Statistical Analysis Plan
WWARN Primaquine Efficacy, Safety and Tolerability Study Group: A pooled analysis investigating the effect of primaquine dose on the efficacy, safety and tolerability of patients with *Plasmodium vivax* malaria
Version 1.3

WorldWide Antimalarial Resistance Network (WWARN)

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Version History Version number	**Revision(s) & reason for amendment**	**Release date**
V1.1		18 August 2021
V1.2	• Clinical methaemoglobinaemia (>10%) added as a secondary safety endpoint • Definition of schizontocidal elimination half-life included • CYP2D6 activity score updated following recent publication • Methaemoglobinaemia dropped as an exposure of interest as will be analysed in a separate study • The relationship between mg/kg dose of primaquine and predicted risk of recurrence altered to include exploration with spline models • Management of missing smears to calculate incidence rate defined in more detail including symptomatic and any recurrence • Composite endpoint for tolerability adjusted to vomiting, diarrhoea and anorexia • Tolerability and safety analysis restricted to analysis of daily mg/kg primaquine dose as the exposure of interest • Linear mixed effect model added to assess impact of primaquine dose on haemoglobin over time. • Haemoglobin at day 0 and G6PD status included as confounders in multivariable safety models	1 October 2021
V1.3	• Updated timing of Safety Analysis primary endpoint to i) day 2/3 and ii) day 6+/-1 given variable sampling of haemoglobin concentrations within studies. • Updated timing of Safety Analysis secondary endpoints to day 2/3 and day 6+/-1 given variable sampling, and for severe adverse events to days 1-14 to ensure early haemolytic events captured. • Updated Tolerability and Safety Analysis inclusion criteria for primaquine to commence within three days of blood schizontocidal treatment. • Updated Tolerability Analysis primary and secondary endpoints to day 6+/-1 given variable sampling of symptom checklist data. • Updated Tolerability Analysis secondary endpoints prior to day 3 from day 0/1 to day 0 and day 1/2 to	30 May 2022

	distinguish acute malaria pre-treatment and schizontocidal impact. • Anaemia definitions were updated to reflect standard definitions • Updated Efficacy Analysis to assess the effect of primaquine duration on risk of recurrence • Supervision definition updated to better distinguish supervision. • Total primaquine very low dose category changed from <2.5 mg/kg to <2 mg/kg to ensure even capture of low dose primaquine (2-5 mg/kg). • GI Tolerability multivariable analyses updated to exclude schizontocidal drugs (as endpoint now after treatment finished, although day 1/2 will analyse as subgroups) and presence of fever (as endpoint now later after acute malaria symptoms resolved and most efficacy studies have fever as inclusion criteria). • Updated Haematological analyses to restrict primary analyses to patients with >=30% G6PD activity as analysis of all patients as a single group includes an unknown mix of patients with differing G6PD activities that prevent generalisation. Given this, G6PD subgroup analyses updated to <30% activity, 30-<70% activity, >=70% activity and unknown G6PD activity. • Haematological analyses updated to exclude G6PD status (as analysis now restricted to specific G6PD activity), presence of fever (as most efficacy studies have fever as inclusion criteria), schizontocidal treatment (as collinear with study site) and relapse periodicity (as variable considered unlikely to substantially impact endpoint).	

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1. Introduction and Rationale

Vivax malaria is associated with recurrent parasitaemia and anaemia with both attributable morbidity and mortality. Recurrent *P. vivax* can arise from recrudescence (treatment failure), reinfection (new infections) and relapse (reactivation from dormant liver stages). Whilst it is currently not possible to differentiate reliably between these alternatives, early recurrence is more likely to be due to relapse or recrudescence, whereas later recurrences are more likely to be due to relapse or reinfection. Relapse periodicity varies markedly with geographical location and can occur many months after the initial infection. Other factors influencing the risk, frequency and timing of recurrence include: antimalarial drug resistance, the pharmacokinetic profile of the antimalarial agents administered, host immunity, the use of hypnozoitocidal drugs and transmission intensity.

