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Single low-dose primaquine for blocking transmission of *Plasmodium falciparum* malaria – a proposed model-derived age-based regimen for sub-Saharan Africa

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Abstract

Background: In 2012, the World Health Organization recommended blocking the transmission of *Plasmodium falciparum* with single low-dose primaquine (SLDPQ, target dose 0.25 mg base/kg body weight), without testing for glucose-6-phosphate dehydrogenase deficiency (G6PDd), when treating patients with uncomplicated falciparum malaria. We sought to develop an age-based SLDPQ regimen that would be suitable for sub-Saharan Africa.

Methods: Using data on the anti-infectivity efficacy and tolerability of primaquine (PQ), the epidemiology of anaemia, and the risks of PQ-induced acute haemolytic anaemia (AHA) and clinically significant anaemia (CSA), we prospectively defined therapeutic-dose ranges of 0.15–0.4 mg PQ base/kg for children aged 1–5 years and 0.15–0.5 mg PQ base/kg for individuals aged ≥6 years (therapeutic indices 2.7 and 3.3, respectively). We chose 1.25 mg PQ base for infants aged 6–11 months because they have the highest rate of baseline anaemia and the highest risks of AHA and CSA. We modelled an anthropometric database of 661,979 African individuals aged ≥6 months (549,127 healthy individuals, 28,466 malaria patients and 84,386 individuals with other infections/illnesses) by the Box–Cox transformation power exponential and tested PQ doses of 1–15 mg base, selecting dosing groups based on calculated mg/kg PQ doses.
(Continued on next page)

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Results: From the Box–Cox transformation power exponential model, five age categories were selected: (i) 6–11 months ($n = 39,886$, 6.03%), (ii) 1–5 years ($n = 261,036$, 45.46%), (iii) 6–9 years ($n = 20,770$, 3.14%), (iv) 10–14 years ($n = 12,155$, 1.84%) and (v) ≥15 years ($n = 328,132$, 49.57%) to receive 1.25, 2.5, 5, 7.5 and 15 mg PQ base for corresponding median (1st and 99th centiles) mg/kg PQ base of: (i) 0.16 (0.12–0.25), (ii) 0.21 (0.13–0.37), (iii) 0.25 (0.16–0.38), (iv) 0.26 (0.15–0.38) and (v) 0.27 (0.17–0.40). The proportions of individuals predicted to receive optimal therapeutic PQ doses were: 73.2 (29,180/39,886), 93.7 (244,537/261,036), 99.6 (20,690/20,770), 99.4 (12,086/12,155) and 99.8% (327,620/328,132), respectively.

Conclusions: We plan to test the safety of this age-based dosing regimen in a large randomised placebo-controlled trial (ISRCTN11594437) of uncomplicated falciparum malaria in G6PDd African children aged 0.5 – 11 years. If the regimen is safe and demonstrates adequate pharmacokinetics, it should be used to support malaria elimination.

Keywords: Primaquine, Age-based dosing, *Plasmodium falciparum*, Malaria, Transmission blocking

Background

In an effort to stem the continuing emergence of artemisinin-resistant *Plasmodium falciparum* (ARPf) from the Greater Mekong Subregion to other parts of the world [1–8], the World Health Organization (WHO) has recommended the addition of single low-dose primaquine (SLDPQ) to artemisinin-based combination therapy (ACTs) for treating acute uncomplicated falciparum malaria in low-transmission areas, particularly where ARPf is prevalent [9, 10]. The recommended target dose of 0.25 mg/kg body weight of PQ base was considered safe, even in patients with severe forms of glucose-6-phosphate dehydrogenase deficiency (G6PDd). Accordingly, the WHO recommended its adoption without G6PDd testing.

While SLDPQ is not currently deployed anywhere in sub-Saharan African (SSA), several low-endemicity countries, including Botswana, Eritrea, Swaziland and Zimbabwe (R. Cibulski, personal communication) have added SLDPQ to their national treatment guidelines and intend to go forward with SLDPQ treatment. The main reasons cited by ministries of health for not enacting the WHO recommendation is the fear that SLDPQ would cause acute haemolytic anaemia (AHA) in G6PDd individuals and the impossibility of widely deploying a suitable G6PDd test to exclude such individuals from receiving SLDPQ [11].

Despite this, there is an increasing body of evidence that SLDPQ is tolerated well by both malaria patients and healthy individuals with good pre-treatment haemoglobin (Hb) concentrations in both Southeast Asia [12] and SSA [13] (SAFEPRIM I and II, and PQSAFETY studies; ClinicalTrials.gov identifiers NCT02174900, NCT02654730, and NCT02535767). Moreover, pilot work has demonstrated the feasibility of deploying SLDPQ in Swaziland's health system [14].

Important hurdles to using SLDPQ include the lack both of user-friendly age- or weight-based regimens and of suitable paediatric formulations and tablet strengths that have been produced to good manufacturing practice standards and registered to international standards. Although the WHO did recommend a weight-based dose regimen in 2015, it relies on tablet fractions [15].

We recently designed an age-based dosing regimen for Cambodia (and by extension several neighbouring countries) using four tablet strengths: 2.5, 5, 7.5 and 15 mg [16]. This regimen resulted in high proportions of patients who would receive an optimised transmission-blocking PQ dose, defined as 0.15–0.38 mg/kg body weight of PQ base, taking into account that the severe G6PDd Viangchan variant is prevalent in Cambodia [17, 18].

Age-based dosing regimens exist for several antimalarial drugs, including artesunate (AS)-mefloquine and AS-amodiaquine (AQ; ASAQ) [19]. They offer the substantial advantage of not requiring weighing scales and are suitable for the rapid distribution of drugs to large numbers of individuals, even under logistically challenging circumstances, for example, the mass drug administration (MDA) of ASAQ in Liberia during a recent Ebola outbreak [20]. Moreover, several SSA countries have adopted home-based management of malaria [21] and many patients buy their drugs from the informal private sector, where reliable weighing scales may not be available [22–25].

The development of an age-based dosing regimen requires a thorough examination of efficacy, tolerability, pharmacokinetics (PK), PK–pharmacodynamic (PD; PKPD) relationships, regimen simplicity, and suitable tablet strength availability. These aspects have been investigated elsewhere [16], but a recent study of PQ PK in *P. falciparum*-infected children aged 2–14 years from Burkina Faso illustrates the independent effects of age, weight and cytochrome 2D6 metaboliser status on PQ and carboxyPQ exposures [26]. Younger children and children with a lower body weight had lower PQ and carboxyPQ exposures, and poor PQ metabolisers had increased PQ but reduced carboxyPQ exposures. In this paper, we review aspects of SLDPQ pertinent to SSA and discuss how we determined our proposed age-based dosing regimen for SLDPQ.

