A phase 1b study to characterise the pharmacokinetic/pharmacodynamic relationship of pyronaridine in healthy adult participants experimentally infected with blood stage *Plasmodium falciparum*.

Protocol Identifying Number: MMV_Pyronaridine_21_01

Principal Investigator: Associate Professor Bridget Barber

Sponsor: Medicines for Malaria Venture

Local Sponsor: Southern Star Research

Version Number: 4.0

27 July 2022

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STATEMENT OF COMPLIANCE

Investigator declaration

I have read the protocol and agree that it contains all necessary details for carrying out the study as described. I will conduct this protocol as outlined herein and will make a reasonable effort to complete the study within the time designated.

I agree to personally conduct or supervise the described study.

The study will be conducted in accordance with the following:

- World Medical Association Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Participants (Fortaleza, Brazil 2013)
- NHMRC National Statement on Ethical Conduct in Human Research (2007, updated 2018)
- Integrated addendum to ICH E6(R1): Guideline for Good Clinical Practice ICH E6(R2) (November 2016) – with introductory comments of the Australian Therapeutic Goods Administration
- Current ethics approved Clinical Trial Protocol.

I agree to inform all participants that the study agents are being used for investigational purposes and I will ensure that the requirements related to obtaining informed consent are in accordance with International Council of Harmonisation (ICH) Guidelines for Good Clinical Practice (GCP) section 4.8 and local requirements.

I agree to report adverse events that occur in the course of the study to the Sponsor in accordance with ICH Guidelines for GCP section 4.11 and local requirements.

I have read and understand the information in the Investigator's Brochure(s), including the potential risks and side effects of the study drug.

I agree to promptly report to the Human Research Ethics Committee (HREC) all changes in the research activity and all unanticipated problems involving risk to participants. I will not make any changes to the conduct of the study without HREC and Sponsor approval, except when necessary to eliminate apparent immediate harm to participants.

I agree to maintain adequate and accurate records and make those records available in accordance with ICH Guidelines for GCP section 4.11 and local requirements.

I agree to ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.

I understand that the study may be terminated or enrolment suspended at any time by the Sponsor, with or without cause, or by me if it becomes necessary to protect the best interest of the participants.

_____ Date: _____

Principal Investigator

Signatories

The undersigned parties agree, that the protocol was written in accordance with the World Medical Association Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Participants (Fortaleza, Brazil 2013), the NHMRC National Statement on Ethical Conduct in Human Research (2007, updated 2018), and the Integrated Addendum to ICH E6 (R1): Guideline for Good Clinical Practice E6 (R2) (November 2016) – with introductory comments of the Australian Therapeutic Goods Administration.

This clinical trial protocol has been reviewed and approved by the Sponsor.

Name	Signature	Date
Medical Director: **Anne Claire Marrast, MD** Medicines for Malaria Venture		
Vice President, Head of Integrated Sciences: **Jörg Moehrle, Associate Professor** Medicines for Malaria Venture		

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	A phase 1b study to characterise the pharmacokinetic/pharmacodynamic relationship of pyronaridine in healthy adult participants experimentally infected with blood stage *Plasmodium falciparum.*
Study Description:	This is an open-label, adaptive study that will utilise the *P. falciparum* induced blood stage malaria (IBSM) model to characterise the pharmacokinetic/pharmacodynamic (PK/PD) profile of pyronaridine. Up to 18 healthy, malaria naïve adult participants are planned to be enrolled into this study, in cohorts of up to six participants each. Following a screening period of up to 28 days, cohorts of up to 6 healthy participants will be enrolled. After confirmation of eligibility, including a Rapid Antigen Test (RAT) for SARS-CoV-2, each participant will be inoculated intravenously on Day 0 with approximately 2,800 viable *P. falciparum*-infected erythrocytes. Participants will be followed up daily via phone call or text message on Days 1 to 3 post-inoculation to solicit any adverse events. Participants will attend the clinical unit once on Days 4, 5, 6 and 7 for clinical evaluation and blood sampling to monitor the progression of parasitaemia, using quantitative polymerase chain reaction (qPCR) targeting the gene encoding 18S rRNA (referred to hereafter as malaria 18S qPCR). Participants will have a nasopharyngeal aspirate (NPA) for SARS-CoV-2 on Day 6 am. Participants will also have blood collected on Day 7 am to monitor haematology and biochemistry. Participants will be admitted to the clinical trial unit on Day 8 for dosing with the investigational medicinal product (IMP; pyronaridine) when parasitaemia for the majority of participants is expected to be above 5,000 parasites/mL. Participants will need to return a negative (RAT) for SARS-CoV-2 prior to admission. Pyronaridine will be administered as a single oral dose. Different doses of pyronaridine will be administered across and within cohorts in order to effectively characterise the PK/PD relationship. Each cohort will comprise up to three dose groups, with participants randomised to a dose group on the day of dosing. The highest dose of pyronaridine administered will be no more than 720 mg; the lowest dose administered will be no less than 180 mg. The proposed dosing regimen for the Cohort 1, assuming six participants are enrolled, is as below. If less than six participants are enrolled the Safety Data Review

Team (SDRT) will meet between Day 0 and Day 8, to decide on the dose/s to be administered.

	Dose group		
	1A (n=2)	1B (n=2)	1C (n=2)
Pyronaridine	360 mg	540 mg	720 mg

Each subsequent cohort will be composed of up to 3 dose groups. If more than one dose is tested in a given cohort, subjects will be randomised after inoculation day (Day 0) but prior to Day 8. The dose/s administered to Cohort 2 and Cohort 3 will be selected based on PK/PD and safety data from the preceding cohort/s. The SDRT will review all available safety and tolerability data and PK/PD analysis outcomes from the previous cohort/s prior to inoculation of the next cohort. The study will conclude when sufficient data have been obtained to define the PK/PD parameters for pyronaridine (the primary endpoint).

A maximum sample size of 18 participants administered pyronaridine is expected to be sufficient to achieve the primary endpoint.

Participants will be confined in the clinical unit for at least 96 h (Days 8 - 12) to monitor the safety and tolerability of pyronaridine dosing and to ensure adequate clinical response against *P. falciparum*. During confinement, regular safety assessments will be performed and blood will be collected to monitor parasite clearance and pyronaridine concentration. If participants are clinically well at the end of the confinement period, they will be discharged and monitored on an outpatient basis up to Day 50±2.

Participants will receive compulsory antimalarial rescue treatment with Riamet® (artemether/lumefantrine) on Day 47±2 or earlier in the following cases:

- Parasitaemia is not reduced by approximately 10-fold at the end of the 96 hour confinement period (Day 12) when compared with peak parasitaemia.
- Parasite regrowth occurs after an initial reduction in parasitaemia, with regrowth defined as ≥5000 parasites/mL and a two-fold parasitaemia increase within 48 hours.
- Volunteer discontinuation/withdrawal from the study.
- Investigator's discretion in the interest of participant safety.

Parasite lifecycle stages (including gametocytes) will be monitored at select time points during the study by collecting blood samples and using quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) targeting parasite lifecycle stage-specific mRNA transcripts.

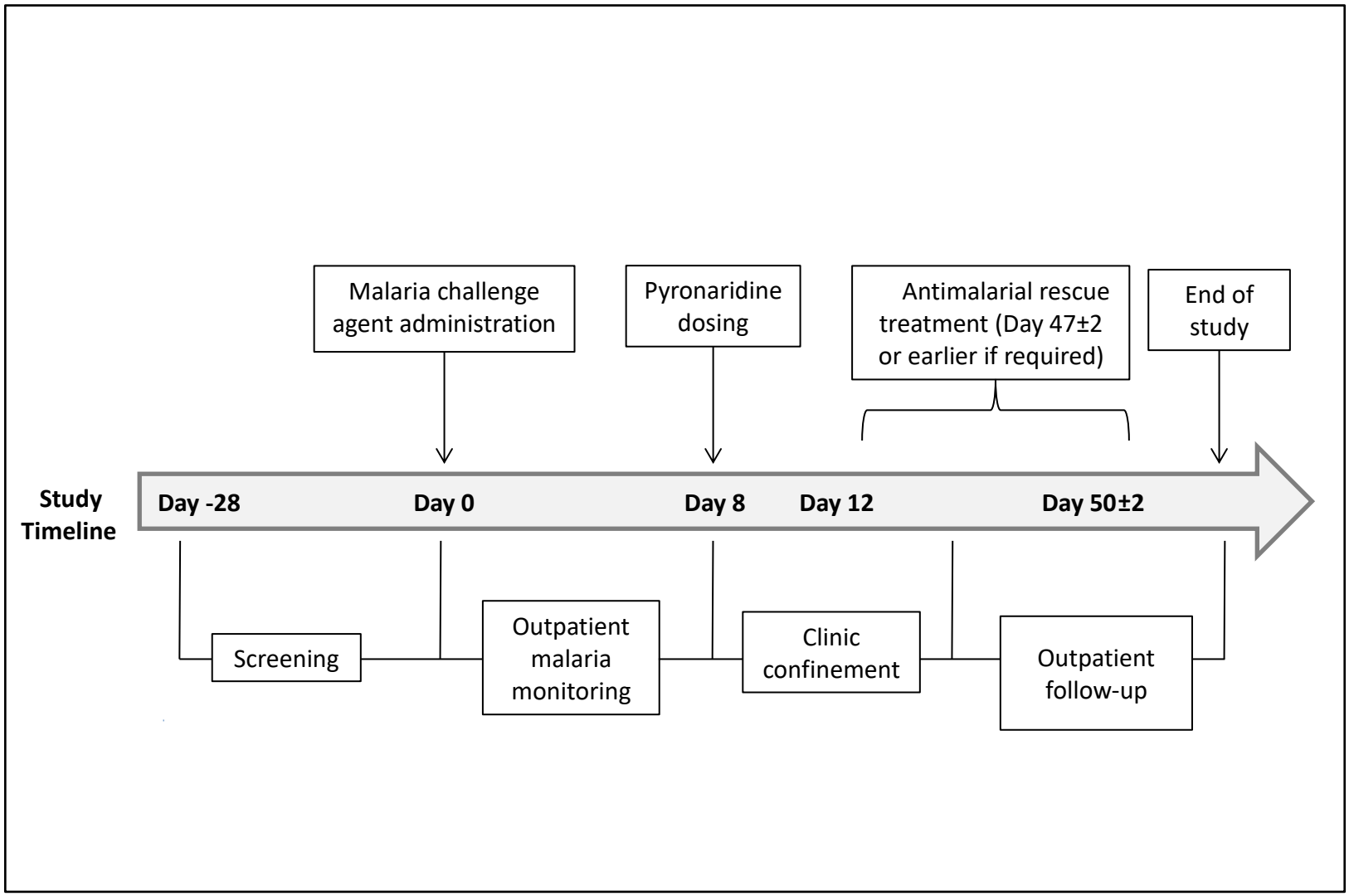
If gametocytes are present as determined by qRT-PCR at any time point after the confinement period, a single dose of Primacin® will be administered at the Investigator's discretion. If an allergy or contraindication to Riamet® develops, Malarone®

