**RESEARCH PROTOCOL**

**A comparison of two artemisinin combination therapies (ACTs) in combination with primaquine for radical cure of *Plasmodium vivax* malaria in the Solomon Islands: the “ACT-radical” study.**

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# PART 1: EXECUTIVE SUMMARY

This protocol details a clinical trial designed to answer a specific question regarding the possibility that a drug-drug interaction (DDI) between a commonly used artemisinin combination therapy (artemether-lumefantrine) and the 8-aminoquinoline drug, primaquine (PQ) may compromise radical cure of *Plasmodium vivax*. This hypothesis is supported by existing knowledge that (1) the activity of PQ is dependent on activation by the cytochrome P450 2D6 (CYP2D6) human hepatic enzyme, (2) that artemether lumefantrine (AL) is a known inhibitor of CYP2D6 and (3) observations of poor radical curative efficacy of PQ when co-administered with AL in the South Pacific. This question has fundamental importance to strategies for eliminating malaria throughout *P.vivax* endemic regions of the world.

The research will be a randomized controlled clinical trial (RCT) of patients with clinical *P.vivax* infection in the Solomon Islands. It will compare the radical curative efficacy of PQ when co-administered with AL (AL+PQ) versus PQ co-administered with an alternative ACT (dihydroartemisinin-piperaquine (DP) – a drug that does not cause significant CYP2D6 inhibition). In addition to the RCT, stratification of results by CYP2D6 genotype derived activity score and an additional nested pharmacokinetic study aim to clarify the mechanism of any interaction.

|  |  |
| --- | --- |
| Start date: 1/11/2016 | End date: 30/04/2018 |
| List of countries where the project will be conducted: Solomon Islands | |
| List of main language(s) used: English AND Melanesian Pigin | |

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**Sponsor:** Bill and Melinda Gates Foundation

**Funding grant ID number:** OPP1151132

**Grant title:** “Could a significant interaction between artemether-lumefantrine and primaquine be seriously undermining drug-based strategies for malaria elimination?”)

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# **PART 3: ABBREVIATIONS AND DEFINITIONS**

|  |  |
| --- | --- |
| **Abbreviation** | **Term** |
| AUC | Area Under Curve |
| AUC∞ | Area Under Curve to infinity |
| AL | Artemether-lumefantrine |
| ACT | Artemisinin Combination Treatment |
| BMGF | Bill and Melinda Gates Foundation |
| CRF | Case Record Form |
| CI | Co-investigator |
| CYP2D6 | Cytochrome P450 2D6 |
| DSMB | Data and Safety Monitoring Board |
| DP | Dihydroartemisinin-piperaquine |
| DOT | Directly Observed Treatment |
| DDI | Drug-drug interaction |
| G6PD | Glucose-6 Phosphate Dehydrogenase |
| g/dL | Grams per decilitre |
| Hb | Haemoglobin |
| HRP | Histidine-Rich Protein |
| LCMS | Liquid Chromatography Mass Spectrometry |
| LDH | Lactate dehydrogenase |
| Met-Hb | Methaemoglobin |
| MOH | Ministry of Health |
| MAS | Multiple Amplicon Sequencing |
| *P.falciparum* | *Plasmodium falciparum* |
| *P.vivax* | *Plasmodium vivax* |
| PCR | Polymerase chain reaction |
| PQ | Primaquine |
| PI | Principal Investigator |
| RCT | Randomized Controlled Trial |
| RDT | Rapid diagnostic test |
| RAM | Rotary Against Malaria |
| EOCRU | The Eijkman-Oxford Clinical Research Unit |
| WEHI | The Walter and Eliza Hall Institute of Medical Research |
| VDBC | Vector-Borne Disease Control |
| wk | week |
| WHO | World Health Organization |

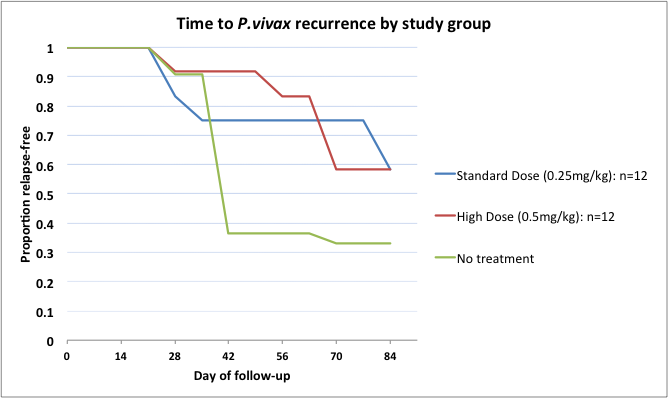
# **PART 4: PROJECT DESCRIPTION**

## 1. Background and Rationale

***P.vivax* is becoming the dominant malaria parasite species in the South Pacific where it has broad and significant effects on population health.** [[2](#_ENREF_2)] It continues to place a huge burden on local health systems. Hypnozoite relapse is estimated to drive up to 80% of malaria transmission due to *P.vivax* in the Melanesian South Pacific. [[3](#_ENREF_3)] Control and elimination strategies directed at *P.vivax* in this region and elsewhere are therefore likely to be highly dependent on the efficacy of the single hypnozoitiicidal drug class available, the 8-aminoquinolines (that includes PQ and tafenoquine). Malaria control is therefore in turn likely to be significantly impacted by any factor that compromises the efficacy of this drug class.

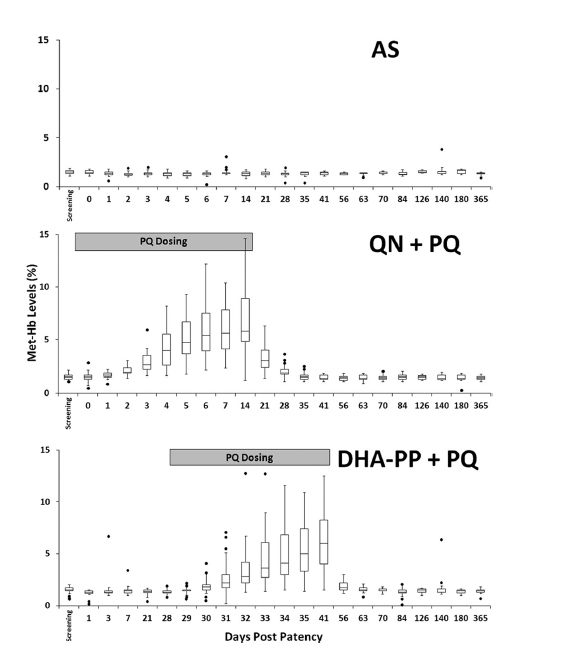
***It has recently become clear that the therapeutic efficacy of PQ is crucially dependent on metabolism by the CYP2D6 human drug metaboliser system.*** [[4](#_ENREF_4)] Although PQ is subject to highly complex metabolic pathways, it has become clear that its therapeutic activity and predictable dose-related toxicity (including both haemolytic effects and the production of methaemoglobin) are dependent on short-lived hydroxylated intermediary metabolites generated by the CYP2D6 system. [[5](#_ENREF_5),[6](#_ENREF_6)] The cruciality of CYP2D6 for PQ bioactivation has now been proven *in vivo* both in mouse models [[5](#_ENREF_5)] and in humans (including notable cases of repeated failure of PQ anti-relapse therapy in individuals later shown to carry genetic polymorphisms at the CYP2D6 gene). [[4](#_ENREF_4)]

***Artemether-lumefantrine is currently guideline-recommended schizonticidal treatment in >50 countries and is a known inhibitor of CYP2D6 in vitro*** [[7](#_ENREF_7)]. However the possibility that this may manifest as a significant interaction with the CYP2D6 substrate PQ appears to have been overlooked, including by policy makers in the South Pacific where all three of the malaria-endemic countries (Papua New Guinea, the Solomon Islands and Vanuatu) now have national guidelines recommending use of both AL and PQ for case-management of *P.vivax.* [[8](#_ENREF_8)] This is also the case in at least 10 other countries (mostly in the Americas and Asia). In addition, at least 43 countries (mostly in Africa) currently use AL, but may be considering combined AL+PQ for adjunctive gametocidal treatment of *P.falciparum* [[8](#_ENREF_8)] and many countries currently using CQ+PQ for *P.vivax* (in the Asia Pacific) may soon consider switching to AL+PQ due to concerns regarding *P.vivax* CQ resistance. [[9](#_ENREF_9)] The AL+PQ combination has never been subject to formal evaluation (of anti-relapse efficacy) in a clinical trial setting. Despite the huge implications of AL’s documented *in vitro* effects on CYP2D6, *in vivo* data characterizing these effects or exploring interactions with other CYP2D6 substrate drugs are completely lacking. In particular, the possibility that this interaction could compromise PQ efficacy has never been addressed and the magnitude and significance of CYP2D6 inhibition by AL have never been quantified *in vivo* with formal phenotypic testing.

***Preliminary clinical data from P.vivax patients in Vanuatu corroborates concerns that AL may in fact significantly impair PQ efficacy and generated a hypothesis that these observations of poor efficacy may reflect******AL’s known inhibitory effects on CYP2D6 enzyme activity.*** This small pilot study showed worryingly high recurrent *P.vivax* parasitemia rates (of 41.7% within 3 months in both standard and high-dose arms: see Figure 1). Although the numbers enrolled in this study are small, 95% confidence intervals around these figures (20%-60% for the recurrence rate) suggest a high likelihood that real rates of treatment failure could be of clinical and public health significance. Even more concerning is corroborating data from this study examining methaemoglobin (met-Hb) concentrations. Because some degree of haemoglobin methylation is thought to be intrinsic to the action of PQ’s active metabolite, methaemoglobinaemia following PQ dosing is such a consistent finding that it has been advocated as a means of assessing compliance (met-Hb concentrations should exceed 3% after 7 days of dosing in compliant individuals). [[1](#_ENREF_1)] However despite directly supervised therapy, none of the 23 subjects treated with PQ had measured met-Hb concentrations ≥3% at day 7 (see Figure 3). Indeed, met-Hb concentrations in the two PQ treatment arms were no higher than those observed in the no PQ control arm. This supports the hypothesis that AL, through its effect on CYP2D6, is impairing conversion of PQ to its active metabolite, raising the question as to whether this effect might explain the observations of high apparent treatment failure rates in Vanuatu.