Low-dose primaquine efficacy in reducing mosquito infectivity

Early work demonstrated a rapid reduction in mosquito infectivity (within 15 hours) when low doses (e.g. 10 mg) of the 8-aminoquinoline pamaquine/plasmoquine were combined with standard antimalarial treatments in falciparum malaria patients [27, 28]. Later work suggested that 15 mg of PQ had a similar effect on mosquito infectivity as 45 mg (0.75 mg/kg in a 60-kg adult) [29, 30].

The comprehensive effect of SLDPQ on mosquito infectivity has been demonstrated by Dicko et al., who studied symptomatic [12/81 (14.8%)] and asymptomatic *P. falciparum*-infected Malian adults and children with ≥2 gametocytes on a thick blood film [31]. Given with the first dose of dihydroartemisinin-piperaquine (DHAPP), the mg/kg dose of PQ base was 0 (control), 0.0625, 0.125, 0.25 and 0.5. Efficacy was assessed by direct mosquito membrane feeding assays.

The primary efficacy endpoint was the mean within-person percentage reduction in mosquito infectivity for a given PQ-dosing group on study day 2 (D2), i.e. 48 hours in patients infected ≥1 mosquito pre- and post-PQ treatment:

100% × (*N* infected individuals at D0 − *N* infected individuals at D2)/*N* infected individuals at D0.

The 0.125-mg/kg dose (7.5 mg in a 60-kg individual) achieved a mean 94.9% [95% confidence interval (CI) 84.6–100] reduction in infectivity ($p = 0.045$) whereas the 0.0625-mg/kg dose (3.75 mg in a 60-kg individual) achieved a non-significant 59.83% reduction in infectivity (95% CI 16.9–100, $p = 0.18$). Consistent results come from a dose-ranging study in DHAPP-dosed Cambodian and Vietnamese *P. falciparum*-infected adults. A significant anti-infectivity effect was seen with 3.75 mg of PQ base and a maximal blocking effect was seen with 7.5 mg of PQ base [9].

Dicko et al. reconfirmed the pre-treatment non-linear gametocyte density–infectivity relationship [32–34], but following treatment there was no relationship between gametocyte density and mosquito infectivity. Consistent with these observations are the findings that febrile adults with patent gametocytaemia are more infectious than those who are afebrile with subpatent gametocytaemia [35], and that asymptomatic *P. falciparum* carriers (QT-NASBA-positive gametocytaemia) have low baseline [36] and post-treatment [artemether-lumefantrine (AL) or DHAPP] infectivity whether or not they also received SLDPQ [36, 37].

Based on the patient mosquito infectivity data, we chose the same minimum 0.15 mg/kg dose of PQ base as we did in Cambodia [16].

Primaquine tolerability and safety

PQ is a well-tolerated and very safe drug. The mortality associated with PQ has been estimated at only 1 in 621,428 (upper 95% CI 1 in 407,807) patients treated, most having been associated with repeated PQ dosing as anti-relapse therapy for vivax malaria [38]. The three principal side effects of PQ, all dose-dependent, are abdominal pain, methaemoglobinaemia and AHA.

Gastrointestinal side effects

Occurrences of abdominal pain and vomiting have been unremarkable in recent trials in asymptomatic *P. falciparum*-infected African children, dosed with PQ on D2. PQ-induced abdominal pain and early vomiting necessitating retreatment were not reported by Goncalves et al. [36] or Eziefula et al. [39], but the former re-dosed 5/360 patients with AL on D0 or D1. Okebe et al. re-dosed 1/344 children with DHAPP and the reported frequencies of abdominal pain in the DHAPP-alone vs. DHAPP + PQ arms (0.2, 0.4 and 0.75 mg/kg) were 12, 14, 15 and 9%, respectively [37].

Similarly, 14 (2.5%) of 564 children treated with sulphadoxine-pyrimethamine (SP), artesunate and PQ on D2 vomited but none had persistent vomiting following retreatment [40]. Dicko et al. reported overall rates of mild abdominal pain of 10% and mild vomiting of 1.3% across all arms but no one required repeat dosing [31] whilst acutely infected falciparum patients of all ages (median 10 years) from Tanzania had identical rates of abdominal pain and vomiting of 6.4% (7/110) and 5.5% (6/110), respectively, in the AL-alone and the AL + SLDPQ arms [13].

When ACTs are given alone, early ACT-induced vomiting in uncomplicated malaria patients occurs primarily in children aged <5 years and, within this age band, the youngest children are at the highest risk. Rates of early vomiting tend to be significantly less frequent on D1 compared to D0. DHAPP is associated with the highest D0 rate of early vomiting (7–10%) whilst AL and ASAQ have overlapping rates (<3–6%) [41–44]. One trial reported very low early vomiting rates in adults and children aged >5 years: 0.4% (2/496) vs. 0% (0/502) in ASAQ (mean age 17.3 years) and AL-treated patients (mean age 16.5 years), respectively [44]. The WHO recommendation is to give SLDPQ with the first dose of ACT. Whether this would increase the rates of early vomiting in younger children, who, therefore, might be better dosed on D1 due to better tolerability, is unknown.

Methaemoglobinaemia

While no published PQ studies from Africa have measured methaemoglobin (metHb) concentrations, they are being measured in an ongoing study (ClinicalTrials.gov identifier NCT02535767). Methaemoglobinaemia manifests clinically as a blue discoloration of the lips and tongue when metHb concentrations are approximately 20%. metHb levels are mildly raised in malaria and correlate positively with disease severity. Mean levels of 2%,

4.1% and 5.8% were reported in healthy controls, uncomplicated malaria patients and severe malaria patients with death or clinical sequelae, respectively [45]. Data from Cambodia show that 0.25 mg/kg of PQ (L. Desoley, unpublished) in *P. falciparum*-infected patients and 0.75 mg/kg in *P. vivax*-infected patients resulted in a maximum metHb concentration of 3.6% and 4.9% [46], respectively. Thus, methaemoglobinaemia is not considered a clinical concern in SLDPQ-treated African children.

