Statistical Analysis Plan
WWARN Primaquine for Radical Cure of *Plasmodium vivax* and *Plasmodium ovale*: A pooled analysis investigating the tolerability, safety and efficacy of primaquine in paediatric patients compared with adults
Version 1.3

WorldWide Antimalarial Resistance Network (WWARN)

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Note: This statistical analysis plan was generated for a detailed report commissioned by the sponsor. A manuscript will be generated using a subset of the analyses described in this plan.

Version number	Revision(s) & reason for amendment	Release date
V1.1		14 Feb 2022
V1.2	• Updated Efficacy Analysis to consider weekly PQ and GI Tolerability Analysis inclusion criteria to daily primaquine as intermittent PQ dosing was not considered within analyses previously • Updated timing of Haematological 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 Haematological Safety Analysis primary and secondary endpoints to day 2/3 and day 6+/-1 given variable sampling, and for severe adverse events to days 1-13 to ensure early haemolytic events captured. • Updated GI Tolerability Analysis primary and secondary endpoints to day 6+/-1 given variable sampling of symptom checklist data. • Updated GI Tolerability Analysis secondary endpoints prior to day 3 from day 0/1 to day 0 and day 1/2 to distinguish acute malaria pre-treatment and schizontocidal impact. • Removed day 7 to day 42 endpoint from Efficacy Analysis as already multiple endpoints (day 90, 180, 365) and day 42 unlikely to provide additional data. • 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), day PQ commenced (as endpoint now focused on specific days), 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). • The definition of serious adverse events was included as this had previously been missing.	30 May 2022

V1.3	For Adverse Event IPD meta-analyses:	21 Jun 2022
	• As gastrointestinal adverse events generally occur shortly after dosing, their occurrence between 2 and 14 days of first primaquine administration will also be analysed.	
	• The total number of adverse events in each of these categories will be summarised for those in primaquine and not primaquine arms.	
	• Adverse event regression analyses will investigate both daily dose and total dose prior to AE start. Categorical dose variables are specified as: Patients receiving no primaquine versus low (<0.375 mg/kg) versus intermediate (0.375-<0.75 mg/kg) versus high (0.375-<0.75 mg/kg) daily doses of primaquine; total dose prior to start of each adverse event will be extrapolated from these daily dose categories and treatment duration.	
	• Inclusion criteria clarified with additions shown in italics:	
	○ *Eligible for randomisation to* at least one treatment arm with primaquine radical cure regimen commencing within seven days of blood schizontocidal treatment	
	○ Adverse event description and start date (*relative to primaquine initiation, or equivalent schedule in placebo / no primaquine arms).*	
	• Covariates added: Total dose prior to start of adverse event as more accurate than total dose or daily dose, particularly for non-GIT adverse events.	
	• Covariates excluded: relapse periodicity	
	• Covariates clarified (changes in italics):	
	○ Given the higher risk of anaemia, a common adverse event among adult women: Patients aged between 6 months and 1 year (data permitting) versus children 1 year to less than 5 years versus children 5 to <15 years old *versus adults (aged 15+ years,* stratified by sex).	
	○ G6PD deficient versus G6PD intermediate versus G6PD normal paediatric patients (genotype and/or phenotype), data permitting	
	• Covariates added to line listing of adverse events: raw adverse event term, sex, total primaquine dose and duration till day of AE	
	Additional analysis: to quantify potential reporting bias in open label studies: The risk of anaemia reported as an adverse event will be compared with that defined based on changes in haemoglobin as defined in 8.5 below among the sub-set of studies that report at least one case of anaemia as an adverse event.	

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Contents

1. Introduction and Rationale

Primaquine, an 8-aminoquinoline (8-AQ), is the only widely available antimalarial that kills dormant liver stages (hypnozoites) of *Plasmodium vivax* and *Plasmodium ovale* [1]. *Plasmodium vivax* remains widespread and is becoming the predominant cause of malaria outside of Africa and is expected to be a greater obstacle to malaria elimination than *Plasmodium falciparum*.

The main burden of malaria is in young children and yet there is no suitable paediatric formulation available. Reliance on the adult tablet formulation may result in inaccurate paediatric dosing, and thus have a higher risk of adverse events and lower efficacy in preventing *P. vivax* recurrence.

Whilst primaquine is highly efficacious against hypnozoites and gametocytes, it can cause severe drug-induced haemolysis in individuals with G6PD deficiency. The risk of haemolysis depends on the duration and dose of primaquine administration, which is almost 20-fold higher for *P. vivax* radical cure than the single low dose administered for *P. falciparum* gametocytocidal activity [2]. At high doses, primaquine induces gastro-intestinal symptoms that often reduce patient adherence.