	(atovaquone/proguanil) will be administered. If in the rare event participants are unable to complete oral antimalarial rescue treatment, they will be admitted to hospital to receive intravenous artesunate.
Objectives:	Primary Objective: • To characterise the PK/PD relationship of pyronaridine in healthy participants experimentally infected with blood stage *P. falciparum*. Secondary Objectives: • To evaluate the safety and tolerability of single oral doses of pyronaridine in healthy participants experimentally infected with blood stage *P. falciparum*. • To characterise the parasite clearance kinetics following single doses of pyronaridine in healthy participants experimentally infected with blood-stage *P. falciparum*. • To characterise the pharmacokinetics of pyronaridine following single oral dose administration in healthy participants experimentally infected with blood stage *P. falciparum*. • To characterise the effect of pyronaridine on *P. falciparum* 3D7 parasite viability using phenotypic and molecular approaches.
Endpoints:	Primary Endpoint: • The PK/PD relationship between pyronaridine blood concentrations and blood stage asexual parasitaemia. Secondary Endpoints: • The safety and tolerability of pyronaridine will be determined by recording the incidence, severity and relationship to pyronaridine of adverse events (as determined by clinical, laboratory and ECG examinations) from Day 8 up to Day 50±2. • The parasite clearance kinetics following dosing with pyronaridine will be determined by calculating the parasite reduction ratio over a 48 hour period (PRR_{48}) and corresponding parasite clearance half-life ($PCt_{1/2}$). • The pharmacokinetics of pyronaridine will be determined by calculating the following parameters using non-compartmental methods: C_{max}, T_{max}, t_{lag}, $AUC_{0\text{-}last}$, $AUC_{0\text{-}\infty}$, CL/F, Vz/F, λz, $t_{1/2}$. • Parasite growth in *ex vivo* cultures.
Population:	Participants will be malaria-naïve healthy males and females, aged between 18-55 years old, who meet all the inclusion criteria and none of the exclusion criteria. Women of childbearing potential (WOCBP) willing to use highly effective double barrier contraception will be eligible. Efforts will be made to ensure a reasonable gender

	balance. Up to 18 participants will be enrolled in cohorts of up to 6 participants, with each cohort comprising up to 3 dose groups.
Phase:	Phase 1b
Sites enrolling participants:	The trial is planned to be performed at the University of Sunshine Coast Clinical Trials Centres in Queensland, Australia. Moreton Bay Health Hub Morayfield 19-31 Dickson Road Morayfield QLD 4506 South Bank Building A2, SW1 Complex 52 Merivale Street South Brisbane QLD 4101
Description of Study Interventions:	**Investigational Medicinal Product:** Pyronaridine tablets (each containing 180 mg pyronaridine tetraphosphate) will be manufactured by Shin Poong Pharmaceutical Co., Ltd (Seoul, South Korea). Each participant will be administered the appropriate dose of tablets orally at the clinical trial unit under direct staff observation. Pyronaridine will be administered after a ≥ 8 hour fast. **Malaria Challenge Agent:** The *P. falciparum* 3D7 master cell bank (MCB) was produced from blood collected from a donor with clinical manifestation of malaria. Each challenge dose will be prepared aseptically at Q-Gen Cell Therapeutics (QIMR Berghofer) from an aliquot of the *P. falciparum* 3D7 MCB. Each participant will be inoculated intravenously with a dose of approximately 2,800 viable *P. falciparum* 3D7-infected erythrocytes in 2 mL of saline for injection. **Antimalarial Medications:** Compulsory Riamet® (each tablet containing 20 mg artemether and 120 mg lumefantrine) is marketed by Novartis Pharmaceutical Australia. A standard treatment course comprises 6 doses of 4 tablets administered orally over 60 hours (total course of 24 tablets). Each dose of tablets should be taken with food or drinks rich in fat (e.g. milk). If required • Primacin® (each tablet containing 13.2 mg primaquine phosphate equivalent to 7.5 mg of primaquine) is marketed by Boucher & Muir Pty Ltd. If gametocytes are present as determined by qRT-PCR at any time point after the confinement period, a single dose of Primacin® will be administered at the Investigator's discretion. A standard treatment course comprises a single dose of 6 tablets administered orally, unless G6PD deficient in which case a low dose of 15 mg will be administered.

	• Malarone® (each tablet containing 250 mg atovaquone and 100 mg proguanil hydrochloride) is marketed by GlaxoSmithKline Australia Pty Ltd. A standard treatment course comprises three doses of 4 tablets administered orally (one dose daily for 3 days; total course of 12 tablets). • Intravenous artesunate is the recommended parenteral treatment for malaria in Australia. The recommended dose regimen is 2.4 mg/kg at approximately 0, 12, 24, 48 hours and then daily for up to 7 days or until able to take oral drugs.
Study Duration:	It is estimated that the clinical portion of the study will be completed in 12 months.
Volunteer Duration:	Approximately 79 days for each participant which includes a screening period (up to 28 days), a period of observation following malaria challenge (8 days), follow-up after administration of the IMP (39 days) and follow up after compulsory antimalarial rescue treatment (3 days).

1.2 SCHEMA


Malaria challenge agent administration
Pyronaridine dosing
Antimalarial rescue treatment (Day 47±2 or earlier if required)
End of study
Study Timeline
Day -28
Day 0
Day 8
Day 12
Day 50±2
Screening
Outpatient malaria monitoring
Clinic confinement
Outpatient follow-up

1.3 SCHEDULE OF ACTIVITIES

The below table summarises the activities and procedures to be conducted as per this protocol during screening, eligibility, malaria inoculation, out patient monitoring, confinement, and at the end of study. This is a summary only, Section 8.1 provides detailed information about the assessments required at each time-point. There may be multiple samples/assessments for some activities on a particular day. Sections 8.2-8.3 provide more information about how to perform the assessments.

	Screening	Eligibility[a]	Malaria inoculation	Phone contact	Malaria monitoring[b]	IMP administration and clinical unit confinement[c]					Out-patient monitoring[d]	EOS
Day	-28 to -1	-3 to -1	0	1 to 3	4 to 7	8	9	10	11	12	13 to 50±2	50±2
Eligibility assessments												
Informed consent	X											
Beck Depression Inventory-II	X											
Cardiovascular risk	X											
Demography	X											
Medical and social history, inc/exc. criteria, & prior medications	X		X									
Drug & alcohol screen	X		X			X						
Body height	X											
Body weight	X											X
Serology & RBC alloantibody	X											X
Coagulation profile	X											
COVID-19 testing[l]		X	X		X (D 6 am)	X						
G6PD	X											
Serum pregnancy test (all females at screening & WOCBP at EOS)	X											X
Urine pregnancy test (WOCBP)			X			X						

	Screening	Eligibility[a]	Malaria inoculation	Phone contact	Malaria monitoring[b]	IMP administration and clinical unit confinement[c]					Out-patient monitoring[d]	EOS
Day	-28 to -1	-3 to -1	0	1 to 3	4 to 7	8	9	10	11	12	13 to 50±2	50±2
FSH (post-menopausal women)	X											
Safety assessments												
Full physical exam	X											X
Abbreviated physical exam			X			X						
Symptom-directed physical exam[e]					X	X	X	X	X	X	X	
Vital signs[f]	X		X		X	X	X	X	X	X	X	X
ECG	X		X			X	X	X	X	X	X	X
Urinalysis	X	X										X
Haem. & Biochem.	X[g]	X			X (D 7 am)	X			X		X	X
Safety serum retention			X									X
Diary card			X	X	X						X	X
AEs & Conmeds.			X	X	X	X	X	X	X	X	X	X
Study interventions												
Challenge agent inoculation			X									
Pyronaridine administration						X						
Definitive/rescue antimalarial treatment											X[h]	
Malaria monitoring and PK blood sampling												
Malaria 18S qPCR[i]			X		X	X	X	X	X	X	X	X
Parasite lifecycle stage qRT-PCR blood sampling											X	
Pyronaridine concentration[j]						X	X	X	X	X	X	X
Other												
Malaria future research (optional)			X			X					X[m]	X
Parasite viability *ex vivo* growth[k]						X	X	X	X	X	X (parasite regrowth only)	

AEs: adverse events; ECG: electrocardiograph; EOS: End of Study; G6PD: glucose-6-phosphate dehydrogenase qPCR: quantitative polymerase chain reaction; qRT-PCR: quantitative reverse-transcriptase polymerase chain reaction; RBC: red blood cell; WOCBP: women of childbearing potential.

[a] This visit is not required in the event that the screening visit is conducted within this period.

[b] Daily visits.

[c] See Section 8.1.5 for details of assessments to be performed prior to and after pyronaridine administration. Also note that there are multiple samples/assessments for some activities on the confinement days.

[d] Follow up visits will occur at least three times per week. See Section 8.1.6 for the specific timings of different activities.

[e] Symptom-directed physical exam will only be performed if clinically indicated.

[f] Supine & standing blood pressure and heart rate required at screening; seated measurements only at other time-points. On Day 0, record vital signs prior to inoculation and prior to leaving the clinical trial unit (approximately 60 minutes after inoculation). Record vital signs 3 times a day whilst confined.

[g] Haematology and biochemistry at screening includes lipids for cardiovascular risk factor.

[h] Riamet® will be administered to all participants on Day 47±2 or earlier as outlined in Section 8.1.7. Participants may be treated with Primacin® if gametocytaemia is suspected from parasite lifecycle stage qRT-PCR at the Investigator's discretion, to ensure clearance of gametocytes. If needed, participants will be administered a single oral dose of six Primacin® tablets (total dose 45 mg primaquine) with food, unless G6PD deficient in which case a low dose of 15 mg (two tablets) will be administered.

[l] Blood samples for malaria 18S qPCR will be collected following pyronaridine treatment at 4, 8, 12, 16, 20, 24, 30, 36, 42, 48, 60, 72, 84 and 96 hours. As outpatients, blood will be collected at least three times per week until antimalarial rescue treatment. Allowed time windows for sample collection are specified in Section 8.4.

[j] Blood samples for drug concentration measurements will be collected following pyronaridine treatment at 1, 2, 3, 4, 6, 8, 12, 16, 20, 24, 30, 36, 42, 48, 60, 72, 84 and 96 hours. As outpatients, blood will be collected on the following days: 13, 15, 17, 20, 22, 24, 27, 29, 32, 39, 44, 47 and 50. Allowed time windows for sample collection are specified in Section 8.4.

[k] Blood samples for parasite viability ex vivo growth will be collected following pyronaridine treatment at 4, 8, 12, 16, 20, 24, 48, 72 and 96 hours.