Acute haemolytic anaemia and African variants of G6PD deficiency

The most-feared PQ side effect is dose-related AHA. Previous experience with AHA induced by chlorproguanil dapsone, resulting in the stopping of its development, is a cautionary lesson [47, 48]. The incidence of AHA is greater in individuals with lower G6PD enzyme activities, so that hemizygous males and homozygous females are more likely to suffer PQ-induced AHA than heterozygous females with intermediate G6PD enzyme activities [49, 50].

The G6PDd A- variant (G202A), which in one study from Kenya accounted for most of the variance in G6PD enzyme activity [51], is the most common G6PD variant in SSA [52], with reported allele frequencies of ~2% in The Gambia, ~17% in Kenya and ~21% in Nigeria [53–55]. Although referred to as a mild variant, enzyme activities of ≤1 U/g Hb are well documented [56–58]. Other mild A- variants, reported from West Africa are G6PDd Mexico City (C680A) and Betica-Selma (A968G) [59].

G6PD Santamaria (T542A), a severe G6PDd variant, is also present in West Africa [59] and has a residual enzyme activity of ~2–3% of wild-type G6PD [60, 61]. It had a reported allele frequency of 6.6% in 2379 Gambian children [62]. In Sudan, the frequencies in males of the severe G6PD Mediterranean (≤3.8%) and G6PD A- variants (≤2.1%) were low in two studies [63, 64].

Primaquine research on transmission blocking in Africa

PQ research in Africa is limited. Studies have included G6PDd and G6PD normal healthy individuals, malaria patients and asymptomatic *P. falciparum* carriers – all with high baseline Hb concentrations, usually ≥8 g/dL. G6PD status has been assessed by the qualitative fluorescent spot test or a qualitative rapid diagnostic test, supplemented by genotypic analysis in some studies.

G6PDd and G6PD normal healthy individuals

One MDA study examined 0.75 mg/kg of PQ base, administered on D2, in Tanzanian children aged <12 years (Hb ≥ 8 g/dL) who also received a standard dose of SP [40]. On D7, genotypically determined homozygous or hemizygous G6PDd children had the greatest fall in mean Hb: -2.5 (95% CI -1.2 to -3.8) g/dL vs. -1.6 (-0.9 to -2.2) g/dL in heterozygous females, and vs. -0.5 (-0.4 to -0.6) g/dL in G6PD normal children. No PQ PK analysis was performed but the authors noted that children aged <5 years were at risk of a greater fall in Hb, independent of G6PD status, if they received higher mg/kg doses of PQ. Indeed, an examination of the PQ-dosing regimen used shows that many children received doses far greater than the 0.75 mg/kg target dose, based on anthropometric data of the local population (Table 1) and those of our modelled database (Table 2).

G6PDd and/or G6PD normal malaria patients

The Tanzanian study of Mwaiswelo et al. [13] showed that predominantly older children (median age 10 years, range 1 – 84) whose mean Hb was ~11 g/dL tolerated SLDPQ (0.25 mg/kg) well when given on D0 with the first dose of AL. The G6PDd heterozygous females ($n = 4$) in the AL-alone arm had the greatest mean absolute decline in Hb of ~1.7 g/dL whereas the G6PDd hemizygous males ($n = 9$) and homozygous females ($n = 5$) in the AL + SLDPQ arm had the greatest fall in Hb, a mean of ~1.5 g/dL with an upper 95% CI of ~2.4 g/dL, representing fractional falls from baseline of 12.6 and 19%, respectively. The corresponding data for these patients in the AL-alone group are 0.35 and 1.1 g/dL, and 2.7 and 9.7%.

In G6PD normal patients aged 1–10 years (Hb ≥ 8 g/dL), AL alone or combined with PQ (0.1, 0.4 or 0.75 mg/kg on D2) resulted in mean falls in Hb on D3 (the nadir in this study) of ~ -0.5 to ~ -0.75 g/dL that were unrelated to PQ dose. Indeed, the mean changes in Hb on D2 before PQ was given were very similar and explain most of the decline in Hb change post-PQ treatment [39]. On further analysis, these authors reported a PQ dose effect in the mean absolute fall in Hb on D7 (not seen on D3 or D10) in the small number of genotypically determined heterozygous females ($n = 14$) who received 0.75 mg/kg of PQ compared to those who received placebo [65]. The difference in the 0.75 vs. placebo arm was ~ -0.67 g/dL ($p = 0.044$).

When *P. falciparum*-infected children (3–15 years) were dosed on D3 with 0.75 mg base/kg of PQ, four G6PDd hemizygous males showed an increasing trend of a higher decline (median ~ -22%) in the fractional Hb on D7 compared to the 39 G6PD normal and nine G6PDd heterozygous females, who had median Hb falls of ~ -5% [66].

G6PD normal P. falciparum carriers

Goncalves et al. recruited children aged 2 – 15 years (Hb ≥ 8 g/dL) and reported small changes in mean Hb concentrations on D3 and D7 using AL alone or AL plus 0.25 or 0.4 mg/kg of PQ on D2 [36]. The absolute mean maximal fall in Hb (measured at the time of maximum fall) was highest in the 0.4-mg/kg arm but this was significantly different

Table 1 The primaquine (PQ) regimen administered to Tanzanian children aged <12 years by Shekalaghe et al. [40] and the mg/kg they received based on anthropometric data of the local population

		Weight (kg)			PQ received (mg/kg body weight)*		
PQ dose (mg base)	Age (years)	25th	Median	75th	25th	Median	75th
7.5	1–2	9.5	11.0	11.6	0.65	0.68	0.79
15	3–4	11.5	12.9	14.5	1.03	1.16	1.30
15	5–7	14.8	16.7	19.0	0.79	0.90	1.01
22.5	8–11	20.5	23.0	27.1	0.83	0.98	1.10

PQ primaquine
* Corresponding mg base/kg doses of PQ are shown for the 25th, 50th (median) and 75th centiles

($p = 0.006$) only when compared to placebo: -1.21 (95% CI -1.45 to -0.97) vs. -0.71 (-0.98 to -0.44). The proportion of patients with Hb falls ≥2 g/dL were broadly similar ($p = 0.5$) between the AL alone [9/62 (14.5%)], and the AL plus 0.25-mg/kg [12/75 (16%)] or 0.4-mg/kg PQ [14/73 (19.2%)] arms.

Okebe et al. also reported low and transient mean declines in Hb concentrations at D7 in just over half of treated asymptomatic *P. falciparum* carriers (DHAPP alone and DHAPP plus three PQ doses). The highest mean decline in Hb occurred on D3 and most subjects recovered their Hb concentration by D10 [37].