Since its introduction into clinical practice more than 60 years ago there is a wealth of data from clinical trials documenting the safety and efficacy of primaquine in patients with *P. vivax* malaria, but relatively limited data in *P. ovale* malaria. Individual patient data (IPD) meta-analyses ensure the best use of available data from which to generate evidence that will inform improved dosing regimens with a new, clinically relevant, age-appropriate formulation. A paediatric formulation will result in improved safety, tolerability, efficacy, and adherence.

2. Aim of the study

The aim of these individual patient data (IPD) meta-analyses is to assess the tolerability, safety, and efficacy of primaquine when used for the radical cure (RC) of *Plasmodium vivax* (Pv) / *Plasmodium ovale* (Po) in paediatric patients, comparing:
   a) Children (<15 years) receiving low versus high daily and total doses of primaquine
   b) Patients aged less than 15 years versus adults
   c) Patients aged between 6 months and 1 year versus young children (aged <5) and versus older children (5 to <15 years)
   d) G6PD deficient versus G6PD normal paediatric patients (data permitting)
   e) Patients with documented CYP2D6 polymorphisms versus with those with wild-type CYP2D6, data permitting.

3. Eligibility criteria

3.1. Eligibility criteria for Gastrointestinal Tolerability IPD meta-analysis

3.1.1. Essential inclusion criteria for GI Tolerability analysis

- Clinical efficacy study published or undertaken from January 2000 to August 2021, inclusive
- Uncomplicated *P. vivax* or *P. ovale* or mixed infection
- A minimum 28 days follow up
- At least one treatment arm with a daily primaquine radical cure regimen commencing within three days of blood schizontocidal treatment
- Information on dose of schizontocidal treatment (at least as per protocol)
- Information on use, timing, mg/kg dose and duration of primaquine (at least as per protocol)

- Study includes at least one patient aged under 15 years
- Tolerability checklist including at least one of vomiting, nausea, anorexia, abdominal pain and/or diarrhoea
- Date of birth or age
- Sex data
- Asexual *P. vivax* or *P. ovale* parasite density at day 0

3.1.2. Desirable inclusion criteria for GI Tolerability analysis

- Baseline weight
- Individual primaquine dosing data (mg/kg dose; actual dose times; treatment supervision)
- Individual schizontocidal dosing data (mg/kg dose; actual dose times; treatment supervision)
- Qualitative or quantitative assessment of G6PD status
- CYP2D6 genotype/phenotype (ie activity score)
- Presence or absence of fever at day 0 or within the previous 24 hours
- Duration of symptoms
- Outcome of malaria treatment according to standardised WHO/CDISC criteria
- Any concomitant medication documented

3.1.3. Exclusion criteria for GI Tolerability analysis

- Study level:
  - *P. falciparum* transmission blocking study
  - Mass drug administration study
  - Human challenge study
  - Healthy volunteer study
  - Prophylaxis study
- Patient level:
  - Severe malaria on enrolment including severe anaemia (Hb<5 g/dL or Hct <15% on day 0)
  - Treatment with an alternative hypnozoiticidal agent (i.e. tafenoquine)
  - Adjunctive treatment with an additional antimalarial in the first 14 days after commencing treatment

3.2. Eligibility criteria for adverse event IPD meta-analysis

3.2.1. Essential inclusion criteria for adverse event analysis

- Clinical efficacy study published or undertaken from January 2000 to August 2021, inclusive
- Uncomplicated *P. vivax* or *P. ovale* or mixed infection
- A minimum 28 days follow up
- Eligible for randomisation to at least one treatment arm with primaquine radical cure regimen commencing within seven days of blood schizontocidal treatment
- Information on dose of schizontocidal treatment (at least as per protocol)
- Information on use, timing, mg/kg dose and duration of primaquine (at least as per protocol)
- Study includes at least one patient aged under 15 years
- Adverse event description and start date (relative to primaquine initiation, or equivalent schedule in placebo / no primaquine arms).
- Date of birth or age

- Sex

3.2.2. Desirable inclusion criteria for adverse event analysis

- Baseline weight
- Individual primaquine dosing data (mg/kg dose; actual dose times; treatment supervision)
- Individual schizontocidal dosing data (mg/kg dose; actual dose times; treatment supervision)
- Nutritional status according to weight/ height/ middle upper arm circumference
- Qualitative or quantitative assessment of G6PD status
- CYP2D6 genotype/phenotype (ie activity score)
- Outcome of malaria treatment according to standardised WHO/CDISC criteria
- Adverse event end date, grade, causality assessment
- Adverse event elicitation methodology specified
- Any concomitant medication documented

