema T, Drakeley C. 2018. Safety of single low-dose primaquine in glucose-6-phosphate dehydrogenase deficient falciparum-infected African males: Two open-label, randomized, safety trials. PLoS One 13:e0190272.
21. Tine RC, Sylla K, Faye BT, Poirot E, Fall FB, Sow D, Wang D, Ndiaye M, Ndiaye JL, Faye B, Greenwood B, Gaye O, Milligan P. 2017. Safety and Efficacy of Adding a Single Low Dose of Primaquine to the Treatment of Adult Patients With *Plasmodium falciparum* Malaria in Senegal, to Reduce Gametocyte Carriage: A Randomized Controlled Trial. Clin Infect Dis 65:535-543.
22. Lin JT, Lon C, Spring MD, Sok S, Chann S, Ittiverakul M, Kuntawunginn W, My M, Thay K, Rahman R, Balasubramanian S, Char M, Lanteri CA, Gosi P, Ubalee R, Meshnick SR, Saunders DL. 2017. Single dose primaquine to reduce gametocyte carriage and *Plasmodium falciparum* transmission in Cambodia: An open-label randomized trial. PLoS One 12:e0168702.
23. Arroyo-Arroyo M, Arango E, Carmona-Fonseca J, Aristizabal B, Yanow S, Maestre A. 2017. Efficacy of Different Primaquine Regimens to Control *Plasmodium falciparum* Gametocytemia in Colombia. Am J Trop Med Hyg 97:712-718.
24. Okebe J, Bousema T, Affara M, Di Tanna GL, Dabira E, Gaye A, Sanya-Isijola F, Badji H, Correa S, Nwakanma D, Van Geertruyden JP, Drakeley C, D'Alessandro U. 2016. The gametocytocidal efficacy of different single doses of primaquine with dihydroartemisinin-piperaquine in asymptomatic parasite carriers in the Gambia: a randomized controlled trial. EBioMedicine 13:348-355.

25. Sutanto I, Suprijanto S, Kosasih A, Dahlan MS, Syafruddin D, Kusriastuti R, Hawley WA, Lobo NF, Ter Kuile FO. 2013. The effect of primaquine on gametocyte development and clearance in the treatment of uncomplicated falciparum malaria with dihydroartemisinin-piperaquine in South sumatra, Western indonesia: an open-label, randomized, controlled trial. Clin Infect Dis 56:685-93.
26. Bancone G, Ménard D, Khim N, Kim S, Canier L, Nguong C, Phommasone K, Mayxay M, Dittrich S, Vongsouvath M, Fievet N, Le Hesran J, Briand V, Newton P, Gorsawun G, Tardy K, Chu C, Rattanapalroj O, Dong L, Quang H, Tam-Uyen N, Thuy-Nhien N, Tran T, Kalnoky M, Nosten F. 2019. Molecular characterization and mapping of glucose-6-phosphate dehydrogenase (G6PD) mutations in the Greater Mekong Subregion. Malar J 18:20.
27. Khim N, Benedet C, Kim S, Kheng S, Siv S, Leang R, Lek S, Muth S, Chea N, Chuor CM, Duong S, Kerleguer A, Tor P, Chim P, Canier L, Witkowski B, Taylor WR, Menard D. 2013. G6PD deficiency in *Plasmodium falciparum* and *Plasmodium vivax* malaria-infected Cambodian patients. Malar J 12:171.
28. Dysoley L, Kim S, Lopes S, Khim N, Bjorges S, Top S, Huch C, Rekol H, Westercamp N, Fukuda MM, Hwang J, Roca-Feltrer A, Mukaka M, Ménard D, Taylor WR. 2019. The tolerability of single low dose primaquine in glucose-6-phosphate deficient and normal falciparum-infected Cambodians. BMC Infectious Diseases 19:250.
29. Kheng S, Muth S, Taylor WR, Tops N, Kosal K, Sothea K, Souy P, Kim S, Char CM, Vanna C, Ly P, Ringwald P, Khieu V, Kerleguer A, Tor P, Baird JK, Bjorge S, Menard D, Christophel E. 2015. Tolerability and safety of weekly primaquine against relapse of *Plasmodium vivax* in Cambodians with glucose-6-phosphate dehydrogenase deficiency. BMC Med 13:203.

