Statistical Analysis Plan

WWARN Primaquine Indian Region Study Group: An individual patient data meta-analysis from Indian subcontinent to inform the local safety and efficacy of primaquine regimens for radical cure of *P. vivax*

Version 1.0

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Version History Version number	**Revision(s) & reason for amendment**	**Release date**
V 1.0		
V 1.1	• Tolerability and haematological outcomes changed to start from day 1 instead of day 2 to ensure all potential PQ-associated events are captured. • PQ supervision definition updated for consistency with other studies • Nomenclature of daily PQ mg/kg dose categories updated to low dose, intermediate dose and high dose instead of very low, low and high dose to avoid confusion • Two stage meta-analysis added	1/10/2022

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1. Introduction and Rationale

P. vivax is the most frequent and widely distributed cause of relapsing malaria. Relapsing malaria leads to recurrent acute febrile illness, anaemia, morbidity and mortality. Relapses are caused by dormant liver stage parasites called hypnozoites, weeks and months after initial infection. Primaquine, an 8-aminoquinoline (8-AQ), is the most widely available antimalarial that kills *P. vivax* hypnozoites. Primaquine has efficacy against hypnozoites and gametocytes, but it can cause drug-induced haemolysis in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency, in addition to other side effects such as methaemoglobinaemia and gastrointestinal disturbance in normal and G6PD deficient individual.

World Health Organization recommends the use of 0.25mg/kg primaquine daily for 14 days in India. The national guidelines of the Indian subcontinent countries also recommends WHO guided dosage and duration of the primaquine. However, this regimen can have poor adherence resulting in reduced effectiveness. Anti-relapse efficacy is related to the total mg/kg dose of primaquine administered, with higher doses of primaquine potentially providing greater efficacy, although daily dosing may be limited by tolerability. Efficacy, safety and tolerability can vary between populations and regions. An individual patient data meta-analysis from India and neighbouring countries will provide improved understanding of the safety, tolerability and efficacy of differing primaquine radical cure regimens for the Indian context; directly informing national malaria control policies in this region.

2. Aim of the study

The overall aim of this individual patient data (IPD) meta-analysis is to determine the most efficacious, safe, and tolerable regimen of primaquine to treat *Plasmodium vivax* (Pv) in the Indian sub-continent.

2.1 Objectives

1. To investigate the effect of different doses of primaquine (mg/kg) and durations on the risk of *P. vivax* recurrence in the Indian region.
2. To investigate the effect of daily primaquine mg/kg dose on tolerability and safety in the Indian sub-continental countries, in patients with normal and deficient G6PD status.

3. Eligibility criteria

3.1 Eligibility criteria for IPD meta-analysis

3.1.1 Essential inclusion criteria for analysis

- Clinical efficacy study of uncomplicated *P. vivax* malaria undertaken from 2000 to August 2021 inclusive with at least 28 days follow up
- Study includes at least one primaquine arm which was commenced within 7 days of the schizontocidal treatment
- Baseline data on age and sex
- Parasitemia on day 0
- Information on the schizontocidal dose
- Information on the timing, duration and dose of primaquine
- Reporting of parasite presence or absence during follow up

3.1.2 Desirable inclusion criteria for analysis

- Baseline weight
- Individual tablet or mg dosing
- G6PD status
- Haemoglobin (hb) or haematocrit (hct) measured on day 0
- Vomiting of primaquine within one hour
- History of malaria within the past 28 days
- History of fever within the last 24 hours at baseline and during follow up
- Parasite genotype
- Documentation on the supervision of drug administration
- CYP2D6 status
- Methaemoglobin measured on day 0 and during follow up
- White cell count measured on day 0 and during follow up
- Adverse event data including serious adverse events and symptoms questionnaires

3.2 Exclusion criteria for analysis

• Study evaluating treatment with primaquine in P. falciparum transmission blocking, Mass Drug Administration, healthy volunteers, or as prophylaxis

4. Data pooling

Relevant studies will be identified following PROSPERO-registered systematic reviews. Trials published or undertaken from January 2000 to August 2021, inclusive, that fulfil the study criteria will be targeted through direct email to the corresponding author and/or principal investigator.

4.1 Data management

Data sets uploaded to the WWARN repository will be standardized using the WWARN Clinical Data Management and Statistical Analysis Plan [4] and IDDO-CDISC nomenclature into quality-assured IPD sets. Meta-data including study design, study site/s, methodology (e.g. Adverse Event collection and elicitation method) will also be recorded. Data will remain the property of the individual data contributors.