3.2.3. Exclusion criteria for adverse event analysis

- Study level:
  - *P. falciparum* transmission blocking study
  - Mass drug administration study
  - Human challenge study
  - Healthy volunteer study
  - Prophylaxis study
- Patient level:
  - Severe malaria on enrolment including severe anaemia (Hb<5 g/dL or Hct <15% on day 0)
  - Treatment with an alternative hypnozoiticidal agent (i.e. tafenoquine)
  - Adjunctive treatment with an additional antimalarial in the first 14 days after commencing treatment

3.3. Eligibility criteria for haematological safety IPD meta-analysis

3.3.1. Essential inclusion criteria for haematological safety analysis

- Clinical efficacy study published or undertaken from January 2000 to August 2021, inclusive
- Uncomplicated *P. vivax* or *P. ovale* or mixed infection
- A minimum 28 days follow up
- At least one treatment arm with primaquine radical cure regimen commencing within seven days of blood schizontocidal treatment
- Information on dose of schizontocidal treatment (at least as per protocol)
- Information on use, timing, mg/kg dose and duration of primaquine (at least as per protocol)
- Study includes at least one patient aged under 15 years
- Haemoglobin or haematocrit measured at least on day 0 and one or more follow up measurements
- Date of birth or age
- Sex

3.3.2. Desirable inclusion criteria for haematological safety analysis

- ≥42 days follow up

- Baseline weight
- Individual primaquine dosing data (mg/kg dose; actual dose times; treatment supervision)
- Individual schizontocidal dosing data (mg/kg dose; actual dose times; treatment supervision)
- Nutritional status according to weight/ height/ middle upper arm circumference
- Qualitative or quantitative assessment of G6PD status
- CYP2D6 genotype/phenotype (ie activity score)
- Outcome of malaria treatment according to standardised WHO/CDISC criteria
- Presence or absence of fever at day 0 or within the previous 24 hours
- Additional haematology measures (including methaemoglobin, transfusion needed, leucopaenia, haemoglobinuria and thrombocytopenia)
- Any concomitant medication documented

3.3.3. *Exclusion criteria for haematological safety analysis*
- Study level:
  - *P. falciparum* transmission blocking study
  - Mass drug administration study
  - Human challenge study
  - Healthy volunteer study
  - Prophylaxis study
- Patient level:
  - Severe malaria on enrolment including severe anaemia (Hb<5 g/dL or Hct <15% on day 0)
  - Treatment with an alternative hypnozoiticidal agent (i.e. tafenoquine)
  - Adjunctive treatment with an additional antimalarial in the first 14 days after commencing treatment

3.4. Eligibility criteria for efficacy IPD meta-analysis

3.4.1. *Essential inclusion criteria for efficacy analysis*
- Clinical efficacy study published or undertaken from January 2000 to August 2021, inclusive
- Follow up duration of $\geq$42 days
- Uncomplicated *P. vivax* or *P. ovale* or mixed infection
- At least one treatment arm with primaquine radical cure regimen commencing within seven days of blood schizontocidal treatment
- Information on dose of schizontocidal treatment (at least as per protocol)
- Information on use, timing, dose and duration of primaquine (at least as per protocol)
- Study includes at least one patient aged under 15 years
- Asexual *P. vivax* or *P. ovale* parasite density at enrolment and presence or absence during follow up
- Date of birth or age
- Sex

3.4.2. *Desirable inclusion criteria for efficacy analysis*
- Baseline weight
- Individual primaquine dosing data (mg/kg dose; actual dose times; treatment supervision)
- Individual schizontocidal dosing data (mg/kg dose; actual dose times; treatment supervision)

- Nutritional status according to weight/ height/ middle upper arm circumference
- Qualitative or quantitative assessment of G6PD status
- CYP2D6 genotype/phenotype
- Haemoglobin or haematocrit at enrolment
- Outcome of malaria treatment according to standardised WHO/CDISC criteria
- Any concomitant medication documented

3.4.3. *Exclusion criteria for efficacy analysis*

- Study level:
  - *P. falciparum* transmission blocking study
  - Mass drug administration study
  - Human challenge study
  - Healthy volunteer study
  - Prophylaxis study
- Patient level:
  - Severe malaria on enrolment including severe anaemia (Hb<5 g/dL or Hct <15% on day 0)
  - Treatment with an alternative hypnozoiticidal agent (i.e. tafenoquine)
  - Adjunctive treatment with an additional antimalarial in the first 14 days after commencing treatment

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. Data from unpublished studies will also be included if available.

4.1. Data management

Data sets uploaded to the WWARN repository will be standardized using the WWARN Clinical Data Management and Statistical Analysis Plan [3] and IDDO-CDISC nomenclature into quality-assured IPD sets. Adverse event raw terms will be coded using the same version of the Medical Dictionary for Regulatory Activities (MedDRA) and summarized by Preferred Term and System Organ Class. 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.

