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

4
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15
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18
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24 **Abstract (250 words)**

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

31 In this randomized controlled trial, 109 Cambodians with acute uncomplicated *P. falciparum*
32 malaria received dihydroartemisinin-piperazine (DP) alone or combined with SLDPQ on the
33 first treatment day. Transmission-blocking efficacy of SLDPQ was evaluated on Days 0, 1, 2, 3,
34 7, 14, 21, 28 and recrudescence by reverse transcriptase polymerase chain reaction (RT-PCR)
35 (gametocyte prevalence) and membrane-feeding assays with *Anopheles minimus* mosquitoes
36 (gametocyte infectivity). Without the influence of recrudescence infections, DP+SLDPQ reduced
37 gametocyte carriage 3 fold compared to DP. Of 48 patients tested on Day 0, only three patients
38 were infectious to mosquitoes (~6%). Post-treatment, three patients were infectious: on D14
39 (3.5%, 1/29), and on the first and seventh day of recrudescence (8.3%, 1/12 for each); this overall
40 low infectivity precluded our ability to assess its transmission blocking efficacy.

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

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

47 **Introduction**

48 Malaria remains a major public health challenge with an estimated 228 million cases recorded in
49 2018 (1). While considerable progress has been made since 2010 when the estimated case burden
50 was 251 million, the recent emergence and spread of both artemisinin and piperaquine resistant
51 *Plasmodium falciparum* lineage (KEL1/PLA1) in the Eastern Greater Mekong subRegion
52 (GMS), threaten this remarkable global achievement (2-7). Eliminating rapidly multidrug
53 resistant *P. falciparum* (MDRPf) is, therefore, the top priority for countries of the Greater
54 Mekong Subregion (GMS). Implementation of novel tools and strategies or repurposing existing
55 tools like primaquine that specifically aim at interrupting malaria transmission are essential to
56 reach this goal.

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

63
64 To date, several clinical studies conducted in Africa, Colombia and Cambodia, have assessed the
65 safety and efficacy of SLDPQ (14-24), mainly by measuring gametocyte carriage [microscopy or
66 Taqman reverse transcriptase polymerase chain reaction (RT-PCR)] as a surrogate marker of its
67 transmission blocking efficacy. This is due to the challenging logistical requirements of carrying
68 out mosquito infectivity studies. These studies showed that SLDPQ, dosed from 0.20 to 0.75
69 mg/kg, clearly reduced gametocyte carriage and increased their clearance in a dose dependent

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

75
76 In Cambodia, glucose-6-phosphate dehydrogenase deficiency (G6PDd) is a common X-linked
77 disorder of the red blood cells with frequencies ranging from 10.8% to 29.6% in males (26) and a
78 prevalence rate in malaria patients seen at health centers estimated at 13.9% (27). This is the
79 main reason why Cambodian policy makers have been reluctant to deploy SLDPQ without local
80 data on the safety and the efficacy of SLDPQ. Therefore, we conducted a trial assessing the
81 tolerability and the safety of SLDPQ (0.25mg/kg) in Cambodia in 2015-2016 (28), which
82 followed on from a safety trial of 0.75 mg/kg/week of PQ in vivax patients (29). Here, we present
83 our investigations on the transmission-blocking efficacy of this treatment through the evaluation
84 of gametocyte prevalence dynamics as well as infectivity measures on a subset of patients using
85 membrane-feeding assays with *Anopheles minimus* mosquitoes, one of the main malaria vectors
86 in Southeast Asia.

87

88 **Methods**

89 Study design

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

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

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

111

112 Mosquito infection

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

115 Mosquitoes were starved for 24h before being provided a blood meal. Venous blood samples
116 were collected in heparinized tubes and 400µl of blood were made available in membrane feeders
117 maintained at 37°C. Females were fed only once on freshly drawn blood. Post-feeding, unfed
118 females were discarded and fed females were kept in cages (20x20x20cm) with constant access to
119 a 10% sucrose solution. Patient infectivity was determined by assessing infection prevalence (i.e.
120 proportion of infected females) and infection intensity (i.e. number of *Plasmodium sp.* oocysts in
121 infected females). Midguts were dissected in a 1% Mercurochrome[®] stain and the presence and
122 the number of oocysts were determined under a microscope (20x magnification). Dissections
123 were performed 7-day post-blood meal. The mean number of dissected females was 49 (range:
124 15-85, median: 50).

