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Safety and efficacy of primaquine in patients with *Plasmodium vivax* malaria from South Asia: a systematic review and individual patient data meta-analysis

Reena Verma ,[1] Robert J Commons,[2,3,4] Apoorv Gupta,[1] Manju Rahi ,[1,5,6] Nitika ,[1] Praveen K Bharti,[1] Kamala Thriemer,[2] Megha Rajasekhar,[7] Sauman Singh-Phulgenda,[8,9,10] Bipin Adhikari ,[10,11] Mohammad Shafiul Alam,[12] Prakash Ghimire ,[13] Wasif A Khan,[12] Rishikesh Kumar,[1,14] Toby Leslie,[15,16] Benedikt Ley,[2] Alejandro Llanos-Cuentas,[17] Sasithon Pukrittayakamee,[11] Komal Raj Rijal ,[13,18] Mark Rowland,[15] Kavitha Saravu ,[19,20] Julie A Simpson,[3,7] Philippe J Guerin ,[8,9,10] Ric N Price,[2,3] Amit Sharma [21]

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RV and RJC contributed equally.

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For numbered affiliations see end of article.

Correspondence to
Dr Robert J Commons; rob.commons@wwarn.org and Dr Amit Sharma; amit.icgeb@gmail.com

ABSTRACT

Background The optimal dosing of primaquine to prevent relapsing *Plasmodium vivax* malaria in South Asia remains unclear. We investigated the efficacy and safety of different primaquine regimens to prevent *P. vivax* relapse.

Methods A systematic review identified *P. vivax* efficacy studies from South Asia published between 1 January 2000 and 23 August 2021. In a one-stage meta-analysis of available individual patient data, the cumulative risks of *P. vivax* recurrence at day 42 and 180 were assessed by primaquine total mg/kg dose and duration. The risk of recurrence by day 180 was also determined in a two-stage meta-analysis. Patients with a >25% drop in haemoglobin to <70 g/L, or an absolute drop of >50 g/L between days 1 and 14 were categorised by daily mg/kg primaquine dose.

Results In 791 patients from 7 studies in the one-stage meta-analysis, the day 180 cumulative risk of recurrence was 61.1% (95% CI 42.2% to 80.4%; 201 patients; 25 recurrences) after treatment without primaquine, 28.8% (95% CI 8.2% to 74.1%; 398 patients; 4 recurrences) following low total (2 to <5 mg/kg) and 0% (96 patients; 0 recurrences) following high total dose primaquine (≥5 mg/kg). In the subsequent two-stage meta-analysis of nine studies (3529 patients), the pooled proportions of *P. vivax* recurrences by day 180 were 12.1% (95% CI 7.7% to 17.2%), 2.3% (95% CI 0.3% to 5.4%) and 0.7% (95% CI 0% to 6.1%), respectively. No patients had a >25% drop in haemoglobin to <70 g/L.

Conclusions Primaquine treatment led to a marked decrease in *P. vivax* recurrences following low (~3.5 mg/kg) and high (~7 mg/kg) total doses, with no reported severe haemolytic events.

PROSPERO registration number CRD42022313730.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The WHO South-East Asia Region accounted for ~2% of malaria cases globally in 2022. India accounted for 79% of cases in the region, of which ~40% of cases were caused by *P. vivax*.

⇒ *P. vivax* malaria is a substantial challenge for malaria control and elimination due to relapsing infections.

⇒ Primaquine is the only approved antimalarial to prevent relapses in this region, however, its optimal dosing regimen remains uncertain.

INTRODUCTION

Plasmodium vivax causes an estimated 4.9 million cases of malaria annually with 2.5 billion people at risk of infection.[1] India is aiming for malaria elimination by 2030, however, in 2020, it was estimated to have contributed 79% of the malaria cases and 83% of malaria deaths to the WHO South-East Asia Region.[1] The Indian National Center for Vector Borne Disease Control recorded a total of 161 753 confirmed malaria cases in 2021, of which 60 187 cases (37.2%) were caused by *P.* vivax.[2]

Unlike *Plasmodium falciparum*, *P. vivax has* dormant liver stages that can cause relapses of malaria weeks or months later. Relapsing vivax malaria leads to recurrent acute febrile illness, anaemia and morbidity, and contributes to ongoing transmission[3]. Successful treatment of *P. vivax* (radical cure) requires treatment of both the blood and liver stages of the parasite.[3 4]

WHAT THIS STUDY ADDS

⇒ Individual data from 791 patients enrolled into 7 trials in South Asia were included in a one-stage meta-analysis.
⇒ Aggregated data from 3259 patients from 9 studies in South Asia were included in a two-stage meta-analysis.
⇒ Low and high total dose primaquine regimens appear to have good antirelapse efficacy in South Asia.
⇒ There were no severe haemolytic events with any primaquine dose in patients with ≥30% glucose-6-phosphate dehydrogenase (G6PD) activity.
⇒ The study highlights a relative lack of available data on primaquine efficacy in patients followed for 180 days or more, and a lack of data on tolerability and safety of different primaquine regimens.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The available data support the current recommendation for low total dose primaquine regimens in South Asia.
⇒ Despite limited data, regimens with higher daily doses appeared to be safe in patients with normal G6PD activity, supporting the introduction of 7 day 0.5 mg/kg primaquine regimens following G6PD testing in the South Asia region.

For more than 70 years, primaquine, an 8-aminoquinoline, has been the only available drug to treat *P. vivax* hypnozoites and prevent vivax relapses. However, primaquine can cause drug-induced haemolysis in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency, in addition to other side effects such as gastrointestinal disturbance and methaemoglobinaemia.[4] Although concerns about haemolysis, coupled with a lack of available bedside G6PD testing, have prevented the widespread implementation of primaquine radical cure,[5] point of care G6PD testing is increasingly available, with a quantitative test now approved in India, paving the way for safer use of primaquine.[6]

The total mg/kg dose of primaquine is related to antirelapse efficacy, with higher doses of primaquine potentially providing greater efficacy.[4] The total dose of primaquine used in South Asia is 3.5 mg/kg given over 14 days (0.25 mg/kg/day), in line with the current WHO recommendation.[7] However, few studies have compared primaquine regimens with higher total doses[8 9] and a recent individual patient data meta-analysis across all vivax-endemic regions suggested a higher 7 mg/kg total dose may improve efficacy.[10] Regimens with long durations may have poor adherence, leading to low effectiveness although this is not consistent among all studies.[11 12] Two recent studies have demonstrated non-inferiority of a 7-day vs 14-day regimen of high total primaquine dose (7 mg/kg).[13 14] Some countries, such as Brazil, use a lower 3.5 mg/kg total dose regimen over 7 days,[15 16] which has recently been recommended by WHO.[7] However, daily dosing may be limited by tolerability and haemolytic safety. Thus, any potential benefits from increasing the total dose or providing an equivalent total dose over a shorter period need to be balanced against the associated risks of haemolysis with higher daily doses.

To better understand the safety, tolerability and efficacy of different total and daily primaquine doses for uncomplicated *P. vivax* in South Asia, we undertook this systematic review and individual patient data meta-analysis of efficacy studies of uncomplicated *P. vivax*.

METHODS

Search strategy and selection criteria

We systematically searched MEDLINE, EMBASE, Web of Science and Cochrane Central according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses Individual Patient Data (PRISMA-IPD) guidelines (online supplemental checklist S1) to identify prospective efficacy studies of uncomplicated *P. vivax* monoinfection published in any language between 1 January 2000 and 23 August 2021 that were undertaken in South Asian countries including Bangladesh, Bhutan, India, Pakistan, Nepal and Sri Lanka. This work is an extension of a previously published review[17] with search terms and criteria described in detail in online supplemental box S1. Studies were included if they included a treatment arm with primaquine commencing within 7 days of schizontocidal treatment, had data on age, sex and parasitaemia on day 0, data on schizontocidal and primaquine dosing and reported parasite presence or absence during follow-up.

Identification of eligible studies was undertaken separately by two investigators (RV and RJC), with disagreement resolved through discussion. Investigators of eligible studies were approached to share data. If available, data from eligible but unpublished studies were also included. Individual patient data were collated in the WorldWide Antimalarial Resistance Network secure repository, where they were standardised into a quality-assured dataset.[18] Metadata, including study design and study site details, were recorded.

Procedures

Individual patients' data were excluded if information on dose and regimen of primaquine were incomplete or if no data were available for baseline parasitaemia, age, sex or weight of the patient. The number of tablets given to each patient were used to calculate primaquine doses. If tablet counts were not available, doses were calculated from age-based or weight-based dosing schemes in the study protocol, or if not available, doses were assumed from planned mg/kg dosing regimens. Total primaquine dose was categorised as very low total dose if <2 mg/kg primaquine, low total dose if 2 to <5 mg/kg corresponding with ~3.5 mg/kg and high total dose if ≥5 mg/kg was given corresponding with ~7 mg/kg. Patients treated with weekly primaquine regimens (0.75 mg/kg/week for 8 weeks) were assessed separately. Primaquine regimens were classified by treatment duration in days into 7-day and 14-day regimens. Daily primaquine mg/kg doses were defined as low daily dose if <0.375 mg/kg/day corresponding with ~0.25 mg/kg/day, intermediate daily

dose if ≥0.375 to <0.75 mg/kg/day corresponding with ~0.5 mg/kg/day and high daily dose if ≥0.75 mg/kg/day corresponding with ~1 mg/kg/day were administered.

Parasite transmission was categorised based on subnational data of *P. vivax* incidence per 1000 persons from the Malaria Atlas Project as low (<1), moderate (1 to <10) or high (≥10).[19] Anaemia was defined as mild (haemoglobin (Hb)≥80 g/L and <110 g/L), moderate (≥50 g/L and<80 g/L) and severe (<50 g/L).[20] In studies where haemotocrit was measured without Hb, haematocrit was converted to Hb using the formula: haematocrit=5.62+0.26×Hb (g/L).[21]

Outcomes

The primary efficacy outcomes of this study were the incidence risk of any *P. vivax* recurrence between (1) day 7 and day 42 and (2) between day 7 and day 180. Primary outcomes for haematological safety were a >25% drop in Hb from baseline to an Hb <70 g/L, and an absolute drop in Hb of >50 g/L between day 0 and days 1–14. A composite gastrointestinal endpoint including the presence of vomiting, anorexia, or diarrhoea between days 1 and 14 was the primary tolerability outcome.