**Figure 1**

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**Figure 2:** Met-Hb concentrations following PQ co-administered with **(A)**, AL in Vanuatu (n=23) and **(B)** DP in Indonesia (reproduced from Sutanto *et al*.[[1](#_ENREF_1)]). The dashed red line represents the proposed lower threshold for expected Met-Hb levels (assessed at day 7) assuming compliance.

**Population genetic factors will also be important determinants of PQ efficacy in any given setting.** These “static” effects will need to be accounted for when examining the possibility of a “dynamic effect” due to a drug-drug interaction. In particular, the gene encoding CYP2D6 is highly polymorphic, with over 40 genotypes having been identified. The prevalence of different genotypes varies significantly between populations. [[10-12](#_ENREF_10)] Many genotypes have been characterized based on their phenotypic associations and can be described as being associated with either “poor” or “null” (linked to PQ failure), “intermediate”, “extensive” and “ultra” metabolizer phenotypes. [[12](#_ENREF_12)] Alternatively, genotypes can be stratified on the basis of a standardized “activity score” that effectively quantifies their likely phenotypic CYP2D6 activity.[[10](#_ENREF_10)] A recent clinical trial in Indonesian military populations by one of our investigator team (unpublished data: JKB) has demonstrated a tight “dose-response” relationship between genotypically derived CYP2D6 activity score and whether or not *P.vivax* relapse occurred. In this population this meant that the odds of *P.vivax* relapse was dramatically higher when CYP2D6 activity score was <1.75. If a clinically significant drug interaction was to further compromise CYP2D6 activity, this “dose response curve” would effectively be shifted laterally – manifesting especially as higher proportions of treatment failures in those with intermediate activity scores.

**In animal models, CYP2D6 inhibition results in slower clearance of PQ and therefore higher area under curve (AUC) estimations for the parent drug**. [[13](#_ENREF_13)] Therefore, a finding of slower clearance and higher PQ AUCs when AL is co-administered would add further evidence to support the CYP2D6 mechanistic hypothesis, especially if this combination was shown to have significantly poorer efficacy than a non-CYP2D6 inhibiting control arm. Moreover, measurement of a CYP2D6 dependent metabolite of PQ (5,6 orthoquinone which is thought to represent a direct indicator of therapeutically active PQ metabolites) suggests that when CYP2D6 activity is impaired, concentrations of this metabolite are lower and that this correlates directly with impaired parasite killing.[[13](#_ENREF_13)] Therefore, a comprehensive analysis is required that bring together (1) genetic characterization and *in vitro* phenotyping of CYP function, (2) well-characterized PK data and (3) definitive clinical efficacy outcomes.

***The Melanesian countries of the South-West Pacific are relatively unique for having the combination of both existing AL-based treatment guidelines for treating P.vivax and highly endemic P.vivax that necessitates widespread 8-aminoquinoline use.*** They therefore represent the ideal setting for an *in situ* evaluation designed to answer these fundamentally important questions. In particular the following are important:

* Existing official national policy-mandated use of the AL+PQ combination precludes ethical complexities regarding investigative evaluation of this combination.
* The direct implications for local policy strengthen its programmatic rationale and relevance to national malaria programs, ensuring that it will be locally supported.
* Characteristics of local Chesson strains in this region confer numerous methodological and logistic advantages (e.g. high rates (70-80%) of early (within 3 months) first hypnozoite relapse).
* Endemicity is still sufficiently high enough to allow acceptable enrolment rates and
* Members of our research group (Karunajeewa and Mueller) have a proven track record of implementing large, logistically complex clinical/PK trials in this region that have lead to direct policy translation at national, regional level and global levels. [[14](#_ENREF_14)]

## **2. Objective and outcomes**

This study is designed to provide a clear answer to the question “Do AL’s effects on CYP2D6 significantly impair the efficacy of PQ when these drugs are co-administered?” The study has therefore been carefully designed to maximize the chance that it provides unambiguous evidence to guide policy decision-making. This will involve demonstrating both (1) that when co-administered with AL, compared with a suitable control arm, therapeutic efficacy of PQ is impaired to a degree that is of clinical and/or public health significance and (2) that there is sufficient evidence to support the mechanistic hypothesis that this is due to a drug-drug interaction caused by CYP2D6 inhibition. Our experimental approach to addressing both of these in a way that supports public health policy decision-making is shown schematically in Figure 3.

**Figure 3:** Schematic outlining experimental approach in relation to objective of informing policy

**Is PQ radical curative efficacy significantly impaired when co-administered with AL (compared with DP)?**

No

Yes

Is this because of AL’s inhibitory effects on CYP2D6?

Policy change not supported

Yes

No

Is another type of pharmacokinetic interaction to blame (eg an effect on PQ absorption)?

Yes

Policy Change is supported

No

Alternative schizonticidal

(eg DP)

Alternative dosing strategy (eg delay PQ after AL)

Further work needed

The aims of this study are therefore as follows:

2.1 Primary Aim:To determine whether *P.vivax* radical curative efficacy of PQ co-administered with AL is significantly poorer than PQ when co-administered with DP.

### 2.2 Secondary Aims:

1. To determine whether AL co-administration results in altered pharmacokinetic disposition that is consistent with inhibition of CYP2D6 (ie lower clearance and higher AUCs of PQ itself, and lower overall concentrations of the 5,6 orthoquinone metabolite).
2. To compare the relationship between CYP2D6 genotype-derived activity score and risk of relapse in participants co-administered AL vs those co-administered DP and determine whether any differences seen are consistent with CYP2D6 inhibition.
3. To compare met-Hb concentrations over 28 days following initiation of PQ therapy between participants co-administered AL vs those co-administered DP and to investigate the relationship between met-Hb measurements and subsequent risk of relapse (thereby exploring the utility of met-Hb as a prognostic marker of treatment response).

## **3. Design and methods**

### 3.1 Study design

A single centre three-arm open-label randomised controlled trial.

### 3.2 Treatment arms

Arm 1: AL + PQ

Arm 2: DP + PQ

Arm 3: AL alone (“no PQ control arm” - PQ treatment deferred until first recurrence or end of 6-month follow-up period, whichever comes first)

### 3.3 Study site

Tetere region of Guadalcanal province (approximately 20km from Honiara), Solomon Islands. Recruitment will be from Good Shepherd Hospital, Tetere and feeding health centres in this area as well as directly from the local community.

### 3.4 Population

Locally living Melanesian adults and children diagnosed with *P.vivax* blood-stage infection.

### 3.5 Sample size

Total enrolment target is 382 based on enrolling into the three arms in a 2:2:1 ratio (ie Arm 1: n=153, Arm 2: n=153, Arm 3: n=76). Further details regarding how this sample size was arrived at are detailed in a later section of this document (section 6.5).

### 3.6 Ascertainment

At each study health facility a register will be maintained of all presentations to the health centre. Those with fever (axillary >37.5 or oral/tympanic >38) or history of fever, chills or rigors in previous 72 hours will have a malaria RDT and microscopy performed in line with standard routine clinical practice. Adults and children diagnosed with *P.vivax* on the basis of field microscopy or RDT will be assessed for eligibility according to inclusion and exclusion criteria (see below).

### 3.7 Eligibility

All individuals with a microscopic diagnosis of *P.vivax* (including mixed infections involving *P. vivax*) will have a checklist completed to determine eligibility. Items on the checklist will be the inclusion and exclusion criteria (below). Verbal consent will be obtained to perform the final eligibility check including (1) urine pregnancy test (for women of childbearing age), (2) Hb estimation and (3) G6PD screening on a finger-prick blood sample.

*Inclusion criteria*

1. Age over 12 months
2. Weight ≥10kg
3. Melanesian background and living in local area
4. Microscopically (based on field microscopy) or RDT confirmed *P.vivax* regardless of parasite density. Mixed infections (*P.falciparum-P.vivax*) can be included.

*Exclusion criteria*

1. Any signs of severe malaria (see WHO definitions)[[15](#_ENREF_15)] including: impaired consciousness, respiratory distress, severe anaemia (Hb<5), multiple seizures, frequent vomiting/ inability to swallow tablets, prostration, jaundice, hypotension, abnormal bleeding or hypoglycaemia.
2. Clinical evidence of non-malarial illness (such as pneumonia or otitis media)
3. Severe malnutrition (weight-for-age nutritional Z score [WAZ] <60th percentile)
4. Permanent disability, which prevents or impedes study participation.
5. Treatment with PQ in the previous 14 days
6. Residence or planned travel outside the study area during the follow-up period (precluding supervised treatment and follow-up procedures)
7. Known or suspected pregnancy
8. Currently breastfeeding
9. A positive rapid test for G6PD deficiency (Binax™ or Carestart™ RDT)

### 3.8 Informed consent

All individuals satisfying all inclusion and exclusion criteria will be invited to provide written informed consent to participate. A verbal explanation of the study and its procedures will be provided in Melanesian Pigin by a local research nurse to all adult participants, all children >5 and parents/ guardians of children <16 using a pictorial flip chart. An information sheet (in Melanesian Pigin) will also be provided to literate adults and children. Adults (>16) to be enrolled and parents of children will provide consent by either signing or providing a mark or thumbprint on a written consent form. Children aged 12-16 will be asked to provide assent.