125
126 Biological investigations

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

136
137 Ethical statement

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

143
144 Statistical analyses
145 Initially, univariate Generalized Linear Mixed Models (GLMMs) with a binomial distribution
146 were fitted to model the PCR measured, gametocyte prevalence rates. In these models, follow-up
147 days (D1, 2, 3, 7, 14, 21 and 28), treatment (DP vs. DP+SLDPQ), presence of gametocytes prior
148 to treatment on D0, recrudescence status (recrudescent vs. cured patient) and hemoglobin
149 concentration prior to treatment on D0 were included in the model as fixed factors. Patient ID
150 was coded as a random factor to account for repeated measures on the same individual. The
151 univariate analyses were followed by a multivariable GLMM to model the dynamics of
152 gametocyte prevalence post-treatment including all the significant factors from the univariate
153 analysis.

154 Gametocyte clearance times were not compared in the study for three principal reasons: (i) of the
155 21 patients with microscopically detected D0 gametocytes, only 7 had complete follow up
156 microscopy data, (ii) Taqman RT-PCR measured gametocyte densities were highly variable
157 during the 28 days follow-up, and, (iii) by D28, 10/34 patients with gametocytes on D0 by
158 Taqman RT-PCR were still gametocytemic (n=1, DP+SLDPQ arm). *P*-values <0.05 were deemed
159 significant. All analyses were performed in R v.3.5.1 (33).

160

161 **Results**

162 One hundred nine patients with uncomplicated falciparum malaria were enrolled and treated with
163 the standard 3-day DP alone (48.6%, 53/109) or DP+SLDPQ (51.4%, 56/109). Seven patients
164 were lost to follow up (6.4%). Of the remaining 102 patients, 28 (27.4%) had PCR-proven *P.*
165 *falciparum* recrudescences; 24 occurred within the 28-day follow up on Days 12 (1), 14 (1), 17
166 (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,
167 and 124. Of the 28 recrudescence patients, 10 were retreated with the standard 3-day DP and 18
168 with quinine or quinine plus tetracycline; six of the 10 patients retreated with DP experienced a
169 second recrudescence.

170 At D0, gametocytes were detected in 47/109 patients (43.1%) by Taqman RT-PCR (Table 1; see
171 30 for further details), with no significant difference between arms ($P=0.3057$).

172

173 *Gametocyte dynamics*

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

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

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

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

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

189

190 *Mosquito infectivity*

191 A total of 55 patients were included in the DMFAs. Amongst the 292 feeding assays, only 113 of
192 14,444 (0.78%) dissected mosquitoes were infected (Table 4). Overall, 3 out of 48 ($6.3 \pm 6.8\%$)
193 individuals infected at least one mosquito at baseline, one individual was infectious on D14 (DP)
194 and two individuals with recrudescence infections (Tables 4 & 5) who were repeat DP treatment
195 failures: (i) at presentation on D124 (DP+SLDPQ), and (ii) D35 (7 days post DP started on D28).
196 The three individuals infectious on D0 were all gametocytemic by microscopy, received
197 DP+SLDPQ and were not infectious on D1 or D3 post-treatment (Table 5).

198

199 **Discussion**

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

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

206 Our results show that SLDPQ, given on the first day of the 3-day standard ACT treatment,
207 significantly decreased gametocyte carriage over time, starting on D3 and was least on D7
208 resulting in a 5.59 fold reduced risk, relative to DP alone, over 28 days in the univariate analysis.
209 This is consistent with recent reports on the efficacy of PQ in reducing gametocyte carriage (15-
210 17, 19, 21-25, 34) and earlier reports showing a lag between reduced gametocyte carriage and
211 rapid mosquito infectivity. The multivariable analysis revealed the significant effect of
212 recrudescence (resistant) infections on gametocyte carriage with a 3 fold reduction in gametocyte
213 carriage in the absence of resistance.