[l] Perform SARS-CoV-2 testing at the eligibility visit (NPA PCR), or at the screening visit if this visit is conducted two days prior to inoculation, Day 0 (RAT), Day 6am (NPA PCR) and Day 8 (RAT). Further details are specified in Section 8.3.7.

[m] Blood samples for future malaria research will be collected during the outpatient monitoring phase on Days 15±1 and 29±1

2 INTRODUCTION

2.1 BACKGROUND AND STUDY RATIONALE

Malaria is a mosquito-borne parasitic disease prevalent in tropical and subtropical regions around the world. It is caused by infection with the *Plasmodium* parasite of which five main species are known to infect humans: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. Of these species, *P. falciparum* is responsible for the majority of the malaria burden, particularly in sub-Saharan Africa. The malaria parasite has a complex life cycle in humans consisting of both a liver stage and a blood stage. Clinical symptoms of malaria infection are due to the asexual blood stage of the parasite's life cycle. Malaria is responsible for significant global morbidity and mortality, with an estimated 229 million new cases and 409,000 deaths worldwide in 2019 [1]. The emergence of antimalarial drug resistance has been a major setback for malaria control and progress towards the goal of elimination. Resistance has emerged to all antimalarials in widespread use, including artemisinin-based combination therapies (ACTs), the current first line treatment for uncomplicated *P. falciparum* malaria. Although resistance to ACTs has not yet been reported in Africa, the emergence of such resistance would likely have devastating consequences [2]. Thus, the development of novel antimalarial therapies are required to control malaria in regions with high prevalence of artemisinin-resistance, and prevent the emergence of resistance in sub-Saharan Africa where the world's malaria morbidity and mortality rates are highest.

Pyronaridine is an antimalarial compound developed in China in the 1970's and 1980's and has been shown to have potent activity towards blood-stage *P. falciparum* parasites, including isolates resistant to chloroquine, quinine, amodiaquine, pyrimethamine or mefloquine [3]. The antimalarial activity of pyronaridine is thought to occur primarily due to inhibition of hemozoin formation, which results in accumulation of toxic hematin in the parasite food vacuole [4]. Pyronaridine has been used as a single agent for the treatment of malaria in China for over 30 years. It was developed as a fixed-dose combination therapy with artesunate (an artemisinin derivative) for the treatment of acute uncomplicated *P. falciparum* malaria and blood stage *P. vivax* (Pyramax®, co-developed by Medicines for Malaria Venture, Geneva, Switzerland and Shin Poong Pharmaceutical Co., Ltd, Seoul, South Korea). Pyramax® received a positive opinion by European Medicines Agency (EMA) under the article 58 process [evaluation of medicinal products intended exclusively for markets outside the European Community]) in 2012 and 2015. Pyramax® was also added to the World Health Organization's (WHO) Model List of Essential Medicines (EML) and Model List of Essential Medicines for Children (EMLc) in 2017 and is now registered in 29 countries worldwide. Its procurement and use at a country level is further supported by a WHO Information Note of October 2019.

Despite the fact that pyronaridine has been used to successfully treat malaria for many years, either in monotherapy or in combination with other antimalarial drugs [3], its antimalarial activity in humans has not been completely characterised. Determining the relationship between pyronaridine pharmacokinetics (PK) and pharmacodynamic (PD) antimalarial activity in humans would inform efforts to broaden its use in new antimalarial combination therapies. Combination therapies are the focus of antimalarial drug

development to reduce the risk of selecting for resistant mutants and to target multiple stages of the parasite lifecycle including the transmissible gametocytes [5]. Further, using a combination of two or more drugs is typically considered a prerequisite for achieving cure without the need for an extended dosing regimen, which would pose compliance challenges. In the case of ACTs, the fast acting artemisinin component rapidly reduces the parasite burden, while a partner drug with a more moderate rate of action but longer elimination half-life is relied upon to clear residual parasites and prevent recrudescence. The rate of action of pyronaridine in clearing malaria parasitaemia in humans is unclear; however clinical pharmacokinetic studies have demonstrated that it has a relatively long elimination half-life of approximately 14 days [3] and thus may be able to offer extended protection from recrudescence.

Volunteer infection studies (VIS) using the induced blood stage malaria (IBSM) model have previously successfully characterised the PK/PD relationship of several experimental antimalarial compounds as well as antimalarial drugs currently in use [6-9]. The IBSM model involves intravenous inoculation of healthy adult participants with blood-stage parasites and administration of the test antimalarial when parasitaemia reaches a predefined threshold. Frequent blood sampling is performed to measure parasitaemia and drug concentration kinetics over the course of the study, thus providing data for PK/PD modelling analyses. Such VIS have been shown to accurately predict the activity of investigational antimalarials in studies in endemic populations [10,11].

The current study aims to determine the PK/PD relationship of single doses of pyronaridine following administration in healthy adult participants using the *P. falciparum* IBSM model. Data obtained in this study will support the use of pyronaridine in new antimalarial combination therapies by informing partner drug selection and dosing considerations.

2.2 RISK/BENEFIT ASSESSMENT

2.2.1 KNOWN POTENTIAL RISKS

Risks associated with the Investigational Medicinal Product (pyronaridine)

Pyronaridine in a fixed dose combination with artesunate received positive scientific opinion from EMA through Article 58 and is registered in many malaria endemic countries in Africa and South-East Asia as Pyramax® for treatment of acute uncomplicated malaria for both adults and children.

Through the extensive use of Pyramax® in many African and South-East Asian countries, the safety and tolerability of pyronaridine when combined with artesunate for the treatment of uncomplicated malaria is well established.

In studies involving patients with malaria or healthy volunteers, pyronaridine and artesunate combination treatment was associated with transient elevations in alanine aminotransferase (ALT) in approximately 6% of subjects, which were considered to be primarily due to pyronaridine [12]. In approximately 1.3% of cases these rises were >5 times the upper limit of normal (x ULN) without clinical sequelae. Transaminase rises occurred within the first 3-7 days and returned to within normal limits generally by Day 14. In a

repeat dose study, there was no increased risk of transaminitis when the pyronaridine/artesunate combination was administered once, or more than once as a repeat dose [12]. In a study which included patients with baseline transaminase levels >2× ULN, there was no clinical evidence of hepatotoxicity. The early onset (Day 3-7) and rapid resolution are consistent with a direct, low-level toxicity and do not indicate a risk of progressive liver injury with 3-day combination treatment [12].

Apart from the transient increase in transaminases., the most frequently reported adverse events (AEs; >1/100 to < 1/10) were headache, eosinophilia, neutropenia, anaemia, increased platelet count, vomiting (2.2 % to 2.5 %), abdominal pain, bradycardia, and hypoglycaemia [13]. Analyses performed in patients with malaria suggest that no clinically concerning QTc prolongation is expected at therapeutic concentrations of pyronaridine [14].

Additional information on non-clinical and clinical studies conducted with pyronaridine is provided in the IB.

Risks associated with the *P. falciparum* induced blood stage malaria model (IBSM)

Risks pertaining to the IBSM model are development of blood borne infections, reaction to the blood sample, severity of malaria infection (including development of haematological adverse events such as neutropenia or leucopenia), development of liver function abnormalities, and occurrence of cardiac AE). These are each discussed in detail below.

Risk management of blood borne infections in IBSM

The *P. falciparum* 3D7 challenge agent will be used to induce blood stage malaria in this trial. Although the challenge agent contains a very small amount of blood, risk of a transfusion-transmissible infection in this trial is extremely low. Firstly, donors were screened and tested negative for the presence of active blood borne infections. Secondly, the Australian Red Cross Blood Service (Blood Service) removed white blood cells from the donor blood to lower the risk of a transfusion-transmissible infection. Thirdly, the volume of blood associated with the malaria challenge inoculum is many thousands of times smaller than in a transfused unit (i.e., a relatively lower risk of infection). As part of the safety monitoring, all participants will be screened for blood borne infections before and after the trial. To date, no blood borne infections have been reported in any of the 435 participants who have received this *P. falciparum* 3D7 challenge agent in IBSM clinical trials.

Risk management of reaction to the blood sample in IBSM

The risk of developing red blood cell (RBC) alloantibodies and/or experiencing an acute haemolytic reaction in this trial is considered extremely low because the donor blood used to produce the challenge agent was blood group O (RhD) negative. People with this blood group are generally considered “universal donors”. However, it is possible that participants could suffer a transfusion reaction after they receive the challenge agent, or could develop alloantibodies to the donor RBCs that may make blood transfusion more difficult in the future. To date, one participant has developed an antibody response to a minor Rh antigen (anti-E antibody) following inoculation with the *P. falciparum* 3D7 challenge agent. However,

there was no laboratory evidence to indicate that the specific Rh phenotype of the donor RBCs in the challenge agent stimulated production of this anti-E alloantibody.

Alloimmunisation has been observed in an IBSM study testing a genetically modified *P. falciparum* 3D7 strain. This study investigated the safety, infectivity and immunogenicity of a strain in which the gene encoding the knob associated histidine rich protein (*kahrp*) had been deleted in order to explore its potential use as a live-attenuated malaria vaccine. In this study, two participants developed alloimmunisation (anti-C and anti-P1 antibodies in one participant and anti-c antibodies in the other participant) after administration of the highest dose of parasites (approximately 1000 fold higher dose of parasites than routinely administered in IBSM studies using the wild-type *P. falciparum* 3D7 strain). However, the total dose of erythrocytes (including non-parasitized) administered was similar to that routinely administered in IBSM studies (approximately 1×10^8 erythrocytes). Although anti-P1 antibodies are not considered clinically important for transfusion reactions, anti-C and anti-c antibodies may result in a delayed transfusion reaction characterized by slow drop in hemoglobin over two weeks post-transfusion. Consultation with transfusion medicine specialists at the completion of the study indicated there was no immediate risk if these participants were to require emergency administration of unmatched Group O Rh (D) negative blood, and in the setting of routine blood transfusion a full cross-match would obviate such a reaction. Additionally, since both antibodies were of low titre, there was a possibility that they may diminish over time. None of the other 6 participants enrolled in this study were found to have developed alloimmunisation; this includes 4 participants administered a lower dose of parasites and 2 participants administered the same dose of parasites as the 2 participants who developed alloimmunisation.

Participants will be monitored for signs and symptoms in the period immediately after administration of the challenge agent to further assess the risk of the challenge agent causing a transfusion reaction. All participants will be tested for RBC alloantibodies at screening and at the end of the trial as part of safety monitoring.

Women of childbearing potential (WOCBP) have a small additional risk if they develop RBC alloantibodies, as this could cause problems during pregnancy. WOCBP who have participated in several IBSM trials with *P. falciparum* isolate 3D7 have had no known issues to date. Including WOCBP in the trial enhances the generalisability of the trial results.