### 3.9 Enrolment procedures and baseline clinical assessment (Day 0)

Following informed consent all enrolled participants would be administered a symptom questionnaire (including recent fever, health centre visits and malarial treatment) and clinical examination (including body temperature, body weight and mid-upper arm circumference) for recording in the standardised case record form. Hb estimation by HemoCue ™ will be performed and recorded. As well as two initial giemsa-stained thick and thin blood slides and a baseline urine sample, an initial 250µl whole blood sample will be taken by finger prick and dried on a filter paper, centrifuged and stored (as red cell pellet and plasma) frozen for later transport. Dates of all follow-up visits would be recorded in the study diary (see study follow-up schema below and in Table 4).

### 3.10 Randomization and treatment allocation

Study participants will be randomly assigned, with the use of a computer-generated random-numbers list, to one of the three study arms. Randomization will be performed in permuted blocks of 12 with an allocation ratio of 2:2:1. The allocation will be concealed from investigators through the use of sequentially numbered, sealed envelopes that will be opened after the decision to enrol a subject has been made by the study team.

### 3.11 Blinding

The study would not be strictly blinded, in that investigators and participants will be aware of which treatment dose is being administered. However treatment allocation will not be known to microscopists evaluating follow-up slides or laboratory staff performing parasite PCR. Therefore the major study outcome measures should be free of potential for bias.

### 3.12 Drug administration

**Artemether-lumefantrine:** All individuals enrolled in arms 1 and 3 will receive a standard 3-day treatment course of AL (Coartem™, Novartis) at standard age-based dosage in accordance with current Solomon Islands and WHO treatment recommendations [[16](#_ENREF_16)] (see Table 1) with the morning dose administered under supervision with 30mls of milk and the evening dose dispensed with a tetra-pack of milk with instructions regarding administration (on study visits on days 0, 1 and 2). See Table 1.

**Table 1. Artemether-lumefantrine dosing (twice daily for 3 days)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Weight (kg)** | 10 – 14.9 | 15 – 24.9 | 25 – 34.9 | ≥ 35 |
| **Number of tablets per dose** | 1 tablet | 2 tablets | 3 tablets | 4 tablets |
| **Artemether/lumefantrine per dose (mg)** | 20/120 | 40/240 | 60/360 | 80/420 |
| **Artemether mg/kg dose range** | 1.3-2.0 | 1.6-2.7 | 1.7-2.4 | ≤2.3 |
| **Lumefantrine mg/kg per dose range** | 8.1-12.0 | 9.6-16.0 | 10.3-14.4 | ≤12.0 |

**Dihydroartemisinin-piperaquine:** Individuals enrolled in arm 2 will receive a standard 3-day treatment course of DP (Eurartesim™, Sigma Tau, Italy) according to body weight based on the latest WHO recommended dosing table [[16](#_ENREF_16)] that ensure that most participants will receive total piperaquine doses close to or >59mg/kg over 3 days.[[17](#_ENREF_17)] Dosing will be administered without food. See Table 2.

**Table 2. Dihydroartemisinin-piperaquine dosing (daily for 3 days)**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Weight (kg)** | 10-10.9 | 11 –16.9 | 17 – 24.9 | 25 – 35.9 | 36-59.9 | 60-79.9 | ≥ 80 |
| **Number of tablets per dose** | ¾ tablet | 1 tablet | 1 ½ tablets | 2 tablets | 3 tablets | 4 tablets | 5 tablets |
| **Dihydroartemisinin-piperaquine per daily dose (mg)** | 30/240 | 40/320 | 60/480 | 80/640 | 120/960 | 160/1,280 | 200/1600 |
| **Dihydroartemisinin mg/kg daily dose range** | 2.8-3.0 | 2.4-3.6 | 2.4-3.5 | 2.2-3.2 | 2.0-3.3 | 2.0-2.7 | ≤2.5 |
| **Piperaquine daily mg/kg per dose range** | 22.0-24.0 | 18.9-29.1 | 19.3-28.2 | 17.8-25.6 | 16.0-26.7 | 16.0-21.3 | ≤20.0 |

**Primaquine:** All individuals in Arms 1 and 2 will receive PQ as a daily dose of 0.25 mg/kg for 14 consecutive days commencing on the day of enrolment. Dosing will be according to current Solomon Islands weight-based categories. PQ will be administered under supervision with food (eg savoury biscuits) on 14 consecutive days (days -0 through to 13). Patients will be observed for 30 minutes following each to ensure that vomiting does not occur. All doses will be directly supervised and observed by either a member of the research team, or a trained community direct observed treatment (DOT) officer. See Table 3.

**Table 3. Primaquine dosing**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Weight** | 10 – 15.9 kg | 16 – 19.9 kg | 20 – 29.9 kg | 30-49.9kg | ≥ 50kg |
| **Number of tablets per daily dose** | ¼ tablet | ½ tablet | 1 tablet | 1 ½ | 2 tablets |
| **Primaquine per daily dose (mg)** | 1.875 | 3.75 | 7.5 | 11.25 | 15 |
| **Primaquine mg/kg daily dose range** | 0.12-0.1875 | 0.19-0.23 | 0.25-0.375 | 0.23-0.375 | ≤0.3 |

### 3.13 Safety and toxicity monitoring during primaquine therapy

At each researcher visit during PQ dosing, the following safety and toxicity evaluation will be performed:

1. Side-effect symptom questionnaire (including abdominal pain, cramping, nausea, vomiting, headache, pruritis, visual disturbance, dyspnoea).
2. Clinical examination for scleral icterus.
3. Urine dispstick for heamoglobinuria
4. Oxygen saturation measurement using Masimo Rad-57 plus oximeter to determine Met-Hb (days, 0, 1, 2, 3, 7, 10, 14 and 28).
5. Haemoglobin estimation using HemoCue™ Reader (finger-prick blood examination on days, 0, 1, 2, 3, 7, 10, 14 and 28).

### 3.14 Treatment stopping rules and rescue procedures

1. Any signs of severe malaria (see WHO definitions) [[15](#_ENREF_15)] including: impaired consciousness, respiratory distress, severe anaemia (Hb<5), multiple seizures, frequent vomiting/ inability to swallow tablets, prostration, jaundice, hypotension, abnormal bleeding or hypoglycaemia.
2. Any symptom consistent with a side-effect which is deemed intolerable by patient or parent (including significant abdominal pain/ cramping, intractable vomiting or severe pruritis).
3. Evidence of significant intravascular haemolysis:
   1. Haemoglobinuria on dipstick examination
   2. Scleral icterus
   3. Haemoglobin concentration fall by more than 25% of baseline or absolute concentration <5g/dL
4. Clinically significant methaemoglobinaemia evidenced by:
   1. Measured met-Hb saturation (using Masimo Rad-57 plus oximeter) >15%
5. Any other severe event deemed by the study clinicians to be plausibly associated with PQ administration
6. Any request by patient or parent to cease treatment and withdraw from the study

In any of these instances, participants will be withdrawn from the study immediately. Further medical management will be instituted as deemed necessary by the study clinician, including if necessary referral and admission to Honiara General Hospital. Participants with features of severe malaria will be managed in accordance with national treatment guidelines (intramuscular artesunate). For participants with significant haemolytic anaemia, facilities for blood transfusion are available at Honiara hospital. Any participant meeting criteria 1-5 (above) will have an adverse event form completed and this will be forwarded to the data and safety monitoring board. For those in whom a significant adverse event is considered related to PQ treatment (criteria 2-5) PQ treatment will be ceased immediately.

### 3.15 Follow-up and monitoring

Both passive and active surveillance will be undertaken to detect relapsed infection.

*Passive surveillance:* All participating study health facilities will maintain surveillance to detect study participants presenting to the health centre or hospital outside of scheduled follow-up days. This will be facilitated by a bright coloured sticker affixed to all participants health record/ clinic booklet that should alert staff that the patient is participating in the study. In this instance, following routine clinical care, the patient will be referred to the study nurse to have a standardised assessment including history of fever, history of any antimalarial use, body temperature, Hb estimation (by Hemocue™), a malaria RDT, giemsa stained thick and thin blood films, urine and a finger-prick blood sample collected onto filter paper.

*Active surveillance:* will be performed by visiting participants over a 6-month follow-up period as detailed in table 4. This will mean participants will be assessed by a member of the research team on study days 0, 1, 2, 3, 7 (wk1), 10, 14 (wk2), 21, 28 (wk 4/ 1 month), 42 (wk 6), 56 (wk 8/ 2 month), 70 (wk 10), 84 (wk 12/ 3 months), 112 (wk 16/ 4 months), 140 (wk 20/ 5 months and 168 days (wk 24/ 6 months).

Follow-ups will be conducted either at the clinic (if feasible, the participant will be asked to return to the clinic) or by the field team visiting the participant at their place of residence or at work. Research teams will endeavour to see participants on the exact day of their scheduled visit. If this is unsuccessful, they will try again the following day, and if necessary on a 3rd occasion in two days from the scheduled date. If 3 daily consecutive unsuccessful attempts at follow-up are made, this will be regarded as a “missed follow-up” and no further attempts will be made until the next scheduled follow-up.

Participants who fail to complete their entire treatment courses (including all 14 days of PQ in Arms 1 and 2 or a full course of AL or DP in any of the study arms) will be regarded as protocol violations and will be withdrawn from the study. They will not be included in the per-protocol analysis but will remain in the intention to treat analyses (see section 6.2).