The secondary outcomes for haematological safety were the maximum absolute reduction in Hb between baseline and days 1–14, an Hb fall below 50 g/L, renal failure, need for blood transfusion, death (between days 1 and 28) and presence of moderate or severe anaemia as separate outcomes. The secondary tolerability outcomes were the presence of vomiting, nausea, anorexia, abdominal pain, dizziness and diarrhoea as separate endpoints between days 2 and 14, and vomiting within 1 hour of primaquine dosing.

Statistical analysis

A one-stage meta-analysis of eligible and available individual patient data was undertaken. Cumulative risks of recurrence at day 42 and day 180 were calculated for different primaquine total dose categories by Kaplan-Meier survival analysis. Patients were right censored based on the day of first recurrence, the last visit, a gap of >60 days between blood smears or the last day of study follow-up. Cox regression analyses compared the rate of recurrence between day 7 and day 42 and day 7 and day 180 comparing different primaquine total dose categories to no primaquine after adjusting for sex and age, with shared frailty for study-site. Estimation of incidence rates of recurrent vivax parasitaemia over 180 days were planned for each total dose primaquine regimen, however, no available studies followed individuals through recurrent parasitaemic events.

A two-stage meta-analysis was undertaken in studies with 180 days or more follow-up.[22] Study-specific aggregated proportions from the one-stage individual patient data meta-analysis were combined with aggregated proportions of patients with vivax recurrence at day 180 from studies that were eligible for inclusion in the one-stage meta-analysis but where data were unavailable. The pooled risk of vivax recurrence at day 180 was estimated using random-effects meta-analysis. Proportions were pooled using the Freeman-Tukey double arcsine transformation, with confidence intervals calculated using exact methods.

Using individual patient data, haematological outcomes were described by primaquine daily mg/kg dose category. A multivariable logistic regression analysis was planned to estimate the effect of primaquine daily mg/kg dose on the odds of the composite gastrointestinal outcome, however, no symptom data were available for the included studies. Comparison of the proportions of acute vomiting within 1 hour were similarly planned between primaquine daily mg/kg dose categories but were not possible.

The effect of heterogeneity in the studies was assessed by removal of one study site at a time from the one-stage meta-analysis, with calculation of the coefficient of variation around day 42 and day 180 cumulative risks. Bias in studies included in the one-stage meta-analysis was assessed by comparison between included and unavailable studies and by undertaking the two-stage meta-analysis which including aggregated data from all eligible studies. Within study bias was assessed by the Cochrane Risk of Bias 2 tool[23] for randomised controlled trials and the Joanna Briggs Institute Case Series tool for single arm studies.[24]

Statistical analyses were conducted in Stata, V.17 (StataCorp) according to an a priori statistical analysis plan.[25] The review protocol was registered in PROSPERO: CRD42022313730.[26]

RESULTS

The systematic review identified 32 prospective efficacy studies of uncomplicated *P. vivax* malaria from South Asia, of which 14 were excluded as they had no primaquine arm. Of the 18 eligible studies, individual patient data were unavailable for 10 studies[8 9 27–34] and minimum required data were unavailable for one study[11] resulting in 7 published studies (38.9%) being included in the individual patient data one-stage meta-analysis (online supplemental tables S1–S5 and figure S1).[35–42] A total of 566 out of 1357 patients from these studies were excluded, mainly due to enrolment outside of South Asia, treatment with tafenoquine or missing data on parasite density, leaving 791 patients who were eligible for inclusion in the one-stage individual patient data meta-analysis (figure 1).

Of the 791 included patients, the median age was 24.0 years (IQR 16.0–37.0, range 1–80 years), with a median weight of 55.0 kg (IQR 42.0–62.0, range 7–116) (table 1). The majority were male (463, 58.5%) and were treated with chloroquine as schizontocidal treatment (782, 98.9%), with a mean total chloroquine dose of 28.7 mg/kg (SD 7.6, range 12.9–93.8). Patients had planned follow up to 28 days in 3 studies,[35–38] 180 days in 2 studies[39 40] and 330–365 days in another 2 studies.[41 42]

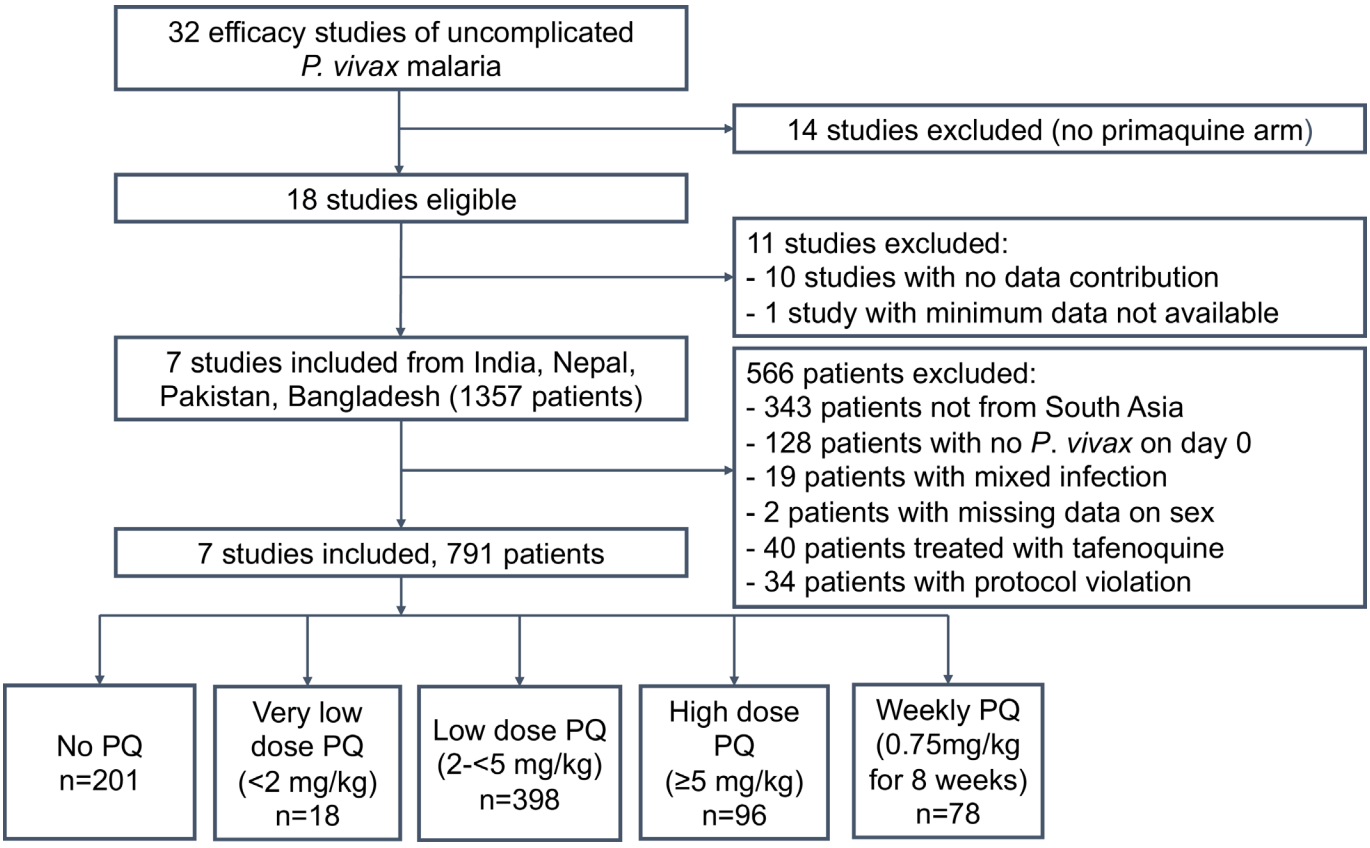


Figure 1 Study flow chart. PQ, primaquine.

Eligible studies that were targeted but not included in the pooled analysis were mainly from India and had longer durations of follow-up and younger patients compared with studies that were included (online supplemental table S6).[8 9 11 27–34] Risk of bias was low or unclear in all included studies (online supplemental tables S7 and S8).

There were 201 (25.4%) patients who were not treated with primaquine, 18 (2.3%) were administered a very low total dose of primaquine (<2 mg/kg), 398 (50.3%) were administered low total dose primaquine (2 to <5 mg/kg), 96 (12.1%) were administered high total dose primaquine (≥5 mg/kg) and 78 (9.9%) were administered weekly primaquine (~0.75 mg/kg per week over 8 weeks totalling 6 mg/kg; online supplemental figure S2). The expected duration of the primaquine treatment was 14 days in all 512 patients treated with a daily primaquine dose regimen. All patients treated with daily primaquine regimens had ≥30% G6PD activity. Due to the small number of patients (n=18) in the very low-dose total primaquine group, this group was excluded from efficacy analyses.

Efficacy of primaquine total mg/kg dose

Between day 7 and 180, 29 recurrences were reported from 713 patients treated without primaquine or with daily primaquine regimens; 25 in patients treated without primaquine, 4 following low total dose primaquine and 0 following high total dose primaquine. Recurrences between day 7 and day 42 were observed in 9 patients treated without primaquine, 2 following low total dose primaquine and 0 following high total dose primaquine. There were 2 recurrences in the 78 patients who were treated with weekly primaquine. The relationship between body weight, mg/kg dose and presence of recurrence between day 7 and 180 is presented in online supplemental figure S3.