<u>Risk management of severity of malaria infection in IBSM</u>

The number of viable blood stage parasites that will be used to infect the participants in this trial (~2,800) is substantially lower than the parasitaemia induced from the bite of a single malaria-infected mosquito (~30,000 parasites are released into the blood when they break out of a single infected liver cell. In this trial, parasite growth and malaria symptoms will be closely monitored in participants following administration of the challenge agent. The threshold for commencement of antimalarial drug treatment defined for Day 8 has been selected because it is before the time-point at which clinical symptoms of malaria are likely to occur.

Participants will be admitted to the clinical trial unit for earlier treatment if a participant experiences an serious adverse event (SAE) or ≥ Common Terminology Criteria for Adverse Events (CTCAE) Grade 3 event deemed related to malaria and not self-resolved or relieved with concomitant medications, or the Investigator considers it necessary for participant safety. Blood will be collected at specified time-points to monitor for haematological adverse events such as neutropenia or leucopenia that may occur as a result of early malaria infection.

Risk management of liver function abnormalities in IBSM

Transient, asymptomatic liver function test (LFT) abnormalities, including rare cases of alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) elevations >10 fold the upper limit of normal (×ULN), have been reported in several participants in IBSM studies. However, changes in bilirubin have only been reported in one participant with unappreciated pre-existing liver disease. The LFT abnormalities did not require treatment, and resolved by the end of the studies. A few cases of the LFT elevations were considered serious AEs (SAEs) by two Pharma sponsors due to internal processes for SAE notifications. An independent review involving drug-induced liver injury experts found these liver function abnormalities are most likely a direct consequence of the malaria infection rather than a direct drug-induced liver injury caused by an investigational antimalarial drug. As a precaution, all participants with LFTS above ULN will be excluded in this trial. All enrolled participants will undergo regular safety monitoring to assess for asymptomatic liver function abnormalities. Participants will be required to minimise intake of possibly hepatotoxic substances, such as alcohol and paracetamol, during the trial. Drugs of abuse are not permitted under any circumstance. Given the fact that pyronaridine has also been associated with LFT abnormalities, participants with an ALT or AST > 2 x ULN, or a total bilirubin > 1 x ULN, will be dosed with compulsory antimalarial treatment rather than pyronaridine (see above, “Risks associated with IMP [pyronaridine]”).

Risk management of cardiac adverse events

There have been four cardiac SAEs reported in healthy participants in the Netherlands participating in malaria challenge studies using sporozoites (i.e., direct feeds by infected mosquitoes rather than IBSM infection). These four cardiac SAEs are described in the *P. falciparum* 3D7 challenge agent IB; two of the events have also been published as case reports [20,21]. No cardiac SAEs caused by the challenge agents have been reported in IBSM studies. However, in a recent trial, 2 participants (one infected with the *P. falciparum* 3D7 challenge agent and one infected with another malaria challenge agent strain [*P. falciparum* K13]) developed ventricular extra systoles that were classified as moderate AEs possibly related to malaria. As a precaution, people at significant risk of cardiovascular disease will be excluded from participating in IBSM studies, and regular safety monitoring, including physical examination and ECG recordings, will take place for all participants. Follow-up with a cardiologist is also available if any cardiac AEs are seen during the IBSM studies.

Risks associated with antimalarial rescue medications

Risks related to use of artemether-lumefantrine (Riamet®), primaquine phosphate (Primacin®) and atovaquone/proguanil (Malarone®) are detailed in the prescribing information provided by manufacturers. Primacin® may cause severe haemolytic anaemia in participants with glucose-6-phosphate dehydrogenase (G6PD) deficiency. Participants will be tested for G6PD deficiency at screening to ensure the safety of Primacin®. Participants with a severe G6PD deficiency will not be eligible for the study, however those with a mild or moderate G6PD deficiency may still be enrolled. Participant's G6PD status will determine how they are treated with Primacin®. A dosing table based on G6PD status will be provided in the Pharmacy Manual. To mitigate any other potential risks, the wellbeing of participants during and after administration of rescue treatment will be appropriately monitored. Participants who have any known contra-indication to any of the rescue medications according to the applicable labelling at screening will be excluded from participating in the study.

The overall risks to the participants in this study will be managed by frequent safety monitoring, including close monitoring during the period of confinement at the clinical unit when a single oral dose of pyronaridine will be administered. Safety monitoring will include clinical laboratory safety tests (biochemistry and haematology), physical examination, vital signs, ECG analysis and adverse event monitoring. Throughout the study, the safety, parasitaemia and PK data will be assessed by the PI, Sponsor Medical Director and Medical Monitor and the SDRT prior to commencing subsequent cohorts.

2.2.2 KNOWN POTENTIAL BENEFITS

There are no expected clinical benefits for the healthy participants who will participate in this trial. There may be benefits to others in the future if the results of this study lead to improved treatment outcomes in preventing people from contracting malaria and in treating patients infected with malaria.

2.2.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

On the basis of the safety provisions and risk minimisation activities outlined in Section 2.2.1, the overall risk to the participants in this trial is considered to be minimal and acceptable, and the potential of future improved treatment for malaria is considered to outweigh these potential risks.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS
Primary	
To characterise the PK/PD relationship of pyronaridine in healthy participants experimentally infected with blood stage *P. falciparum.*	The PK/PD relationship between pyronaridine blood concentrations and blood stage asexual parasitaemia.
Secondary	
To evaluate the safety and tolerability of single oral doses of pyronaridine in healthy participants experimentally infected with blood stage *P. falciparum*.	The safety and tolerability of pyronaridine will be determined by recording the incidence, severity and relationship to pyronaridine of adverse events (as determined by clinical, laboratory and ECG examinations) from Day 8 up to Day 50±2.
To characterise the parasite clearance kinetics following single doses of pyronaridine in healthy participants experimentally infected with blood-stage *P. falciparum*.	The parasite clearance kinetics following dosing with pyronaridine will be determined by calculating the parasite reduction ratio over a 48 hour period (PRR_{48}) and corresponding parasite clearance half-life ($PCt_{1/2}$).
To characterise the pharmacokinetics of pyronaridine following single oral dose administration in healthy participants experimentally infected with blood stage *P. falciparum*.	The pharmacokinetics of pyronaridine will be determined by calculating the following parameters using non-compartmental methods: C_{max}, T_{max}, t_{lag}, $AUC_{0\text{-}last}$, $AUC_{0\text{-}\infty}$, CL/F, Vz/F, λz, $t_{1/2}$.
To characterise the effect of pyronaridine on *P. falciparum* 3D7 parasite viability using phenotypic and molecular approaches.	Parasite growth in *ex vivo* cultures.

4 STUDY DESIGN

4.1 OVERALL DESIGN

This is an open-label, adaptive study that will utilise the *P. falciparum* IBSM model to characterise the PK/PD profile of pyronaridine.

Up to 18 healthy, malaria naïve adult participants are planned to be enrolled into this study, in cohorts of up to six participants each. Following a screening period of up to 28 days, cohorts of up to 6 healthy participants will be enrolled. After confirmation of eligibility, including a Rapid Antigen Test (RAT) for SARS-CoV-2, each participant will be inoculated intravenously on Day 0 with approximately 2,800 viable *P. falciparum*-infected erythrocytes. Participants will be followed up daily via phone call or text message on Days 1 to 3 post-inoculation to solicit any adverse events.

Participants will attend the clinical unit once on Days 4, 5, 6 and 7 for clinical evaluation and blood sampling to monitor the progression of parasitaemia, using quantitative polymerase chain reaction (qPCR) targeting the gene encoding 18S rRNA (referred to hereafter as malaria 18S qPCR). Participants will have a nasopharyngeal aspirate (NPA) for SARS-CoV-2 on Day 6 am. Participants will also have blood collected on Day 7 am to monitor haematology and biochemistry.

Participants will be admitted to the clinical trial unit on Day 8 for dosing with the investigational medicinal product (IMP; pyronaridine) when parasitaemia for the majority of participants is expected to be above 5,000 parasites/mL. Participants will need to return a negative (RAT) for SARS-CoV-2 prior to admission.

Pyronaridine will be administered as a single oral dose. Different doses of pyronaridine will be administered across and within cohorts in order to effectively characterise the PK/PD relationship. Each cohort will comprise up to three dose groups, with participants randomised to a dose group on the day of dosing. The highest dose of pyronaridine administered will be no more than 720 mg; the lowest dose administered will be no less than 180 mg. The proposed dosing regimen for the Cohort 1, assuming six participants are enrolled, is as below. If less than six participants are enrolled the Safety Data Review Team (SDRT) will meet between Day 0 and Day 8, to decide on the dose/s to be administered.

	Dose group		
	1A (n=2)	1B (n=2)	1C (n=2)
Pyronaridine	360 mg	540 mg	720 mg

Each subsequent cohort will be composed of up to 3 dose groups. If more than one dose is tested in a given cohort, subjects will be randomised after inoculation day but prior to Day 8. The dose/s administered to Cohort 2 and Cohort 3 will be selected based on PK/PD and safety data from the preceding cohort/s. The SDRT will review all available safety and tolerability data and PK/PD analysis outcomes from the previous cohort/s, prior to inoculation of the next cohort. The study will conclude when sufficient data have been obtained to define the PK/PD parameters for pyronaridine (the primary endpoint).

A maximum sample size of 18 participants administered pyronaridine is expected to be sufficient to achieve the primary endpoint.

Participants will be confined in the clinical unit for at least 96 h (Days 8 - 12) to monitor the safety and tolerability of pyronaridine dosing and to ensure adequate clinical response against *P. falciparum*. During confinement, regular safety assessments will be performed and blood will be collected to monitor parasite clearance and pyronaridine concentration. If participants are clinically well at the end of the confinement period, they will be discharged and monitored on an outpatient basis up to Day 50±2.

Participants will receive compulsory antimalarial rescue treatment with Riamet® (artemether/lumefantrine) on Day 47±2, or earlier in the following cases:

- Parasitaemia is not reduced by approximately 10-fold at the end of the 96 hour confinement period (Day 12) when compared with peak parasitaemia.
- Parasite regrowth occurs after an initial reduction in parasitaemia, with regrowth defined as ≥5000 parasites/mL and a two-fold parasitaemia increase within 48 hours.
- Volunteer discontinuation/withdrawal from the study.
- Investigator's discretion in the interest of participant safety.

Parasite lifecycle stages (including gametocytes) will be monitored at select time points during the study by collecting blood samples and using quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) targeting parasite lifecycle stage-specific mRNA transcripts.