Participants who miss 3 consecutive scheduled follow-ups from Day 21 onwards will be deemed to be permanently lost to follow-up.

Table 4 details assessment at each visit that will include direct observation of medication administration, administration of side-effect questionnaire, measurement of temperature, Hb measurement by Haemaccue™, Met-Hb measurement (by Massimo Rad57™ spectrophotometer: til day 28), Giemsa-stained blood slide for malaria microscopy, filter paper blood spot for parasite PCR and blood samples for plasma drug assays (see pharmacokinetic sub study below).

**Table 4.** Monitoring and procedures during 6-month follow-up period

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **DAY** | **0** | **1** | **2** | **3** | **4**  **-6** | | | **7** | **8**  **-9** | | **10** | **11**  **-13** | | | **14** | **21** | **28** | **42** | **56** | **70** | **84** | **112** | **140** | **168** |
| Baseline history and exam |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| G6PD RDT |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| CYP2D6, G6PD genotype/ gene sequencing. |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Primaquine (Research team) |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Primaquine (community DOT) |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| AL/ DP (Research team)1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Side-effect check |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Temperature |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Examination |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Hb (Haemaccue) |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Meth-Hb (Massimo Rad57) |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Microscopy |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Parasite PCR |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| PQ drug levels | Various times as determined by population PK sampling schedule (see section 4) | | | | | | | | | | | | | | |  |  |  |  |  |  |  |  |  |
| Lumefantrine drug levels |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

1. Morning dose of AL would be supervised by researcher and co-administered with milk. Evening dose would be dispensed with a tetra pack of milk for self-administration.).

### 3.16 Specimen storage, handling and transport

*Venous blood samples (Day 0, Day 7 and various other times according to population PK sampling regimen)*:

All venous blood samples (including those for the pharmacokinetic sub-study, see section 4, below) will be collected into EDTA vacutainers, centrifuged on site (3000rpm for 5 minutes) with two aliquots of plasma transferred by pipette into labelled eppendorf tubes and two aliquots of the red cell pellet saved in the original EDTA tube and a 2nd EDTA tube. Labeling will include participant’s unique study number, date/time and study day. Plasma and red cell pellets will have interim storage in a -20°C freezer at the health facility, prior to transport to Honiara hospital for storage at -70°C. One aliquot each of plasma and red cell pellet will then be transported by dry shipper to a drug assay laboratory (either in the USA, Thailand or Australia) with the second aliquot maintained as a backup in the -70°C freezer in Honiara.

In addition, for venous blood samples collected at baseline and day 7, prior to sample processing, 10 drops of whole blood will be expressed directly onto Nucleosave™ (Macherey-Nagel, United States) filter paper that will be labelled and stored as below. A few more drops of blood will be used to prepare two thick and thin blood films and for Haemaccue™ estimation of Hb.

*Finger-prick blood samples (Day 14, 21, 28, 42, 56, 70, 84, 112, 140, 168):*

A lancet will be used to prick the participants’ finger. The first few drops of blood expressed will be used to prepare two thick and think blood films (for Giemsa staining) and then for Haemaccue™ testing. Finally, a further 4 or more large drops of blood will need to be collected onto Nucleosave™ (Macherey-Nagel, United States) filter paper cards, ensuring that all four 3mm collection circles are completely filled. This may require a 2nd finger-prick, or even a small venous blood sample to be taken if not enough blood is available from the first finger-prick. After labelling (with study code, indicating unique study number and study day), filter paper cards will be placed in a zip-lock plastic bag with desiccant and stored at 18-25°C prior to them being shipped to Australia.

### 3.17 Diagnostic assays and laboratory methods

*Point of care testing:*

G6PD rapid diagnostic test: Finger prick blood samples will be applied to CareStart™ G6PD rapid test kit cards in accordance with the manufacturer’s instructions.

Malaria rapid diagnostic testing: Finger prick blood samples will be applied to CareStart™ HRP2/pLDH(PfPv) Combo rapid diagnostic kits in accordance with the manufacturer’s instructions.

Haemoglobin estimation: Will be by calibrated Hemocue™ portable spectrophotometer in accordance with the manufacturer’s instructions.

Methaemoglobin concentration and oxygen saturation: Will be by Massimo Rad57 portable spectrophotometer-based non-invasive testing (probe applied to one finger).

Malaria microscopy: Thick and thin films will be stained with Giemsa according to conventional methodology and directly read by a Level 2 microscopist in the field. Parasites will be speciated and parasites and white cells counted in 100 fields counted (to generate a parasite count per white cell). Parasite density will then be derived based on assumed white cell count of 8 x 109. All field microscopy will be verified for positivity and speciation by being independently read by a second Level 2 microscopist (based in Honiara, blinded to the 1st microscopy result) within 24 hours. When these two results are discrepant (either for positivity or speciation), the result will be adjudicated by a third level 2 microscopist (also blinded to the results of both microscopists). At the conclusion of the trial, all microscopy results will be “corrected” for speciation based on the results of species-specific PCR.

Pregnancy testing: A proprietary βHCG-based urine dipstick test will be used according to the manufacturer’s instructions to rule out pregnancy.

Urine dipstick for haemoglobinuria. A proprietary urine dipstick test will be used according to the manufacturer’s instructions to establish the presence of haemoglobin in urine.

*Parasite PCR*

Parasite PCR and genotyping will be performed on filter paper samples at the Walter and Eliza Hall Institute, Melbourne.

Malaria species-specific PCR: Following sample transport to WEHI, Melbourne, DNA extraction will be performed by standard methods, followed by application of ligase detection reaction (LDR) using multiplex species specific DNA probes as previously performed by our research group. [[18](#_ENREF_18)]

Genotypic analysis: Samples from those with *P.vivax* detected at follow-up will be compared with baseline samples as previously described by our group [[19](#_ENREF_19)] for genotypic homology in order to determine the probability of re-infection versus hypnozoite relapse or recrudescence.

*CYP2D6 and G6PD genotyping*

Characterization of CYP2D6 and G6PD genes will be undertaken at WEHI using a recently developed novel molecular approach, termed “multiplex amplicon sequencing” (MAS), to fully sequence both gene loci in all participants as follows: First, using existing published primer sets, full-length copies of each of these genes will be amplified from a subset of known control samples. These amplicons will be individually bar-coded and then pooled and sequenced on a next generation platform (Illumina™ and PacBIO™) to generate highly accurate, long reads sufficient to cover the entire length to ensure accurate identification of all SNPs, linkage to their specific gene of origin and to each individual participant. Following validation, gene-specific PCR primers will be multiplexed such that both genes can be amplified simultaneously in a single reaction prior to sequencing. This will enable a rapid, cost-effective means of characterizing all SNPs in these genes.

CYP2D6 genotypes will be mapped to a phenotypic “activity score” as previously described. [[10](#_ENREF_10)]

*Drug assays*

Concentrations of lumefantrine, primaquine and a number of its metabolites (including 5,6 orthoquinone) will be performed using liquid chromatography mass-spectometry as previously described. [[13](#_ENREF_13)] This work will be performed under sub-contract at a laboratory either in the US, Thailand or Australia.

## 4. Pharmacokinetic sub-study

A pharmacokinetic study will be nested within the main study in order to define disposition of PQ and its main metabolite of interest (5,6-orthoquinone). For logistic reasons, this study will commence once the main study has been enrolling for 4 weeks. This sub-study will be limited to participants aged 5 and over and require a further 6 venous blood samples be taken from each participant over the first 14 days of the study. Sampling will be conducted on days 3, 7 and 14 of the study and include one baseline sample (taken prior that day’s dose) and one more sample taken over the next 24 hours. The timing of the second sample in each participant will be pre-determined by a randomization process to ensure that a dataset is generated from samples taken over a range of times in relation to each dose. These will include samples taken at the following times in relation to the most recent dose: 0h (baseline prior to dose), 0.25h, 0.5h, 1h, 1.5h, 2h, 3h, 4h, 6h, 8h, 18h and 24h (prior to next scheduled dose). This will results in samples taken from a total of 30 time-points as shown in Table 5.

**Table 5:** Sampling times for pharmacokinetic study. Each participant will have a total of 6 samples taken from these sampling times.

|  |  |  |  |
| --- | --- | --- | --- |
| **Study Day** | **Day 3** | **Day 7** | **Day 14** |
| Timing in relation to last dose | 0 (pre-dose) | 0 (pre-dose) | 0 (pre-dose) |
| 0.5 | 0.5 | 0.5 |
| 1.0 | 1.0 | 1.0 |
| 1.5 | 1.5 | 1.5 |
| 2.0 | 2.0 | 2.0 |
| 3.0 | 3.0 | 3.0 |
| 4.0 | 4.0 | 4.0 |
| 6.0 | 6.0 | 6.0 |
| 8.0 | 8.0 | 8.0 |
| 18.0 | 18.0 | 18.0 |
| 24.0 | 24.0 | 24.0 |

Samples would be processed by centrifugation to separate plasma and red cell pellets, with two aliquots of each immediately placed in a -20°C freezer prior to transfer to interim storage in a -70°C freezer in Honiara, then transferred by dry shipper to the drug assay laboratory (either in Australia, Thailand or the USA) for drug assays by LCMS.

Population pharmacokinetic analysis of concentration-time data derived from this study would employ mixed non-linear effects compartmental modelling using dedicated NONMEM population pharmacokinetic software to derive estimates of primary (absorption coefficients, volumes of distribution and clearance) and secondary (maximal concentrations, AUCs and elimination half-lives) pharmacokinetic parameters and explore the influence of co-variates including treatment arm, study day, age, sex, mg/kg dose and baseline parasitaemia.