Recent pooled analyses through WWARN have identified the risk factors associated with early recurrence up to day 42. However, risk factors associated with recurrence, including multiple recurrences, over a longer duration have not been evaluated comprehensively.

Antirelapse efficacy is related to the total mg/kg dose of primaquine administered. Although higher doses of primaquine may provide greater efficacy, daily dosing is limited by tolerability particularly gastrointestinal symptoms and haemolysis. The comparative safety of different primaquine doses on adverse events needs additional investigation to inform development and implementation of high dose short course regimens.

1.2. Aim of the study

The aim of this study is to assess the effect of primaquine dose and regimen on i) efficacy, and ii) safety and tolerability in patients with *P. vivax* malaria.

1.3. Eligibility criteria for inclusion in efficacy pooled analysis

1.3.1 Essential data for inclusion in the analysis

- Prospective clinical efficacy studies of uncomplicated vivax malaria
- A minimum follow up of 42 days
- Treatment with chloroquine or one of four common artemisinin-based combination therapies (artemether-lumefantrine, artesunate-mefloquine, artesunate-amodiaquine, dihydroartemisinin-piperaquine)
- Information on dose of schizontocidal treatment
- At least one treatment arm with primaquine given over multiple days and commencing within seven days of blood schizontocidal treatment
- Information on use, timing and mg/kg dose of primaquine
- Study meta-data as described in the Clinical Data Management and Statistical Analysis Plan (1)
- Baseline data on patient age and sex
- Asexual parasite density at day 0
- Reporting of parasite presence or absence during follow up

1.3.2 Desirable data for inclusion in the analysis

- Baseline weight
- Individual tablet or mg dosing
- Documentation on the supervision of drug administration
- Data on food administration with primaquine
- Follow up through multiple malaria recurrence events
- Follow up for 180 days or longer
- Haemoglobin (hb) or hematocrit (hct) measured on day 0
- Malnutrition as gauged by weight and age
- Qualitative or quantitative assessment of G6PD status
- CYP2D6 genotype/phenotype
- Data on methaemoglobinaemia
- Primaquine/Carboxyprimaquine drug levels during follow up
- History of malaria within the past 28 days
- History of fever within the last 24 hours at baseline and during follow up
- Data on vomiting post administration
- Genotyping to potentially distinguish recrudescence and reinfection

1.3.3 Exclusion criteria

- Pregnancy
- Retrospective studies
- Studies of severe malaria
- Studies without active follow up
- Adjunctive antimalarial treatments

1.4. Eligibility criteria for inclusion in tolerability and safety pooled analysis

1.4.1 Essential data for inclusion in the analysis

- Prospective clinical efficacy studies of uncomplicated vivax malaria
- A minimum follow up of 28 days
- Treatment with chloroquine, one of four common artemisinin-based combination therapies (artemether-lumefantrine, artesunate-mefloquine, artesunate-amodiaquine, dihydroartemisinin-piperaquine) or primaquine alone
- Information on dose of schizontocidal treatment
- At least one treatment arm with primaquine given over multiple days and commencing within three days of blood schizontocidal treatment
- Information on use, timing and mg/kg dose of primaquine
- Data on adverse events from routine symptom questionnaires (tolerability analysis)
- Qualitative or quantitative assessment of G6PD status (safety analysis)
- Haemoglobin (hb) or hematocrit (hct) measured on day 0 (safety analysis)
- Study meta-data as described in the Clinical Data Management and Statistical Analysis Plan (1)
- Baseline data on patient age and sex
- Asexual parasite density at day 0

1.4.2 Desirable data for inclusion in the analysis

- Baseline weight
- Individual tablet or mg dosing
- Data on dose adherence
- Documentation on the supervision of drug administration
- Data on food administration with primaquine
- Haemoglobin (hb) or hematocrit (hct) measured during follow up
- Malnutrition as gauged by weight and age
- CYP2D6 genotype/phenotype
- History of malaria within the past 28 days
- History of fever within the last 24 hours
- Data on vomiting post administration
- Methaemoglobin measured on day 0 and during follow up

1.4.3 Exclusion criteria

- Pregnancy
- Retrospective studies
- Studies of severe malaria
- Studies without active follow up
- Adjunctive antimalarial treatments

1.5. Data Pooling

A systematic review of all prospective clinical efficacy trials involving *Plasmodium vivax* mono-infection will be performed. Trials published or undertaken since the year 2000 inclusive, that fulfil the study criteria will be targeted through direct email to the corresponding author and/or principal investigator. Data from unpublished and ongoing clinical studies will also be included if available. Once data are uploaded into the WWARN repository, they will be curated and standardised using the WWARN Data Management and Statistical Analysis Plans (1) for clinical data and pooled into a single database of quality-assured individual patient data.