If gametocytes are present as determined by qRT-PCR any time point after the confinement period, a single dose of Primacin® will be administered at the Investigator's discretion.
If an allergy or contraindication to Riamet® develops, Malarone® (atovaquone/proguanil) will be administered. If in the rare event participants are unable to complete oral antimalarial rescue treatment, they will be admitted to hospital to receive intravenous artesunate.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

The study design of this trial using the IBSM model has been used previously to safely and effectively determine the PK/PD relationship of several antimalarial agents [6-9]. The *P. falciparum* 3D7 challenge agent used to initiate blood-stage malaria infection in this study has been well characterised, with consistent and reproducible parasite growth occurring following intravenous inoculation of healthy participants in previous studies. Experience from these studies indicates that parasitaemia will be above 5,000 parasites/mL on Day 8 following inoculation, but clinical symptoms of malaria will either be absent or only mild in severity.

The duration of confinement within the clinic following dosing with pyronaridine on Day 8 (96 hours) is considered appropriate to ensure close monitoring of participants for safety purposes and to enable frequent blood sampling for parasitaemia and drug concentration measurements. Blood sampling time points have been selected by taking into account data from previous clinical PK studies, as well as

preclinical data on the antimalarial activity of pyronaridine. These time points may be adjusted during the study, by means of a protocol amendment, based on the emerging data in order to optimally determine the exposure/response relationship of pyronaridine.

The total follow-up period following pyronaridine dosing (42±2 days) is equal to three times the elimination half-life of pyronaridine (approximately 14 days). This duration was selected to allow sufficient time for pyronaridine blood concentrations to fall to a level amenable to parasite regrowth, which is required to calculate the PK/PD parameters. Further, this duration will ensure that participants have a negligible blood concentration of the IMP at the completion of the study.

Compulsory treatment with the registered antimalarial Riamet® (artemether/lumefantrine) will occur for all participants to ensure all parasites are cleared at the completion of the study. *P. falciparum* 3D7 parasites are known to be sensitive to artemether/lumefantrine, and Riamet® has been effective in clearing parasitaemia in previous IBSM studies using this challenge agent. A single dose of Primacin® will be used to ensure clearance of gametocytes (the life cycle stage responsible for transmission to mosquitoes) if these are present at any time point after the confinement period at the Investigator's discretion. An alternate registered antimalarial treatment Malarone® (atovaquone/proguanil) will be used in the event of contraindication to Riamet® Malarone has also previously been effective in clearing *P. falciparum* 3D7 parasitaemia [22]. If in the rare event participants are unable to complete oral antimalarial rescue treatment, they will admitted to hospital to receive intravenous artesunate, the recommended parenteral treatment of malaria in Australia.

4.3 JUSTIFICATION FOR DOSE

Pyronaridine will be administered as a single oral dose on Day 8, with different doses to be tested across and within cohorts in order to effectively characterise the PK/PD relationship. Each cohort will comprise up to three dose groups, with participants randomised to a dose group on the day of dosing. For each cohort, the SDRT will meet between Day 0 and Day 8, to decide on the dose/s to be administered. Dose selection will be decided based on number of participants inoculated with the malaria challenge agent, and when applicable available data from previous cohorts.

The highest dose of pyronaridine administered will be no more than 720 mg. This dose is equal to the maximum single daily dose of pyronaridine administered to adult patients ≥65 kg treated with Pyramax® (pyronaridine/artesunate combination) in accordance with manufacturer's product information for the treatment of acute uncomplicated malaria. Pyramax® is administered as a multiple dose treatment course (daily dosing with 720 mg pyronaridine/ 240 mg artesunate over three days for adults ≥65 kg), thus the total exposure to pyronaridine following administration of a single dose of 720 mg in the current study is expected to be lower than occurs during a course of Pyramax® treatment. A dose of 720 mg may be required to determine the maximum killing rate (E_{max}) of pyronaridine in the IBSM model.

The lowest dose of pyronaridine administered in this study will be no less than 180 mg. Modelling studies using clinical PK data and preclinical PD data (*in vitro* and murine studies) have indicated that a dose as low as 180 mg may be required for parasite regrowth to occur following initial parasite clearance. Parasite regrowth is required in this study to calculate all PK/PD parameters (primary endpoint).

4.4 END OF STUDY DEFINITION

A participant is considered to have completed the trial if they have completed all phases of the trial including the last visit or the last scheduled procedure shown in the Schedule of Activities (SoA), Section 1.4. For the purposes of clarity, a participant may still be considered to have completed the trial if they have not completed all scheduled procedures. The end of the trial globally is defined as the time at which all participants have completed the trial

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

Individuals must fulfil all of the following criteria to be eligible for inclusion in this trial:

Demography

1. Male or female (non-pregnant, non-lactating) aged 18 to 55 years inclusive who will be contactable and available for the duration of the trial and up to two weeks following the EOS visit.
2. Total body weight greater than or equal to 50 kg, and a body mass index (BMI) within the range of 18 to 32 kg/m^2 (inclusive). BMI is an estimate of body weight adjusted for height. It is calculated by dividing the weight in kilograms by the square of the height in metres.

Health status

3. Certified as healthy by a comprehensive clinical assessment (detailed medical history and full physical examination).
4. Fully vaccinated (meaning first, second and booster dose) against COVID-19 within 14 days of planned inoculation date.
5. Vital signs at screening (measured after 5 min in the supine position):
    - Systolic blood pressure (SBP) - 90–140 mmHg,
    - Diastolic blood pressure (DBP) - 40–90 mmHg,
    - Heart rate (HR) 40–100 bpm.
6. At Screening and pre-inoculation with the malaria challenge agent: normal standard mean of triplicate 12-lead electrocardiogram (ECG) parameters after 5 minutes resting in supine position in the following ranges:
    a. *QTcF ≤450 msec (male participants); QTcF ≤470 msec (female participants);*
    b. *QRS 50–120 msec*
    c. *PR interval ≤ 210 msec for both males and females, and*
    d. *Normal ECG tracing unless the PI or delegate considers an ECG tracing abnormality to be not clinically relevant.*
7. Women of childbearing potential (WOCBP) who anticipate being sexually active with a male during the trial must agree to the use of a highly effective method of birth control (see below) combined with a barrier contraceptive from the screening visit until 100 days after the last dose of pyronaridine (covering a full menstrual cycle of 30 days starting after 5 half-lives of last dose pyronaridine) and have a negative result on urine pregnancy test performed before inoculation with the malaria challenge agent.

Note:

a. *Highly effective birth control methods include: combined (oestrogen and progestogen containing) oral/intravaginal/transdermal/implantable hormonal contraception associated with inhibition of ovulation, progestogen-only oral/injectable/implantable hormonal contraception associated with inhibition of ovulation, intrauterine device, intrauterine hormone-releasing system, bilateral tubal occlusion, vasectomised partner, or sexual abstinence or same sex relationship.*

b. *Female participants who are abstinent (from penile-vaginal intercourse) must agree to start a double method if they start a sexual relationship with a male during the study. Female participants must not be planning in vitro fertilisation within the required contraception period.*

8. Women of non-childbearing potential (WONCBP) are defined as:

a. *Natural (spontaneous) post-menopausal defined as being amenorrhoeic for at least 12 months without an alternative medical cause with a screening follicle stimulating hormone level (FSH) >25 IU/L (or at the local laboratory levels for post-menopause)*

b. *Premenopausal with irreversible surgical sterilization by hysterectomy and/or bilateral oophorectomy or salpingectomy at least 6 months before screening (as determined by participant medical history)*

9. Males who have, or may have, female sexual partners of child bearing potential during the course of the study must agree to use a double method of contraception including condom plus diaphragm, or condom plus intrauterine device, or condom plus stable oral/transdermal/injectable/implantable hormonal contraceptive by the female partner, from the time of informed consent through to 70 days (covering a spermatogenesis cycle) after pyronaridine administration. Abstinent males must agree to start a double method if they begin sexual relationship with a female during the study and up to 70 days after the last dose of pyronaridine. Males with female partners of child-bearing potential that are surgically sterile, or males who have undergone sterilisation and have had testing to confirm the success of the sterilisation, may also be included and will not be required to use above described methods of contraception.

Regulations

10. Completion of the written informed consent process prior to undertaking any trial-related procedure.
11. Must be willing and able to communicate and participate in the whole trial.
12. Agreement to adhere to Lifestyle Considerations (see Section 5.3) throughout trial duration
13. Agreement to provide current contact details (two telephone numbers including a mobile number and a responsible adult as an emergency contact) and a relevant email address.

5.2 EXCLUSION CRITERIA

1. Individual who lives alone, OR, does not satisfy the following criteria: Participants who live alone may be included on a case-by-case basis, following discussion with the Principal Investigator. Participants who live alone must identify and provide contact details of a support person who is aware of the participant's participation in the study and is available to provide assistance if required (for example with contacting the participant in the event that study staff are unable to, or with transporting the participant to and from the study site if required).
2. Known hypersensitivity to pyronaridine, artesunate or any of its derivatives, artemether, lumefantrine or other artemisinin derivatives, proguanil/atovaquone, primaquine, or 4-aminoquinolines.
3. Haematology, biochemistry or urinalysis results at screening or at the eligibility visit that are outside of Sponsor-approved clinically acceptable laboratory ranges (appendix) or are considered clinically significant by the PI or their delegate.

4. Participation in any investigational product trial within the 12 weeks preceding pyronaridine administration.
5. Symptomatic postural hypotension at screening (confirmed on two consecutive readings), irrespective of the decrease in blood pressure, or asymptomatic postural hypotension defined as a decrease in systolic blood pressure ≥20 mmHg within 2–3 min when changing from supine to standing position.
6. Any history of anaphylaxis or other severe allergic reactions including face, mouth, or throat swelling or any difficulty breathing, or other food or drug allergy that the Investigator considers may impact on participant safety. Participants with seasonal allergies/hay fever or allergy to animals or house dust mite that are untreated and asymptomatic at the time of dosing can be enrolled in the trial.
7. History of convulsion (including drug or vaccine-induced episodes). A medical history of a single febrile convulsion during childhood (< 5 years) is not an exclusion criterion.
8. Presence of current or suspected serious chronic diseases such as cardiac or autoimmune disease, diabetes, progressive neurological disease, severe malnutrition, hepatic or renal disease. Acute or progressive hepatic or renal disease, porphyria, psoriasis, rheumatoid arthritis, asthma (excluding childhood asthma, or mild asthma with preventative asthma medication required less than monthly), or epilepsy.
9. History of malignancy of any organ system (other than localised basal cell carcinoma of the skin or *in situ* cervical cancer), treated or untreated, within five years of screening, regardless of whether there is evidence of local recurrence or metastases.
10. Individuals with history of schizophrenia, bipolar disorder psychoses, disorders requiring lithium, attempted or planned suicide, or any other severe (disabling) chronic psychiatric diagnosis including generalised anxiety disorder.
11. Individuals who have been hospitalised within five years prior to enrolment for either a psychiatric illness or due to danger to self or others.