5. Study Group governance, co-ordination and membership

The Primaquine Indian Region Study Group comprises participating investigators who contribute relevant data sets to the pooled analysis. Data sets will remain the property of the investigator and will not be shared without their consent. Dr. Reena Verma, and Dr. Rob Commons, will oversee the statistical analyses. The Study Group collectively makes decisions with respect to including additional studies and plans for publication. The Study Group will assign a Writing Committee to coordinate activities including data analysis and drafting of publications and reports for complete group review. The Writing Committee will comprise a group of interested investigators undertaking the data analysis and preparation of the manuscript, including Dr. Reena Verma, Dr. Apoorv Gupta Dr. Amit Prakash Sharma Dr. Nitika, Dr. Praveen Bharti, Dr. Rob Commons, Prof. Ric Price. Authors will be recognized according to the ICMJE guidelines and the WWARN publication policy.

6. Endpoints

Primary:

- Efficacy – Incidence risk of any *P. vivax* recurrence between day 7 and day 42 and day 7 and day 180
- Tolerability - A composite endpoint including any of the following symptoms of gastro-intestinal disturbance between days 1-14: vomiting, anorexia, diarrhoea.
- Safety - >25% drop in haemoglobin to below 7g/dL between day 0 and days 1 - 14

Secondary Efficacy Endpoints:

- Incidence risk of symptomatic *P. vivax* recurrence between days 7 to 42 and days 7 to 180
- Incidence rate of any *P. vivax* recurrence between days 7 and 180
- Incidence rate of symptomatic *P. vivax* recurrences between days 7 and 180

Secondary Tolerability Endpoints:

- Presence of the following symptoms as separate endpoints between days 1-14: vomiting, nausea, anorexia, abdominal pain, diarrhoea and dizziness.
- Vomiting within 1 hour of primaquine dosing

Secondary Safety Endpoints:

- Maximum absolute reduction in haemoglobin between baseline and days 1 – 14 after primaquine
- Presence of any of the following: haemoglobin fall below 5 g/dL or haemoglobin fall >5 g/dL from baseline between days 1-14 or renal failure needing dialysis, blood transfusion or death between days 1-28.
- Presence of moderate and severe anaemia as separate endpoints at days 1–14

6.1 Study endpoints

6.2 Endpoint definitions

P. vivax recurrence before day X is defined as any recurrence of *P. vivax* parasitaemia between day 7 and X irrespective of symptoms.
Anaemia will be defined as:

- Mild (Hb ≥8 g/dL and <11g/dl),
- Moderate (Hb ≥5 g/dL and <8g/dl)
- Severe (Hb <5g/dL)

Clinical methaemoglobinaemia will be defined as a methaemoglobin level >10% [1,2].
Severe methaemoglobinaemia will be defined as a methaemoglobin level >20% [1,2].

7. Covariate definitions

7.1 Patient and study characteristics

The following baseline characteristics will be examined:

Site: regional relapse periodicity, transmission intensity, geographical location (region and country)
Patient: age, sex, weight, history of malaria in the last 28 days, history of fever in the last 24 hours, fever (≥37.5°C axillary), G6PD status, CYP2D6 status
Drug: schizontocidal treatment and mg/kg dose, primaquine use, start day, duration and mg/kg dose, association with food intake, supervision of drug intake (full or partial), early vomiting of primaquine (within 1 hour)
Laboratory: parasitaemia, haemoglobin concentration, methaemoglobinaemia, white cell count

Schizontocidal treatment will be classified as supervised if all doses were directly observed, partially supervised if at least the morning doses of a bd regimen were observed, and not-supervised if fewer doses were observed.
Primaquine treatment supervision will be classified as:

- *Supervised* if all doses were directly observed
- *Partially supervised* if <100% but >1 dose were observed
- *Unsupervised* if 0 or 1 dose were observed.