30. Vantaux A, Samreth R, Piv EP, Khim N, Kim S, Berne L, Chy S, Lek D, Siv S, Taylor WR, Menard D. 2018. Contribution to malaria transmission of symptomatic and asymptomatic parasite carriers in Cambodia. *J Infect Dis* 217:1561-1568.
31. Canier L, Khim N, Kim S, Sluydts V, Heng S, Dourng D, Eam R, Chy S, Khean C, Loch K, Ken M, Lim H, Siv S, Tho S, Masse-Navette P, Gryseels C, Uk S, Van Roey K, Grietens K, Sokny M, Thavrin B, Chuor C, Deubel V, Durnez L, Coosemans M, Menard D. 2013. An innovative tool for moving malaria PCR detection of parasite reservoir into the field. *Malar J* 12:405.
32. Lawaly YR, Sakuntabhai A, Marrama L, Konate L, Phimpraphi W, Sokhna C, Tall A, Diène Sarr F, Peerapittayamongkol C, Louicharoen C, Schneider BS, Levescot A, Talman A, Casademont I, Menard D, Trape J-F, Rogier C, Kaewkunwal J, Sura T, Nuchprayoon I, Arieu F, Baril L, Singhasivanon P, Mercereau-Puijalon O, Paul R. 2010. Heritability of the Human Infectious Reservoir of Malaria Parasites. *PLOS ONE* 5:e11358.
33. Team RC. 2018. R: A language and environment for statistical computing. <https://www.R-project.org/>, R Foundation for Statistical Computing, Vienna, Austria.
34. Smithuis F, Kyaw MK, Phe O, Win T, Aung PP, Oo APP, Naing AL, Nyo MY, Myint NZH, Imwong M, Ashley E, Lee SJ, White NJ. 2010. Effectiveness of five artemisinin combination regimens with or without primaquine in uncomplicated falciparum malaria: an open-label randomised trial. *Lancet Infect Dis* 10:673-681.
35. Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, Sreng S, Anderson JM, Mao S, Sam B, Sopha C, Chuor CM, Nguon C, Sovannaroeth S, Pukrittayakamee S, Jittamala P, Chotivanich K, Chutasmit K, Suchatsoonthorn C, Runcharoen R, Hien TT, Thuy-Nhien NT, Thanh NV, Phu NH, Htut Y, Han KT, Aye KH, Mokuolu OA, Olaosebikan RR, Folaranmi

OO, Mayxay M, Khanthavong M, Hongvanthong B, Newton PN, Onyamboko MA, Fanello CI, Tshefu AK, Mishra N, Valecha N, Phyo AP, Nosten F, Yi P, Tripura R, Borrmann S, Bashraheil M, Peshu J, Faiz MA, Ghose A, Hossain MA, Samad R, et al. 2014. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. N Engl J Med 371:411-23.

36. Sawa P, Shekalaghe SA, Drakeley CJ, Sutherland CJ, Mweresa CK, Baidjoe AY, Manjurano A, Kavishe RA, Beshir KB, Yussuf RU, Omar SA, Hermsen CC, Okell L, Schallig HDFH, Sauerwein RW, Hallett RL, Bousema T. 2013. Malaria transmission after artemether-lumefantrine and dihydroartemisinin-piperaquine: a randomized trial. J Infect Dis 207:1637-1645.

37. Bassat Q, Mulenga M, Tinto H, Piola P, Borrmann S, Menendez C, Nambozi M, Valea I, Nabasumba C, Sasi P, Bacchieri A, Corsi M, Ubben 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.

38. Onyamboko MA, Fanello CI, Wongsan K, Tarning J, Cheah PY, Tshefu KA, Dondorp AM, Nosten F, White NJ, Day NP. 2014. Randomized comparison of the efficacies and tolerabilities of three artemisinin-based combination treatments for children with acute *Plasmodium falciparum* malaria in the Democratic Republic of the Congo. Antimicrob Agents Chemother 58:5528-36.

39. WWARN Gametocyte Study Group. 2016. Gametocyte carriage in uncomplicated *Plasmodium falciparum* malaria following treatment with artemisinin combination therapy: a systematic review and meta-analysis of individual patient data. BMC Med 14:1-18.