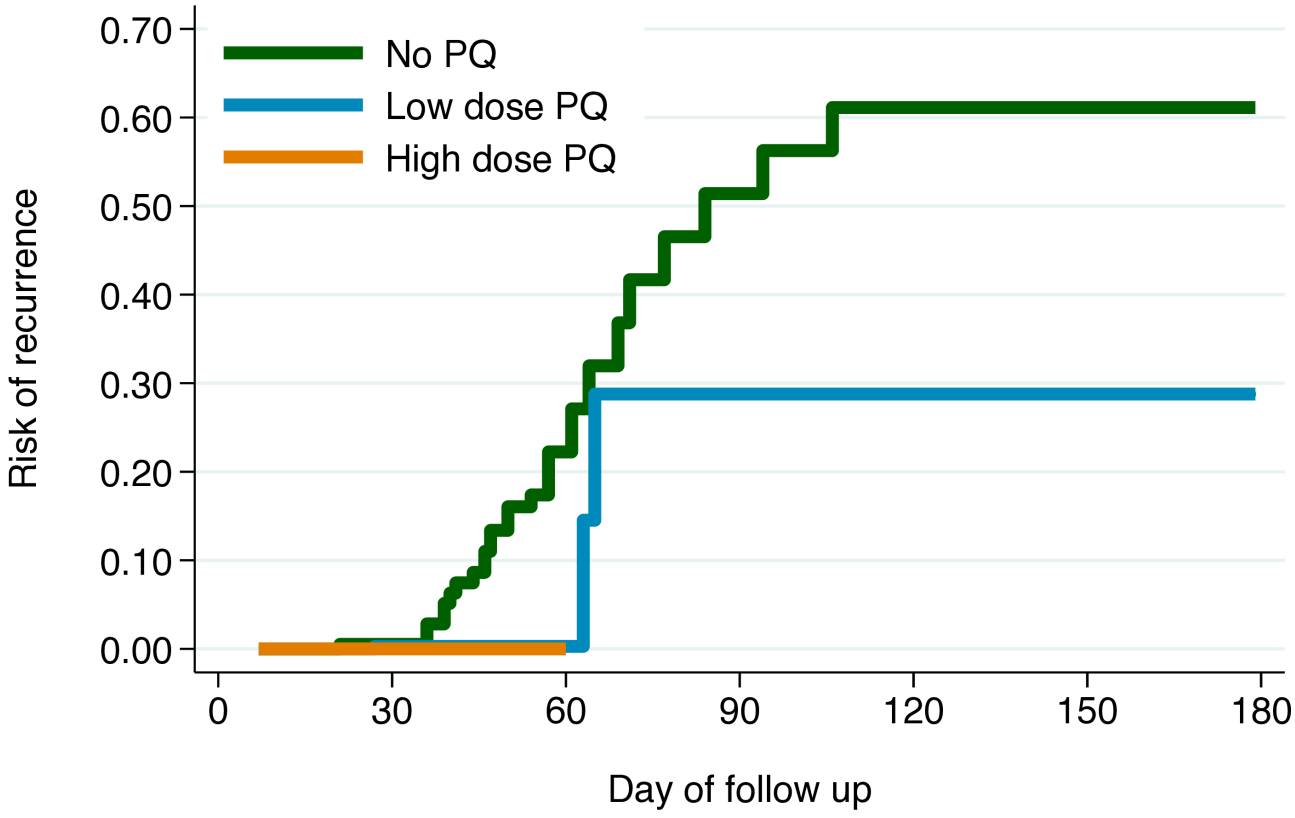
The cumulative risk of recurrence for patients to day 42 was 7.5% (95% CI 3.6% to 15.2%) in patients treated without primaquine compared with 0.2% (95% CI 0.0% to 2.0%) following low total dose primaquine and 0% following high total dose primaquine. The cumulative risk of recurrence for patients at day 180 was 61.1% (95% CI 42.2% to 80.4%) in patients treated without primaquine compared with 28.8% (95% CI 8.2% to 74.1%) in patients with low total dose primaquine and 0% following high total dose primaquine (figure 2); however, despite longer planned follow-up periods no patients receiving high total dose and few patients receiving low total dose primaquine had data available for inclusion past day 60. Results were similar in sensitivity analyses removing one study at a time (online supplemental table S9). The cumulative risk of recurrence at day 42 or day 180 could not be calculated for the 78 patients treated with weekly primaquine, since 76 (97.4%) were only followed until their final day of treatment on day 56.

As the proportional hazards assumption was violated for Cox regression analysis of the risk of recurrence by

Table 1 Demographic and baseline characteristics

	Total N=791	No primaquine N=201	Daily primaquine			Weekly primaquine (6 mg/kg total over 8 weeks) N=78
			Very low dose total primaquine (<2 mg/kg) N=18	Low-dose total primaquine (2 to <5 mg/kg) N=398	High-dose total primaquine (≥5 mg/kg) N=96	
Sex (%)						
Male	463 (58.5)	74 (36.8)	18 (100)	266 (66.8)	63 (65.6)	42 (53.8)
Female	328 (41.5)	127 (63.2)	0 (0)	132 (33.2)	33 (34.4)	36 (46.2)
Age (years)	24.0 (16.0–37.0)	21.0 (10.0–30.0)	28.5 (25.0–42.0)	30.0 (22.0–42.0)	12.0 (7.0–22.5)	11.0 (8.0–18.0)
Weight (kg)	55.0 (42.0–62.0)	50.0 (25.0–56.0)	90.0 (86.0–103.0)	58.0 (51.0–65.0)	29.5 (18.0–45.0)	30.0 (20.0–48.0)
Presence of fever on day 0 (%)*	559 (94.4)	124 (94.7)	18 (100)	372 (95.6)	40 (81.6)	5 (100%)
Maximum parasitaemia on day 0	2610 (649–7046)	6400 (3180–14000)	1352 (864–5330)	1995 (520–5390)	258 (55–718)	270 (256–810)
Haemoglobin on day 0 (g/L)	126 (20)	120 (18)	144 (13)	129 (20)	122 (20)	126 (18)
Schizontocidal administered (%)						
ACT	9 (1.1)	0 (0)	0 (0)	5 (1.2)	4 (4.2)	0 (0)
CQ	782 (98.9)	201 (100)	18 (100)	393 (98.7)	92 (95.8)	78 (100)
Expected duration of PQ (%)						
14 days	512 (87.2)	0 (0)	18 (100)	398 (100)	96 (100)	0 (0)
7 days	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
8 weeks	78 (12.8)	0 (0)	0 (0)	0 (0)	0 (0)	78 (100)
Country (%)						
Bangladesh	55 (7.0)	0 (0)	0 (0)	33 (8.3)	22 (22.9)	0 (0)
India	332 (42.0)	30 (14.9)	18 (100)	256 (64.3)	23 (24.0)	5 (6.4)
Nepal	206 (26.0)	101 (50.2)	0 (0)	100 (25.1)	5 (5.2)	0 (0)
Pakistan	198 (25.0)	70 (34.8)	0 (0)	9 (2.3)	46 (47.9)	73 (93.6)

Data are presented as mean (SD) or median (IQR) for continuous measures, and n (%) for categorical measures.
*Data only available for 592 patients.
ACT, artemisinin-based combination therapy; CQ, chloroquine; PQ, primaquine.



Number at risk

No PQ	195	86	16	10	7	6	0
Low dose PQ	367	35	10	5	5	5	0
High dose PQ	81	42	5	0	0	0	0

Figure 2 Kaplan-Meier risk of first *Plasmodium vivax* recurrence over 180 days by treatment arm. High dose, ≥5 mg/kg total primaquine dose; low dose, 2 to <5 mg/kg total primaquine dose; PQ, primaquine.

day 180, due to an unequal proportion of patients being available for analysis beyond day 60 in different treatment groups, the analysis was restricted to days 7–42. Compared with treatment without primaquine, the hazard ratio for first recurrence between days 7 and 42 was 0.4 (95% CI 0.1 to 2.8) following low-dose primaquine. High-dose primaquine was not included in the model as there were no recurrences in this group (table 2).

From the original systematic review, there were 12 studies with 180 days follow-up or more. After exclusion of three studies (one with a single treatment arm of very low total dose primaquine[31] and two[11 29] that did not report the risk of recurrence at day 180), 3529 patients from nine studies were included in a two-stage meta-analysis. This included four studies used in the one-stage meta-analysis[39–42] and five studies that were targeted for inclusion in the one-stage meta-analysis but for which data were not available[8 9 27 32 33] (online supplemental figure S4). Risk of bias was low or unclear in all included studies (online supplemental tables S7 and S8). The pooled risk of first *P. vivax* recurrences in 1667 patients treated without primaquine was 12.1% (95% CI 7.7% to 17.2%) compared with 2.3% (95% CI 0.3% to 5.4%) in 1401 patients treated with low total dose primaquine and

Table 2 Multivariable Cox regression analysis assessing the effect of primaquine treatment arm on risk of *Plasmodium vivax* recurrence between day 7 and day 42

Treatment arm	No of patients	No of recurrences	Adjusted HR* (95% CI)
No primaquine	201	9	Reference
Low-dose total primaquine (2 to <5 mg/kg)	398	2	0.41 (0.06 to 2.77)
High-dose total primaquine (≥5 mg/kg)	96	0	†

*Adjusted for age and sex with random effect for study site.
†Patients with high total dose primaquine were not included in the model as there were no recurrences in this group.