## 5. Pre-specified study endpoints

### 5.1 Primary endpoint:

The primary endpoint will be microscopically confirmed, PCR verified *P.vivax* infection occurring at any time during the designated 6-month follow-up period. This “microscopy endpoint” will require that the following criteria be met:

1. A finding of *P.vivax* parasitaemia on field-based examination of a Giemsa-stained thick and thin blood film (by a level 2 microscopist) at any scheduled or unscheduled visit between days 21 and 168, inclusive.
2. Confirmation of initial field microscopy diagnosis of *P.vivax* by a 2nd Level 2 microscopist,

or

1. Confirmation of a diagnosis of *P.vivax* by a 3rd Level 2 microscopist (only when 1st and 2nd reads are discrepant).
2. Malaria species specific PCR confirms the presence of *P.vivax* DNA in dried blood spot taken at the same time-point as the positive slide.

In addition, the primary endpoint includes the following circumstances:

1. Both asymptomatic and symptomatic infections.
2. Mixed infections: PCR-confirmed *P.vivax* infections where there is additional evidence of *P.falciparum* co-infection (on either PCR or microscopy).

### 5.2 Secondary endpoints:

*Parasitological/ treatment efficacy:*

1. Positive species specific PCR for *P.vivax* (regardless of microscopy results) at any scheduled or unscheduled visit between days 14 and 168: The “PCR endpoint”.
2. *P.vivax* recurrent parasitaemia during the follow-up period*,* genotypically corrected forreinfections. Following genotyping of baseline-recurrent PCR positive paired samples, those with discrepant genotypes (suggesting re-infection) will be censored, leaving only those recurrences with identical genotypes as meeting this endpoint [[19](#_ENREF_19)]: The “Genotypically-corrected PCR endpoint”.

*Pharmacokinetic indices and surrogates of active drug exposure:*

1. Population pharmacokinetic model-derived per-treatment group estimate of primaquine clearance.
2. Population pharmacokinetic model-derived per-treatment group estimate of primaquine area under curve from time 0 to ∞ (AUC0-∞) for full 14-day course.
3. Population pharmacokinetic model-derived per-treatment group estimate of 5,6 orthoquinone area AUC0-∞ for full 14-day course.
4. Methaemoglobin levels (%Hb saturation) measured at:
   1. Day 3
   2. Day 7
   3. Day 14
   4. As total AUC from day 0-28, calculated by linear trapezoidal interpolation of measurements at day 0, 1, 2, 3, 7, 10, 14, 21 and 28.

*Safety and toxicity*

1. Adverse events reported and defined as:
   1. Absent (=0)
   2. Minor (=1: not bad enough to interfere with daily activity)
   3. Moderate (=2: bad enough to interfere with daily activity)
   4. Severe or life-threatening (=3: requiring hospitalization or associated with risk of death)
2. Likely drug-induced haemolytic anaemia – defined as >25% reduction in Hb from baseline any time from day 1-28, together with evidence of haemoglobinuria (on urinary dipstick) or jaundice.
3. Haemoglobin concentrations (g/dL) measured at:
   1. Day 2 (usual post-treatment nadir)
   2. Day 14 (final PQ dose)
   3. Day 28 (recovery)

## **6. Data management and statistical analysis**

### 6.1 Data management

All data will be collected on paper case report forms (CRFs). There will be separate individual CRFs for each for the 16 study visit days (baseline/day 0 to 168) and these will all be completed by research workers in the field. Rather than waiting until each participant completes the 6-month study observation period, each participants’ paper CRFs will be transported to Honiara for data entry in the Ministry of Health after the first 14 days of the study have been completed (including day 0, 1, 2, 3, 7, 10 and 14 forms) and then after each visit from then on (day 21, 28, 42, 56, 70, 84, 112, 140, 168). This will ensure that data entry can keep up with enrolments, thereby facilitating real-time auditing and quality control processes. CRFs will be kept in a locked filing cabinet at all times other than when data is actually being entered.

Data will be entered into a purpose-designed relational database that will be developed with the help of collaborators at the Institut Pasteur. This database will include data entry checking rules and in-built verification processes. It will be designed to be able to quickly generate reports for the purposes of auditing and quality control. It will be a cloud-based system, ensuring remote back-up (to the “cloud”) and remote access by principal investigators for the purposes of auditing and monitoring the progress of data entry.

Data entry progress will be assessed every week by the on-site clinical research manager and every month by the principal investigator to ensure that all data is entered within a benchmark of 28 days from the time of collection. Aggregate data reports, detailing numbers of participants enrolled into each treatment arm, follow-up rates, data completeness and any adverse events will be generated and forwarded to the Data and Safety Monitoring Board (DSMB) as set out in section 9.

Once each participant reaches the conclusion of their 6-month study period and their final visit CRF has been entered into the database, all their CRFs will be collated into the one file to be stored securely in a locked filing cabinet in the Ministry of Health. Data forms will be stored on site here until the conclusion of the study and then for another 5 years.

Separate forms for screening and eligibility completed at each participating health facility will be saved separately and transported to Honiara once a week. They will be entered into a separate file that will be used to generate this study’s CONSORT diagram.

### 6.2 *A priori* statistical analysis plan

*Primary endpoint therapeutic efficacy analysis:* This will compare rates of *P.vivax* recurrence in each treatment arm based on time-to-event analysis over the 6-month follow-up period. The following approaches will be utilized:

* The primary comparison of therapeutic efficacy (ie between the AL+PQ arm and the DP+PQ arm) will be defined as the proportional difference in the *P.vivax* recurrence incidence rates based on Cox-regression (assuming proportional hazard).
* *P.vivax* recurrence incidence rates will be calculated as the number of participants in each treatment arm with recurrent *P.vivax* parasitaemia divided by the total duration for which they are at risk of recurrence – with this denominator equating to the total number of person-years of follow-up.
* For each individual, the total measured duration at which they are at risk will depend on duration for which they are actively followed-up in the study. This will vary between 1 and 154 days (from the final PQ dose) and will depend on the following:
  1. Any individual meeting criteria for the “microscopy endpoint” (see section 5.1) will be considered to have met the study endpoint. So no further data will be collected in any individual following a documented *P.vivax* recurrence. Therefore participants will effectively be removed/ censored once they reach the study endpoint (recurrence or 6 months follow-up, whichever comes first).
  2. Any individual who is re-treated with PQ for reasons other than a documented *P.vivax* recurrence (eg inadvertent re-treatment due to initial misdiagnosis of *P.falciparum*) will be censored from the time of PQ treatment onwards.
  3. Participants who are diagnosed and treated for *P. falciparum* malaria during the follow-up period will be excluded from time at risk for 3 weeks following their AL re-treatment (based on estimated period of post-treatment schizonticidal prophylaxis).
  4. The approach to missing data (due to missed scheduled visits or complete loss to follow-up) will be as follows:
     + Primary analysis will be “per-protocol”, meaning that participants lost to follow-up will effectively be censored from the time of their last follow-up.
     + When one or more scheduled follow-ups are missed and followed by a successful follow-up, the microscopy result during the missed visits will be assumed to be the same as on that of the next successful follow-up. For instance for a participant who is seen on Day 56 and has a negative blood slide, then misses the Day 70 visit and is seen on Day 84 with a negative blood slide, then the assumed blood slide result on Day 70 will be negative. If the Day 84 blood slide was positive, then the Day 70 result would also be assumed to be positive.

Further sub-analyses based on the primary endpoint will include the following:

1. Intention to treat analysis based on “worst case scenario”. This assumes that any participant lost to follow-up had a *P.vivax* recurrence at the time of their next scheduled follow-up.
2. Intention to treat analysis based on a “best case scenario”. This assumes that any participant lost to follow-up remained free of *P.vivax* recurrence for the entire 6-month follow-up. Together with (1), this provides a sensitivity analysis around the per-protocol estimate. [[14](#_ENREF_14)] *P.vivax* recurrence incidence rates documented in the delayed PQ control arm (Arm 3) will be used to re-calculate an estimate of radical curative efficacy in Arms 1 and 2 according to the formulas:

Radical curative efficacy Arm 1= 1 – *P.vivax* recurrence incidence rate Arm 1

*P.vivax* recurrence incidence rate Arm 3

Radical curative efficacy Arm 2= 1 – *P.vivax* recurrence incidence rate Arm 2

*P.vivax* recurrence incidence rate Arm 3

1. Recurrence incidence rates in each treatment group will be stratified by genotypically derived activity score (AS)
2. In order to explore a range of factors that may contribute to treatment failure, binary logistic regression will be conducted, using *P.vivax* recurrence (yes/no) as the dependent variable and the following independent variables:
   1. Genotypically-derived CYP2D6 activity score (ordinal variable 0-4)
   2. Age
   3. Sex
   4. Treatment arm (1, 2 or 3)
   5. Baseline parasite density
   6. Baseline temperature
   7. Baseline Hb concentration (g/dL)
   8. Met-Hb level at day 7

*Analyses of secondary endpoints:*

1. PCR endpoint (alternative parasitological/ treatment efficacy endpoint) – will use exactly the same methods as set out for the primary efficacy analysis, except that when a participant has a negative blood slide but a positive *P.vivax* PCR at a given time-point, this will be regarded as a recurrence, meaning that they will be considered to have reached the study endpoint and all data from subsequent follow-ups will be censored.
2. Genotypically-corrected PCR endpoint (alternative parasitological/ treatment efficacy endpoint) – will use exactly the same methods as set out for the primary efficacy analysis, except that when a participant has recurrent *P.vivax* parasitaemia with genotype that is discrepant with their baseline sample (consistent with a reinfection) then for the purposes of this analysis, this result will be re-classified as a negative blood slide.
3. Met-Hb levels and Hb concentrations at specified time-points will be compared between treatment arms using either independent T tests or (if non-normally distributed data) Mann-Whitney-U tests.
4. Incidence of adverse events (including haemolytic anaemia) would be compared between the treatment arms using Chi Squared testing.
5. Per-treatment group estimates of primary and secondary pharmacokinetic parameters would be determined by applying mixed non-linear effects modelling using NONMEM population pharmacokinetic modelling methods as previously described. [[20](#_ENREF_20)]

Statistical analyses will be performed using STATA by Sophie Zaloumis.