40. Burgess RW, Bray RS. 1961. The effect of a single dose of primaquine on the gametocytes, gametogony and sporogony of *Laverania falciparum*. Bull World Health Organ 24:451-6.

- 450 41. Delves MJ, Ruecker A, Straschil U, Lelievre J, Marques S, Lopez-Barragan MJ, Herreros E,
451 Sinden RE. 2013. Male and female *Plasmodium falciparum* mature gametocytes show
452 different responses to antimalarial drugs. *Antimicrob Agents Chemother* 57:3268-3274.
- 453 42. White N, Ashley E, Recht J, Delves M, Ruecker A, Smithuis F, Eziefula A, Bousema T,
454 Drakeley C, Chotivanich K, Imwong M, Pukrittayakamee S, Prachumsri J, Chu C, Andolina
455 C, Bancone G, Hien T, Mayxay M, Taylor W, von Seidlein L, Price R, Barnes K, Djimde A,
456 ter Kuile F, Gosling R, Chen I, Dhorda M, Stepniewska K, Guerin P, Woodrow C, Dondorp A,
457 Day N, Nosten F. 2014. Assessment of therapeutic responses to gametocytocidal drugs in
458 *Plasmodium falciparum* malaria. *Malar J* 13:483.
- 459 43. Jeffery GM, Eyles DE. 1955. Infectivity to mosquitoes of *Plasmodium falciparum* as related
460 to gametocyte density and duration of infection. *Am J Trop Med Hyg* 4:781-789.
- 461 44. Churcher TS, Bousema T, Walker M, Drakeley C, Schneider P, Ouédraogo AL, Basáñez M-
462 G. 2013. Predicting mosquito infection from *Plasmodium falciparum* gametocyte density and
463 estimating the reservoir of infection. *Elife* 2:e00626.
- 464 45. Leang R, Khu NH, Mukaka M, Debackere M, Tripura R, Kheang ST, Chy S, Kak N, Buchy
465 P, Tarantola A, Menard D, Roca-Felterer A, Fairhurst RM, Kheng S, Muth S, Ngak S,
466 Dondorp AM, White NJ, Taylor WR. 2016. An optimised age-based dosing regimen for single
467 low-dose primaquine for blocking malaria transmission in Cambodia. *BMC Med* 14:171.
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469 **Tables**

470 **Table 1.** Baseline characteristics of the two study groups of patients

	DP	DP + SLDPQ
No. enrolled	53	56
No. recrudescent	18	10
Male: Female	44:9	44:12
Mean age, yrs (SD)	25 (16)	26 (14)
G6PD deficient: G6PD normal	3:50	6:50
Number of gametocyte positive slide	14	7
Mean gametocytemia / μ l (SD, range)	64 (250, 0-1432)	40 (149, 0-787)
Number of positive gametocyte Taqman RT-PCR	26	21
Hemoglobin concentration (g/dL)	12.63	13.19
Anemia (Hb<11g/dL)	10/51	9/56

471

472 **Table 2.** Independent factors associated with change in gametocytemia over 28 days by
 473 univariate analysis. Odds ratio < 1 indicates a negative association.

Factor		OR	95% CI	P-value
Treatment	DP+SLDPQ			
	DP	5.59	1.71 , 18.27	0.0044
D0 status	D0 negative			
	D0 positive	15.83	4.83 , 51.91	<0.0001
Recrudescence status	Cured patient			
	Recrudescent patient	11.11	2.78 , 44.36	0.0006
D0 hemoglobin		0.46	0.34 , 0.63	<0.0001
Day of follow-up		0.94	0.91 , 0.96	<0.0001

474

475

476 **Table 3.** Multivariable models (with and without recrudescence) of the factors associated with
 477 changes in gametocytemia over 28 days.