2. Outline of Statistical Analysis

2.1 Specific objectives of the study

1. To investigate the effect of total primaquine mg/kg dose and regimen duration on the risk of *P. vivax* recurrence and how these effects change in different populations
2. To investigate the correlation between primaquine and carboxyprimaquine concentrations and the risk of *P. vivax* recurrence and how these effects change in different populations
3. To investigate the effect of daily primaquine mg/kg dose on drug tolerability

4. To investigate adverse effects associated with primaquine use and daily dose

2.2 Study endpoints

Primary:

- Efficacy - *P. vivax* recurrence between day 7 and day 180 or day 365 (PCR unadjusted)
- Tolerability - A composite endpoint including any of the following symptoms of gastro-intestinal disturbance on day 6+/-1: vomiting, anorexia, diarrhoea.
- Safety - > 25% drop in haemoglobin to below 7g/dL between baseline and days 1 - 14

Secondary Efficacy Endpoints:

- *Symptomatic P. vivax* recurrence between day 7 and day 180 or day 365 (PCR unadjusted)
- Parasite positivity on day 1, 2 and 3
- Delayed parasite clearance

Secondary Tolerability Endpoints:

- Presence of the following symptoms as separate endpoints on days 6+/-1: vomiting, nausea, anorexia, abdominal pain, diarrhoea and dizziness.
- Presence of a composite endpoint of any of the following symptoms of gastro-intestinal disturbance on i) day 0 and ii) on day 1-2: vomiting, anorexia, diarrhoea
- Vomiting within 1 hour of primaquine dosing

Secondary Safety Endpoints:

- Presence of clinical methaemoglobinaemia (>10%) or severe methaemoglobinaemia (>20%) 1-14 days after starting primaquine treatment
- Maximum absolute change in haemoglobin between baseline and days 2/3 after primaquine
- Maximum absolute change in haemoglobin between baseline and day 6+/-1 after primaquine
- Presence of <u>any</u> of the following: haemoglobin fall below 5 g/dL or haemoglobin fall of >5 g/dL from baseline between days 1-14 or renal failure needing dialysis, blood transfusion or death between days 1-28.
- Development of mild, moderate or severe anaemia by day 2/3 or separately by day 6+/-1 (defined in 2.3 below)

2.3 Definitions of Endpoints

Primary

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

Secondary

Symptomatic P. vivax recurrence will be defined as any recurrence of *P. vivax* parasitaemia in patients with a temperature ≥37.5°C or a history of fever.

Patient's early parasitological response (*parasite clearance*) will also be evaluated (a) positivity on Day 1; (b) positivity on Day 2; (c) positivity on Day 3. *Parasite positivity* is defined as the proportion of people with positive parasite counts on day X compared to the number assessable on this day. Definitions are detailed on page 15 of the Clinical Module DMSAP v1.2 (1). *Delayed parasite clearance* is classified as parasite positivity on day 2 or later.

Serious adverse events will be defined i) according to guidelines for Good Clinical Practice from the International Conference for Harmonization and ii) according to individual study-based reports.

Anaemia will be defined as:

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

Clinical methaemoglobinaemia will be defined as a methaemoglobin level >10%. Severe methaemoglobinaemia will be defined as a methaemoglobin level >20% (2).