## **7. Justification of study design**

### **7.1 Choice of treatment arms**

Given the hypothesis underlying the rationale for this study, the most appropriate comparator arm should employ an alternative schizonticidal agent to AL that is not associated with significant CYP2D6 inhibition. DP does not inhibit CYP2D6 and is one of four other currently licensed ACTs. It is the most widely-used alternative to AL in the Asia Pacific region. There is also good evidence from the region (PNG and Indonesian West Papua) supporting its excellent schizonticidal efficacy as treatment of *P.vivax*. [[14](#_ENREF_14),[21](#_ENREF_21)] It therefore represents the most practical policy alternative to AL. Arm 2 (PQ+DP) can be considered the “control” arm in this study for the purposes of testing the hypothesis that Arm 1 (PQ+AL) will be significantly inferior to this control arm.

### 7.2 Inclusion of a delayed PQ arm

Defining the radical curative efficacy of treatments for *P.vivax* is extremely challenging in field-based clinical trials. It is virtually impossible to distinguish *P.vivax* recurrences as being either (1) failed schizonticidal treatment (resulting in recrudescent parasitaemia after a period of partial suppression), (2) failed hypnozoiticidal treatment (resulting in hypnozoite relapse) or (3) re-infections due to new mosquito inoculations occurring during the follow-up period. Genotypic approaches have been employed to address this issue but still have fundamental limitations (particularly relating to dealing with multi-strain infections). Therefore the only way to quantify absolute radical curative therapeutic efficacy is to employ a control arm so that reduction in *P.vivax* recurrence can be judged relative to the situation in which no radical curative treatment is given, but where effective schizonticidal treatment is administered. Other studies have attempted to deal with this issue by employing historical control groups [[22](#_ENREF_22)] – but this clearly has important limitations due to the potential for confounding due to changes in transmission intensity in a particular region over time.

### 7.3 Follow-up duration

Our total follow-up duration is limited by logistical and cost constraints. However data from Vanuatu and PNG suggest that most first relapses occur within 6 months of a primary infection, of which the majority (>90%) occur within the first 3 months and after which the survival curve “flattens out”. [[3](#_ENREF_3)] Therefore the 6-month follow-up should be more than adequate.

### 7.4 Choice of endpoints

**For reasons discussed in section 7.2, measuring and defining radical curative efficacy in P.vivax is complex and subject to a number of challenges. We have therefore included a range of alternative endpoints that aim to best address these challenges, including the use of a no PQ control arm to calculate absolute treatment efficacy, a PCR endpoint to deal with insensitivity and operator dependence of microscopy and genotyping techniques to account for re-infections. However we have chosen unadjusted P.vivax recurrence based on a PCR-corrected microscopy endpoint as our primary endpoint for analysis because this represents the most unambiguous metric and is the one that has been most widely reported in recent literature in this field. [**[**23**](#_ENREF_23)**]**

**Given this study’s underlying hypothesis, pharmacokinetic metrics and surrogates of active drug exposure are clearly important and have therefore been included as secondary endpoints. At the current time, 5,6 orthoquinone concentrations probably represent the best surrogate of active drug metabolites and, importantly these are directly dependent on CYP2D6 activity. Dose response relationships in PQ suggest that efficacy is most closely associated with the total dose administered (regardless of how long it takes to administer the course). Therefore AUC seems the most appropriate metric. Current evidence suggests that with CYP2D6 inhibition, PQ parent drug clearance will be impaired and AUCs will increase. Therefore measuring parent drug (PQ itself) and defining clearance and AUC as pre-specified endpoints also has value in addressing our hypothesis. There is a strong biological rationale to suggest that Met-Hb levels will also correlate with active drug exposure and therefore therapeutic efficacy. Similarly AUC would seem a plausible metric to employ here, although measuring Met-Hb at specific time-points (day 3, day 7 and day 14) may also have practical value as a simple, easily implemented prognostic marker.**

### 7.5 Sample size calculation and statistical power

Sample size calculation has utilized the following approach and assumptions:

1. Based on survival analysis.
2. Designed to detect inferiority of Arm 1 (AL+PQ) compared with Arm 2 (DP+PQ). In other words it is *not* based on a non-inferiority or equivalence design.
3. Significance (α) of 0.05 and a one-sided test. A one-sided test is appropriate given our hypothesis is that AL+PQ will be inferior to DP+PQ and we have no reason to suspect that DP+PQ could be worse than AL+PQ. Therefore a 2-sided test is not necessary)
4. Power (β) of 0.8
5. Assuming a *P.vivax* recurrence rate in the DP+PQ arm of 25% (including 10% “failure” rate due to hypnozoite relapse and 15% re-infection rate).
6. Designed to detect a treatment difference of 15% (in other words a 15% higher recurrence rate (ie 40%) in the AL+PQ arm).

This approach yielded a sample size calculation of 123 for each of the two treatment arms.

was calculated.

Assuming an attrition rate of 25% due to withdrawals, loss to follow-up, post-hoc exclusions, this would mean an enrolment target of 153 per treatment arm

A third delayed PQ control arm would require a relatively small sample (as this would not be subject to statistical comparison with the other treatment arms). A sample of 51 would be sufficient to define the background rate of hypnozoite relapse (expected to be 0.75) with a confidence level of 0.9 and precision of 0.1. Therefore enrolling in a 2:2:1 ratio would be ideal (=122, 122 and 61 in AL+PQ, DP+PQ and AL+delayed PQ groups respectively). Assuming 25% attrition rate in this arm results in an enrolment target of 76

Enrolment targets in Arms 1, 2 and 3 therefore become 153, 153 and 76, summing to a total of 382 participants.

### 7.6 Feasibility and logistics

*Logistic considerations*

Logistic challenges for this study include a lack of laboratory facilities (including freezers and specimen processing capability) in proximity to both study sites, lack of established clinical research infrastructure, limited local practitioner experience in conducting studies of this nature and a relatively intense study protocol requiring a high level of supervision of participants with multiple follow-up visits. The follow-up procedures are resource intensive, requiring a large transport commitment in order to achieve the >5000 follow-up visits that will be required across all participants over the duration of this study. For these reasons significant investments have been directed towards supporting transport infrastructure (purchase of a new dedicated study vehicle and ongoing maintenance of a second existing vehicle) and field staff (at least 12 local staff deployed as 3 separate teams).

*Feasibility of achieving sample size/ enrolment targets.*

Achieving our total enrolment target of 382 within within our planned 9-month enrolment period would require an average of approximately 9.7 enrolments per week. This is probably close to the limit of what is feasible give our resource constraints and *P.vivax* clinical case numbers at our field site. Enrolments at Health Facilities in our field site area over a 9-month period in 2015 are shown in Table 6. We aim to enrol from both Good Samaritan Hospital, Tetere clinic and at least two other health centres (from Lunga, Ngalimbiu or Mbalasuna). This data suggests we will need to be able to enrol 70% of *P.vivax* presentations at these 4 health facilities in order to meet enrolments. In previous studies we have been able to enrol between 85% and 100% of eligible cases. [[14](#_ENREF_14)]

**Table 6. Microscopy-confirmed clinical presentations of *P.vivax* malaria at health facilities in the Tetere region over 9 months (January to September) in 2015.**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Health Facility** | **Jan** | **Feb** | **March** | **April** | **May** | **June** | **July** | **Aug** | **Sept** |  | **TOTAL** |
| **Good Samaritan** | 25 | 18 | 51 | 33 | 46 | 26 | 37 | 14 | 18 |  | 268 |
| **Tinaghulu** |  | 5 |  | 6 |  | 6 |  |  |  |  | 17 |
| **Konga** |  | 1 | 3 | 3 | 4 | 2 |  |  |  |  | 13 |
| **Lunga** | 20 | 26 |  | 31 | 37 | 16 | 10 | 16 | 26 |  | 182 |
| **Ngalimbiu** | 4 | 4 | 12 | 15 | 16 | 10 | 0 | 5 | 9 |  | 75 |
| **Tetere** | 5 | 8 | 16 | 14 | 19 | 10 | 14 | 13 | 11 |  | 110 |
| **Mbalasuna** | 21 | 12 | 13 | 12 | 9 | 8 | 5 | 8 | 6 |  | 94 |
|  |  |  |  |  |  |  |  |  |  |  |  |
| **ALL** | 75 | 74 | 95 | 114 | 131 | 78 | 66 | 56 | 70 |  | 759 |

## 8. Ethical considerations

### 8.1 Potential benefits to participants

Prospective and enrolled participants would potentially benefit from:

1. **Curative treatment for malaria that is equal to or better than that recommended in current guidelines and local best practice:** Participants enrolled in Arm 1 or Arm 2 of this study would receive either management that is in-line with current in-country policy guideline (Arm 1 patients receiving AL+PQ) or a treatment that we expect to be at least as effective as, or more effective than (if our hypothesis proves to be correct) this standard treatment. Therefore, treatment received in these arms could be considered at least as good, or better than they would ordinarily receive.
2. **Routine G6PD point-of-care testing prior to enrolment to minimize risks of haemolytic anaemia:** Although WHO recommends that G6PD deficiency be ruled out using an appropriate test before PQ is prescribed, this has not been feasible in the Solomon Islands and is usually not performed. This means that under usual circumstances, patients are either treated without having G6PD testing (thereby being potentially put at risk of haemolytic anaemia if they were, unknowingly G6PD deficient) or, as is more common, not being treated with PQ at all (and therefore being subject to further relapsed vivax infections). Results of G6PD testing will be clearly documented on each patient’s own health clinic book by way of a red sticker – this will alert health care providers in the future if the individual is G6PD deficient and therefore that PQ is contraindicated. Therefore the results generated in this study from this testing approach could have benefits that they would not ordinarily enjoy and, by potentially preventing future harm, that extend beyond the period of the study.
3. **Intensive monitoring of compliance, drug safety and active detection of disease relapse.** This includes a scheduled 15 encounters with health care workers in the research team over a 6-month period during which treatment doses will be directly observed, side effect questionnaires administered, Met-Hb and Hb measured and blood slides taken to detect recurrent malaria infection. Therefore participants are more likely to have effective treatment, to have side effects detected (or detected earlier) and to have recurrent malaria infection detected (or detected earlier) and then treated appropriately compared with if they were undergoing conventional treatment outside the study.