Model	Factors	adjOR	95% CI	P-value
Model 1	Treatment	2.39	0.81 , 7.08	0.1152
	D0 status	7.35	2.27 , 23.78	0.0009
	Recrudescence	6.02	1.70 , 21.26	0.0053
	D0 hemoglobin	0.57	0.42 , 0.77	0.0003
	Day of follow-up	0.93	0.90 , 0.96	<0.0001
Model 2	Treatment	3.15	1.09 , 9.11	0.0344
	D0 status	6.63	2.10 , 20.87	0.0012
	D0 hemoglobin	0.55	0.41 , 0.75	0.0001
	Day of follow-up	0.93	0.90 , 0.96	<0.0001

478

479

Table 4. Infectivity rates (No. of positive isolates/No. of tested isolates) defined by treatment assessed by direct-membrane feeding assays during the 28-day follow-up.

Follow-up day	DP	DP+SLDPQ	Total	Infectivity rate
D0	0/15	3/33	3/48	6.3 ± 6.8 %
D1	0/1	0/6	0/7	0
D2	0/12	0/26	0/38	0
D3	0/15	0/31	0/46	0
D7	0/11	0/24	0/35	0
D14	1/10	0/19	1/29	3.5 ± 6.6 %
D21	0/1	0/4	0/5	0
D28	0/6	0/22	0/28	0
rec1D0	0/4	1/8	1/12	8.3 ± 15.6 %
rec1D3	0/4	0/6	0/11	0
rec1D7	1/6	0/6	1/12	8.3 ± 15.6 %
rec1D14	0/4	0/1	0/4	0
rec1D28	NA	0/1	0/1	0
rec1D35	NA	0/1	0/1	0
rec2D0	0/4	0/1	0/5	0
rec2D3	0/4	0/1	0/5	0
rec2D7	0/4	0/1	0/5	0
Total DMFA	101	191	292	2 ± 1.6 %

DP – dihydroartemisinin piperazine
25

484 SLDPQ – single low dose primaquine

485 Rec1 and Rec2 correspond to the first and second recrudescences, respectively.

486 Positive isolates are isolates which were able to infect at least one mosquito from direct-
487 membrane feeding assay (DFMA).

488

Table 5. Details of the five patients, infectious to *A. minimus* by direct-membrane feeding assays during the 28-day follow-up.

Day	Patient ID	Treatment Given	Sex	Age	Gametocytemia (gametocytes/μl)	Gametocytes detected by Taqman RT-PCR	Infection prevalence (proportion of infected females ± 95%CI)	Infection intensity (mean number of oocysts ± SE)
D0	D_002	DP +SLDPQ	F	29	562	Positive	41.7 ± 14	4.7 ± 0.97
D1					336	Positive	0	NA
D2					48	Positive	0	NA
D3					0	Positive	0	NA
D7					NA	Negative	0	NA
D14					NA	Negative	0	NA
D21					NA	Negative	0	NA
D28					0	Positive	0	NA
Rec1D0 (D124)					24	Positive	10 ± 8.3	2.6 ± 1.4
Rec1D3 (D127)	D_002	QN+TE			64	Positive	0	NA
Rec1D7 (D131)					168	Positive	0	NA
D0								
D0	N_015	DP+ SLDPQ	M	23	787	Positive	16±10	2.12±0.4

D1					2208	Positive	0	NA
D2					40	Positive	0	NA
D3					176	Positive	0	NA
D7					NA	Negative	0	NA
D14					NA	Negative	0	NA
D28					NA	Negative	0	NA
Rec1D0 (D28)	N_035	QN	F	8	1823	Positive	0	NA
Rec1D3 (D31)					11912	Positive	0	NA
Rec1D7 (D35)					7904	Positive	94.3 ± 7.7	82.6 ± 5.5
D0	N_046	DP	M	8	1432	Positive	0	NA
D2					2904	Positive	0	NA
D3					3952	Positive	0	NA
D7					1952	Positive	0	NA
D14					432	Positive	6 ± 6.5	1
D28					16	Positive	0	NA
D0	N_074	DP +SLDPQ	M	25	540	Positive	86.3 ± 9.4	11.3 ± 1.7
D3					8	Positive	0	NA