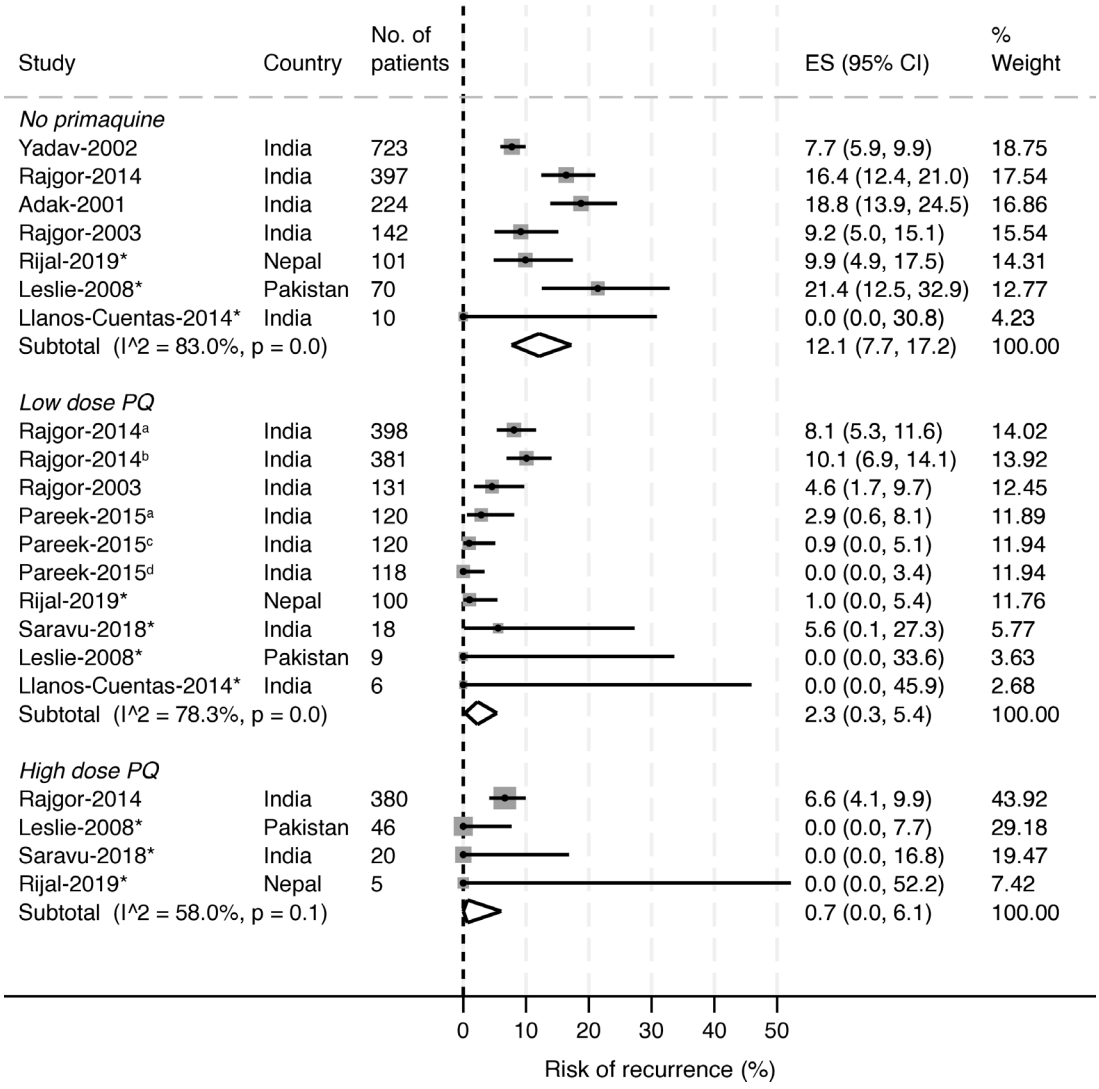


Figure 3 Pooled risk of recurrence by study and treatment arm. Numbers refer to total number of patients available for analysis from the study in that treatment arm. Study-specific treatment estimates were generated from individual patient data where possible (*asterisked studies) and aggregated data from the manuscripts. Pooled estimates for treatment arms were estimated using random-effects meta-analysis with proportions pooled using the Freeman-Tukey double arcsine transformation without adjustment to observed values and with exact methods to calculate confidence intervals. Analysis was restricted to studies with at least 180 days follow-up. a: 15 mg primaquine given for 14 days; b: 30 mg primaquine given for 7 days; c: 30 mg slow release primaquine given for 7 days; d: 15 mg slow release primaquine given for 14 days. ES, effect size; high dose, ≥5 mg/kg total primaquine dose; low dose, 2 to <5 mg/kg total primaquine dose; PQ, primaquine.

0.7% (95% CI 0% to 6.1%) in 451 patients treated with high total dose primaquine (figure 3).

Safety analysis

The studies did not report any deaths or incidents of blood transfusion. Haematological safety analyses, as part of the one-stage individual patient data meta-analysis, were restricted to the 713 patients treated without primaquine or with daily primaquine; all patients treated with daily primaquine regimens had ≥30% G6PD activity. A day 0 Hb concentration was available for 712 patients with a mean Hb at baseline of 126 g/L (SD 20), including 4 (0.6%) patients with an Hb less than 70 g/L. There were 401 patients with available Hb concentrations between days 1 and 14, of whom 55 (13.7%) had an Hb measured four or more times, 342 (85.3%) had two or three measurements and 4 (1.0%) had a single measurement.

No patients had a fall in Hb of >25% from baseline to <70 g/L, a fall of >50 g/L from day 0 or developed severe anaemia (<50 g/L) (table 3). The absolute and percentage change in Hb from day 0 for different categories of daily doses of primaquine are summarised in table 3. The unadjusted mean absolute change in Hb from day 0 to the minimum Hb between day 1 and 14 was −3 g/L (SD 10) in patients treated without primaquine, −6 g/L (SD 10) following low daily dose primaquine, and −10 g/L (SD 12) following intermediate daily dose primaquine. Data were only available from two patients who received high daily dose primaquine, their fall in Hb was 9 and

Table 3 Haematological safety outcomes between days 1 and 14 by primaquine daily dose

	No primaquine N=181	Low-dose daily primaquine: <0.375 mg/kg N=150	Intermediate-dose daily primaquine: ≥0.375 to <0.75 mg/kg N=68	High-dose daily primaquine: ≥0.75 mg/kg N=2
Drop in Hb of >25% and to Hb<70 g/L, n/N (%)	0/181 (0)	0/150 (0)	0/68 (0)	0/2 (0)
Drop in Hb of >50 g/L from baseline between days 1 and 14, n/N (%)	0/181 (0)	0/150 (0)	0/68 (0)	0/2 (0)
Absolute change to nadir*, mean (SD) (range) g/L	−3 (10) (−38 to 20)	−6 (10) (−44 to 9)	−10 (12) (−32 to 36)	(−9 to 6)†
Percentage change to nadir*, mean (SD) (range) %	−2.4 (7.8) (−31.4 to 17.8)	−4.3 (6.8) (−26.0 to 7.8)	−7.9 (10.7) (−28.4 to 37.1)	(−9.2 to 5.2)†

*Nadir considered to be lowest measurement between day 1 and day 14.
†As there are only two patients in the high daily dose primaquine group, only the range is given.
Hb, haemoglobin.

6 g/L. Of 399 patients starting with an Hb≥80 g/L at day 0, 2 (0.5%) developed moderate anaemia (<80 g/L to ≥50 g/L) between days 1 and 14, both of whom were treated with intermediate daily dose primaquine.

No data were available on the presence of vomiting, anorexia, diarrhoea, nausea or abdominal pain following primaquine or acute vomiting within 1 hour of primaquine administration, therefore, no tolerability analysis could be performed.

DISCUSSION

Primaquine remains the only widely available antimalarial to prevent relapses from liver hypnozoites caused by *P. vivax*, the most common species of malaria in South Asia. However, the relative benefits and drawbacks of the primaquine dose and duration of the currently used regimen of 3.5 mg/kg total dose over 14 days compared with alternative regimens in this region are unclear. This meta-analysis, including individual patient data from 791 patients from seven studies combined with an aggregated two-stage analysis of 3259 patients from nine studies, provides a detailed assessment of efficacy in South Asia, demonstrating good efficacy of low total dose (~3.5 mg/kg) and high total dose (~7 mg/kg) primaquine regimens, without any severe haemolytic events across all regimens. The analysis also highlights the lack of available comparative individual patient data on regimens of different durations and doses with prolonged follow-up and the lack of detailed safety and tolerability data.

Antirelapse efficacy is determined by the total primaquine mg/kg dose administered. Five studies have compared high-dose versus low-dose primaquine, including two from India.[8 40 43–45] The results of the current meta-analysis support the findings from these studies, suggesting a minimal benefit in raising the total dose of primaquine from 3.5 to 7 mg/kg in preventing relapses in South Asia. This contrasts with other regions of the world, with a recent individual patient data meta-analysis of 6879 patients from all vivax-endemic countries suggesting a benefit of 7 mg/kg total dose primaquine regimens.[10] WHO currently recommends low total dose primaquine regimens for South Asia and these recommendations are supported by the current meta-analysis.[7 46 47]

Primaquine radical cure regimens are generally administered over 14 days, however, the WHO recently revised treatment guidelines to include a 7-day 3.5 mg/kg total dose primaquine regimen.[7] Shorter course regimens are expected to improve patient adherence and thus improve antirelapse effectiveness. Two studies from India compared such regimens, although one gave a sustained release formulation of primaquine and the other enrolled patients between 2001 and 2004.[8 9] All studies in the current individual patient data meta-analysis administered primaquine over 14 days.

In contrast with the studies included in this meta-analysis, most patients treated with primaquine in South Asia are currently not tested for G6PD deficiency. The risk of severe haemolytic events in G6PD deficient individuals is associated with the daily dose. Thus, the increase in the daily primaquine dose with a 7-day regimen from 0.25 mg/kg/day to 0.5 mg/kg/day potentially increases the risk of severe events if G6PD screening is not undertaken. There is a need to further understand the feasibility and risks of a 7-day regimen following G6PD testing compared with a 14-day regimen to better inform national policies in this region.