Unpublished G6PDd healthy individuals and P. falciparum carriers – SAFEPRIM and PQSAFETY studies

Three studies have assessed the tolerability of SLDPQ in G6PDd males (determined by the fluorescent spot test) aged 18–45 years with asymptomatic *P. falciparum* infection (Burkina Faso, $n = 50$) or healthy volunteers aged ≥10 years (The Gambia, $n = 50$), who had baseline Hb concentrations ≥11 g/dL and were treated with either AL (Burkina Faso, SAFEPRIM I) or DHAPP (The Gambia, SAFEPRIM II). The PQ doses (0.25 and 0.4 mg/kg) used were tolerated well and the falls in Hb were generally small when assessed at D7 (U. D'Alessandro, personal communication). The Hb dynamics were characterised by an initial decline in mean Hb followed by recovery, a pattern similar to that in patients with acute uncomplicated falciparum malaria.

In a small PQ PK study (ClinicalTrials.gov identifier NCT02535767) of healthy G6PDd Malian adults with Hb concentrations ≥10 g/dL, PQ doses of 0.4, 0.45 and 0.5 mg/kg were also tolerated well (I. Chen, personal communication), with overlap of the fractional and absolute falls in Hb in the G6PD normal and G6PDd groups given 0.5 mg base/kg of SLDPQ.

Summary of PQ research in Africa

These studies, in which individuals had relatively high pre-treatment Hb concentrations, have provided useful data on Hb dynamics in patients of all ages as well as healthy G6PD normal and G6PDd *P. falciparum* carriers. The declines in Hb appeared well tolerated despite falls ≥2 g/dL in some patients. These data show that the declines in Hb in PQ-treated hemizygous males and homozygous females overlap with heterozygous females and normal individuals who do not receive PQ but that the distribution in the decline of Hb shifts to the left, i.e. PQ induces greater absolute and fractional falls in Hb. The

Table 2 Calculated mg/kg of primaquine (PQ) base that would be received by children aged <12 years using the PQ regimen of Shekalaghe et al. [40] based on anthropometric data of the modelled database

	Modelled weights (kg)										
Age band (years)	Minimum	1st	5th	10th	25th	Median	75th	90th	95th	99th	Maximum
1–2	5.5	6.5	7.4	7.9	8.9	10.0	11.5	12.8	13.6	15.2	18.2
3–4	7.8	9.2	10.4	11.1	12.4	13.8	15.3	16.8	17.9	19.9	23.9
5–7	10.0	11.5	13.0	14.0	15.2	17.0	19.2	21.0	22.8	26.0	32.1
8–11	14.7	16.6	18.4	19.5	21.5	24.0	27.4	31.0	33.2	38.0	46.0
	PQ dose (mg base/kg)										
PQ dose (mg base)	Minimum	1st	5th	10th	25th	Median	75th	90th	95th	99th	Maximum
7.5	0.41	0.49	0.55	0.59	0.65	0.75	0.84	0.95	1.01	1.15	1.37
15	0.63	0.75	0.84	0.89	0.98	1.09	1.21	1.35	1.44	1.63	1.92
15	0.47	0.58	0.66	0.71	0.78	0.88	0.99	1.07	1.15	1.30	1.50
22.5	0.49	0.59	0.68	0.73	0.82	0.94	1.05	1.15	1.22	1.36	1.53

PQ primaquine

Hb cost of PQ depends on the dose with a doubling (from ~10% to ~20%) of the upper 95% CI fractional fall seen in Tanzanian patients [13], to an approximately six-fold higher difference in the upper 95% CI of the absolute fall in Hb (3.8 vs. 0.6 g/dL) in the MDA study in which many children received toxic doses of PQ [40]. The latter study is a salutary reminder of the toxicity of high mg/kg PQ doses, possibly augmented by sulphadoxine, when given to G6PDd A- children [40]. One key group lacking PQ data is young, moderately anaemic (Hb < 8 g/dL) children with acute uncomplicated falciparum malaria.

Background anaemia in African children

In African children, anaemia is common and its aetiology is frequently multifactorial [67]. The rates of background anaemia in Africa are high (e.g. 40–60%) across all malaria transmission intensities [68–73], but tend to be lower in low-transmission settings [69, 72]. Anaemia is inversely related to age in healthy individuals, asymptomatic falciparum carriers and malaria patients independent of malaria transmission intensity [68, 74, 75]. Table 3 shows Hb data in children aged <5 years from a community survey in the Kenyan highlands (low transmission) [68], and Table 4 shows Hb data in *P. falciparum*-infected patients of all ages from several African countries (database used in this analysis).

Younger children would be the prime recipients of SLDPQ in a programme that targets patients with symptomatic malaria. Although their median Hb concentrations at presentation may seem adequate (Table 4), a sizeable minority will have moderate anaemia and a small number will have severe anaemia (Hb < 5 g/dL). Younger children treated with ACT suffer greater post-treatment falls in Hb compared to older children. From our database, children aged <5 years had significantly lower Hb concentrations at baseline (9.3 vs. 10.9 g/dL) and D7 (8.6 vs. 10.5 g/dL) compared to children aged 5–11 years (p values < 0.001).

The risks of *P. falciparum*-infected children experiencing PQ-induced AHA and/or clinically significant anaemia (CSA), including profound anaemia (Hb < 4 g/dL) and severe anaemia (Hb < 5 g/dL) with features of severe malaria, are unknown. Limited data from a trial comparing ACTs [42] and a trial comparing SP vs. atovaquone-proguanil [76] suggest risk factors for a blood transfusion were: (i) high parasite biomass in ACT-treated children (clinical indication was decompensated anaemia), and, for the SP-treated children (clinical indications were Hb <3 g/dL or the development of respiratory distress or extreme lethargy), (ii) pre-treatment Hb < 5 g/dL, (iii) younger age, and (iv) high parasite biomass.

Summary of SLDPQ risk and background anaemia

There are two main reasons to be cautious in setting the upper dose limit of SLDPQ. Firstly, all the PQ studies that have recruited G6PDd patients have had high baseline Hb concentrations; therefore, we cannot extrapolate with confidence these findings to young symptomatic anaemic *P. falciparum*-infected children who have lower Hb concentrations (Table 4) and, secondly, the initial reduction in Hb level in PQ-treated asymptomatic *P. falciparum* carriers is similar to uncomplicated falciparum malaria patients, with some individuals experiencing Hb drops >2 g/dL [36, 37].