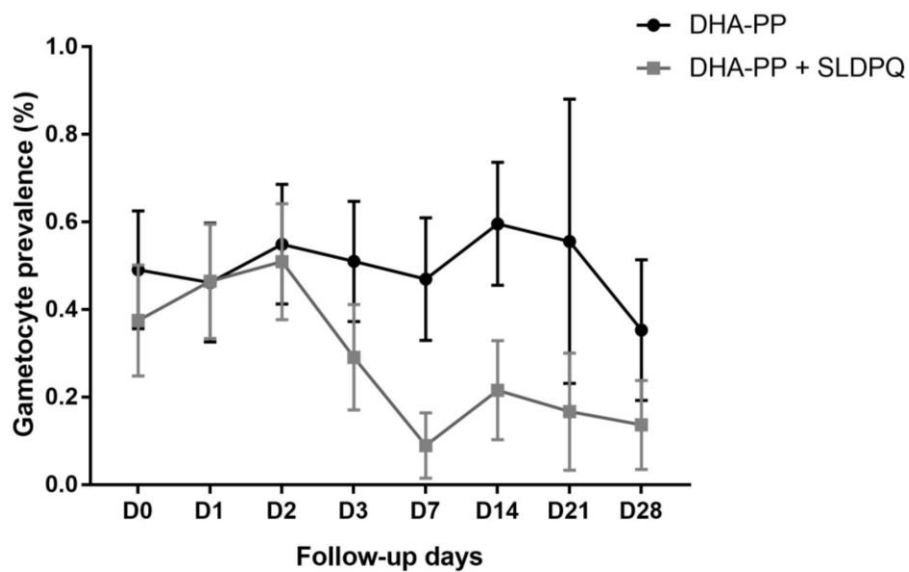
491

492 QN+TE: Quinine + tetracycline

493 Rec1 corresponds to the first recrudescence, Day post initial infection is indicated in brackets.

494 NA: Data not available

496 **Figures**



497

498 Figure 1.

499 Gametocyte prevalence by Taqman RT-PCR by follow-up day and treatment group. Data show

500 proportion \pm 95% confidence intervals.

501

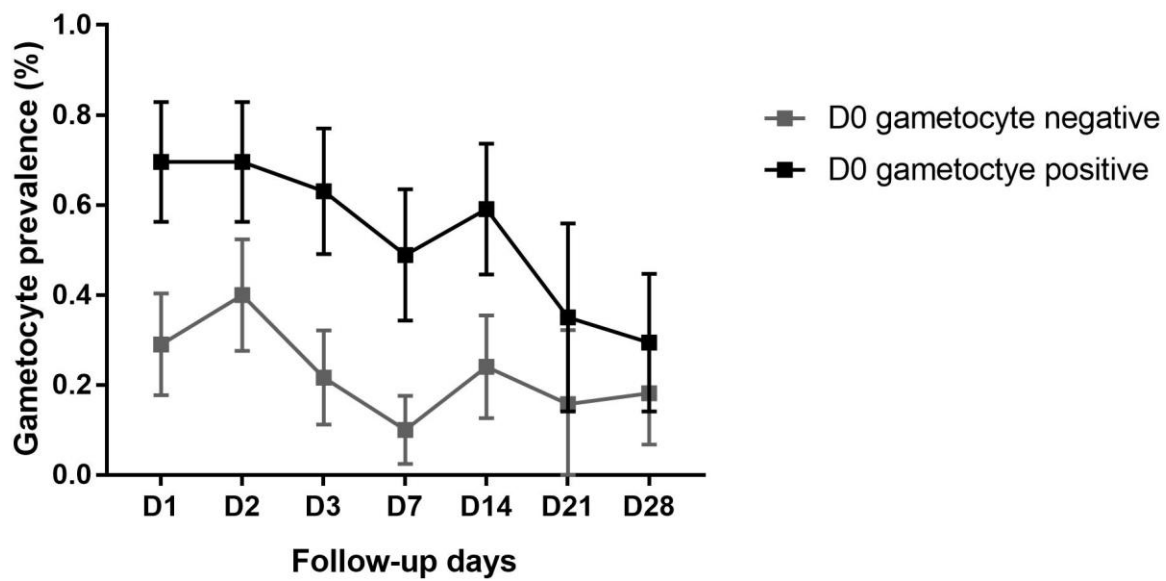


Figure 2.

Gametocyte prevalence by Taqman RT-PCR by follow-up day and presence of gametocyte prior to treatment on D0 detected by Taqman RT-PCR. Data show proportion \pm 95% confidence intervals.