12. History of an episode of mild/moderate depression lasting more than 6 months that required pharmacological therapy and/or psychotherapy within the last 5 years; or any episode of major depression.

    The Beck Depression Inventory-II (BDI-II) will be used as a validated tool for the assessment of depression at screening. In addition to the conditions listed above, participants with a score of 20 or more on the BDI-II and/or a response of 1, 2 or 3 for item 9 of this inventory (related to suicidal ideation) will not be eligible for participation. These participants will be referred to a general practitioner or medical specialist as appropriate. Participants with a BDI-II score of 17 to 19 may be enrolled at the discretion of an Investigator if they do not have a history of the psychiatric conditions mentioned in this criterion and their mental state is not considered to pose additional risk to the health of the participant or to the execution of the trial and interpretation of the data gathered.
13. History of recurrent headache (e.g. tension-type, cluster, or migraine) with a frequency of ≥2 episodes per month on average and severe enough to require medical therapy, during the 2 years preceding screening.
14. Presence of clinically significant infectious disease or fever (e.g., sublingual temperature ≥38.5°C) within the five days prior to inoculation.
15. Blood product donation to any blood bank during the 8 weeks (whole blood) or 4 weeks (plasma and platelets) prior to admission in the clinical unit on Day 8.
16. History or presence of alcohol abuse (alcohol consumption more than 40 g/4 units/4 standard drinks per day), or drug habituation, or any prior intravenous usage of an illicit substance.
17. Any individual who currently (within 14 days prior to inoculation) smokes >5 cigarettes/day.
18. Breastfeeding or lactating; positive serum pregnancy test at screening, positive urine pregnancy test upon admission or at other time points as specified by schedule of activities tables.
19. Any COVID-19 vaccine within 14 days of inoculation, any other vaccination within 28 days of IMP intake, and any vaccination planned up to the final follow-up visit.
20. Any corticosteroids, anti-inflammatory drugs (excluding commonly used over-the-counter anti-inflammatory drugs such as ibuprofen, acetylsalicylic acid, diclofenac), immunomodulators or anticoagulants within the past three months. Any individual currently receiving or having previously received immunosuppressive therapy (including systemic steroids, adrenocorticotrophic hormone or inhaled steroids) at a dose or duration potentially associated with hypothalamic-pituitary-adrenal axis suppression within the past year.
21. Use of prescription drugs (excluding contraceptives) or non-prescription drugs or herbal supplements (such as St John's Wort), within 14 days or five half-lives (whichever is longer) prior to inoculation. Limited use of other non-prescription medications or dietary supplements, not believed to affect participant safety or the overall results of the trial, may be permitted on a case-by-case basis following approval by the Sponsor in consultation with the PI. Participants are requested to refrain from taking non-approved concomitant medications from recruitment until the conclusion of the trial.
22. Cardiac/QT risk:
    - Family history of sudden death or of congenital prolongation of the QTc interval or known congenital prolongation of the QTc interval or any clinical condition known to prolong the QTc interval.

- History of symptomatic cardiac arrhythmias or with clinically relevant bradycardia.

23. Any history of malaria or participation in a previous malaria challenge trial or malaria vaccine trial.
24. Must not have had malaria exposure that is considered by the PI or their delegate to be significant. This includes but is not limited to: history of having travelled to or lived (>2 weeks) in a malaria-endemic region during the past 12 months or planned travel to a malaria-endemic region during the course of the trial; history of having lived for >1 year in a malaria-endemic region in the past 10 years; history of having ever lived in a malaria-endemic region for more than 10 years inclusive. For endemic regions see https://malariaatlas.org/explorer/#/, Bali is not considered a malaria-endemic region.
25. Has evidence of increased cardiovascular disease risk (defined as >10%, 5-year risk for those greater than 35 years of age, as determined by the Australian Absolute Cardiovascular Disease Risk Calculator [http://www.cvdcheck.org.au/]). Risk factors include sex, age, systolic blood pressure (mm/Hg), smoking status, total and HDL cholesterol (mmol/L), and reported diabetes status.
26. History of splenectomy.
27. Individual unwilling to defer blood donations to the Blood Service for at least twelve months after the EOS visit.
28. Individual who has ever received a blood transfusion.
29. Any recent (<6 weeks) therapy with an antibiotic or drug with potential antimalarial activity (e.g. chloroquine, piperaquine phosphate, benzodiazepine, flunarizine, fluoxetine, tetracycline, azithromycin, clindamycin, doxycycline etc.).

General conditions

30. Any individual who, in the judgement of an Investigator, is likely to be non-compliant during the trial, or is unable to cooperate because of a language problem or poor mental development.
31. Any individual in the exclusion period of a previous trial according to applicable regulations.
32. Any individual who is an Investigator, research assistant, pharmacist, trial coordinator, or other staff thereof, directly involved in conducting the trial.
33. Any individual without good peripheral venous access.

Biological status

34. Positive result on any of the following tests: hepatitis B surface antigen (HBs Ag), anti-hepatitis B core antibodies (anti-HBc Ab), anti-hepatitis C virus (anti-HCV) antibodies, anti-human immunodeficiency virus 1 and 2 antibodies (anti-HIV1 and anti-HIV2 Ab).
35. Positive urine drug test. Any drug listed in the urine drug screen unless there is an explanation acceptable to an Investigator (e.g., the participant has stated in advance that they consumed a prescription or over-the-counter product that contained the detected drug) and the participant has a negative urine drug screen on retest by the pathology laboratory.
36. Severe G6PD deficiency.
37. Positive alcohol breath test.
38. Positive for SARS-CoV-2 by PCR or RAT.

39. Positive for red cell antibodies

5.3 LIFESTYLE CONSIDERATIONS

While participating in this trial, participants are asked to:

- Refrain from alcohol consumption of more than 20 g/2 units/2 standard drinks per day from 24 hours before inoculation until the EOS.
- Abstain from any illicit drug habituation until the EOS.
- Abstain from any alcohol and tobacco use for the duration of clinical trial unit confinement.
- Refrain from tobacco use of more than five cigarettes or equivalent per day until the EOS.
- Refrain from excessive consumption of beverages or food containing xanthine bases including Red Bull, chocolate, coffee etc. (more than 400 mg caffeine per day, equivalent to more than 4 cups of coffee per day).
- Refrain from consumption of Seville oranges, and grapefruit or grapefruit juice from 7 days prior to inoculation until EOS.
- Refrain from consumption of quinine containing foods/beverages such as tonic water and lemon bitter from inoculation until the EOS.
- Abstain from strenuous exercise for 24 h before each blood collection for clinical laboratory tests. Participants may participate in light recreational activities during studies.

5.4 SCREEN FAILURES

Healthy candidate participants who do not fulfil all the inclusion criteria, and/or fulfil any of the exclusion criteria should not be enrolled, however unscheduled visits may be planned to assess, confirm, and follow-up on out-of-range clinical laboratory test, vital sign, or ECG values that determine a participant's eligibility. A positive urine drug screen should only be re-tested if there is a strong rationale for doing so (for example, false positive). The result of the re-test must be considered for participant eligibility and must be available prior to inoculation or pyronaridine administration. In case of doubt, the PI is to confer with the Medical Monitor for agreement.

If a participant does not meet all eligibility criteria (is a screen failure) but at some point in the future is expected to meet the eligibility criteria, the participant may be rescreened on one occasion only. Participants who are rescreened should be assigned the same screening number as for the initial screening.

A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE). Screen failure information will not be entered into the eCRF. Participants who fail screening due to an underlying medical condition previously unknown to them will be reimbursed for their time, and provided with the appropriate referrals for guidance and counselling for their condition.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

It is anticipated that up to 18 healthy, adult, malaria- naïve participants will need to be dosed with pyronaridine in this trial. It is anticipated that a total of 6 reserve participants will be required to ensure adequate participant numbers.

Participants will be recruited by general or trial specific advertising via print, radio, social media, or poster media, as approved by the HREC(s). No restrictions will apply for ethnic or racial categories.

Participants who withdraw or are withdrawn from the trial will be compensated on a fractional basis for their involvement unless they are withdrawn as a consequence of their misconduct.

Reserve participants who do not participate in the trial will be compensated for the inconvenience associated with their attendance for screening and attendance on Day 0.

The monetary value of the compensation for each trial part is documented in the respective Participant Information Sheet.

6 STUDY INTERVENTIONS

6.1 STUDY INTERVENTIONS ADMINISTRATION

The following sections describe the study interventions. A pharmacy manual with detailed information will be finalised and approved prior to study start.

6.1.1 STUDY INTERVENTIONS DESCRIPTION

IMP

Pyronaridine is a benzonaphthyridine derivative first synthesized in China in 1970. Pyronaridine has been used in China for the treatment of malaria as a single agent for more 50 years. The antimalarial activity of pyronaridine is thought to occur primarily due to inhibition of hemozoin formation, which results in accumulation of toxic hematin in the parasite food vacuole.

It was developed as an oral fixed-dose combination therapy with artesunate (an artemisinin derivative) for the treatment of acute uncomplicated *P. falciparum* malaria and blood stage *P. vivax* (Pyramax®, co-developed by Medicines for Malaria Venture, Geneva, Switzerland and Shin Poong Pharmaceutical Co., Ltd, Seoul, South Korea). Pyramax® received a positive opinion by European Medicines Agency (EMA) under the article 58 process [evaluation of medicinal products intended exclusively for markets outside the European Community] in 2012 and 2015. Pyramax® was also added to the World Health Organization's (WHO) Model List of Essential Medicines (EML) and Model List of Essential Medicines for Children (EMLc) in 2017. Its procurement and use at a country level is further supported by a WHO Information Note of October 2019. Pyramax® is currently approved for use in 29 countries, although it has not yet been registered for use in Australia. For the current study, pyronaridine tablets for oral

administration will be manufactured by Shin Poong Pharmaceutical Co., Ltd, licence holder and manufacturer of Pyramax.

Challenge agent

A *P. falciparum* 3D7 master cell bank (MCB) was produced from a participant with type O Rh(D) negative blood who was infected with the parasite by mosquito bite. Blood was collected and aliquoted into cryovials and stored in liquid nitrogen under controlled conditions. Refer to the *P. falciparum* 3D7 IB for more details. A cryovial will be retrieved from storage, thawed, and used to aseptically prepare the intravenous inoculum at Q-Gen Cell Therapeutics (QIMR Berghofer).

Rescue Antimalarial medications

Riamet® (Novartis Pharmaceuticals Australia Pty Ltd) is an oral fixed dose combination therapy containing artemether and lumefantrine. Riamet® is registered in Australia for the treatment of acute uncomplicated malaria.

Primacin® (Boucher & Muir Pty Ltd) contains the active ingredient primaquine phosphate. Primaquine phosphate is an 8-aminoquinoline effective against the sexual forms (gametocytes) of *Plasmodium* species which are responsible for transmission to mosquitoes. Primacin® is registered for use in Australia.