Predicting the fall in Hb in young PQ-treated malaria patients is challenging, but based on: (i) the published data cited above, (ii) unpublished summarised post-treatment Hb dynamics data for African patients aged <5 years (kindly provided by the WorldWide Antimalarial Resistance Network), (iii) the inverse relationship between baseline Hb and post-treatment Hb decline, and (iv) avoiding the tendency to underplay the toxicity of SLDPQ, we hypothesise that an average population of *P. falciparum*-infected African children aged <5 years may experience Hb declines of: (i) 1 g/dL (median), (ii) 1.5 g/dL (lower interquartile range), and (iii) 3 g/dL (lower 5%).

On the other hand, Shekalaghe et al. provide some reassurance that toxic doses of PQ were tolerated well in apparently healthy Tanzanian children aged 1–12 years with Hb concentrations ≥8 g/dL [40]. Moreover, in areas of low malaria transmission, the rates of background anaemia are less, so the risk of SLDPQ-induced AHA should be less compared to areas of high transmission and high background rates of anaemia.

We adopted a risk-stratified approach to setting the dose of SLDPQ. Given the uncertainty of the haemolytic potential of PQ in very young *P. falciparum*-infected

Table 3 Haemoglobin concentrations in g/dL in healthy children aged 6 months to 4 years from low-transmission areas in western Kenya [68], where only 0.3% (5/1697) were polymerase chain reaction (PCR) positive for *P. falciparum* in the community

Age (years)	1st	5th	10th	25th	Median	75th	90th	95th	99th	No.
<1	6.1	7.8	8.1	9.3	10.5	11.0	12.0	12.3	13.0	37
1	5.9	6.5	7.3	9.2	10.2	10.9	11.6	12.1	13.3	59
2	5.8	7.7	8.7	9.7	10.5	11.3	12.7	13.0	14.0	73
3	6.8	8.4	9.1	9.9	11.4	12.15	12.8	13.2	14.0	80
4	5.3	9	9.7	10.9	12.0	12.7	13.4	13.6	14.8	79

Table 4 Baseline haemoglobin concentrations in g/dL in patients of both sexes with acute uncomplicated falciparum malaria enrolled in trials in several African countries

Age (years)	1st	5th	10th	25th	Median	75th	90th	95th	99th	No.
0.5 to <1	4.8	5.5	6.0	6.9	8.2	9.7	10.7	11.7	12.8	1693
1	4.8	5.9	6.4	7.4	8.9	10.2	11.5	12.1	13.6	3247
2	4.8	5.9	6.7	7.8	9.4	10.7	11.7	12.7	14.0	2262
3	5.0	6.1	7.1	8.6	9.9	11.2	12.4	12.8	14.2	1838
4	5.0	6.3	7.3	8.9	10.2	11.5	12.5	13.2	14.3	1528
5	5.5	7.1	7.5	9.1	10.5	11.8	12.8	13.3	14.1	602
6	5.5	7.1	7.6	9.0	10.3	11.6	12.6	13	14.3	429
7	5.0	7.1	7.8	9.3	10.6	11.9	13.0	13.8	15.1	357
8	5.1	6.8	7.7	9.4	10.9	12.1	13.2	14.0	15.5	297
9	6.3	7.5	8.6	9.7	11.0	12.5	13.2	13.6	14.8	232
10	6.7	7.7	8.9	10.1	11.3	12.7	13.8	14.2	15.1	227
11	6.8	7.4	8.9	10	11.6	12.7	14.0	14.8	15.5	133
12	6.4	8.2	9.4	10.5	11.6	12.9	14.0	14.8	15.8	100
13	6.5	7.8	8.5	10.1	11.5	12.6	14.0	15.1	15.4	57
14	7.1	7.9	9.2	10.4	11.6	12.8	13.7	14.3	15.5	62
15	7.8	8.0	9.0	9.7	11.1	12.5	14.4	15.2	15.6	33
16	8.0	8.4	9.2	10.5	12.0	13.5	14.7	16.1	17.3	33
17	7.8	7.8	8.6	10.2	12.0	13.6	15.8	15.9	16.1	24
≥18	6.7	8.2	9.1	10.9	12.3	13.9	15.1	16	17.3	574

children aged 6 – 11 months and the need to be cautious, we decided to under-dose them and arbitrarily set a dose of 1.25 mg PQ base, for a median PQ dose of 0.16 mg/kg (i.e. ~60% of the WHO recommendation). For children aged 1–5 years, we set an upper limit of 0.4 mg/kg of PQ base but increased this upper limit to 0.5 mg/kg for older children because they have less post-treatment declines in Hb and appear to tolerate SLDPQ well.

Methods

Assembling the African anthropometric database

We obtained permission to download anthropometric databases from the Demographic Health Survey website (https://dhsprogram.com/), which include a broad range of socioeconomic, geographical and other randomly collected data; however, the anthropometric data are confined to girls and boys aged <60 months and women aged 15 – 45 years. Other databases obtained from academic researchers, Médecins Sans Frontières, France, and Epicentre, Paris, contained data from malaria patients and field surveys of individuals from malaria-endemic areas.

The first author approached individuals to explain the purpose of this project and requested anonymised anthropometric data. Later, haematological data were also sought. All those who freely gave data would be co-authors and were expected to provide feedback on this paper.

Data obtained from KEMRI-WT (Kenya) followed a formal request to a data access committee. We considered ethical approval unnecessary for this project, which was confirmed by the Oxford Tropical Research Ethics Committee.

Modelling the weight-for-age data

The raw weight-for-age data were modelled into a growth curve (Additional file 1: Figure S1) by excluding outlying weight-for-age data, which were defined as values falling outside the 1st and 99th centiles (as was done for the Cambodian database). Modelled weight-for-age centiles were obtained using the three-parameter Box–Cox power exponential distribution [77] and the centile growth curves smoothed using the cubic spline smoothing technique [78]. The modelling was performed in Stata using the 'xriml' macro Stata module [77] with a cubic spline.

The ages of children aged 6 – 11 months were expressed as a decimal of 1 year. Ages ≥12 months were rounded down to whole integers (e.g. 2 years and 5 months was rounded down to 2 years) and ages ≥18 years were grouped together. The mg base/kg dose of PQ received for a given age group was calculated as PQ dose/all the weights for that age group.