214
215 Our patients had a high rate of baseline gametocytemia (19% by microscopy, just under 45% by
216 Taqman RT-PCR), which is similar to that reported previously in 2010 in artemisinin resistant
217 western Cambodia (~19%) but is three fold higher than the 6% reported earlier in Rattanakiri
218 province (35). Given the high prevalence of *PfKelch13* mutant *P. falciparum* parasites (63%) in
219 our study and the very high recrudescence rate (~28%), our high gametocyte carriage rate is
220 consistent with the presence of ARPF, which has also led to piperaquine resistance (7). This is
221 consistent with another study in Cambodia which showed that DP treated patients from Oddar
222 Meanchey province (northern Cambodia) also had prolonged gametocyte carriage, irrespective of
223 the slow asexual parasite clearance time, due to ARPF, and in patients with recrudescence
224 infections (22, 35).

225

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

231
232 Overall, we observed a biphasic pattern of gametocyte prevalence with a drop on D7, a rebound
233 on D14, followed by a decrease on D21 and D28. However, this effect was mainly driven by the
234 DP treated patients as their gametocyte carriage rate was similar over the 28-day follow-up
235 whereas it decreased markedly in the DP+SLDPQ treated patients (Figure 1).

236
237 We were not able to assess the transmission-blocking efficacy of SLDPQ due to the very low
238 infectivity rate observed in only three patients (~6%) at baseline and three infectious events
239 during follow up. This is a major limitation of DFMA, previously reported by Lin *et al.* (22) at
240 baseline using *Anopheles dirus* mosquitoes and on D7, without assessing baseline, by Okebe *et al*
241 (24). In another study in African children, despite a higher infectivity rate at enrollment after
242 microscopy-based selection of gametocyte positive asymptomatic children (38%), Gonçalves *et*
243 *al.* (17) were also unable to detect the added value of SLDPQ due to very low infectivity post-
244 treatments in all arms. As our clinical trial aimed primarily at assessing SLDPQ tolerability and
245 safety, we did not particularly select patent or high-density gametocyte carriers and thus would
246 have needed substantially more individuals to assess transmission-blocking efficacy. Only two
247 studies in Mali statistically confirmed high anti-infectivity efficacy (>90%) at 48h of SLDPQ,
248 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.

269

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283

284

285 **References**

- 286 1. World Health Organization. 2019. World malaria report 2019. Geneva, Switzerland: 232p.
- 287 2. Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, Fukuda MM, Consortium ARCS. 2008.
288 Evidence of Artemisinin-Resistant Malaria in Western Cambodia. N Engl J Med 359:2619-
289 2620.
- 290 3. Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J. 2009. Artemisinin resistance in
291 *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.

- 313 9. Sinclair D, Zani B, Donegan S, Olliaro P, Garner P. 2009. Artemisinin-based combination
314 therapy for treating uncomplicated malaria. *Cochrane Database Syst Rev*
315 doi:10.1002/14651858.CD007483.pub2:Cd007483.
- 316 10. Dechy-Cabaret O, Benoit-Vical F. 2012. Effects of antimalarial molecules on the gametocyte
317 stage of *Plasmodium falciparum*: the debate. *J Med Chem* 55:10328-10344.
- 318 11. Targett G, Drakeley C, Jawara M, von Seidlein L, Coleman R, Deen J, Pinder M, Doherty T,
319 Sutherland C, Walraven G, Milligan P. 2001. Artesunate reduces but does not prevent
320 posttreatment transmission of *Plasmodium falciparum* to *Anopheles gambiae*. *J Infect Dis* 183.
- 321 12. Drakeley CJ, Jawara M, Targett GA, Walraven G, Obisike U, Coleman R, Pinder M,
322 Sutherland CJ. 2004. Addition of artesunate to chloroquine for treatment of *Plasmodium*
323 *falciparum* malaria in Gambian children causes a significant but short-lived reduction in
324 infectiousness for mosquitoes. *Trop Med Int Health* 9:53-61.
- 325 13. World Health Organization. 2012. Updated WHO policy recommendation (October 2012).
326 Single dose primaquine as gametocytocide in *Plasmodium falciparum* malaria. Geneva WHO,
327 2012.
- 328 14. Mwaiswelo R, Ngasala BE, Jovel I, Gosling R, Premji Z, Poirot E, Mmbando BP, Björkman
329 A, Mårtensson A. 2016. Safety of a single low-dose of primaquine in addition to standard
330 artemether-lumefantrine regimen for treatment of acute uncomplicated *Plasmodium*
331 *falciparum* malaria in Tanzania. *Malar J* 15:1-11.
- 332 15. Eziefula AC, Bousema T, Yeung S, Kanya M, Owaraganise A, Gabagaya G, Bradley J,
333 Grignard L, Lanke KHW, Wanzira H, Mpimbaza A, Nsobya S, White NJ, Webb EL, Staedke
334 SG, Drakeley C. 2014. Single dose primaquine for clearance of *Plasmodium falciparum*