4.2. Study Group governance, co-ordination and membership

The Study Group comprises participating investigators who contribute relevant IPD sets to the IPD meta-analysis. The Study Group collectively makes decisions with respect to data analysis and plans for publication. Participating investigators will be recognised in publication as contributors under the banner of the Paediatric Primaquine Study Group. A Writing Committee will coordinate activities, undertaking the data analysis and preparation of the reports and publications for review by the study group and sponsor. Authors will be recognised according to the ICMJE guidelines and the WWARN publication policy.

5. Objectives

5.1. Paediatric Primaquine Gastro-Intestinal (GI) Tolerability

PRIMARY: to assess GI tolerability in children (<15 years) and how this relates to daily dose of primaquine.
SECONDARY: to assess if the relationship between daily dose of primaquine and GI tolerability differs for young children, older children and adults

5.2. Paediatric Primaquine Adverse Events

PRIMARY: to compare the incidence, and nature of serious adverse events within 28 days in young children (<5 years) to that in older children and adults.
SECONDARY:
To describe any adverse event within 28 days and specifically haemoglobinuria, anaemia, leucopoenia, methaemoglobinaemia, blood transfusions, vomiting, anorexia, diarrhoea, abdominal pain, QT prolongation, and compare the frequency and severity of these in young children (<5 years) to that in older children and adults, data permitting.

5.3. Paediatric Primaquine Haematological Safety

PRIMARY:
i. To describe and compare the acute absolute reductions in haemoglobin between a) baseline and day 2/3 and b) baseline and day 6 +/- 1 in children (<15 years) and how this relates to daily dose of primaquine.
ii. To describe and compare falls in haemoglobin by >25% to below 7g/dL between baseline and any day between 1 – 13 in children (<15 years) and how this relates to daily dose of primaquine.
SECONDARY:
i. To assess if the relationship between daily dose of primaquine and reductions (absolute and relative) in haemoglobin differs between young children, older children and adults
iii. To describe cases of methemoglobinemia and leucopoenia and compare the frequency and severity of these cases in young children (<5 years) to that in older children and adults, data permitting.

5.4. Paediatric Primaquine Efficacy

PRIMARY: to assess efficacy in terms of the prevention of *P. vivax* / *P. ovale* recurrence between day 7 and day 180 in children (<15 years) and how this relates to total dose of primaquine
SECONDARY:
i. To assess efficacy in terms of the prevention of *P. vivax* / *P. ovale* recurrence between day 7 and day 90 and 365 in children (<15 years) and how this relates to total dose of primaquine
ii. To assess if the relationship between total primaquine dose and the incidence risk of *P. vivax* (or *P. ovale*) recurrence between day 7 and days 90, 180 and 365 differs for young children, older children and adults

6. Endpoints

6.1. Study endpoints

6.1.1. Primary

- Gastro-intestinal tolerability:
    - A <u>composite</u> endpoint including any of the following symptoms of gastro-intestinal disturbance on day 6+/-1: vomiting, anorexia, diarrhoea
- Adverse events
    - Serious adverse events within 28 days of first primaquine administration
- Haematological safety
    - >25% drop in haemoglobin to below 7g/dL between baseline and days 1 – 13
    - Maximum absolute reduction in haemoglobin between baseline and day 2/3
    - Maximum absolute reduction in haemoglobin between baseline and day 6+/-1
- Efficacy
    - Incidence risk of *P. vivax* or *P. ovale* recurrence between day 7 and day 180 (PCR unadjusted)

6.1.2.   *Secondary gastro-intestinal tolerability endpoints:*

- Presence of the following symptoms as separate endpoints on day 6+/-1: vomiting, anorexia and diarrhoea.
- 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

6.1.3.   *Secondary adverse event endpoints:*

- Any adverse event within 28 days of first primaquine administration
- The following adverse events of special interest within 28 days of first primaquine administration: haemoglobinuria, anaemia, leucopoenia, methaemoglobinaemia, blood transfusions, QT prolongation, vomiting, anorexia, diarrhoea and abdominal pain.
- As gastrointestinal adverse events generally occur shortly after dosing, their occurrence between 2 and 14 days of first primaquine administration will also be analysed.