Data analysis and determining optimal age-dosing categories

Within each age-dosing group, we calculated the proportions of males and females who would receive therapeutic doses for a given age in years as well as for the age-dosing group overall. Male–female differences were compared by Fisher's exact test, differences in the distribution of skewed data were compared using Mann Whitney U test and mean differences in normally distributed data were compared using an unpaired *t*-test. A *p* value ≤0.05 was considered significant.

PQ doses of 1–15 mg were tested across different age groups and the results analysed. The final age categories were selected based on: (i) the need to be especially cautious in children aged 6 – 11 months, (ii) the mg base/kg dose received, (iii) the proportions receiving a therapeutic dose, (iv) how well a given dosing band fitted in with the next dosing band, and (v) a desire to minimise the number of dosing groups and to use the same tablet strengths as those in the Cambodian regimen (i.e. 2.5, 5, 7.5 and 15 mg of PQ base).

Results

Features of the anthropometric database

The modelled database numbered 661,979 individuals aged ≥6 months. The female/male ratio is 2:1 [missing data = 95 (0.01%)] and the rural/urban ratio is 2.6:1. Individuals aged 6–11 months, 1 – 4 years, 5–14 years and ≥15 years represent approximately 6%, 38%, 6% and 50% of the database. The Demographic Health Survey data accounted for 83.0% ($n = 549,127$) of the database whilst malaria ($n = 28,466$), other infections ($n = 30,009$) and miscellaneous data from rural clinics ($n = 54,377$) accounted for 4.3%, 4.5%, and 8.2%, respectively. The distributions of these categories by countries are shown in Additional file 2: Table S1. The weight distributions in children aged ≤5 years with and without malaria overlapped substantially (Fig. 1).

Dosing regimen

There are five age-dosing groups (Table 5). Overall, the model predicts that ~94–99% of patients in dosing bands from 1 year upwards would receive a PQ dose within the defined therapeutic range, and that their median doses would range from 0.23 to 0.29 mg/kg of PQ base. The median dose for children aged 6–11 months is 0.16 mg/kg. Based on our minimally efficacious transmission-blocking dose of 0.15 mg/kg PQ, 27% of infants will be under-dosed but this falls to 3.3% (1,322/39,886) if using the minimum dose of 0.125 mg/kg of PQ base, as reported by Dicko et al.

Primaquine dose breakdown by individual ages and sex

The proportions of females and males predicted to receive subtherapeutic, supratherapeutic and therapeutic doses are shown in Figs. 2 and 3, respectively. Rates of supratherapeutic dosing are low, a maximum of 1.3% in females aged 1 year. Age groups at risk of under-dosing exceeding 5% are females and males aged 6 to <12 months, 4 and 5 years, and females aged 14 years. Altogether, 73.5% to 100% of females (Fig. 2) and 67.5% to 100% of males (Fig. 3) would receive a therapeutic dose.

The median mg base/kg dose of PQ base ranged from 0.17 to 0.32 in females (Fig. 4) and from 0.17 to 0.33 in males (Fig. 5). The age groups with the lowest median

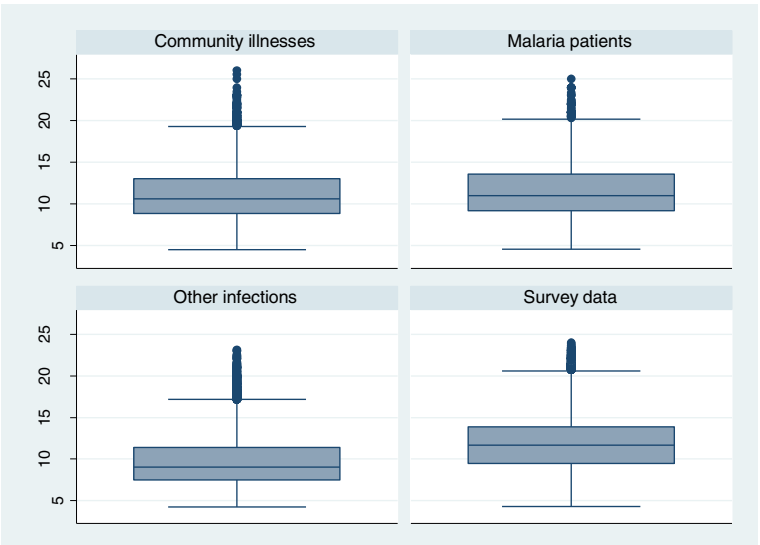


Fig. 1 Box plots of modelled weights in children aged less than five years by health status

Table 5 The proposed age-based dosing regimen showing the proportions of individuals who would receive a subtherapeutic, therapeutic or supratherapeutic dose and the corresponding mg base/kg doses of primaquine (PQ)

	PQ dose (mg base)	Subtherapeutic dose <0.15 mg/kg, *n* (%)	Therapeutic dose 0.15–0.5 mg/kg, *n* (%)	Supratherapeutic dose >0.5 mg/kg, *n* (%)	Modelled weights by centile (kg)			PQ dose by centile (mg base/kg)		
					1st	Median	99th	1st	Median	99th
0.5 to <1 (*n* = 39,886)	1.25	10,778 (27.0)	29,180 (73.2)	0	5.0	7.6	10.8	0.12	0.16	0.25
1–5 (*n* = 261,036)	2.5	15,744 (6.0)	244,537 (93.7)	755 (0.3)	6.8	12.0	19.2	0.13	0.21	0.37
6–9 (*n* = 20,770)	5	80 (0.4)	20,690 (99.6)	0	13.2	20.0	31.2	0.16	0.25	0.38
10–14 (*n* = 12,155)	7.5	69 (0.6)	12,086 (99.4)	0	20.0	29.0	49.0	0.15	0.26	0.38
≥15 (*n* = 328,132)	15	469 (0.14)	327,620 (99.8)	43 (0.01)	37.8	55.3	90.2	0.17	0.27	0.40

PQ primaquine

mg/kg dose were 6–11 months, 4 years, and 5 years (0.17), and 3 years and 14 years (0.19) for females, and 6–11 months and 5 years (0.16), 4 years (0.17), and 3 years (0.19) for males.

Discussion

This age-dosing SLDPQ regimen for SSA was developed from an anthropometric database of more than 660,000 individuals from across the continent. It has five dosing groups and how optimised they are will be tested in a large randomised trial to assess its safety in G6PDd and G6PD normal African children with acute uncomplicated falciparum malaria (ISRCTN11594437). If this trial shows acceptable safety and adequate PK profiles, it will provide substantial reassurance to African ministries of health in accelerating the uptake of SLDPQ.