2.4 Study and patient characteristics

The following baseline characteristics will be examined:

Site: regional relapse periodicity, transmission intensity, geographical location (region and country)

Patient: age, sex, weight, nutritional status, 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, schizontocidal elimination half life, primaquine use, start day, duration and mg/kg dose, association with food intake, supervision of drug intake (full or partial), early vomiting of drug (within 1 hour)

Laboratory: parasitaemia, haemoglobin concentration, methaemoglobinaemia

Children will be considered as aged <15 years with childhood stratified into patients <5 years and those 5 to <15 years.

The nutritional status of children aged <5 years of age will be calculated as a weight-for-age z-score, using the igrowup package developed by WHO (3). Those with weight-for-age z-scores < -2 (i.e. below the 3rd centile) will be classified as underweight-for-age (termed underweight). Weight-for-age Z scores will be set to missing if the score is less than -6 or greater than 6.

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 >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 (4):

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

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 (5).
b) *Low (long) and high (short) periodicity of relapses* according to Battle's regions (6), 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 (7, 8) 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 ultrarapid metabolisers, respectively. Patients with AS = 1.25, 1.5, 2 and 2.25 are designated as normal metabolisers (9).

Schizontocidal elimination half-life will be defined as i) short (<1 day), ii) intermediate (1 to 7 days) and long (>7 days)(10).

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2.5 Exposures of interest

The doses of treatment received i.e. primaquine, chloroquine, ACT 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. 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 (11).

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. Regimens from South America that treat patients for 7 to 9 days depending on their bodyweight will be considered with the 7 day treatment arms.

Primaquine and carboxyprimaquine blood concentrations from the last day of planned treatment (day 6 or day 13) will be used as a continuous variable for most analyses. To assess the risk of recurrence using Kaplan-Meier plots, concentrations will be categorised into three groups corresponding to the tertiles of the plasma primaquine and carboxyprimaquine concentrations.

2.6 Summary of statistical analyses

2.6.1 Description and baseline characteristics of study sample:

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

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

2.6.1.3. 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.

2.6.2 Baseline characteristics of patients:

2.6.2.1. 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 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 timing of primaquine (ie first day of treatment), 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.

2.6.3 Baseline efficacy and treatment related analyses:

2.6.3.1. Primaquine treatment dosing

- 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 drugs, dosing strategies (age based and weight based) and regions (6) 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).

- Summary statistics of primaquine dosing strategies used in children (e.g. % studies using quarter/half tablets, % dissolving tablets, suspension or paediatric formulation) will be reported.

- Each patient will be categorised according to whether the actual PQ dose administered fell within the bounds of the corresponding study target dose (low: 2-5mg/kg or high: 5-9mg/kg). Those outside of these bounds will be classified as SubTarget primaquine dose or SupraTarget dose. Patients falling outside of these bounds will be described with factors associated with patients receiving sub or supra target doses explored through a descriptive table including age, sex, weight, protocol type, target dose, nutritional status and location.

- A summary of the distribution of primaquine and carboxyprimaquine concentrations at day 6 and 13 will be presented. The distributions will be calculated separately for different age groups, blood schizontocidal drugs, and primaquine regimens (total dose and duration).

2.6.3.2. Time to first P. vivax recurrence within 180/365 days

- 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 90, day 180 and day 365 for treatment arms with and without primaquine for each study site where there was a minimum of 42 days follow up. 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, in addition to tertiles of day 6 or day 13 primaquine and carboxyprimaquine concentrations. Subgroup analyses for geographic region, relapse periodicity region, age group and primaquine regimen will be undertaken.

- The proportion of patients who had a recurrent vivax parasitaemia before day 90, between day 90 and 180 and between day 180 and 365 will be presented. In addition, the median time to presentation with recurrent infection will be calculated with subgroup analyses for geographic region, relapse periodicity region, transmission intensity, age group and primaquine regimen will be undertaken.

- **Cox regression analysis** for the time to first vivax recurrence during follow-up (180 and 365 days) will be performed, with shared frailty for study-site to account for additional variation related to study sites (Annex 1). Studies with 42 days follow up or more will be included. Primaquine dose (exposure of interest) 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 elimination half-life (eg AL vs DP/CQ/Mq). Co-linearity between relapse periodicity and geographical region and transmission intensity will be examined. Additional covariates will be examined including blood schizontocidal mg/kg dose level of treatment supervision, baseline temperature and haemoglobin, acute vomiting of the drug and GI tolerability. Some variables will have less complete data and are also expected to have less impact on efficacy (eg baseline Hb and temperature) and thus will not be included in the primary multivariable model. Variation in the effect of total primaquine dose groups over time will be explored. 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. Subgroup analyses for geographic region, relapse periodicity region, transmission intensity and age group will be undertaken.