6.1.4.   *Secondary haematological safety endpoints:*

- Relative reduction in haemoglobin between i) baseline and the minimum measurement on day 2/3 and ii) baseline and the minimum measurement on day 6+/-1
- Receiving blood transfusion within 14 days of last primaquine treatment
- >5g/dL drop in haemoglobin between baseline and the minimum measurement on days 1 - 13.
- >2g/dL/day fall in haemoglobin between baseline and the minimum measurement on days 1 - 13
- Development of anaemia by day 2/3 or separately by day 6+/-1:
    - mild (Hb ≥8 g/dL and <11g/dl),
    - moderate anaemia (Hb ≥5 g/dL and Hb <8g/dl)
    - severe anaemia (Hb <5g/dL)
- Leucopoenia between day 3 and day 13
- Presence of clinical methaemoglobinaemia (>10%) and severe methaemoglobinaemia (>20%) 1-14 days after starting primaquine treatment
- Day of peak methaemoglobin value (percentage)

6.1.5.   *Secondary efficacy endpoints:*

- Incidence risk of *P. vivax* or *P. ovale* recurrence between day 7 and day 90, and day 7 and day 365 (PCR unadjusted)
- Incidence rate of symptomatic *P. vivax* or *P. ovale* recurrences between day 7 and day 90, day 7 and day 180, and day 7 and day 365 (PCR unadjusted)
- Incidence rate of any *P. vivax* or *P. ovale* recurrences between day 7 and day 90, day 7 and day 180, and day 7 and day 365 (PCR unadjusted)

6.2. Endpoint definitions

P. vivax or *P. ovale recurrence before day X* is defined as any recurrence of *P. vivax* or *P. ovale* parasitaemia between day 7 and X irrespective of symptoms. Defined separately for each species.

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% [4].

Severe methaemoglobinaemia will be defined as a methaemoglobin level >20% [4].

Leucopaenia, neutropaenia and thrombocytopaenia will be defined as > Grade 2 in NIH Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Paediatric Adverse Events [5].

Haemolysis will be defined as haemoglobinuria or "dark urine" [6].

Adverse Events and Serious Adverse Events will be considered according to the primary studies' categorisations, assumed to be the following unless otherwise indicated:

- A*dverse events*: Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6).

- Serious adverse events: A serious adverse event (experience) or reaction is any untoward medical occurrence that at any dose results in death, is life-threatening, *NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe*, requires inpatient hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect. Medical and scientific judgement should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above. *These should also usually be considered serious.* Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalisation; or development of drug dependency or drug abuse (ICH E2A).

Only adverse events known to occur after the administration of primaquine will be considered (i.e. treatment-emergent). If day of onset is missing, or time of onset in relation to the primaquine dose is not recorded we will contact the investigator for clarification.

Grading and causality classification of adverse events are as by the primary study investigator, standardised as mild (grade 1), moderate (grade 2), severe (grade 3) and life-threatening (grade 4) as necessary.

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, 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, 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); total dose prior to adverse event.

Laboratory: parasitaemia, haemoglobin concentration, methaemoglobinaemia, white cell count, platelet count

Children will be considered as aged <15 years with childhood stratified into patients <1 year, 1 to <5 years and 5 to <15 years where possible.

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 [7]. 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:
- o *Supervised* if all doses were directly observed
- o *Partially supervised* if ≥1 dose were observed
- o *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 [8]:

Haematocrit (ht) = 5.62 + 2.60 * 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 [9].

b) *Low (long) and high (short) periodicity of relapses* according to Battle's regions [10], 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 [11, 12] 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 [13].

Association with primaquine will be considered as not related / possibly related. In studies with 1-5 ratings, adverse events rated as 3, 4, 5 (or terms such as possibly, probably, definitely related to primaquine) will be considered as possibly related. If the association is not assessed specifically for primaquine, but to study drugs, it will be assumed to be relevant to both schizontocidal drug and primaquine.

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 [14].

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.

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, geographical area, treatment given, food intake with primaquine, follow up duration, study populations, description of location by country, transmission intensity, and regional relapse periodicity.

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:

A summary of relevant baseline patient characteristics will be presented for the whole paediatric population compared to adults and by subgroups as follows:
   a) Children (<15 years) receiving low versus high daily and total doses of primaquine;
   b) Patients aged between 6 months and 1 year (data permitting) versus children 1 year to less than 5 years versus children 5 to <15 years old;
   c) G6PD deficient versus G6PD normal paediatric patients (genotype and/or phenotype), data permitting;
   d) Paediatric patients with documented CYP2D6 polymorphisms versus with those with wild-type CYP2D6, data permitting.
   e) Male versus female sex
   f) Geographical area and season (if available)

Variables presented (data permitting) 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 or within prior 24 hours) 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. Gastro-intestinal tolerability related analyses:

8.3.1. Risk of intolerance following primaquine

The percentage and 95% confidence intervals of patients with each of the following symptoms reported separately on day 6+/-1 (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 on day 6+/-1 will also be presented. Results will be presented for the whole paediatric population compared to adults and by subgroups as follows:

a) Children (<15 years) receiving low versus intermediate versus high daily doses of primaquine;

b) Patients aged between 6 months and 1 year (data permitting) versus children 1 year to less than 5 years versus children 5 to <15 years old;

c) Paediatric patients with documented CYP2D6 polymorphisms versus with those with wild-type CYP2D6, data permitting.