### 8.2 Potential risks to participants

Potential risks to participants include:

1. **Higher risk of recurrent *P.vivax* in Arm 3:** Because participants enrolled in Arm 3 will have their PQ withheld until they reach the study endpoint, they will be more likely to experience a recurrence of *P.vivax* within the 6-month study period. In reality, the situation in this treatment arm is analogous to current standard practice in the Solomon Islands: because G6PD testing is not routinely available in the Solomon Islands, most patients with *P.vivax* malaria do not receive PQ at all. Therefore participants in this treatment arm receive treatment that is equivalent to current local standard practice but that may be inferior to that in other treatment arms. This risk is offset by the regular and intensive monitoring procedures of the study (that mean that recurrent *P.vivax* infection is likely to be detected early (almost always whilst it is still asymptomatic). All participants who do relapse will be re-treated according to national guidelines, including with PQ. This means that most participants in this arm will probably receive PQ within the first 3 months and the remainder will receive it either before or at the conclusion of the 6-month follow-up period. Therefore, overall treatment in this arm will be superior to current standard practice in the Solomons (where PQ is not given at all due to lack of G6PD testing). We have used this approach in a previous study (“Evaluation of safety and efficacy of two primaquine dosing regimes for the treatment of *Plasmodium vivax* malaria in the Solomon Islands and Vanuatu”: Clinical Trials Registration number NCT 018379992) that was approved by 5 separate Human Research Ethics Committees (The Walter and Eliza Hall Institute (13/02), the Menzies Institute for Medical Research (HREC 2013-1993), The WHO Western Pacific Regional Office Ethical Review Committee (2013.6.Van.2.MVP) the Solomon Islands National Human Research Ethics Committee (HRC13/06) and the Vanuatu Ministry of Health). In this previous study, all cases of *P.vivax* relapse in the delayed PQ control group were asymptomatic and therefore only detected due to the active monitoring employed in the study.
2. **Drug side effects:** AL and DP are generally well-tolerated and are conventional treatments administered to millions of people throughout the region as part of routine care. However PQ is somewhat more problematic. Although local treatment guidelines recommend its use, because this guideline is uncommonly implemented, any PQ side-effect in a study participant could be considered an adverse event that may not ordinarily have occurred if the patient had not been enrolled in this study. Possible side-effects from PQ include:
3. Haemolytic anaemia due to G6PD deficiency. All patients will be screened prior to enrolment and deficient individuals excluded from the study. Previous studies from PNG and Vanuatu suggest that if this is done, the drug is extremely safe in this regard. However there still exists a small possibility of a false negative G6PD test (current point of care tests such as BinaxNOW™ have sensitivity of ≈95%) that could result in G6PD deficient individual being inadvertently enrolled into the study and treated with PQ. This risk is offset by the intensive monitoring in this project including regular checks of Hb (Haemaccue™) and haemoglobinuria (urinary dipstick testing) that would ordinarily not be part of routine care and should ensure early cessation of treatment if this side-effect were to arise. Facilities exist at Honiara hospital for blood transfusion in the extremely unlikely event that a significant episode of haemoloysis occurred in a participant.
4. Unpleasant side-effects (especially cramping abdominal pain and vomiting). These can be almost completely prevented through the co-administration of food as we will do in this study and therefore are unlikely to be significantly problematic at the doses currently used. However should they be present in spite of these measures, the patient has the option to cease treatment and withdraw from the study should they become intolerable.
5. Methaemoglobinaemia. Methaemoglobinaemia is relatively common but generally of a mild magnitude. Methaemoglobin levels up to 25% can be well tolerated in otherwise healthy individuals. We will monitor carefully for this with daily pulse oximetry during treatment, with a threshold methaemoglobin concentration of 15% (whether directly measured or based on an oxygen saturation of 15%) to be used as a trigger for treatment cessation. In our previous study in Vanuatu, Met-Hb levels were much lower than expected and no participant experienced significant methaemoglobinaemia.

All risks and benefits (above) will be fully disclosed in the Patient Information and Consent Form (PICF) (see Appendix).

## **9. Quality assurance, reporting and risk management**

### 9.1 Identified risks and mitigation strategy:

The following risks have been identified that could impede the successful completion of this study:

1. **Challenges in enrolling planned RCT sample size in the time-frame specified:** This could be subject to unpredictable variations in malaria transmission and logistic challenges in managing the very high follow-up numbers.

*Mitigation strategy:*

We aim to ameliorate this risk by ensuring that the study runs over an entire high transmission season (January til April) and by augmenting passive case detection methods with some active case detection (especially during the low transmission season) to ensure a steady flow of enrolments over the RCT enrolment period. We have allocated a high proportion of our resources to support the follow-up teams, so that enrolments are not too heavily constrained by this workload. This includes two dedicated follow-up teams (including vehicle and driver) that mean it should be feasible to perform up to 30 follow-ups per day. This should mean there is capacity to meet our enrolment targets (10 enrolments per week could result in a maximum of 30 follow-ups per day on some days). As well as having a dedicated enrolment team based at Good Samaritan hospital, we would also develop a phone referral network with other participating health centres to ensure good enrolment rates. Prior to commencing the study, a series of community meetings will be organised to ensure the study is has community support and therefore high participation rates.

1. **Political instability and governance issues in the Solomon Islands:** The Solomon Islands has a history of ethnic conflict and political instability that lead to a complete breakdown of government and law and order between the years of 1998 and 2003. However the situation has steadily improved since external intervention by the Australian and New Zealand governments in 2003 and things are now calm, including in the proposed study areas. The last major incident of unrest was rioting in Honiara in 2007. Major issues of political unrest are therefore not considered likely, but smaller scale conflicts can occur occasionally– (eg landowner disputes) that have the potential to interfere with study activities – eg by making travel to certain areas difficult or unsafe for follow-up teams. At the current time, a much more important issue is one of financial governance within the Ministry of Health. Recently reports of financial mismanagement have lead to institution of very stringent internal accounting procedures. Unfortunately these processes are time consuming and difficult to navigate, leading to long delays in moving money around within the ministry.

*Mitigation strategy:*

In partnership with Rotary Against Malaria (RAM) we will establish a sub-contract with a private accounting firm (Morris and Sojnicki) based in Honiara. They would administer project funds in the Solomon Islands, including receiving regular tranches from the administering institution (WEHI), administering a staff payroll for local field staff, and handling in-country procurement (eg vehicles) and ongoing costs (eg vehicle maintenance). This firm has previously administered funds for the Global Fund in a similar manner and is well-regarded.

1. **Natural disasters.** The Solomon Islands, including the Honiara area proposed for this study, is prone to a number of natural disasters including cyclones, flooding, earthquakes and volcanic eruption. Major flooding has affected the region three times in the last 20 years and therefore probably presents the highest statistical risk for a natural disaster with potential to interfere with study activities. These can lead to loss of roads and bridges that could make travel in and out of the study area and to nearby villages difficult or impossible. Under these circumstances it is possible that study activities could be disrupted for a number of weeks or even months. Honiara’s latitude is at the northerly limit of cyclonic activity, so damage from cyclones tends to be from heavy rain leading to flooding rather than from high winds. Major earthquakes (magnitude 8.0) have affected the Solomon Islands in 2007 and 2013 but had minimal effects on the Honiara area that is not geographically prone to tsuanmis. The nearby volcano (Mt Savo) last erupted in the 1850’s.

*Mitigation strategy:*

In the instance of any serious natural disaster including that required suspension or cessation of study activities, the sponsor (BMGF) and DSMB would be fully briefed and kept updated regularly. In a worst case scenario, study activities could require cessation for a number of months, necessitating a request to extend the funding period for the study.

### 9.2 Quality assurance activities

Quality assurance activities would be the primary responsibility of the in-country project supervisor (working closely with CI Wini) and of the principal investigator (PI Karunajeewa). The in-country project supervisor and Dr Wini would be responsible for the following structured activities:

*Pre-study (2 weeks)*

Training of field staff in study activities including all procedures and data collection (case record forms).

Training of community-based compliance officers in DOT procedures.

*Weeks 1-4 (conducted together with principal investigator)*

Direct supervision of screening and enrolment procedures (1st two weeks of study).

Direct supervision of point-of-care testing (Hb, Met-Hb, G6PD, malaria RDTs, urine dipstick testing).

Direct supervision of specimen processing (centrifugation, aliquoting and labelling of samples for drug concentration assays, preparation of thick and thin blood films, filter paper specimens).

Setting up a follow-up calendar and directing follow-up teams.

Direct supervision of follow-up procedures, including direct observed treatment, compliance checks, side effect monitoring.