- A separate Cox regression analysis will be repeated with expected primaquine duration as the exposure of interest. The model will be controlled for potential confounders including primaquine mg/kg dose, sex, age and baseline parasitaemia.

- Additional Cox regression analysis will be undertaken to investigate primaquine and carboxyprimaquine concentrations (on day 6 and 13) as an exposure variable of interest to determine their association with risk of recurrence independent of primaquine dose.

- Depending on feasibility, **network meta-analyses** will be undertaken to synthesise the direct and indirect evidence separately for locations with low and high relapse periodicity to compare treatment with no primaquine use, ow dose total primaquine (including very low dose and low dose regimens) and high dose total primaquine. The network meta-analyses will be undertaken for the outcomes of time to first vivax recurrence during follow-up (180 and 365 days). If enough study data are available that

meet the transitivity assumption then individual patient data from studies with two or more of these treatment arms and a follow up of 42 days or more will be included in a one-stage network meta-analysis model. The transitivity assumption requires that all competing interventions are jointly randomizable (12). The model will be fitted with random effects to allow for between-study heterogeneity. A frequentist approach will be undertaken. Consistency (ie coherence) between direct and indirect effects will be assessed.

- Using the final overall multivariable model and the subgroup models, the relationship between the mg/kg dose of primaquine (mg/kg) and the predicted risk of recurrence by days 90 and 180 will be explored using either natural spline models or categorisation of mg/kg dose.

- Subgroup analyses will be undertaken for the following:
  - Patients treated with chloroquine
  - Studies with a minimum 6 months follow up
  - Studies with high vs low relapse periodicity
  - Studies with treatment arms comparing primaquine to no primaquine
  - Individual countries where feasible

- **Cox regression analyses** and **Network meta-analyses** will be repeated with symptomatic recurrence as the endpoint of interest.

2.6.3.3. Incidence rate of P. vivax recurrence within 180/365 days

- Incidence rates of recurrent vivax parasitaemia over 180 and 365 days will be calculated for treatment without primaquine, treatment with very low dose primaquine, 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 age, sex, baseline parasitaemia, and relapse periodicity. Incidence Rate Ratio (IRR) derived from a negative

binomial regression model with clustering by study site. 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. Subgroup analyses for geographic region, relapse periodicity region, blood schizontocidal drug, and age group will be undertaken.

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.

- Depending on feasibility network meta-analyses will be undertaken separately for locations with low and high relapse periodicity similar to Section 2.6.3.2. However, these network meta-analyses will be undertaken for the outcomes of incidence rates of recurrent vivax parasitaemia over 180 and 365 days.

- Additional analyses will be undertaken to investigate primaquine and carboxyprimaquine concentrations (on day 6 and 13) as an exposure variable of interest to determine their association with incidence rates of recurrent vivax parasitaemia at 180 days independent of primaquine dose.

2.6.3.4. Mixture model to assess efficacy of primaquine

- To better estimate the efficacy of primaquine a time to event model will be used that takes into account the unknown but estimatable proportion of recurrences that are reinfections. Data from studies with a minimum of 180 days follow up that follow patients through multiple epsiodes of malaria will be included in the model. Time to recurrence will be modelled as a mixture of four distributions, with mixture weights depending on the treatment of the previous episode and the mixture distributions corresponding to the different recurrence states (13).

2.6.3.5. Parasite clearance following treatment

The proportions of patients positive for parasitaemia at days 1, 2 and 3 will be assessed. The associations between positive parasitaemia at day 1, 2 and 3 or delayed parasite clearance, and the time to first vivax recurrence within 90 and 180 days will be assessed separately using Cox regression analysis. The effect of daily primaquine mg/kg dose and primaquine use on the odds of delayed parasite clearance (still parasitaemia on day 2 or later) will be assessed using a multivariable logistic regression model with study sites fitted as a random effect. Primaquine use and mg/kg dose (exposures) will be controlled for potential confounders including age, sex, baseline parasitaemia and relapse periodicity. The effect of baseline haemoglobin as a confounding covariate will be explored.