The current study did not identify any severe haemolytic events in 401 patients treated with or without primaquine. However, the unadjusted absolute reduction in Hb in patients treated with primaquine was greater than those without (6 g/L following low daily dose primaquine, 10 g/L following intermediate daily dose and 3 g/L without primaquine; note only 2 patients received a high daily dose of primaquine). This relationship could be impacted by the baseline Hb concentration. A pooled analysis including 3421 patients from 29 studies found that the nadir Hb was similar in patients with G6PD activity ≥30% treated with and without primaquine at a population level.[48] A subsequent individual patient

data meta-analysis of 5462 patients found a similar risk of haemolysis in patients with ≥30% G6PD activity who were treated with 0.25 mg/kg/day or 0.5 mg/kg/day primaquine compared with patients treated without primaquine.[49]

The relapse behaviour of *P. vivax* varies between regions. *P. vivax* in South Asia has a biphasic phenotype where the duration between initial infection and subsequent relapse is generally either around 1–2 months or >6 months.[50–54] The current one-stage meta-analysis was limited by relatively few patients treated with low-dose total primaquine and no patients treated with high-dose total primaquine having data available beyond 60 days. Furthermore, since few of the included studies had prolonged follow-up beyond 6 months the relative antirelapse efficacy of low total dose and high total dose primaquine regimens could not be compared against late (>6 months) vivax malaria relapses. Even if studies with prolonged follow-up had been available, assessment of antirelapse efficacy would be potentially confounded by an increasing proportion of recurrences being due to reinfection. The two-stage meta-analysis demonstrated good antirelapse efficacy of low total dose and high total dose primaquine regimens within 180 days.

Gastrointestinal symptoms are a recognised side effect of primaquine, although they are also associated with acute malaria and schizontocidal drugs. Their association with primaquine dose has not been clearly defined, although it appears that the risk of developing them may be reduced by taking primaquine with food.[55 56] No data were available to assess the risk of gastrointestinal symptoms with different primaquine regimens in the current analysis. Future studies in the region should consider assessing gastrointestinal symptoms and recording whether primaquine was coadministered with food or not.

This study was limited by inclusion of only 39% of eligible studies in the individual patient data meta-analysis, although 7 of 11 studies that were not available to share individual patient data were published before 2010 and 10 of 11 studies that were not included were from India. The routine collation and sharing of data from future clinical efficacy studies would enable greater power to investigate differences in the comparative risks and benefits of antirelapse regimens going forward. Primaquine mg/kg dosing was derived from study protocols in 91% of patients. These mg/kg doses may have differed to the actual doses administered. In addition, there was a significant difference in the population that received high-dose total primaquine compared with low-dose primaquine or were treated without primaquine. Patients receiving high-dose primaquine were younger, however, their young age would have been expected to put them at greater risk of recurrence[10] and this is, therefore, unlikely to have impacted the results of the analysis. Although our analysis aimed to evaluate the antirelapse efficacy of primaquine, it is not possible to determine whether recurrent parasitaemia arises from recrudescences (schizontocidal treatment failures), relapses (hypnozoiticidal treatment failures) or reinfections. For this reason, the risk of recurrence rather than relapse was used as the primary efficacy endpoint; which is confounded by reinfection and background malaria endemicity.

CONCLUSIONS

In summary, the risk of *P. vivax* recurrence was low at day 180 following low and high total dose primaquine, supporting the current recommendation for low total dose primaquine regimens in South Asia. In general, there was a lack of available individual patient data to assess efficacy, tolerability and safety across this region, with no individual patient data available to assess the relative efficacy of 7-day vs 14-day primaquine regimens or the effect of primaquine daily dose on gastrointestinal symptoms. No severe haemolytic events were identified following any treatment.

As countries in the region progress towards malaria elimination by 2030, relapsing malaria caused by hypnozoites poses a major challenge to achieving this goal. The introduction of shorter course regimens may improve adherence and effectiveness and fast track this progress. However, implementation of such courses needs to occur in conjunction with routine G6PD testing at the point of care given the potential for an increased haemolytic risk in G6PD deficient patients with higher daily dose primaquine regimens.

Author affiliations
[1]ICMR-National Institute of Malaria Research, New Delhi, India
[2]Global Health Division, Menzies School of Health Research, Charles Darwin University, Tiwi, Northern Territory, Australia
[3]WorldWide Antimalarial Resistance Network, Asia Pacific Regional Hub - Australia, Melbourne, Victoria, Australia
[4]General and Subspecialty Medicine, Grampians Health Ballarat, Ballarat, Victoria, Australia
[5]Indian Council of Medical Research, New Delhi, India
[6]Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, Uttar Pradesh, India
[7]Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, Victoria, Australia
[8]WorldWide Antimalarial Resistance Network (WWARN), Oxford, UK
[9]Infectious Diseases Data Observatory (IDDO), Oxford, UK
[10]Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, UK
[11]Mahidol Oxford Tropical Medicine Research Unit (MORU), Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand
[12]International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh
[13]Central Department of Microbiology, Tribhuvan University, Kirtipur, Nepal
[14]ICMR-Rajendra Memorial Research Institute of Medical Sciences, Patna, Bihar, India
[15]Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK
[16]HealthNet TPO, Kabul, Afghanistan
[17]Unit of Leishmaniasis and Malaria, Instituto de Medicina Tropical "Alexander von Humboldt", Universidad Peruana Cayetano Heredia, Lima, Peru
[18]Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand
[19]Department of Infectious Diseases, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, Karnataka, India

[20]Manipal Centre for Infectious Diseases, Prasanna School of Public Health, Manipal Academy of Higher Education, Manipal, Karnataka, India
[21]International Centre For Genetic Engineering and Biotechnology, New Delhi, India

Twitter Manju Rahi @RahiManju and Bipin Adhikari @Bipinadhikar

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Contributors RV and RJC conceived the study, analysed and interpreted the data and drafted the manuscript. AS and RNP provided conceptualisation, supervision, editing and review of the manuscript. AG, MRahi, Nitika, PKB, KT, MRajasekhar, SSP, PJG and JAS provided critical editing and review of manuscript. MRajasekhar and JAS contributed to data interpretation and analysis. KT, BA, MSA, PG, WAK, RK, TL, BL, ALC, SP, KRR, MRowland, KS, JAS and RNP conceived and undertook the individual studies, enrolled the patients and contributed the individual patient data. All authors revised the manuscript. RJC acted as guarantor.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

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Ethics approval All of the data in this analysis were acquired in accordance with national ethical regulations and approvals. Ethics approval for this analysis was not required according to the Oxford Human Research Ethics Committee.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. The data that support the findings of this study are available for access via the WorldWide Antimalarial Resistance Network (WWARN.org). Requests for access will be reviewed by a Data Access Committee to ensure that the use of data protects the interests of the participants and researchers according to the terms of ethics approval and principles of equitable data sharing. Requests can be submitted by email to malariaDAC@iddo.org via the Data Access Form available at WWARN.org/accessing-data. The WWARN is registered with the Registry of Research Data Repositories (re3data.org).

ORCID iDs
Reena Verma http://orcid.org/0000-0001-9870-6017
Manju Rahi http://orcid.org/0000-0003-0932-0935
Bipin Adhikari http://orcid.org/0000-0001-8981-3910
Prakash Ghimire http://orcid.org/0000-0003-2503-412X
Komal Raj Rijal http://orcid.org/0000-0001-6281-8236
Kavitha Saravu http://orcid.org/0000-0001-6399-1129
Philippe J Guerin http://orcid.org/0000-0002-6008-2963
Amit Sharma http://orcid.org/0000-0002-3305-0034

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ADDITIONAL FILE 1

Verma R *et al*, Safety and efficacy of primaquine in patients with *P. vivax* malaria from South Asia: A systematic review and individual patient data meta-analysis

Verma R, *et al. BMJ Glob Health* 2023; 8:e012675. doi: 10.1136/bmjgh-2023-012675

Checklist S1. PRISMA-IPD Checklist of items to include when reporting a systematic review and meta-analysis of individual participant data (IPD)

PRISMA-IPD Section/topic	Item No	Checklist item	Reported on page
Title			
Title	1	Identify the report as a systematic review and meta-analysis of individual participant data.	1
Abstract			
Structured summary	2	Provide a structured summary including as applicable:	
		Background: state research question and main objectives, with information on participants, interventions, comparators and outcomes.	4
		Methods: report eligibility criteria; data sources including dates of last bibliographic search or elicitation, noting that IPD were sought; methods of assessing risk of bias.	4
		Results: provide number and type of studies and participants identified and number (%) obtained; summary effect estimates for main outcomes (benefits and harms) with confidence intervals and measures of statistical heterogeneity. Describe the direction and size of summary effects in terms meaningful to those who would put findings into practice.	4
		Discussion: state main strengths and limitations of the evidence, general interpretation of the results and any important implications.	4,5
		Other: report primary funding source, registration number and registry name for the systematic review and IPD meta-analysis.	5
Introduction			
Rationale	3	Describe the rationale for the review in the context of what is already known.	8
Objectives	4	Provide an explicit statement of the questions being addressed with reference, as applicable, to participants, interventions, comparisons, outcomes and study design (PICOS). Include any hypotheses that relate to particular types of participant-level subgroups.	8

Methods			
Protocol and registration	5	Indicate if a protocol exists and where it can be accessed. If available, provide registration information including registration number and registry name. Provide publication details, if applicable.	11, Ref 28
Eligibility criteria	6	Specify inclusion and exclusion criteria including those relating to participants, interventions, comparisons, outcomes, study design and characteristics (e.g. years when conducted, required minimum follow-up). Note whether these were applied at the study or individual level i.e. whether eligible participants were included (and ineligible participants excluded) from a study that included a wider population than specified by the review inclusion criteria. The rationale for criteria should be stated.	9-10, ref 27,28
Identifying studies - information sources	7	Describe all methods of identifying published and unpublished studies including, as applicable: which bibliographic databases were searched with dates of coverage; details of any hand searching including of conference proceedings; use of study registers and agency or company databases; contact with the original research team and experts in the field; open adverts and surveys. Give the date of last search or elicitation.	9, Box S1
Identifying studies - search	8	Present the full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	9 and Box S1
Study selection processes	9	State the process for determining which studies were eligible for inclusion.	9,10 and Box S1
Data collection processes	10	Describe how IPD were requested, collected and managed, including any processes for querying and confirming data with investigators. If IPD were not sought from any eligible study, the reason for this should be stated (for each such study).	Table S1, S2 and S5
		If applicable, describe how any studies for which IPD were not available were dealt with. This should include whether, how and what aggregate data were sought or extracted from study reports and publications (such as extracting data independently in duplicate) and any processes for obtaining and confirming these data with investigators.	
Data items	11	Describe how the information and variables to be collected were chosen. List and define all study level and participant level data that were sought, including baseline and follow-up information. If applicable, describe methods of standardising or translating variables within the IPD datasets to ensure common scales or measurements across studies.	10 and Box S1
IPD integrity	A1	Describe what aspects of IPD were subject to data checking (such as sequence generation, data consistency and completeness, baseline imbalance) and how this was done.	Ref 19