Direct supervision of data collection into case record forms.

*Weeks 5 and onwards*

Weekly audit of screening records, reconciled with health centre records to ensure optimal enrolment rates.

Weekly audit of all case record forms for data completion, legibility and integrity. This will require data collected onto each form at each visit will need to be countersigned to indicate that it has been checked by the project supervisor or by Dr Wini.

Weekly audit and updating catalogue of collected specimens (drug concentration assays, filter paper specimens and blood slides). This will include checking that labelling is legible, accurate and reconciling with CRFs.

Weekly progress reports on data entry (ie number of CRFs for which data entry has been completed for each visit). This will be benchmarked against pre-set data entry targets that aim to have all data collected within 4 weeks of collection.

Completion of adverse event forms

Data from these weekly audits (9-13) will be collated at the end of each month to generate a monthly report that includes the following:

Screening and enrolment report (detailing adequacy of screening relative to health centre records, reasons for ineligibility)

Follow-up completeness report – demonstrating missed follow-ups.

Adverse events reported over the previous month.

Protocol violations and withdrawals occurring over this month.

CRF audit.

Specimen catalogue and audit.

Monthly calibration of Haemaccue machines

Monthly audits of thick and thin blood film quality and dried blood spot quality.

In addition the principal investigator (PI Karunajeewa) would perform the following structured quality assurance activities to monitor progress and data integrity.

Direct overall supervision of project start-up (1st 2 weeks of project – see points 3-8 above).

Site visits at 1 month, 2 months, 4 months, 6 months, 9 months, 12 months and 15 months (for 3-5 days each visit).

Monthly audits of data, including application of data checking/ cleaning algorithms and review of self-generated reports on aggregate data, for overall progress (according to enrolment and data entry targets).

Review of monthly accounting statements from Morris and Sojnicki.

Monthly review of reports generated by the study supervisor (Screening and enrolment report, Follow-up completeness report, Adverse Event Report, Protocol Violations Report, CRF audit, Specimen catalogue and audit).

### 9.3 Data and Safety Monitoring Board (DSMB)

A Data and Safety Monitoring Board (DSMB) including 2 clinicians and 1 statistician will be established under the chairmanship of Professor Dennis Shanks (Australian Army Medical Research Unit, Enogarah, Queensland, Australia). The PI will provide the DSMB with a monthly progress report detailing:

1. A summary of enrolment progress including numbers screened, enrolled and details of any withdrawals or protocol violations.
2. The latest aggregate data summary extracted directly from the study database. This will include information on participant characteristics (age, sex, parasitaemia, temperature, treatment arm enrolled into, side-effect reporting (classified as “none”, “mild”, “moderate” or “severe”), G6PD testing results, Met-Hb data, Hb data and urine dipstick testing).
3. All adverse event reports (entered into a standardized adverse event form: see appendix).

In addition, in the event of any adverse event being noted during the study period that meets a definition as “severe” (requiring hospitalization or considered to carry a risk of death) a standardized adverse event form (see appendix) will be completed and immediately (within 2 working days) forwarded to all members of the DSMB.

### 9.4 Reporting to Sponsor

An annual reporting schedule is in place, with reports due to the BMGF on 30 April 2017, 30 April 2018 and 30 November 2018

## **10**. Dissemination of results

**10.1 Reports and presentations**

Following the conclusion of the RCT and completion of data cleaning, a preliminary analysis conducted by PI Karunajeewa and CI Zaloumis will be conducted and presented at an analysis meeting attended by all primary investigators in 2018. This will be followed by definitive analysis with results to be disseminated as follows:

1. Report to the Solomon Islands Ministry of Health that will clearly set out the investigator team’s considerations as to what the implications of the study’s results should be for treatment policy in the Solomon Islands. This report will also be made available to the Solomon Islands WHO country office. Depending on the study’s findings and significance for policy, this report may also be forwarded to the WHO Western Pacific Region Office and the Ministries of Health in Vanuatu and Papua New Guinea.
2. Presentations at scientific, program and network meetings, including for example:

* American Society of Tropical Medicine and Hygiene annual conference
* Asia Pacific Malaria Elimination Network annual meeting (including the Vivax working group meeting)
* The International Conference of *Plasmodium vivax* Research
* The American Society of Microbiology annual meeting (formerly ICAAC)
* Australasian Society of Infectious Diseases Annual Meeting
* A final report to the sponsor (BMGF) due November 2018

### 10.2 Publication plan and data sharing

All publications arising from this work will be submitted to peer-reviewed journals with open access (consistent with BMGF requirements). We envisage a major manuscript from this work submitted to a high impact medical general and possibly a number of smaller publications submitted to specialty journals examining particular aspects (eg pharmacokinetics, pharmacogenomics, value of Met-Hb as a prognostic indicator) in more detail.

It is also likely that data will be made available for pooling with other studies through multinational collaborative networks including the Worldwide Antimalarial Resistance Network (WWARN) and the Cochrane collaboration.

## **11.** Project team and governance structure

**The project team and governance structure is outlined in Figure 4**

****Figure 4. Project governance structure.****

PI Mueller (WEHI)

PI Karunajeewa (WEHI)

PI Baird (Eijkman)

**Principal Investigators**

CI Jex (WEHI)

Pharmaco- genomics

Clinical trial statistics and PK modelling

CI Zaloumis (UniMelb)

CI Simpson (UniMelb)

Microscopist 2

Microscopist 1

Nurse 6

Nurse 5

Screening and enrolment team manager (research nurse 1)

Nurse 7

Driver 2

Driver 1

PK sampling manager (research nurse 4)

Follow-up team 2 manager (research nurse 3)

Follow-up team 1 manager (research nurse 2)

Field staff

CI Wini (SI MOH/ WEHI

CI Bobogare (SI MOH)

In-country project supervisor (WEHI)

In-country management

Field team leaders

**TECHNICAL/ LABORATORY SUPPORT**

**FIELD SITE**

# **PART 5: PARTNERSHIPS AND LEVERAGE**

## **1. Participating institutions**

**The four participating institutions (WEHI, Solomon Islands Ministry of Health, EOCRU and University of Melbourne) bring together complementary expertise. WEHI has capacity for high-level molecular biological diagnostics including for human and parasite genetic analyses (CI Jex). WEHI has worked with the Solomon Islands Ministry of Health over the last 6 years on field based epidemiological studies of malaria, as part of the US National Institutes of Health (NIH) International Centres for Excellence in Malaria Research (ICEMR) program. PI Mueller has previously collaborated with PI Baird and this study will further develop strategic partnerships between WEHI and EOCRU. The School of Population and Global Health is situated in the same suburb of Melbourne as WEHI, facilitating regular face-to-face meetings with PIs at WEHI. It will bring world-class expertise in population pharmacokinetic modelling (CIs Zaloumis and Simpson) and build on existing links with WEHI.**

## **2. Partner organizations**

**Partnerships have also been developed with the following organizations that will play some part in this study as follows:**

1. ****Rotary Against Malaria, Solomon Islands (Mr Wayne Morris):** This non-government organization has played an important part in malaria control activities in the Solomon Islands over the last 10 years. This has included links especially with the Global Fund for AIDS, Malaria and Tuberculosis. They have a valuable practical inside knowledge regarding political and logistic matters in the Solomon Islands and will be an important partner in helping to manage local relationships and dealing with complex logistical issues.**
2. ****Institut Pasteur, Paris:** PI Mueller has a joint appointment here. They will provide a clinical research database platform adapted specifically for this study’s needs.**
3. ****Great Lakes Drug Development (Dr David Wesche):** This organizationworks closely with the sponsor (BMGF) as consultants providing ongoing technical advice on clinical pharmacology matters relating to infectious diseases that are current priorities in global health. David Wesche is a Clinical Pharmacologist who has participated in the study design workshop that was held to develop this protocol. We will continue to seek his advice as required over the course of the project and he will also be invited to the planned analysis meeting in 2018.**

## **3. Sub-contracts:**

**The following sub-contracts will be established as part of this study:**

1. ****Morris and Sojnicki Chartered Accountants, Honiara, Solomon Islands:** This firm will be responsible for receiving funding tranches transferred from the administering institution (WEHI) for disbursement in the Solomon Islands. This will include setting up and managing a payroll, and handling staff entitlements (eg superannuation) and government taxation and other legistlative requirements. They will organize in-country purchasing (eg study vehicle) and ongoing costs (accommodation and vehicle maintenance). They will also provide regular financial statements and reports.**
2. ****Drug assays.** This service will be contracted to an institution with the highly specialised expertise necessary to perform these assays.Quotes for this service will be obtained from a number of potential providers before deciding on the institution that will be awarded this sub-contract.**

## **4. Strategic objectives for future partnerships and funding opportunities**

**If successfully implemented, the work undertaken as part of this study will establish research infrastructure and expertise in the Solomon Islands and demonstrate that the collaboration is able to deliver large, complex clinical research projects in this setting. Therefore it may provide a platform to leverage future funding and resources for ongoing research, including from the following organizations:**

* **Medicines for Malaria Venture (MMV)**
* **Bill and Melinda Gates Foundation (BMGF)**
* **Pharmaceutical Industry (eg Glaxo Smith Kline)**
* **Australian National Health and Medical Research Council (NHMRC)**
* **United States National Institutes of Health (NIH)**
* **Asia Pacific Malaria Elimination Network (APMEN)**
* **Rotary Against Malaria (RAM)**

# **PART 6: LIST OF APPENDICES**

1. **Appendix 1: Participant Information Sheet**
2. **Appendix 2: Participant Consent Form**
3. **Appendix 3: Possible Adverse Event Report Form**
4. **Appendix 4: Protocol for Overseas Sample Testing**

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