Malarone® (GlaxoSmithKline Australia Pty Ltd) is an oral fixed dose combination therapy containing atovaquone and proguanil hydrochloride. Malarone® is registered in Australia for the treatment of acute uncomplicated malaria.

Artesunate (powder and diluent for reconstitution, imported by Link Pharmaceuticals) is an artemisinin derivative; the intravenous formulation is typically used to treat severe malaria. Although parenteral artesunate does not currently have marketing approval in Australia, the Therapeutic Goods Administration (TGA) allows importation of artesunate for Category A use in patients with severe malaria under the Special Access Scheme (SAS).

6.1.2 DOSING AND ADMINISTRATION

IMP

Pyronaridine tablets will be administered after an overnight fast of ≥ 8 hours as a single oral dose with 240 mL of water on Day 8. Pyronaridine will not be administered if any of the criteria described in 7.1.1 has been met. In this situation participants will be administered antimalarial rescue treatment. Different doses of pyronaridine will be tested across and within cohorts, with the doses to be determined by the SDRT based on the emerging data during the study. The maximum dose to be tested in the study will be no more than 720 mg (4 tablets). The minimum dose to be tested in the study will be no less than 180 mg (1 tablet).

Challenge Agent

The malaria challenge agent, containing an estimated ~2,800 viable *P. falciparum* 3D7 parasite-infected erythrocytes in a volume of 2 mL, will be administrated intravenously on Day 0. The actual number of

parasites inoculated will take into account the loss of viability resulting from cryopreservation, storage and thawing. Participants will undergo intravenous cannulation with a 20 or 22 gauge cannula. Placement and patency will be checked by flushing the vein with 5-10 mL of clinical grade saline. The inoculum will be injected, and the cannula again flushed with 5-10 mL of clinical grade saline. The cannula will then be removed, and haemostasis ensured by use of an appropriate dressing. An extra syringe will be prepared to quantify the parasite count of the challenge agent by malaria 18S qPCR.

Rescue Antimalarial medications

Compulsory definitive antimalarial treatment with Riamet® for all participants will be initiated on Day 47±2. Treatment may be initiated earlier in accordance with the criteria described in Section 4.1. Riamet® tablets will be administered as six oral doses of four tablets (total course of 24 tablets) given over a period of 60 hours (total dose of 480 mg artemether and 2.88 g lumefantrine). Participants will be reminded of the potential side effects of Riamet® and will be given the CMI for Riamet®.

Participants may be treated with Primacin® if gametocytaemia is suspected from parasite lifecycle stage qRT-PCR at the Investigator's discretion, to ensure clearance of gametocytes. If needed, participants will be administered a single oral dose of six Primacin® tablets (total dose 45 mg primaquine) with food, unless G6PD deficient in which case a low dose of 15 mg (two tablets) will be administered.
Participants will be reminded of the potential side effects of Primacin® and will be given the CMI for Primacin®.

If an allergy or contraindication to Riamet® develops, Malarone® may be administered at the Investigator's discretion. A treatment course consists of four tablets administered once daily for three days (total dose of 3 g atovaquone and 1.2 g proguanil hydrochloride). Participants will be reminded of the potential side effects of Malarone® and will be given the CMI for Malarone®.

Intravenous artesunate may be administered if the participant vomits or cannot tolerate oral drugs. In this rare event, the participant will be admitted to hospital for treatment. Artesunate will be administered as 2.4 mg/kg IV bolus on admission, with repeat dosing at 12 hours and 24 hours, then once daily until oral therapy is possible.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

The clinical trial unit pharmacist or delegate, as nominated by the PI, is responsible for maintaining accurate study intervention accountability records throughout the study. Study interventions include the malaria challenge agent, the IMP (pyronaridine), and the antimalarial rescue treatments. Dispensing, accountability and documentation will be in accordance with the clinical unit standard procedures.

All products will be inventoried upon receipt by the clinical trial unit pharmacist. The condition of the products at the time of receipt by the pharmacist will be documented, as will the time restrictions of use for the syringes containing the challenge agent. The lot numbers and expiry dates of all study interventions will be documented. The clinical trial unit pharmacist or delegate will ensure that the received products are the specified formulation.

The storage, handling and the disposal of the challenge agents will be in accordance with approved procedures. All dosages prescribed and dispensed to the participants and all dose changes during the study must be recorded in the eCRFs and accountability logs. All drug supplies are to be used only in accordance with this protocol, and not for any other purpose. All used medications will be fully documented. Used and unused drug containers must be destroyed at the unit once drug accountability is final and has been checked by the Sponsor or its delegate, and written permission for destruction has been obtained from the Sponsor.

Study products and study accountability logs will be available to the Sponsor or their representative as part of the study monitoring procedures. Upon completion of the study, copies of all study drug management records will be provided to the Sponsor. Original records will be maintained at the clinical unit with the rest of the study records.

IMP

Pyronaridine tablets will be manufactured by Shin Poong Pharmaceutical Co., Ltd, Seoul, South Korea and acquired by the clinical unit.

Challenge agent

Aliquots of the *P. falciparum* 3D7 MCB are stored in liquid nitrogen under controlled conditions. On the day of inoculation, cryovials will be retrieved from storage and used to aseptically prepare the challenge agent at Q-Gen Cell Therapeutics (QIMR Berghofer). The clinical trial unit pharmacist or designee will be responsible for maintaining the accurate *Plasmodium falciparum* 3D7 Challenge Agent Accountability Log as per clinical trial unit standard operating procedures (SOPs).

Rescue Antimalarial medications

Riamet® (distributed by Novartis Pharmaceuticals Pty Ltd), Primacin® (distributed by Boucher & Muir Pty Limited), and Malarone® (distributed by GlaxoSmithKline Australia Pty Ltd) will be acquired by the clinical

trial unit. Artesunate for intravenous administration is available within the Central Pharmacy of Queensland Health.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

The contents of the dispensing labels for the IMP, the malaria challenge agent, and the antimalarial rescue treatments will be in accordance with all applicable regulatory requirements.

IMP

Pyronaridine tablets, each containing 180 mg pyronaridine tetraphosphate, are orange, round, film-coated scored tablets. Excipients include: microcrystalline cellulose, crospovidone, mannitol, magnesium stearate, talc, Hypromellose 2910, macrogol 6000 and Opadry OY-S-23068. Tablets are packaged in aluminium/PVC/aluminium-oPA foil blisters containing 9 tablets per blister. The blisters are packed into cartons.

Challenge agent

The malaria challenge agent will contain parasitised and unparasitised erythrocytes, resuspended in 0.9% Sodium Chloride Intravenous Infusion, in a total volume of 2 mL in syringes. The syringes will be double contained following preparation and labelled in accordance with GCP guidelines. Labelling is in accordance with PIC/S Annex 13.

Rescue Antimalarial medications

Riamet® tablets (each containing 20 mg artemether/120 mg lumefantrine) are yellow, round, flat tablets marked with N/C and a score line on one side and CG on the other side. Each carton contains 24 tablets.

Primacin® tablets (each containing 13.2 mg primaquine phosphate equivalent to 7.5 mg primaquine) are round, flat, orange uncoated tablets available in bottles of 28 or 56 tablets.

Malarone® tablets are round, pink and film-coated, and are engraved with “GX CM3”. Malarone® tablets are supplied in blister packs of 12 or 24 tablets.

Artesunate powder for reconstitution is supplied in vials containing 60 mg artesunate. Each vial is reconstituted with an ampoule (1 mL) of sodium bicarbonate 5%. Sodium chloride 0.9 % (5 mL) is then added to reconstituted artesunate vial to create a 10 mg/mL solution (total volume 10 mL).

6.2.3 PRODUCT STORAGE AND STABILITY

All drugs will be held in appropriate locked storage conditions at the clinical trial unit until required.

IMP

Pyronaridine tablets are to be stored below 25°C in the original package. The shelf life is 18 months.

Challenge agent

The *P. falciparum* 3D7 challenge agent is prepared at Q-Gen on inoculation day (Day 0). The time between preparation of the challenge agent and administration to the participant will be a maximum of 4 hours. The syringes containing the challenge agent will be stored in a temperature monitored validated transport container at 2-15°C during transportation from Q-Gen to the clinical trial unit and will be immediately transferred to a temperature monitored onsite refrigerator (2-15°C). The clinical trial unit pharmacist will document receipt conditions and time restrictions of use. The challenge agent will then be dispensed to participants as per written prescription. Transporter logs should be provided to the Sponsor.

Rescue Antimalarial medications

Riamet® tablets are to be stored below 30°C and protected from moisture. Primacin® tablets are to be stored below 25°C. Malarone® tablets are to be stored below 30°C. The reconstituted artesunate for intravenous administration should be prepared freshly for each administration and should not be stored.

6.2.4 PREPARATION

IMP

No preparation is required for the pyronaridine tablets for oral administration.

Challenge agent

The challenge agent will be prepared aseptically at Q-Gen from frozen cryovials of the *P. falciparum* 3D7 MCB by nominated QIMR Berghofer staff members. The erythrocytes will be thawed, washed, resuspended in saline, diluted in a final volume of 2 mL of clinical grade saline, and dispensed into syringes. Any remaining unused erythrocytes will be discarded as per approved SOPs.

Rescue Antimalarial medications

Riamet®, Primacin® and Malarone® are available as tablets and no preparation is required. Artesunate for intravenous administration will be prepared according to manufacturer's instructions within the hospital setting immediately prior to administration.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMISATION

All participants will receive an identification number (screening number) as soon as they have signed the informed consent form. Participants who meet the eligibility criteria and are enrolled in the study will be assigned an enrolment number on Day 0 immediately prior to administration of the malaria challenge agent. Participants will be randomised within each cohort to a dose group on Day 8, immediately prior to administration of the IMP. This is an open label study and therefore no blinding will be performed.

6.4 STUDY INTERVENTIONS COMPLIANCE

The investigator will administer the malaria challenge agent intravenously at the clinical trial unit on Day 0.

The IMP (single oral dose of pyronaridine) will be administered at the clinical trial unit on Day 8 under direct observation by staff.

The first dose of Riamet® will be administered at the clinical trial unit under direct observation by staff. The subsequent five doses may be taken at home. Participants will receive a phone call or text message from the clinical trial unit staff to ensure compliance.

If Primacin® is required at any time point after the confinement period, it will be administered at the clinical trial unit under direct observation by staff.

If Malarone® is required, the first dose will be administered at the clinical trial unit under direct observation by staff. The subsequent two doses may be taken at home.

Participants will receive a phone call or text message from the clinical trial unit staff to ensure compliance. If a participant requires intravenous artesunate, the participant will be admitted to hospital for treatment.