8.3.2. In view of the confounding effect of malaria and coadministration of schizontocidal drug/s, the percentage of patients with the composite endpoint in 8.3.1 will also be assessed in patients on day 0 and on days 1-2 and compared to the percentages on day 6+/-1.

8.3.3. A **multivariable logistic regression** analysis will be undertaken to determine whether age modifies the effect of <u>daily</u> 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 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 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 and parasitaemia on day 0 with study site included as a random effect. The effect of nutritional status will be explored.   ity analyses will be undertaken for each gastrointestinal symptom excluding patients presenting with that symptom on day 0. Following comparison of the whole paediatric population compared to adults, subgroup analysis will be undertaken within the paediatric population to compare patients aged between 6 months and 1 year (data permitting) versus children 1 year to less than 5 years versus children 5 to <15 years old. Data permitting, additional analyses will be undertaken comparing i) G6PD deficient versus G6PD normal paediatric patients (genotype and/or phenotype) and ii) patients with documented CYP2D6 polymorphisms versus with those with wild-type CYP2D6.

8.3.4. The **multivariable logistic regression** analysis in 8.3.3 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 days 0. The effect of primaquine dose will be controlled for potential confounders including 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. Following comparison of the whole paediatric population with adults, subgroup analysis will be undertaken within the paediatric population to compare patients aged between 6

months and 1 year (data permitting) versus children 1 year to less than 5 years versus children 5 to <15 years old.

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 presented on days 0 to 2 and days 3-13 for the whole paediatric population compared to adults and by subgroups as follows:

a) Children (<15 years) receiving no versus low versus intermediate versus high daily doses of primaquine;
b) Patients aged between 6 months and 1 year (data permitting) versus children 1 year to less than 5 years versus children 5 to <15 years old;
c) G6PD deficient versus G6PD normal paediatric patients (genotype and/or phenotype), data permitting;
d) Paediatric patients with documented CYP2D6 polymorphisms versus with those with wild-type CYP2D6, data permitting

8.3.6. A **multivariable logistic regression** analysis will be undertaken to determine whether age modifies 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 13. The effect of primaquine dose will be controlled for potential confounders including sex, day 0 parasitaemia, schizontocidal drug 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. Following comparison of the whole paediatric population with adults, subgroup analysis will be undertaken within the paediatric population to compare patients aged between 6 months and 1 year (data permitting) versus children 1 year to less than 5 years versus children 5 to <15 years old. Data permitting, additional analyses will be undertaken comparing i) G6PD deficient versus G6PD normal paediatric patients (genotype and/or phenotype) and ii) patients with documented CYP2D6 polymorphisms versus with those with wild-type CYP2D6.

8.4. Adverse event related analyses:

8.4.1. Risk of adverse events following primaquine

The percentage of patients (and 95% CI) with at least one AE within each of the following categories (SAEs, AEs, haemoglobinuria, anaemia, leucopoenia, methaemoglobinaemia, blood transfusions, QT prolongation, vomiting, anorexia, diarrhoea, abdominal pain) reported within 28 days after the first primaquine administration will be presented in a tabular format. The total number of adverse events in each of these categories will also be summarised for those in primaquine and not primaquine arms. Results will be presented for the whole paediatric population compared to adults and by subgroups as follows:

a) Patients receiving no primaquine versus low (<0.375 mg/kg) versus intermediate (0.375-<0.75 mg/kg) versus high (0.375-<0.75 mg/kg) daily doses of primaquine; total dose prior to start of each adverse event will be extrapolated from these daily dose categories and treatment duration.
b) Patients aged between 6 months and 1 year (data permitting) versus children 1 year to less than 5 years versus children 5 to <15 years old versus adults (aged 15+ years, stratified by sex);

c) G6PD deficient versus G6PD intermediate versus G6PD normal paediatric patients (genotype and/or phenotype), data permitting;

d) Paediatric patients with documented CYP2D6 polymorphisms versus with those with wild-type CYP2D6, data permitting.

8.4.2. A listing of adverse events by MedDRA system organ class and preferred term and raw term will be presented detailing sex, schizontocidal drug, total primaquine dose and duration till day of AE, primaquine daily dose, day and timing in relation to primaquine dose, age, G6PD status, severity, relatedness and outcome.