These data overlapped substantially with data from malaria patients and other survey data conducted in predominantly rural areas. Thus, our database is representative of target populations for SLDPQ. Although the WHO currently recommends that SLDPQ be used in symptomatic malaria patients, it is likely to be used as an elimination tool in asymptomatic parasite carriers, who contribute substantially to malaria transmission [79].

Setting the upper therapeutic range of PQ is crucial for safety and challenging in the absence of a robust dose–response relationship of PQ PK and Hb decline. In contrast to our approach for Cambodia, where malaria is mostly a disease of adults and where background rates of anaemia and malaria-related anaemia are lower [18], we placed much emphasis on the risk of young paediatric patients developing CSA following ACT + PQ because they have lower pre-treatment Hb concentrations and greater falls in Hb, and so a higher risk of CSA compared to older children. In addition, two studies support high parasite biomass as another risk factor for CSA [42, 76].

Therefore, we decided to be cautious and deliberately under-dose infants with 1.25 mg of PQ base, resulting in a mg/kg dose range of 0.1–0.3 (median of 0.16). In this scenario, ~60% of infants would receive less than the WHO-recommended PQ dose of 0.25 mg/kg. What effect this would have on mosquito infectivity is unknown and more research is needed to establish the dose–response (i.e. PQ PK–infectivity) relationship and refine the minimum PQ dose.

We set a minimum PQ dose of 0.15 mg/kg, based on data from the field experience of MDA in Cambodia [80], which is higher than the 0.125 mg/kg suggested by Dicko et al. in their mosquito infectivity experiments

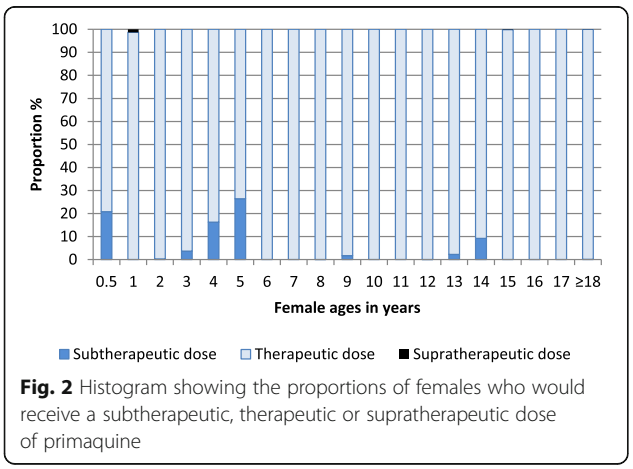


Fig. 2 Histogram showing the proportions of females who would receive a subtherapeutic, therapeutic or supratherapeutic dose of primaquine

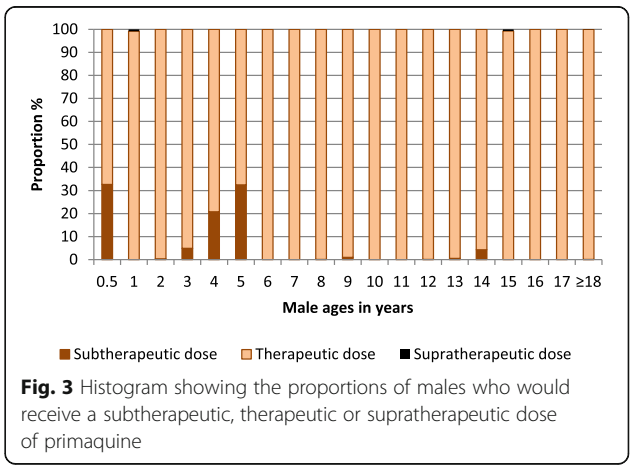


Fig. 3 Histogram showing the proportions of males who would receive a subtherapeutic, therapeutic or supratherapeutic dose of primaquine

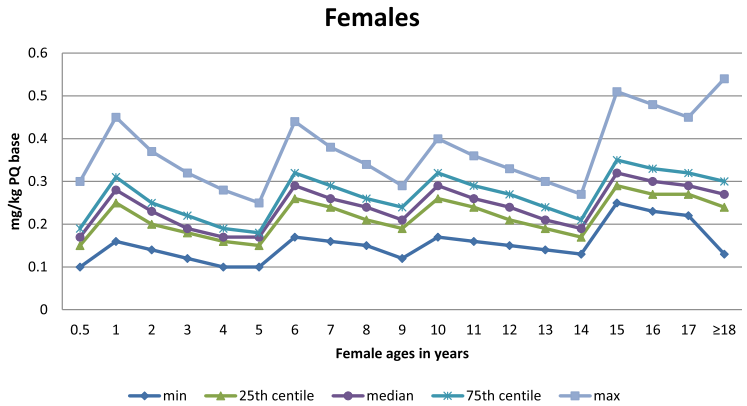


Fig. 4 The mg base/kg dose of primaquine (PQ) by centile that females would receive as a function of age. The proposed therapeutic range of PQ base in mg/kg body weight is 0.15–0.4 (1–5 years) to 0.5 (≥6 years). PQ primaquine

[81]. Which of the two is the more accurate value is unknown. Our threshold predicts ~25% under-dose rate in infants compared to 3% if we use the threshold of Dicko et al. This emphasises the need to define accurately the minimum transmission-blocking dose and to consider how much of a transmission reservoir such young children represent and, therefore, the need to give them SLDPQ.

Much emphasis and anxiety have been placed on the potential dangers of PQ-induced AHA in treated *P. falciparum*-infected G6PDd individuals but there are many other factors that may play an important role in post-treatment Hb dynamics and the risk of post-treatment CSA, such as sickle-cell trait/disease, alpha-thalassaemia, HIV infection, hookworm infection, vitamin A deficiency, poor nutritional state, schistosomiasis, baseline parasitaemia and Hb, and PQ PK [42, 67, 73, 76, 82].

Insights will be forthcoming from our safety trial of SLDPQ and from the large multicentre TRACT study (Transfusion and treatment of severe anaemia in African children trial [83]), which is designed to address optimal blood transfusion strategies in children admitted to hospital with Hb ≤6 g/dL. Data from both trials will inform strategies aimed at identifying children at risk of CSA before they are treated with ACT + SLDPQ and how this may be best achieved in challenging settings, e.g. small remote clinics that may not have accurate point-of-care tests to measure Hb concentration or the time or means to quantify parasitaemias.