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

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

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

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

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

- 358 20. Bastiaens GJH, Tiono AB, Okebe J, Pett HE, Coulibaly SA, Goncalves BP, Affara M,
359 Ouedraogo A, Bougouma EC, Sanou GS, Nebie I, Bradley J, Lanke KHW, Niemi M, Sirima
360 SB, d'Alessandro U, Bousema T, Drakeley C. 2018. Safety of single low-dose primaquine in
361 glucose-6-phosphate dehydrogenase deficient falciparum-infected African males: Two open-
362 label, randomized, safety trials. PLoS One 13:e0190272.
- 363 21. Tine RC, Sylla K, Faye BT, Poirot E, Fall FB, Sow D, Wang D, Ndiaye M, Ndiaye JL, Faye
364 B, Greenwood B, Gaye O, Milligan P. 2017. Safety and Efficacy of Adding a Single Low
365 Dose of Primaquine to the Treatment of Adult Patients With *Plasmodium falciparum* Malaria
366 in Senegal, to Reduce Gametocyte Carriage: A Randomized Controlled Trial. Clin Infect Dis
367 65:535-543.
- 368 22. Lin JT, Lon C, Spring MD, Sok S, Chann S, Ittiverakul M, Kuntawunginn W, My M, Thay K,
369 Rahman R, Balasubramanian S, Char M, Lanteri CA, Gosi P, Ubalee R, Meshnick SR,
370 Saunders DL. 2017. Single dose primaquine to reduce gametocyte carriage and *Plasmodium*
371 *falciparum* transmission in Cambodia: An open-label randomized trial. PLoS One
372 12:e0168702.
- 373 23. Arroyo-Arroyo M, Arango E, Carmona-Fonseca J, Aristizabal B, Yanow S, Maestre A. 2017.
374 Efficacy of Different Primaquine Regimens to Control *Plasmodium falciparum*
375 Gametocytemia in Colombia. Am J Trop Med Hyg 97:712-718.
- 376 24. Okebe J, Bousema T, Affara M, Di Tanna GL, Dabira E, Gaye A, Sanya-Isijola F, Badji H,
377 Correa S, Nwakanma D, Van Geertruyden JP, Drakeley C, D'Alessandro U. 2016. The
378 gametocytocidal efficacy of different single doses of primaquine with dihydroartemisinin-
379 piperazine in asymptomatic parasite carriers in the Gambia: a randomized controlled trial.
380 EBioMedicine 13:348-355.