8.4.3. A **multivariable logistic regression** analyses will be undertaken to determine whether age modifies the effect of <u>daily</u> mg/kg primaquine dose (both as a continuous variable and a categorical variable categorised as i) low, intermediate or high daily dose and ii) no primaquine, very low dose, low dose and high total dose until start of adverse event) on the odds of developing AEs in each of the specified categories (separate analyses). Effect of primaquine dose will be controlled for potential confounders including sex, parasitaemia, schizontocidal drug/s, presence of fever, g6pd status, and baseline haemoglobin with study site included as a random effect. The effect of nutritional status (using a weight for age Z-score, WAZ<-2 to define underweight-for-age in young children) will be explored, data permitting. Following comparison of the whole paediatric population compared to adults, subgroup analysis will be undertaken within the paediatric population to compare patients aged between 6 months and 1 year (data permitting) versus children 1 year to less than 5 years versus children 5 to <15 years old. Data permitting, additional analyses will be undertaken comparing patients with documented CYP2D6 polymorphisms versus with those with wild-type CYP2D6.

Results will be presented for the whole paediatric population compared to adults and by subgroups as follows:

a) Children (<15 years) receiving low (<0.375 mg/kg) versus intermediate (0.375-<0.75 mg/kg) versus high (0.375-<0.75 mg/kg) daily doses of primaquine;

b) Children (<15 years) receiving very low versus low versus high total doses of primaquine prior to start of adverse event (total dose prior to start of each adverse event will be extrapolated from these daily dose categories and treatment duration;

c) Patients aged between 6 months and 1 year (data permitting) versus children 1 year to less than 5 years versus children 5 to <15 years old.

The risk of anaemia reported as an adverse event will be compared with that defined based on changes in haemoglobin as defined in 8.5 below among the sub-set of studies that report at least one case of anaemia as an adverse event.

8.5. Haematological safety related analyses:

8.5.1. 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) for the whole paediatric population compared to adults in patients with G6PD activity ≥30%:

- $\geq$ 25% drop in haemoglobin to below 7g/dL between baseline and the minimum measurement on days 1 - 13
- Blood transfusion –within 14 days of last primaquine treatment
- >5g/dL drop in haemoglobin between baseline and days 1 - 13.
- >2g/dL/day fall in haemoglobin between baseline and the minimum measurement on days 1 - 13
- Renal failure needing dialysis between days 1 - 28
- Death between days 1 - 28
- Development of mild, moderate or severe anaemia by day i) 2/3 or ii) day 6+/-1
- Presence of severe leucopoenia
- Presence of severe thrombocytopaenia
- Presence of severe methaemoglobinaemia between days 1 – 14

8.5.2. The following endpoints will also be summarised by these categories:
- Maximum absolute reduction in haemoglobin between i) baseline and the minimum measurement on day 2/3 or ii) baseline and the minimum measurement on day 6+/-1
- Maximum relative reduction in haemoglobin between i) baseline and the minimum measurement on day 2/3 or ii) baseline and the minimum measurement on day 6+/-1
- Day of peak methaemoglobin value (percentage)

Additional subgroup analyses will be undertaken within the paediatric population to compare i) patients aged between 6 months and 1 year (data permitting) versus children 1 year to less than 5 years versus children 5 to <15 years old, ii) G6PD deficient (<30% activity) versus G6PD intermediate (30-<70%) versus G6PD normal (>=70%) versus G6PD unknown paediatric patients and iii) patients with documented CYP2D6 polymorphisms versus with those with wild-type CYP2D6.

8.5.3. Linear mixed effects modeling will be used to assess the impact of primaquine dose and age on haemoglobin over time, with estimation of the effect of age on haemoglobin at the day of nadir and day 6 derived from separate models of low, intermediate and high dose primaquine 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 sex, parasitaemia on day 0, and haemoglobin at day 0; with random effects fitted to the terms for time according to an individual within each site. The inclusion of transmission intensity and/or relapse periodicity will be explored based on their correlation. The interaction between age category and time will be included, in order to capture the different time course of Hb responses across age groups.

8.5.4. The maximum absolute reduction in haemoglobin between baseline and day 2/3 and day 6+/-1 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) for patients with G6PD activity >=30%. Similarly, the maximum absolute reduction in haemoglobin at day 6+/-1 will be presented for patients with G6PD activity >=30% by cumulative total mg/kg dose to day 5+/-1 (no primaquine, very low total dose, low total

dose and high total dose). Following comparison of the whole paediatric population to adults, subgroup analyses will compare i) patients aged between 6 months and 1 year (data permitting) versus children 1 year to less than 5 years versus children 5 to <15 years old, ii) G6PD deficient (<30% activity) versus G6PD intermediate (30-<70%) versus G6PD normal (>=70%) versus G6PD unknown paediatric patients and iii) paediatric patients with documented CYP2D6 polymorphisms versus with those with wild-type CYP2D6.