In studies with haematocrit measured instead of haemoglobin, haematocrit will be converted to haemoglobin using the following relationship [3]:

$$\text{Haematocrit (ht)} = 5.62 + 2.60 * \text{Haemoglobin}$$

For each study, locations of study sites will be recorded. Each location will be categorised into:

a) *Low, moderate and high transmission settings* based on the observed study site reinfection rate, and the malaria endemicity estimates obtained for subnational regions and year from the Malaria Atlas Project [4].
b) *Low (long) and high (short) periodicity of relapses* according to Battle's regions [5], with high periodicity considered to include regions where the median periodicity was ≤42 days. Thus regions with the two highest periodicities (region 10 and 12) where the

median periodicity is <47 days will be categorised as “high” and others will be categorised as “low”.

G6PD deficiency will be classified as severely deficient (<30% activity or a positive qualitative test (eg FST)) vs normal (>=30% activity or a negative qualitative test (eg FST)). A second categorisation will be explored to assess patients with intermediate deficiency: severely deficient (<30% activity or a positive qualitative test (eg FST)), intermittently deficiency (>=30% to <70% activity) or normal (>=70% activity).
CYP2D6 status will be classified by expected phenotype using the activity score system [6,7] to estimate phenotype from genotype. The activity score assigns values of 0 to 2 to the CYP2D6 alleles identified in the patient as follows: zero, no-function alleles (*4, *4xN, *5); 0.25, substantially decreased-function (*10); 0.5, decreased-function (*9, *17, *29, *41); 1, normal-function (*1, *2, *39) and 2, increased function (*1xN, *2xN). The AS of diplotypes results from the sum of the assigned value to each allele. Patients with AS = 0 are designated as poor metabolisers. Patients with AS = 0.25, 0.5, 0.75 and 1 are designated as intermediate metabolisers. Patients with AS > 2.25 are designated as ultra-rapid metabolisers, respectively. Patients with AS = 1.25, 1.5, 2 and 2.25 are designated as normal metabolisers [8].

7.2 Exposures of interest

The doses of primaquine received will be calculated from the number of daily tablets administered to each patient. If the daily tablet counts are not available, doses will be back-calculated using the dosing scheme available from study protocols. If neither of these are available doses will be assumed according to the planned dosing regimen. For each component, a total dose per weight will be calculated for each patient.
Total primaquine dose will be assessed as a categorical variable primarily to enable comparison of low vs high dose regimens. Additional analyses will be undertaken with primaquine dose as a continuous variable to investigate the impact of small changes in dose. Total primaquine dose categories will be considered *very low dose* if <2 mg/kg primaquine is given, *low dose* if 2 to <5 mg/kg is given and *high dose* in ≥5 mg/kg is given [9].
Daily PQ mg/kg dose will be defined as low dose if <0.375 mg/kg/day, intermediate dose if ≥0.375 and <0.75 mg/kg/day and high dose if ≥0.75 mg/kg/day.

Primaquine regimen will be classified as the duration of treatment in days and will be explored in treatment arms with a similar total mg/kg dose. Seven- and 14-day regimens will be compared.

8. Statistical Analyses

8.1 Description and baseline characteristics of study sample:

8.1.1 A summary (study profile) of the relevant trials uploaded to the WWARN repository will be presented to highlight potential selection bias.

8.1.2 A summary of the relevant studies will be presented, including (but not restricted to) study design, treatment given, food intake with primaquine, follow up duration, study populations, description of location by country, transmission intensity.

8.1.3 A comparison table of the summary statistics of studies that were targeted but not included will be presented to allow evaluation of inclusion bias related to study selection.

8.2 Baseline characteristics of patients:

8.2.1 A summary of relevant baseline patient characteristics will be presented for all patients, those not treated with PQ and those receiving very low, low or high dose of PQ.

Variables presented will include: age and age group, sex, weight, nutritional status, haemoglobin concentration at baseline, asexual parasitaemia at baseline, presence of fever (temperature ≥37.5°C or fever recorded) at baseline, blood schizontocidal treatment and mg/kg dose, mg/kg dose and dose category, timing (i.e. first day of treatment) and duration of primaquine, percentage of primaquine administered with food, and host variants (G6PD status, CYP2D6 status).

The distribution of continuous variables will be described using the mean and standard deviation if the data are normally distributed, geometric mean and 95% reference range if the data are normally distributed following a log transformation, or the median and interquartile range if the data are non-normally distributed. Tests of statistical significance will not be undertaken for baseline characteristics; rather the clinical importance of any differences in the baseline distributions will be noted.