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

- 404 30. Vantaux A, Samreth R, Piv EP, Khim N, Kim S, Berne L, Chy S, Lek D, Siv S, Taylor WR,
405 Menard D. 2018. Contribution to malaria transmission of symptomatic and asymptomatic
406 parasite carriers in Cambodia. *J Infect Dis* 217:1561-1568.
- 407 31. Canier L, Khim N, Kim S, Sluydts V, Heng S, Dourng D, Eam R, Chy S, Khean C, Loch K,
408 Ken M, Lim H, Siv S, Tho S, Masse-Navette P, Gryseels C, Uk S, Van Roey K, Grietens K,
409 Sokny M, Thavrin B, Chuor C, Deubel V, Durnez L, Coosemans M, Menard D. 2013. An
410 innovative tool for moving malaria PCR detection of parasite reservoir into the field. *Malar J*
411 12:405.
- 412 32. Lawaly YR, Sakuntabhai A, Marrama L, Konate L, Phimpraphi W, Sokhna C, Tall A, Diène
413 Sarr F, Peerapittayamongkol C, Louicharoen C, Schneider BS, Levescot A, Talman A,
414 Casademont I, Menard D, Trape J-F, Rogier C, Kaewkunwal J, Sura T, Nuchprayoon I, Arie
415 F, Baril L, Singhasivanon P, Mercereau-Puijalon O, Paul R. 2010. Heritability of the Human
416 Infectious Reservoir of Malaria Parasites. *PLOS ONE* 5:e11358.
- 417 33. Team RC. 2018. R: A language and environment for statistical computing. [https://www.R-](https://www.R-project.org/)
418 [project.org/](https://www.R-project.org/), R Foundation for Statistical Computing, Vienna, Austria.
- 419 34. Smithuis F, Kyaw MK, Phe O, Win T, Aung PP, Oo APP, Naing AL, Nyo MY, Myint NZH,
420 Imwong M, Ashley E, Lee SJ, White NJ. 2010. Effectiveness of five artemisinin combination
421 regimens with or without primaquine in uncomplicated falciparum malaria: an open-label
422 randomised trial. *Lancet Infect Dis* 10:673-681.
- 423 35. Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, Sreng S, Anderson JM,
424 Mao S, Sam B, Sopha C, Chuor CM, Nguon C, Sovannaroeth S, Pukrittayakamee S, Jittamala
425 P, Chotivanich K, Chutasmit K, Suchatsoonthorn C, Runcharoen R, Hien TT, Thuy-Nhien NT,
426 Thanh NV, Phu NH, Htut Y, Han KT, Aye KH, Mokuolu OA, Olaosebikan RR, Folaranmi

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

431 36. Sawa P, Shekalaghe SA, Drakeley CJ, Sutherland CJ, Mweresa CK, Baidjoe AY, Manjurano
432 A, Kavishe RA, Beshir KB, Yussuf RU, Omar SA, Hermsen CC, Okell L, Schallig HDFH,
433 Sauerwein RW, Hallett RL, Bousema T. 2013. Malaria transmission after artemether-
434 lumefantrine and dihydroartemisinin-piperaquine: a randomized trial. J Infect Dis 207:1637-
435 1645.

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

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

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

448 40. Burgess RW, Bray RS. 1961. The effect of a single dose of primaquine on the gametocytes,
449 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-Feltrer 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.
- 468

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

480 **Table 4.** Infectivity rates (No. of positive isolates/No. of tested isolates) defined by treatment
 481 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 % |

482

483 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

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

| Day | Patient ID | Treatment Given | Sex | Age | Gametocytemia (gametocytes/ μ l) | Gametocytes detected by Taqman RT-PCR | Infection prevalence (proportion of infected females \pm 95%CI) | Infection intensity (mean number of oocysts \pm SE) |
|------------------|------------|-----------------|-----|-----|--------------------------------------|---------------------------------------|---|---|
| D0 | D_002 | DP +SLDPQ | F | 29 | 562 | Positive | 41.7 \pm 14 | 4.7 \pm 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) | D_002 | QN+TE | | | 24 | Positive | 10 \pm 8.3 | 2.6 \pm 1.4 |
| Rec1D3 (D127) | | | | | 64 | Positive | 0 | NA |
| Rec1D7 (D131) | | | | | 168 | Positive | 0 | NA |
| D0 | N_015 | DP+ SLDPQ | M | 23 | 787 | Positive | 16 \pm 10 | 2.12 \pm 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 |