8.5.5. **Multivariable logistic regression analyses** will be undertaken to determine how age modifies the effect of daily mg/kg on the odds of a $\geq$25% drop in haemoglobin to below 7g/dL between baseline and days 1 – 13 in patients with >=30% G6PD activity. The effect of primaquine dose will be controlled for potential confounders including sex, parasitaemia at day 0 and haemoglobin at day 0 with study site included as a random effect. The effect of nutritional status will be explored. Following comparison of the whole paediatric population compared to adults, subgroup analyses will be undertaken within the paediatric population to compare patients aged between 6 months and 1 year (data permitting) versus children 1 year to less than 5 years versus children 5 to <15 years old. Data permitting, additional analyses will be undertaken comparing i) G6PD deficient (<30% activity) versus G6PD intermediate (30-<70%) versus G6PD normal (>=70%) versus G6PD unknown paediatric patients and ii) patients with documented CYP2D6 polymorphisms versus with those with wild-type CYP2D6.

8.5.6. **Multivariable linear regression analyses** will be undertaken to determine how age modifies 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 separately between baseline and 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 sex, parasitaemia at day 0 and haemoglobin at day 0 with study site included as a random effect. The effect of nutritional status will be explored. Following comparison of the whole paediatric population compared to adults, subgroup analysis will be undertaken within the paediatric population to compare patients aged between 6 months and 1 year (data permitting) versus children 1 year to less than 5 years versus children 5 to <15 years old. Data permitting, additional analyses will be undertaken comparing i) G6PD deficient (<30% activity) versus G6PD intermediate (30-<70%) versus G6PD normal (>=70%) versus G6PD unknown paediatric patients and ii) patients with documented CYP2D6 polymorphisms versus with those with wild-type CYP2D6.

8.6. *Efficacy related analyses*

8.6.1. 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).

8.6.2. Time to first recurrent vivax or ovale parasitaemia will be used to compute the **Kaplan-Meier (K-M) estimates** of risk of *P. vivax* or *P. ovale* recurrence until 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 or ovale 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 (including weekly primaquine as a separate category) and no primaquine use will be presented for the whole paediatric population compared to adults. Subgroup analyses will be undertaken within the paediatric population to compare i) patients aged between 6 months and 1 year (data permitting) versus children 1 year to less than 5 years versus children 5 to <15 years old, ii) G6PD deficient versus G6PD normal paediatric patients (genotype and/or phenotype) and iii) patients with documented CYP2D6 polymorphisms versus with those with wild-type CYP2D6.

8.6.3. The proportion of patients who had a recurrent vivax or ovale parasitaemia before day 42, between day 42 and 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 as above for 7.6.2.

8.6.4. **Cox regression analysis** for the time to first vivax or ovale recurrence during follow-up (180 days) will be performed, with shared frailty for study-site to account for additional variation related to study sites. Studies with 42 days follow up or more will be included and patients treated with daily primaquine or no primaquine. The interaction between age and primaquine dose will be controlled for potential confounders including 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). 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, GI tolerability and nutritional status. 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. Subgroup analyses will be undertaken within the paediatric population to compare i) patients aged between 6 months and 1 year (data permitting) versus children 1 year to less than 5 years versus children 5 to <15 years old, ii) G6PD deficient versus G6PD normal paediatric patients (genotype and/or phenotype) and iii) patients with documented CYP2D6 polymorphisms versus with those with wild-type CYP2D6.

8.6.5. A separate Cox regression analysis will be repeated similar to section 8.6.4 with an interaction between age and expected primaquine duration. The model will be controlled for potential confounders including primaquine mg/kg dose, sex and baseline parasitaemia.

8.6.6. 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 spline models or categorisation of mg/kg dose. The dose which results in the 95th percentile of the predicted risk to be <5% i.e. estimated efficacy to be ≥95% will be reported.

8.6.7. Incidence rates of recurrent vivax or ovale parasitaemia over 180 and 365 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 or ovale parasitaemia. Incidence rates will be calculated by dividing the number of *P. vivax* or *P. ovale* 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 and compared between paediatric and adult patients 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. Subgroup analyses will be undertaken within the paediatric population to compare i) patients aged between 6 months and 1 year (data permitting) versus children 1 year to less than 5 years versus children 5 to <15 years old, ii) G6PD deficient versus G6PD normal paediatric patients (genotype and/or phenotype) and iii) patients with documented CYP2D6 polymorphisms versus with those with wild-type CYP2D6.

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.

9. References

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