8.3.1 *Risk of intolerance following primaquine*
The percentage and 95% confidence intervals of patients with each of the following symptoms reported on days 2 to 14 (vomiting, anorexia, diarrhoea) according to data collected from symptom checklists will be presented in a tabular format. A composite endpoint including any of these symptoms of gastrointestinal disturbance between days 1-14 will also be presented. Results will be presented by daily primaquine mg/kg dose (no primaquine, very low dose, low dose and high dose).

8.3.2 In view of the confounding effect of malaria and co-administration of schizontocidal drug/s, the percentage of patients with the composite endpoint in 8.3.1 will also be assessed in patients between days 0-1 and compared to the percentages between days 2-14.

8.3.3 A **multivariable logistic regression** analysis will be undertaken to determine whether age mediates the effect of daily mg/kg primaquine dose (both as a continuous variable and a categorical variable categorised as no primaquine, low dose, intermediate dose and high dose) on the odds of fulfilling criteria for the composite gastrointestinal endpoints at any day between 1 and 14. A separate sensitivity analysis will be undertaken to establish the robustness of this result against results from composite scores generated from individuals asked about all three symptoms vs. any two symptoms vs. one symptom. Separate analyses will be undertaken for each gastrointestinal symptom. The effect of primaquine dose will be controlled for potential confounders including sex, parasitaemia, schizontocidal drug/s and presence of fever with study site included as a random effect.

8.3.5 Risk of acute vomiting following primaquine
The percentage of patients vomiting within an hour of primaquine dosing will be presented in a tabular format. Results will be categorised by daily primaquine mg/kg (no primaquine, low dose, intermediate dose and high dose) and the risk presented on day 0 to 2 and days 3-14.

8.3.6 A multivariable logistic regression analysis will be undertaken to determine the effect of daily mg/kg primaquine dose (both as a continuous variable and a categorical variable categorised as no primaquine, low dose, intermediate dose and high dose) on the odds of vomiting within an hour of primaquine dosing at any day between 0 and 14. Primaquine dose will be controlled for potential confounders including age, sex, parasitaemia, schizontocidal drug and presence of fever, with study site included as a random effect. The effect of nutritional status will be explored.

8.4 Haematological safety related analyses:

8.4.1 The percentage of patients with each of the following will be presented categorised by daily primaquine mg/kg dose

Haematological safety

- >25% drop in haemoglobin to below 7g/dL between baseline and days 1 - 14
- Maximum absolute reduction in haemoglobin between baseline and days 1 – 14 after primaquine
- Receiving blood transfusion within 14 days of last primaquine treatment
- >5g/dL drop in haemoglobin between baseline and days 1 - 14.
- Development of anaemia between day 1 and day 14:
  - mild anaemia (Hb ≥8 g/dL and <11g/dl),
  - moderate anaemia (Hb ≥5 g/dL and Hb <8g/dl)
  - severe anaemia (Hb <5g/dL)
- Day of haemoglobin nadir
- Presence of clinical methaemoglobinaemia (10%) and severe methaemoglobinaemia (>20%) 1-14 days after starting primaquine treatment

8.4.2 The maximum absolute reduction in haemoglobin between baseline and days 1 – 14 after primaquine will be presented visually (histogram and/or box and whisker plot) by daily primaquine mg/kg dose (no primaquine, low dose, intermediate dose and high dose) and the maximum absolute reduction in haemoglobin at day 7 after primaquine by cumulative total mg/kg dose (no primaquine, low dose and high dose).

8.4.3 Multivariable linear regression analyses will be undertaken to determine how age mediates the effect of daily mg/kg primaquine dose (both as a continuous variable and a categorical variable categorised as no primaquine, low dose, intermediate dose and high dose) on the maximum absolute reduction in haemoglobin between baseline and days 1-14. The effect of primaquine dose will be controlled for potential confounders including sex, parasitaemia, schizontocidal drug/s, presence of fever, haemoglobin at day 0, G6PD status and relapse.

8.5 Efficacy related analyses

8.5.1 A summary of the distribution of mg/kg primaquine dose will be presented. The distributions will be calculated separately for different age groups, blood schizontocidal drug/s,

dosing strategies (age based and weight based) and regions [7] and presented in tables (mean (SD)) as well as visualised using box and whisker plots, histograms or scatter plots (e.g. mg/kg dosing vs age or weight).