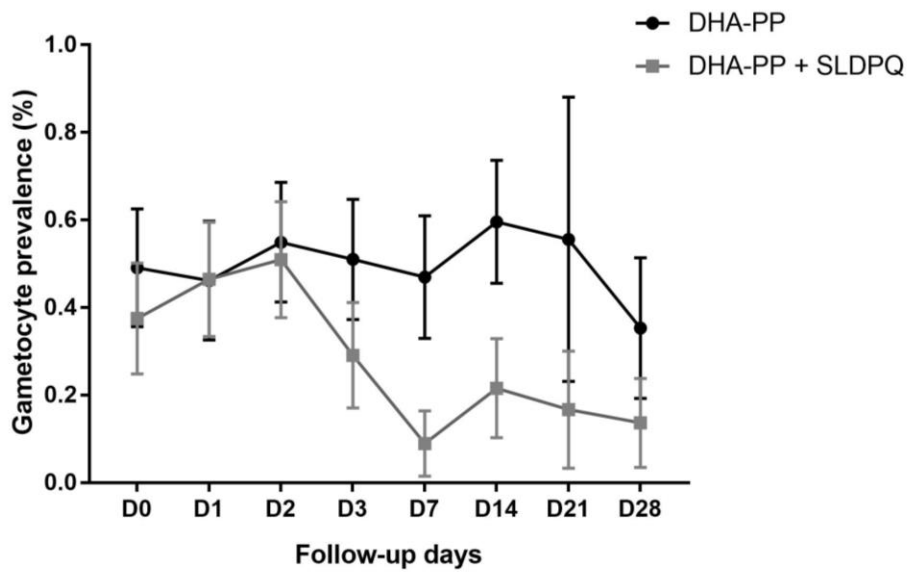
491

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**

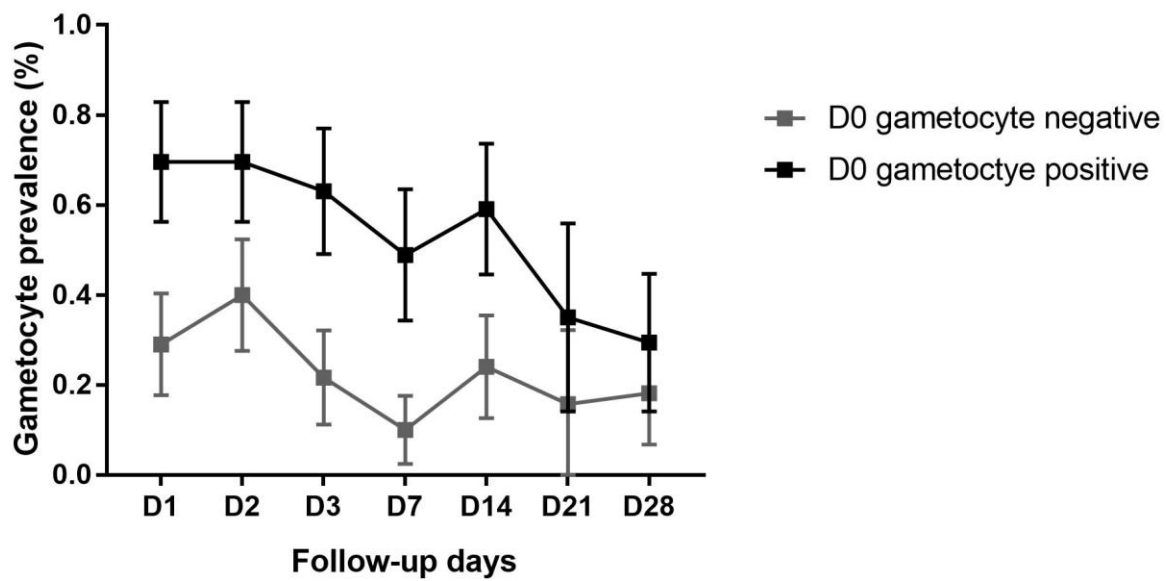


497

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



502
 503 Figure 2.
 504 Gametocyte prevalence by Taqman RT-PCR by follow-up day and presence of gametocyte prior
 505 to treatment on D0 detected by Taqman RT-PCR. Data show proportion \pm 95% confidence
 506 intervals.
 507