2.6.4 Tolerability related analyses:

2.6.4.1. Risk of intolerance following primaquine

- The percentage of patients with each of the following symptoms reported on day 6+/-1 (vomiting, nausea, anorexia, abdominal pain, diarrhoea, dizziness) will be presented in a tabular format. A composite endpoint including any of vomiting, diarrhoea and anorexia on day 6+/-1 will also be presented. Results will be presented by daily primaquine mg/kg dose (no primaquine, low dose, intermediate dose and high dose).

- In view of the confounding effect of malaria and coadministration of schizontocidal drugs, the composite endpoint will be repeated to identify the presence of any gastrointestinal symptoms in patients on day 0 and on days 1-2 and compared to the percentages on day 6+/-1.

- 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 fulfilling criteria for the composite gastrointestinal endpoint a on day 6+/-1. 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 versus any two symptoms versus one symptom. Separate analyses will be undertaken for each gastrointestinal symptom. Primaquine dose will be controlled for potential confounders including age, sex, and parasitaemia on day 0, with study site included as a random effect. The effect of nutritional status will be explored. Sensitivity analyses will be undertaken for each gastrointestinal symptom adjusting for presence of that symptom on day 0.

- Subgroup analyses will be undertaken for individual countries where feasible.
- The **multivariable logistic regression** analysis will be repeated for the composite gastrointestinal endpoint on day 1/2. This analysis will be restricted to patients in a treatment arm without primaquine or starting primaquine on day 0. The effect of primaquine dose will be controlled for potential confounders including age, sex and parasitaemia on day 0 with study site included as a random effect. To investigate the effect of schizontocidal treatment, which is collinear with study site, separate subgroup analyses will be undertaken for different schizontocidal treatments.

2.6.4.2. 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 dose (no primaquine, low dose, intermediate dose and high dose) and the risks presented on days 0 to 2 and days 3-14.

- 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 on any day between 0 and 14. Primaquine dose will be controlled for potential confounders including age, sex, day 0 parasitaemia, schizontocidal drug and and day primaquine was commenced, with study site included as a random effect. The effect of nutritional status and vomiting on day 0 will be explored.

- Subgroup analyses will be undertaken for individual countries where feasible.

2.6.5 Safety related analyses:

2.5.5.1. Risk of additional adverse events

- The percentage of patients with each of the following will be presented, categorised by daily primaquine mg/kg dose (no primaquine, low dose, intermediate dose and high dose) in patients with G6PD activity ≥30%:
  - ≥25% drop in haemoglobin to below 7g/dL between baseline and days 1 - 14
  - Blood transfusion on days 1 - 28
  - >5g/dL drop in haemoglobin between baseline and days 1 - 14.
  - Haemoglobin fall to below 5 g/dL between days 1 - 14
  - Renal failure needing dialysis between days 1 - 28
  - Death between days 1 - 28
  - Development of mild, moderate or severe anemia by day i) 2/3 or ii) day 6+/-1
  - Presence of clinical and severe methaemoglobinaemia between days 1 – 14
  - Maximum change in haemoglobin between i) baseline and the minimum measurement on day 2/3 or ii) baseline and the minimum measurement on day 6+/-1

  Subgroup analysis will be undertaken to compare G6PD deficient (<30% activity) versus G6PD intermediate (30-<70%) versus G6PD normal (>=70%) versus G6PD unknown patients.

- Linear mixed effects modelling will be used to assess the impact of primaquine dose on haemoglobin over time, with estimation of the effect of dose on haemoglobin between day 1 and day 45 in patients with >=30% G6PD activity. The mean Hb-time response following treatment will be estimated using a linear mixed effects model with non-linear terms, derived by fractional polynomial regression; with fixed effects for age, sex, parasitaemia on day 0, and haemoglobin at day 0; with random effects for the terms for time, individuals and site. The inclusion of transmission intensity and/or relapse periodicity will be explored based on their correlation. The interaction between primaquine dose category (no primaquine, low, intermediate and high daily dose) and time will be included, in order to capture effect modification of the Hb profile over time across primaquine doses.