8.5.2 Time to first recurrent vivax parasitaemia will be used to compute the **Kaplan-Meier (K-M) estimates** of risk of *P. vivax* recurrence at day 42, day 90, and day 180 for treatment arms with and without primaquine for each study site. Patients will be censored at time of recurrent vivax parasitaemia, first malaria treatment, lost to follow up, >60 days blood smear gap, last day of study or the day the outcome is being assessed. The K-M curves stratified by categories of primaquine total dose and no primaquine use will be presented for the whole population.

8.5.3 The proportion of patients who had a recurrent vivax parasitaemia before day 42, between day 42 and 90 and between day 90 and 180 will be presented. In addition, the median time to presentation with recurrent infection will be calculated.

8.5.4 Cox regression analysis for the time to first vivax recurrence during follow-up (180 days) will be performed, with shared frailty for study-site to account for additional variation related to study sites. Primaquine dose will be controlled for potential confounders including age, sex, and baseline parasitaemia. Weight will be explored but will likely be excluded due to collinearity with age. Models will be stratified by schizontocidal treatment half-life (eg AL vs DP/CQ/Mq). Transmission intensity will be examined, depending on available data. Additional covariates will be examined including blood schizontocidal mg/kg dose, level of treatment supervision, baseline temperature and haemoglobin, acute vomiting of the drug, GI tolerability Some variables will have less complete data and are also expected to have less impact on efficacy (e.g. baseline Hb and temperature) and thus will not be included in the primary multivariable model. To investigate the impact of small changes in total mg/kg primaquine dose, analyses will be repeated with total mg/kg primaquine dose as a continuous exposure variable.

8.5.5 Incidence rates of recurrent vivax parasitaemia over **180** days will be calculated for treatment without primaquine, treatment with very low dose primaquine, treatment with low dose primaquine and treatment with high dose primaquine from studies with a minimum 180 days follow up that followed patients through multiple episodes of vivax parasitaemia. Incidence rates will be calculated by dividing the number of *P. vivax* episodes by the number of person-years of observation (PYO) in the study population. The start date for PYO will be the day of enrolment into the study and the stop date the last visit performed (either completed study or any last visit before loss to follow up and/or censoring). The period between start and stop dates for each patient will be calculated in days and divided by 365 to determine approximate PYO. The incidence rate of symptomatic recurrences will use the entire period between start and stop dates to determine the PYO. To calculate the PYO for the incidence rate of any recurrence (symptomatic or asymptomatic), if the cumulative time over which the exposure was assessed includes non-consecutive periods when smears were not undertaken, these periods will be excluded from the PYO. Non-consecutive periods will be determined when >15 days exists before or after assessment with a blood smear (i.e. if 32 days exists between blood smear 2 days will be excluded from the PYO). Incidence rate ratios comparing very low, low and high dose primaquine with no primaquine and high dose with low dose and very low dose primaquine will be calculated after controlling for potential confounders

including sex, baseline parasitaemia, and relapse periodicity. Incidence Rate Ratio (IRR) derived from a negative binomial regression model with robust SEs to account for differences in variation at the cluster-level. The effect of blood schizontocidal treatments with different half-lives will be explored with incidence rate ratios estimates pooled across groups if differences are present.

The effect of post-treatment prophylaxis on the time of observation will be assessed as follows in two sensitivity analyses:

- Patients receiving antimalarial treatment during follow up will be assumed to have a period of 28 days of post-treatment prophylaxis. This period will thus be subtracted from their total period of follow up.
- Patients receiving antimalarial treatment during follow up will be assumed to have no period of post-treatment prophylaxis and no time period will therefore be subtracted from their total period of follow up.

8.5.6 A **two stage-meta-analysis** will be undertaken if considerable data are missing from the one-stage meta-analysis assessing the risk of recurrence by day 180. This will be restricted to studies with ≥180 days follow up. Aggregated proportions of patients with vivax recurrence at day 180 will be pooled with data from studies that are available using a random effects meta-analysis stratified by total mg/kg primaquine dose (no primaquine, low dose and high dose). Proportions will be pooled using the Freeman-Tukey double arcsine transformation and with exact methods to calculate confidence intervals.

8.6.1 Exploration of variation in effects

- Sensitivity analyses will be carried out to assess heterogeneity of studies by removing one study site at a time and calculation of the coefficient of variation (CV) around the parameter estimates will be presented.

8.6.2 Risk of bias relating to individual studies

- Potential bias relating to individual studies will be assessed using the ROBINS-I tool (14).

9. References

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