Another key safety issue is the haemolytic effect of SLDPQ in individuals carrying the severe G6PDd Santamaria and Mediterranean variants. Limited data in *P. falciparum*-infected Cambodians with G6PDd Viangchan (median G6PD activity 0.8 U/g Hb [17, 46]) are encouraging, but are only partially applicable to

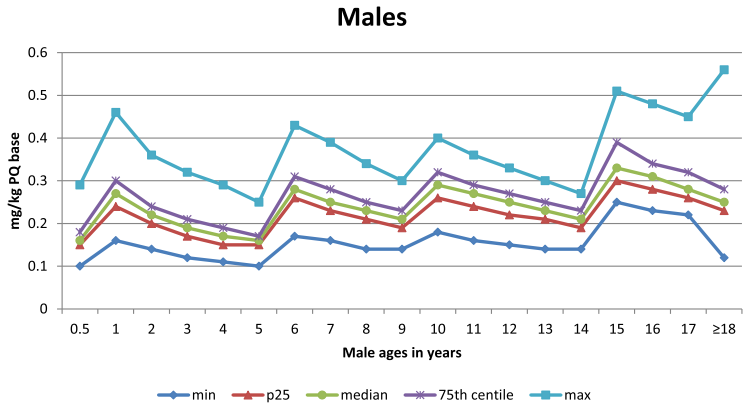


Fig. 5 The mg base/kg dose of primaquine (PQ) base by centile that males would receive as a function of age. The proposed therapeutic range of PQ base in mg/kg body weight is 0.15–0.4 (1–5 years) to 0.5 (≥6 years). PQ primaquine

Africa. Fractional declines in Hb on D7 overlapped in G6PDd and G6PD normal patients (mostly adults) who were treated with DHAPP + SLDPQ or DHAPP alone (L. Desoley, unpublished data). One reassuring aspect of our age-based regimen is that we have restricted the upper limits of PQ and the proportions of individuals receiving supratherapeutic doses is very small, <1.4% in those aged 1 or 15 years.

Our SLDPQ regimen has five dosing bands and the first (6–11 months) has the most restricted age limits. Anecdotally, concern is expressed that age-based dosing is intrinsically inaccurate because patients or guardians are unable to report ages accurately and birth certificates or 'Road to Health' cards may not be widely available in some areas. This was not an issue in the MDA of ASAQ during the Ebola outbreak in which 98% (983/1005) of individuals or guardians in Monrovia were able to tell their ages and take the correct dose of ASAQ [20]. Nevertheless, this is an aspect that needs to be considered when SLDPQ is deployed.

This SLDPQ regimen shares the same tablet strengths (aside from the 6–11 months age band) as the one designed for Cambodia [16]. This overlap in regimens between Africa and Cambodia (and by extension the Greater Mekong Subregion) may reassure the pharmaceutical industry regarding the market for SLDPQ. Indeed, the 2012 WHO recommendation was global. Moreover, if the same table strengths could be used in regimens for *P. vivax* radical cure, this should stimulate the pharmaceutical industry to produce 2.5- and 5-mg tablet strengths and paediatric formulations. This would have a positive effect on access. Substantial evidence on the safety of SLDPQ should be forthcoming in the next 2 years from our trial and other studies, and should allay anxieties that pharmaceutical companies may have regarding PQ toxicity and their liability.

Our analysis has limitations. Similar to others [19, 84], our database was dominated by children aged <5 years and women of childbearing age because most data from the Demographic Health Survey and malaria patients, especially in SSA, are from these groups. Although children aged 5–17 years represented 12% of the total database, each individual age group contained >2500 individuals (except ages 13 and 14 years).

The dosing regimen is not generalisable to countries outside of SSA and, like the Cambodian regimen, does not dovetail nicely with the age–weight dosing categories of commonly used ACTs. DHAPP has eight weight-based dosing bands with an emphasis on children who weigh <25 kg to ensure they receive sufficient dihydroartemisinin. AL, currently the most widely used ACT in Africa, has four weight-based dosing bands and ASAQ has four age-based bands (2–11 months, 1–5 years, 6–13 years and ≥14 years) that share two of the five age-based bands of our SLDPQ regimen. Suitable training would be essential before SLDPQ is implemented by programmes.

We designed this SLDPQ regimen based on current but incomplete knowledge, especially PQ PK in young children. Goncalves et al. have contributed useful data in asymptomatic falciparum carriers [26] and we hope to close the PK gap further. With this new knowledge and, possibly, the eventual definition of the dose–response relationship between PQ's oxidative metabolites and haemolysis and mosquito infectivity, our proposed SLDPQ regimen may need to be amended.

Conclusions

This age-based regimen of SLDPQ was designed to block *P. falciparum* transmission in SSA and will be tested for safety in a large trial in Uganda and the Democratic Republic of Congo in 2017. Depending on the results, this dose regimen may require fine-tuning but could also be readily adopted by national malaria control programmes.

Additional files

Additional file 1: Weight-for-age growth curves. (PDF 1202 kb)
Additional file 2: Table S1. Data type by country. (DOCX 14 kb)

Abbreviations

ACT: Artemisinin-based combination therapy; AHA: Acute haemolytic anaemia; AL: Artemether-lumefantrine; AQ: Amodiaquine; AS: Artesunate; ASAQ: Artesunate-amodiaquine; CI: Confidence interval; CSA: Clinically significant anaemia; D: Day; DHAPP: Dihydroartemisinin-piperaquine; G6PDd: Glucose-6-phosphate dehydrogenase deficiency; Hb: Haemoglobin; MDA: Mass drug administration; metHb: Methaemoglobin; MQ: Mefloquine; PD: Pharmacodynamics; PK: Pharmacokinetics; PKPD: Pharmacokinetics–pharmacodynamics; PQ: Primaquine; SLDPQ: Single low-dose primaquine; SP: Sulphadoxine-pyrimethamine; SSA: Sub-Saharan Africa; WHO: World Health Organization

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Authors' contributions

WRT had the original idea of an age-dosing SLDPQ regimen and was the study principal investigator. The core team (HKN, MMu, NJW and WRT) managed and analysed the data, and designed the regimen. WRT wrote the first draft of the paper. HKN, MMo, NJW, KM, TNW, MK, UA, CF, MMu, JM, GM, MCH, PBr, CJ, RMF and ZY critically reviewed the paper. All other authors

donated data. All co-authors have seen the final draft of the paper, and read and approved it.

Ethics approval and consent to participate

The Oxford Tropical Research Ethics Committee confirmed that this study was exempt from ethical review.

Competing interests

The authors declare that they have no competing interests.

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