Risk of bias assessment in individual studies.	12	Describe methods used to assess risk of bias in the individual studies and whether this was applied separately for each outcome. If applicable, describe how findings of IPD checking were used to inform the assessment. Report if and how risk of bias assessment was used in any data synthesis.	11-12 and Ref 24 and 27
Specification of outcomes and effect measures	13	State all treatment comparisons of interests. State all outcomes addressed and define them in detail. State whether they were pre-specified for the review and, if applicable, whether they were primary/main or secondary/additional outcomes. Give the principal measures of effect (such as risk ratio, hazard ratio, difference in means) used for each outcome.	11
Synthesis methods	14	Describe the meta-analysis methods used to synthesise IPD. Specify any statistical methods and models used. Issues should include (but are not restricted to): • Use of a one-stage or two-stage approach. • How effect estimates were generated separately within each study and combined across studies (where applicable). • Specification of one-stage models (where applicable) including how clustering of patients within studies was accounted for. • Use of fixed or random effects models and any other model assumptions, such as proportional hazards. • How (summary) survival curves were generated (where applicable). • Methods for quantifying statistical heterogeneity (such as I^2 and t^2). • How studies providing IPD and not providing IPD were analysed together (where applicable). • How missing data within the IPD were dealt with (where applicable).	11-12
Exploration of variation in effects	A2	If applicable, describe any methods used to explore variation in effects by study or participant level characteristics (such as estimation of interactions between effect and covariates). State all participant-level characteristics that were analysed as potential effect modifiers, and whether these were pre-specified.	12
Risk of bias across studies	15	Specify any assessment of risk of bias relating to the accumulated body of evidence, including any pertaining to not obtaining IPD for particular studies, outcomes or other variables.	Box S1, Ref. 24,27
Additional analyses	16	Describe methods of any additional analyses, including sensitivity analyses. State which of these were pre-specified.	12 and Table S9
Results			

Study selection and IPD obtained	17	Give numbers of studies screened, assessed for eligibility, and included in the systematic review with reasons for exclusions at each stage. Indicate the number of studies and participants for which IPD were sought and for which IPD were obtained. For those studies where IPD were not available, give the numbers of studies and participants for which aggregate data were available. Report reasons for non-availability of IPD. Include a flow diagram.	13-15 , Tables S1, S4 and S5
Study characteristics	18	For each study, present information on key study and participant characteristics (such as description of interventions, numbers of participants, demographic data, unavailability of outcomes, funding source, and if applicable duration of follow-up). Provide (main) citations for each study. Where applicable, also report similar study characteristics for any studies not providing IPD.	13, Table S1, S2 and S5
IPD integrity	A3	Report any important issues identified in checking IPD or state that there were none.	
Risk of bias within studies	19	Present data on risk of bias assessments. If applicable, describe whether data checking led to the up-weighting or down-weighting of these assessments. Consider how any potential bias impacts on the robustness of meta-analysis conclusions.	Tables S7 and S8
Results of individual studies	20	For each comparison and for each main outcome (benefit or harm), for each individual study report the number of eligible participants for which data were obtained and show simple summary data for each intervention group (including, where applicable, the number of events), effect estimates and confidence intervals. These may be tabulated or included on a forest plot.	18 and Figure 3
Results of syntheses	21	Present summary effects for each meta-analysis undertaken, including confidence intervals and measures of statistical heterogeneity. State whether the analysis was pre-specified, and report the numbers of studies and participants and, where applicable, the number of events on which it is based.	14-20
		When exploring variation in effects due to patient or study characteristics, present summary interaction estimates for each characteristic examined, including confidence intervals and measures of statistical heterogeneity. State whether the analysis was pre-specified. State whether any interaction is consistent across trials.	
		Provide a description of the direction and size of effect in terms meaningful to those who would put findings into practice.	
Risk of bias across studies	22	Present results of any assessment of risk of bias relating to the accumulated body of evidence, including any pertaining to the availability and representativeness of available studies, outcomes or other variables.	Ref. 24,27 Table S1, S2, S5 and S6

Additional analyses	23	Give results of any additional analyses (e.g. sensitivity analyses). If applicable, this should also include any analyses that incorporate aggregate data for studies that do not have IPD. If applicable, summarise the main meta-analysis results following the inclusion or exclusion of studies for which IPD were not available.	Tables S6 and S9
Discussion			
Summary of evidence	24	Summarise the main findings, including the strength of evidence for each main outcome.	22-25
Strengths and limitations	25	Discuss any important strengths and limitations of the evidence including the benefits of access to IPD and any limitations arising from IPD that were not available.	22-25
Conclusions	26	Provide a general interpretation of the findings in the context of other evidence.	6, 24-25
Implications	A4	Consider relevance to key groups (such as policy makers, service providers and service users). Consider implications for future research.	24-25
Funding			
Funding	27	Describe sources of funding and other support (such as supply of IPD), and the role in the systematic review of those providing such support.	25

Box S1. Search strategy

Search strategy

All prospective antimalarial efficacy studies of uncomplicated *P. vivax* with a minimum of 28 days follow up, published between Jan 1, 2000 and August 23, 2021 were identified by the application of the key terms (listed below) through Medline (Pubmed), Web of Science, Embase and the Cochrane Central. Abstracts of all references containing any mention of antimalarial drugs were manually checked to confirm prospective clinical trials, with review of full text when needed. Studies on prevention, prophylaxis, reviews, animal studies, patients with severe malaria, where schizontocidal treatment was unsupervised or where data were extracted retrospectively from medical records outside of a planned trial were excluded. Inclusion criteria included studies undertaken in South Asia including Bangladesh, Bhutan, India, Nepal, Pakistan, Sri Lanka. Studies were included if they had at least one treatment arm with primaquine commencing within 7 days of schizontocidal treatment, had data on age, sex and parasitemia on day 0, data on schizontocidal and primaquine dosing and reported parasite presence or absence during follow up. The year of the study was taken as the year in which the paper was published, although the start and end date of patient enrolment were also recorded. The review process was undertaken by two independent investigators who also performed data extraction (RV and RJC), and the original review process is documented in more detail in, Commons RJ, Thriemer K, Humphreys G, et al. *Int J Parasitol Drugs Drug Resist*. 2017;7(2):181-190 [17].

Key terms:

Literature search (conducted March 2022) with the following key terms (version undertaken in Pubmed): (malaria OR plasmod*) AND (amodiaquine OR atovaquone OR artemisinin OR arteether OR artesunate OR artemether OR artemotil OR azithromycin OR artekin OR chloroquine OR chlorproguanil OR cycloguanil OR clindamycin OR coartem OR dapsone OR dihydroartemisinin OR duo-cotecxin OR doxycycline OR halofantrine OR lumefantrine OR lariam OR malarone OR mefloquine OR naphthoquine OR naphthoquinone OR piperaquine OR primaquine OR proguanil OR pyrimethamine OR pyronaridine OR quinidine OR quinine OR riamet OR sulphadoxine OR tetracycline OR tafenoquine).

Table S1. Studies included in the one stage individual patient data meta-analysis

Author-year	Country	Recruitment Period	Age range (years)	Follow up (days)	Included treatment arms*	PQ supervision	Randomised	Patients available	Included in GI tolerability analysis	Included in haematology analysis	Included in efficacy analysis
Leslie-2008 (42)	Pakistan	2004 – 2006	4-80	330	Cq, Cq_Pq_7.0_14d_D0	Full	Yes	198	No	Yes	Yes
Llanos Cuentas-2013 (39)	India	2011-2013	>16	180	Cq, Cq_Pq_3.4_14d, Cq_Tq	Partial	Yes	16	No	Yes	Yes
Rishikesh-2015 (36,37)	India	2012-2014	18-76	28#	Cq_Pq_3.5_14d	Partial	No	117	No	No	Yes
Ley-2016 (38)	Bangladesh	2014 – 2015	1-66	30	Cq_Pq_3.5_14d_D2	No	No	55	No	Yes	Yes
Saravu-2016 (35, 36)	India	2012-2015	> 18	28#	Cq_Pq_3.5_14d, D0	No	No	161	No	No	Yes
Saravu-2018 (40)	India	2012-2014	>18	180	Cq/ACT_Pq_7_14d, Cq/ACT_Pq_3.5_14d, D2	Partial	Yes	38	No	Yes	Yes
Rijal-2019 (41)	Nepal	2015 - 2016	5-75	365	Cq, Cq_Pq_3.5_14d_D0	Partial	Yes	206	Yes	Yes	Yes

ACT – artemisinin-based combination treatment; Cq – chloroquine; GI – gastrointestinal; PQ/Pq – primaquine; Primaquine treatment was classified as *supervised* if all doses were directly observed, *partially supervised* if >1 dose but not all doses were observed, and *not-supervised* if ≤1 dose was observed; *Treatment code describes (schizontocidal drug)_(hypnozoitocidal drug)_(total primaquine dose)_(duration of primaquine treatment eg 14d = 14 days)_(primaquine start day); # Reference 36 is a follow up study up to 450 days including some of the patient cohort in references 35 and 37.