  Multivariable logistic regression analyses will be undertaken to determine the effect of daily mg/kg primaquine dose on the odds of the above adverse events in patients with >=30% G6PD activity. Primaquine dose will be controlled for potential confounders including age, sex, parasitaemia at day 0, and haemoglobin at day 0, with study site

included as a random effect. Subgroup analysis will be undertaken to compare G6PD deficient (<30% activity) versus G6PD intermediate (30-<70%) versus G6PD normal (>=70%) versus G6PD unknown patients.

- Further multivariable logistic regression analyses will be undertaken to determine the effect of the total mg/kg primaquine dose (categorised as no primaquine, low dose and high dose) on the odds of a subset of the above adverse reactions occurring after day 14:
  - Blood transfusion day 15 - 28
  - Renal failure needing dialysis between days 15 - 28
  - Death between days 15 – 28

- The maximum absolute change in haemoglobin separately between baseline and day 2/3 and day 6+/-1 in patients with >=30% G6PD activity will be calculated and presented visually (histogram and box and whisker plot) by daily primaquine mg/kg dose (no primaquine, low dose, intermediate dose and high dose). Subgroup analysis will be undertaken to compare G6PD deficient (<30% activity) versus G6PD intermediate (30-<70%) versus G6PD normal (>=70%) versus G6PD unknown patients.

- **Multivariable linear regression analyses** will be undertaken to determine the effect of daily mg/kg primaquine dose (categorised as no primaquine, low dose, intermediate dose and high dose) on haemoglobin separately at day 2/3 and day 6+/-1 in patients with >=30% G6PD activity. The effect of primaquine dose will be controlled for potential confounders including age, sex, parasitaemia at day 0 and haemoglobin at day 0, with study site included as a random effect. Subgroup analysis will be undertaken to compare G6PD deficient (<30% activity) versus G6PD intermediate (30-<70%) versus G6PD normal (>=70%) versus G6PD unknown patients.

- Subgroup analyses will be undertaken for individual countries where feasible.

2.6.6 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.
- Sensitivity analyses will be carried out by restricting the analysis to patients for whom the actual administered primaquine dose is known and comparing with the overall estimates.

2.6.7 Risk of bias relating to individual studies

- Potential bias relating to individual studies will be assessed using the ROB2 tool (14) for randomised controlled trials and the Joanna Briggs Institute Checklist for Case Series for single arm efficacy studies.

3. PRISMA Statement

The analysis will adhere to the PRISMA-IPD guidelines for reporting systematic reviews and meta-analyses of individual patient data (15).

4. Tools

All statistical analyses will be carried out using Stata (StataCorp, College Station, Texas). However, when equivalent statistical methods are applied in a different statistical software package (e.g. R statistical software), changing the use of statistical software will not require amendment of this SAP.

5. Study Group Governance, Management, Coordination and Publication Policy

The Vivax Primaquine Efficacy and Safety 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. Rob Commons, Megha Rajasekhar, Ric Price and Julie Simpson will oversee the statistical analyses. Participating investigators will be recognised in publication as contributors under the banner of the **Vivax Primaquine Efficacy and Safety Study Group**. A Writing Committee will coordinate activities including data analysis, and drafting of publications and reports for complete group review. The Writing Committee will comprise Rob Commons, Megha Rajasekhar, Julie Simpson, Ric Price, James Watson, Nick White, Bob Taylor and other interested investigators. They are responsible for undertaking the data analysis and preparation of the manuscript. Authors will be recognised according to the ICMJE guidelines and the WWARN publication policy (16).

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7. Annex 1 – Model assumptions

The association between primaquine dose and the log rate of recurrence will be checked visually, and the proportional hazards assumption tested using Schoenfeld residuals. If non-proportional hazards are present, interactions between terms and time will be evaluated.