Table S2. Study testing and inclusion criteria

Author-year	Hb exclusion criteria	G6PD testing undertaken	G6PD activity included	G6PD data available	Days symptom checklist undertaken and data available	Days Hb/Hct measured and data available
Leslie-2008 (42)	Hb <7 g/dL	Colorimetric test	All activities (if deficient treated with 8 weekly 0.75 mg/kg PQ)	Yes	-	0, 7, 14
Llanos-Cuentas-2013 (39)	Hb< 7 g/dL	Spectrophotometric semiquantitative assay (Trinity Biotech. Bray, County Wicklow, Ireland or Pointe Scientific, Cnaton, MI, USA	All activities (excluded if <70% G6PD activity)	Yes	0	0, 3, 5, 8, 11, 15, 22, 29
Rishikesh-2015 (36,37)*	No exclusion stated	Spectrophotometry	All activities included (if deficient treated with 8 weekly 0.75 mg/kg PQ)	No	0	0
Ley-2016 (38)	Hb <8 g/dL	Spectrophotometry	All activities (if deficient not given PQ)	Yes	-	0, 2, 9
Saravu-2016 (35,36)*	No exclusion stated	Spectrophotometry	All activities (if deficient treated with 8 weekly 0.75 mg/kg PQ)	Yes	0	0, 7, 14, 28
Saravu-2018 (40)	No exclusion stated	Spectrophotometry	All activities (if deficient not given PQ)	No	0	0, 7, 14, 28
Rijal-2019 (41)	No exclusion stated	Carestart RDT	≥30% activity	Yes	0	0, 1, 3, 7

Hb – haemoglobin; Hct – haematocrit. * Reference 36 is a follow up study up to 450 days including some of the patient cohort in references 35 and 37.

Table S3. Study sites included in the one stage individual patient data meta-analysis

Author-year	Study site	Country	Latitude	Longitude	Year Start	Year End	MAP Incidence rate (per 1000 persons)	Transmission intensity*
Leslie-2008 (42)	Adizai	Pakistan	33.79	71.58	2004	2006	2.48	Moderate
Leslie-2008 (42)	Baghicha	Pakistan	34.23	72.16	2004	2006	2.48	Moderate
Leslie-2008 (42)	Khagan	Pakistan	34.54	73.32	2004	2006	2.48	Moderate
Llanos-Cuentas-2014 (39)	Lucknow	India	26.85	80.94	2011	2013	0.16	Low
Llanos-Cuentas-2014 (39)	Bikaner	India	28.02	73.31	2011	2013	0.56	Low
Llanos-Cuentas-2014 (39)	Chennai	India	13.08	80.27	2011	2013	1.18	Low
Rishikesh-2015 (36,37)	Manipal	India	12.96	77.65	2012	2014	2.5	Moderate
Ley-2016 (38)	Alikadam	Bangladesh	21.65	92.31	2014	2015	0.59	Low
Saravu-2016 (35, 36)	Udupi	India	13.34	74.74	2012	2015	2.5	Moderate
Saravu-2018 (40)	Udupi	India	13.34	74.74	2017	2018	2.5	Moderate
Rijal-2019 (41)	Jhapa	Nepal	26.55	87.89	2016	2016	0.12	Low
Rijal-2019 (41)	Kailali	Nepal	28.83	80.90	2016	2016	0.22	Low

MAP – malaria Atlas Project; *Transmission intensity is classified as low (an incidence rate of <1 per 1000 persons), moderate (1 to <10 per 1000 persons), high (≥10 per 1000 persons).

Table S4. Reasons for studies not being included in the one stage efficacy meta-analysis

Reason	Efficacy analysis	
	Number of studies	Studies
Data not available	5	8, 27, 28, 33-34
Investigators unable to be contacted	1	29
No response from investigators	4	9, 30-32
Minimum data not available	1	11

Table S5. Studies targeted for the one stage efficacy individual patient data meta-analysis but not included

First Author	Treatment Arms	Number of Sites	Country	Follow up (days)	Randomised	Recruitment period	Treatment arms	Pv patients enrolled	Treated with PQ	Female (%)	Mean Age (SD)	Median Age (range)	Reasons for exclusion
Adak-2001 (27)	3	1	India	365	Yes	Not stated	Cq; Cq_Pq_1.25_5d_D3; Cq_Bq	663	220	Not stated	Not stated		Data not available
Dua-2001 (31)	1	4	India	540	No	1987-2000	Cq_Pq_1.25_5d_D2	5541	5541	Not stated	Not stated		No response from investigators
Mohapatra-2002 (29)	1	1	India	365	No	1998-2000	Cq_Pq_3.5_14d_DX	110	110	36.4	Not stated		Unable to be contacted
Yadav-2002 (32)	2	1	India	365	Yes	1988-1991	Cq; Cq_Pq_1.25_5d_D2	1482	759	Not stated	Not stated		No response from investigators
Rajgor-2003 (33)	2	1	India	180	Yes	1998-2000	Cq; Cq_Pq_3.5_14d_D4	273	131	12.1	Not stated		Data not available
Leslie-2004 (11)	3	1	Pakistan	270	Yes	2000-2001	Cq; Cq_Pq_3.5_14d_D0(sup); Cq_Pq_3.5_14d_D0(unsup)	595	383	50.7	12.9 (-)		Minimum data not available
Dunne-2005 (30)	2	6	India	28	Yes	1998-2001	Cq _Pq_3.5_14d_D3 ; AZ_Pq_3.5_14d_D3	200	102	20	32		No response from investigators
Ganguly-2013 (34)	2	1	India	42	Yes	2011-2012	Cq; Cq_Pq_3.5_14d_D0	250	125	10.8	25.2 (-)		Data not available
Rajgor-2014 (8)	4	1	India	180	Yes	Not stated	Cq; Cq_Pq_3.5_14d_D4; Cq_Pq_3.5_7d_D4; Cq_Pq_7.0_14d_D4	1556	1159	4.8	31.2 (-)		Data not available
Pareek-2015 (9)	3	8	India	180	Yes	Not stated	Cq_Pq_3.5_14d_D3; Cq_Pq_3.5_14d_D3; Cq_Pq_3.5_7d_D3	358	358	17.3	Not stated	20	No response from investigators
Valecha-2016 (28)	2	9	India	42	Yes	2011-2013	Cq_Pq_3.5_14d_D3; ArtmPip_Pq_3.5_14d_D3	317	317	8.2	33.7 (13.5)		Data not available

Artm – arterolane maleate; AZ – azithromycin; Bq – bulaquine; Cq – chloroquine; Pip – piperaquine; PQ/Pq – primaquine; Pv – *P. vivax*; SD – standard deviation; Tnd – tinidazole; Tq – tafenoquine; *Treatment code describes (schizontocidal drug)_(hypnozoitocidal drug)_(total primaquine dose)_(duration of primaquine treatment eg 14d = 14 days)_(primaquine start day) (supervision status)

Table S6: Comparison of baseline characteristics between studies included and studies targeted for the one stage meta-analysis

Characteristic	Included studies N = 7	Targeted studies that were not included N = 11
Year of Enrolment		
Pre 2010	1 (14.2%)	7 (63.6%)
2010 or later	6 (85.7%)	4 (36.4%)
Countries		
India	4 (57.0%)	10 (90.9%)
Bangladesh	1 (14.2%)	0 (0%)
Pakistan	1 (14.2%)	1 (9.1%)
Nepal	1 (14.2%)	0 (0%)
Age (years), median (inter-quartile range)	21.3 (16-35.6)	31.2 (20-31.2)*
Female, %	34.8%	16.5%#

Age and female percentage of targeted studies frequency weighted according to number of patients treated with chloroquine alone (No primaquine) and different dosages of primaquine. Year of the enrolment defined as the year study enrolment completed. Age, and female percentage of targeted studies calculated using frequency weighted mean or median according to number of patients. * Mean or median age not available for 5 studies. # Percentage not available for 3 studies.

Table S7. Risk of bias assessment in randomised controlled studies in one or two stage meta-analyses

Author-year	Bias from randomisation	Bias due to deviation from intervention	Bias from missing outcome	Bias in measurement of the outcome	Bias in selection of the reported results	Overall bias	Follow up to 180 days
Adak-2001 (27)							
Yadav-2002 (32)							
Rajgor-2003 (33)							
Leslie-2008 (42)							
Llanos-Cuentas-2014 (39)							
Rajgor-2014 (8)							
Pareek-2015 (9)							
Saravu-2018 (40)							
Rijal-2019 (41)		†					

Green – low risk of bias; Red – high risk of bias; Orange – unclear risk of bias; Grey – not applicable; Assessed according to the Cochrane Risk of Bias 2 tool for randomised controlled trials (23); † Study analysed per protocol but all data available for these meta-analyses; PQ – primaquine.

Table S8. Risk of bias assessment in single arm observational studies in one or two stage meta-analyses

Author-year	Clear criteria for inclusion	Condition measured in reliable way	Valid methods for condition	Consecutive inclusion	Complete inclusion	Demographics reported	Clinical information reported	Outcomes reported	Site description	Analysis appropriate	Follow up to 180 days
Rishikesh-2015 (37)											
Ley-2016 (38)											
Saravu-2016 (35)											

Green – yes (low risk of bias); Red – no (higher risk of bias); Orange – unclear; Grey – not applicable; Assessed according to the Joanna Briggs Institute Case Series tool (24) for single arm studies; The appropriateness of analysis was considered appropriate for all studies given that the individual patient data were re-analysed as part of these meta-analyses; PQ – primaquine.

Table S9: Sensitivity analysis for cumulative risk of first *P. vivax* recurrence between day 7 to 42 and between day 7 to 180 for the patients receiving different dosage of primaquine

Variable	Range of cumulative risk (%)	Coefficient of Variation (%)
Day 42		
No primaquine	2.28-11.66	26.58
Low dose primaquine	0.00-0.45	35.39
High dose primaquine	0.00-0.00	
Day 180		
No primaquine	46.68-79.62	13.57
Low dose primaquine	16.97-50.14	28.67
High dose primaquine	0.00-0.00	

Sensitivity analyses were generated by removing one study site at a time from the Kaplan-Meier cumulative risk calculation. The coefficient of variation is calculated as standard deviation divided by the mean of the estimates of the $\log_e$(Cumulative Risk). Low dose = 2 - <5 mg/kg; High dose = ≥5 mg/kg.

Figure S1: Location of study sites in efficacy analysis

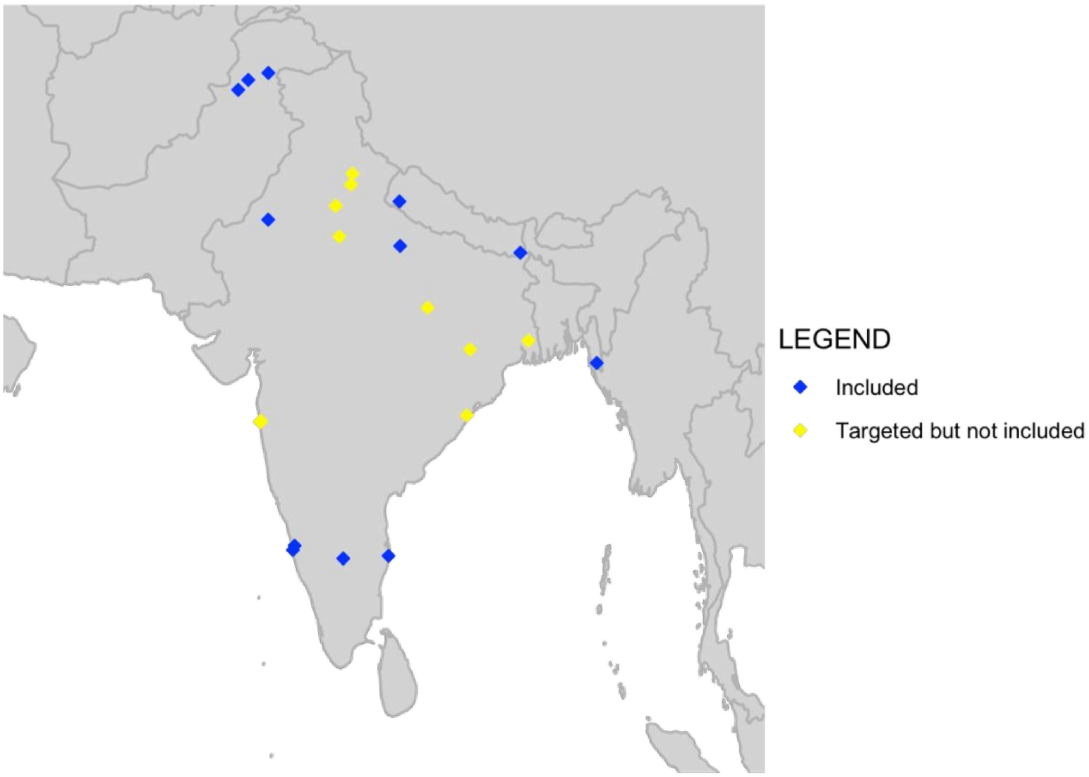


Figure S2: Mg/kg total dose of total primaquine administered (n=512)

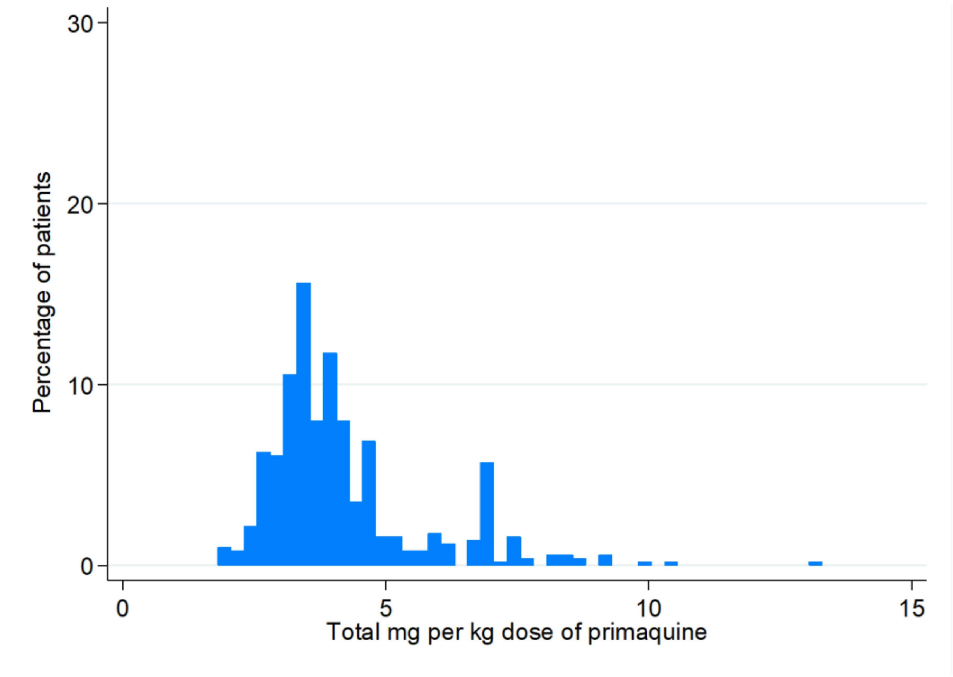


Figure S3: Mg/kg drug dosing of primaquine by body weight (n=512)

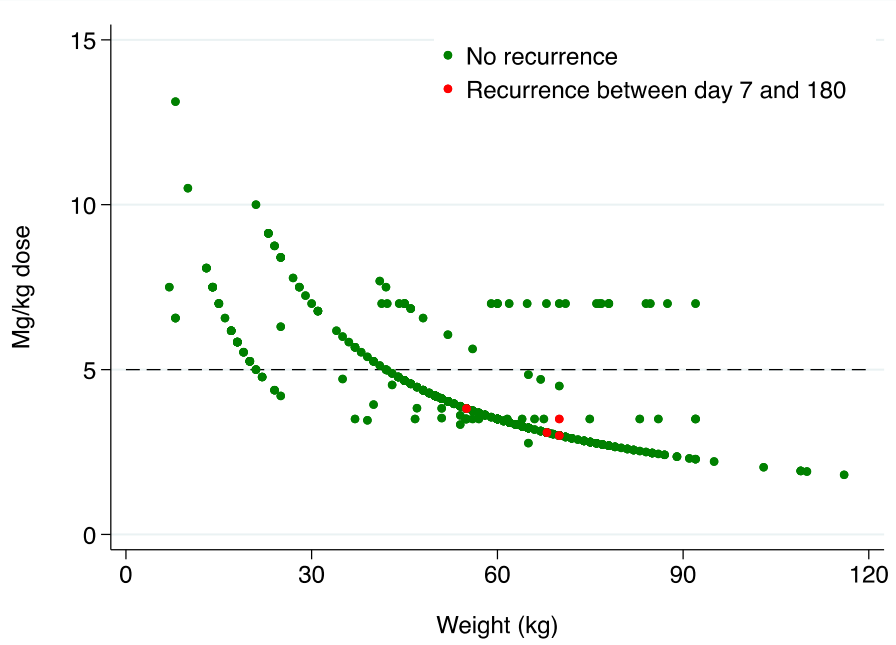
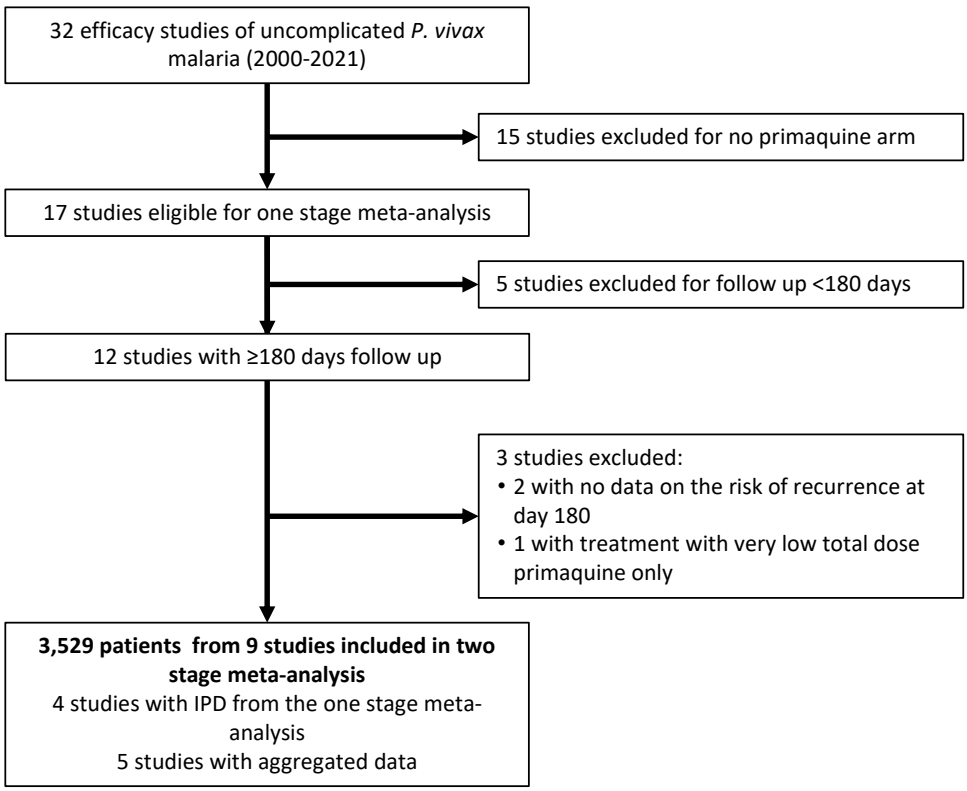


Figure S4: Flowchart for two stage meta-analysis



IPD – individual patient data