6.5 CONCOMITANT THERAPY

Concomitant medications, treatments and procedures are those occurring from the time of administration of the malaria challenge agent (Day 0), until the final study visit. Those occurring prior to Day 0 are classified as prior medications, treatments and procedures. Medications taken within 28 days before Day 0 will be recorded as prior medication.

On Day 0, participants will be questioned in relation to relevant aspects of compliance with the study protocol, including drug intake since their screening visit. Details of all drugs taken (prescription and over-the-counter, systemic and topical administration) will be recorded at this time and appropriate action taken.

From Day 0 until the EOS visit, no medications will be permitted to be taken without the approval of the PI (with the exception of contraceptives and ibuprofen). Ibuprofen (preferred) may be administered at doses of up to 1.2 g/24 hours, or paracetamol up to 4 g/24 hours with Investigator approval, for treatment of headache or other pain if required. Ibuprofen is the preferred treatment for headache or pain. To minimise the risk of liver enzyme elevation, paracetamol is to be avoided if possible; however paracetamol may be required by some participants and as such is not a prohibited substance. All other medications will be assessed by the PI on a case by case basis with regard to the participant's wellbeing and potential for the medication to interfere with study interventions.

Any medication taken during the study for treatment of a medical condition or AE is to be recorded in the concomitant medication pages in the eCRF. The exact dose, route and timing of each dose should be recorded.

6.5.1 RESCUE MEDICINE

Antimalarial rescue treatments used in this study are Riamet®, Primacin® (if required), Malarone® (if required), and IV artesunate (if required). Details are provided in Sections 6.1 and 6.2.

7 STUDY INTERVENTIONS DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTIONS

The Sponsor, SDRT, PI, approving HREC(s), and the TGA independently reserve the right to discontinue the trial at any time for safety or other reasons. This will be done in consultation with the Sponsor where practical. In the event of premature trial termination or suspension, the above-mentioned parties will be notified in writing by the terminator/suspender stating the reasons for early termination or suspension (with the exception of the TGA as it is the Sponsor's responsibility to notify regulatory authorities). After a decision to prematurely terminate the trial or to suspend the trial, the Sponsor and the PI will ensure that adequate consideration is given to protecting the participants' interest and safety. The PI must review all participants as soon as practicable and complete all required records.

7.1.1 CRITERIA FOR ADMINISTRATION OF RESCUE MEDICATION INSTEAD OF IMP DOSING ON DAY 8

Once inoculated, it is expected that some study participants will experience malaria symptoms and biological changes (i.e. mild ALT/AST elevation, mild decrease in neutrophil count). Therefore, the following criteria should be considered as guidance for the decision not to proceed with pyronaridine dosing on Day 8 and preference for rescue medication instead:

1. A participant experiences an SAE or ≥ CTCAE Grade 3 event (e.g. inoculum-related events such as lymphopenia or neutropenia).
2. A participant has an ALT or AST > 2 x ULN or total bilirubin > 1 x ULN.
3. A participant with positive alcohol or urine drug screen.
4. A participant who tests positive for SARS-CoV-2 (section 8.3.7).
5. A participant with one of the following ECG abnormalities:
   - QTcF at any time >480 msec,
   - Bundle branch block (except right bundle branch block that was present prior to pyronaridine administration),
   - Any arrhythmia, except:
     a. *Sinus bradycardia that is clinically asymptomatic, and not associated with any other relevant ECG abnormalities,*
     b. *sinus tachycardia that is clinically asymptomatic, and not associated with any other relevant ECG abnormalities,*
     c. *Respiratory sinus arrhythmia,*
     d. *Wandering atrial pacemaker,*

   e. *Isolated, single premature atrial/ventricular complex (i.e., no bigeminy, trigeminy, couplets, triplets or salvos) that does not occur more than once in a particular ECG tracing.*
6. The Investigator and delegates may also decide not to proceed with pyronaridine dosing based on other safety signals (clinical, ECG, vitals and laboratory tests) not included in the above criteria if there is a risk perceived for the study participants.

In the event that participants are dosed with antimalarial rescue treatment instead of pyronaridine, participants will be administered their first dose of antimalarial rescue treatment at the clinic, but will then be able to return home. The participant will be contacted daily via phone to check that each dose of antimalarial treatment is being taken, and to ascertain any adverse events. Following antimalarial rescue treatment, blood will be taken for 18S qPCR until at least one negative result is obtained. No further bloods will be collected for drug concentration. Blood sampling for haematology and biochemistry and for future malaria research (if the participant has consented) will occur as scheduled until day 29±1. The EOS visit may be brought forward to day 29±1, at the discretion of the PI.

If more than two discontinuations pre-IMP administration occur, additional participants may be recruited to subsequent cohorts to replace the discontinued participants on agreement with the study Sponsor.

7.2 VOLUNTEER DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the trial at any time upon request.

An Investigator may discontinue or withdraw a participant from the trial for the following reasons:

- Pregnancy
- Significant trial intervention non-compliance
- If any clinical AE, laboratory abnormality, or other medical condition or situation occurs (or an exclusion criteria is revealed that was not previously recognised) such that continued participation in the trial would not be in the best interest of the participant

The reason for participant discontinuation or withdrawal from the trial will be recorded on the eCRF. Participants who sign the informed consent form and are randomised but do not receive malaria inoculation may be replaced. Participants who sign the informed consent form, and are randomised and receive malaria inoculation, and subsequently withdraw, or are withdrawn or discontinued from the trial, may be replaced after mutual agreement between the Sponsor and the PI. The decision regarding the replacement of participants will be documented.

Participants who have been inoculated and indicate they wish to withdraw from the trial must complete the full course of definitive antimalarial treatment.

Participants will be reminded of the importance of completing their antimalarial treatment. Additionally, the PI will demonstrate due diligence in following up with the participant to ensure antimalarial therapy has been completed successfully. Participants may also be treated with Primacin™ if gametocytaemia is suspected from parasite lifecycle qRT-PCR or by the presence of stable low-level parasitaemia to ensure complete clearance of gametocytes. The decision to administer Primacin™ will be made by the PI in consultation with the medical monitor.

If a participant is withdrawn from the trial, the Sponsor will be informed immediately. If there is a medical reason for withdrawal, the participant will remain under the supervision of the PI until satisfactory health has returned, or medical/clinical care has been transferred to the participant's general practitioner or to a hospital consultant.

The PI will make every effort to determine the primary reason for a participant's withdrawal from the trial and record this information in the eCRF. If the participant is withdrawn from the trial procedures or follow-up for any reason, with the participant's permission, medical care will be provided for any SAEs that occurred during participation in the trial until the symptoms of any SAEs are resolved and the participant's condition becomes stable.

7.3 LOST TO FOLLOW-UP

If a participant does not attend a scheduled visit, the Investigator will apply due diligence by documenting all steps taken to contact the participant (e.g., dates of phone calls, registered letter, home visit, etc.) in the source documents.

It will be explained to the participants during the consenting process and throughout the trial that they must be readily contactable. The period from inoculation to definitive antimalarial treatment is the critical period for participant safety because they may have blood stage parasites. A participant will be deemed "missing" if they fail to reply to communication to their personal mobile phone and nominated contact's number after 24 h. If after 36 h the participant fails to respond then an Investigator will organise a home visit. Subsequently, if the participant is still absent, an Investigator will request assistance from the local police to locate the missing participant. Once the participant is found, the participant will be administered Riamet if this has not already been administered®, and Primacin® if required.

A participant will be deemed lost to follow-up in the unlikely event that the participant is unable to be contacted or located despite all of the above measures. This is not anticipated to occur prior to administration of antimalarial rescue treatment, as in this situation, all efforts to locate the participant, including with assistance from local police, would continue until the participant is located and treated.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 STUDY CONDUCT SCHEDULE

8.1.1 SCREENING VISIT (DAY -28 TO DAY -1)

A screening visit will be scheduled after an initial phone interview conducted by clinical trial unit staff has occurred to review background information. For the screening visit, potential participants will be asked to come to the clinical trial unit after an overnight fast of ≥8 hours. During this initial screening visit, an Investigator will discuss the details of the trial with the potential participant, and the participant will read the Participant Information Sheet and be encouraged to ask questions. The potential participant will be fully informed of the nature of the trial at this time. Individuals willing to be considered for inclusion may

sign the Informed Consent Form during the screening visit or may return to the clinical trial unit after further consideration of the trial and the Informed Consent Form. The participant will be given a copy of the Participant Information Sheet and signed Informed Consent Form for their records. The consenting process will be documented in the source notes.

The signed and dated originals will be held on file by the clinical trial unit. Participation consent must be obtained from all individuals prior to screening tests. Once the potential participant has provided written consent to participate, the pre-trial screening may be initiated.

Participants who complete all screening procedures and satisfy all entry criteria will be considered eligible to participate in this trial. For eligibility parameters, unscheduled visits may be planned to assess, confirm, and follow-up on out-of-range clinical laboratory test, vital sign, or ECG values that determine a participant's eligibility. In case of doubt, the PI is to confer with the Medical Monitor for agreement.

If screening laboratory results are abnormal (e.g. HIV testing), the participant will be referred for appropriate counselling. If any clinically significant abnormalities are detected during screening, the participant will be referred to a general practitioner or medical specialist for follow-up tests as appropriate.

Participants will be advised of the requirement to repeat some screening tests during the Day -3 to Day -1 eligibility confirmation visit (unless screening occurs within eligibility Day -3 to -1 window) and/or on inoculation day to determine their continuing eligibility.

The screening will be conducted within 28 days prior to the inoculation day and will include all activities listed below:

- A screening number will be assigned to each participant (as per clinical trial unit standard format).
- Elicit a complete medical history and use of medications.
- Elicit a social history including recreational drug, alcohol and tobacco use.
- Elicit demographic data.
- Record body height and weight.
- Perform alcohol breath test.
- Perform urine drug screen.
- Perform complete physical examination.
- Ask participant to complete the Beck Depression Inventory.
- Assess the cardiovascular disease risk (as per the Australian Absolute Cardiovascular Disease Risk Calculator).
- Record vital signs (supine and standing blood pressure and heart rate).
- Obtain a 12-lead ECG.
- Collect urine for urinalysis.
- Collect NPA for SARS-CoV-2 testing (if screening visit is conducted two days prior to inoculation).
- Collect blood samples for haematology and biochemistry (including lipids for cardiovascular risk factor), red cell antibodies, quantitative G6PD, serology and coagulation profile.
- Perform serum β-hCG pregnancy test for all females, and FSH test for post-menopausal females.

- Verify participant meets inclusion/exclusion criteria.

8.1.2 ELIGIBILITY CONFIRMATION VISIT (DAY -3 TO DAY -1)

The participants (including reserve participants) will report to the clinical trial unit between Day -3 to Day -1 for the following baseline assessments, unless screening laboratory assessments were conducted within this period, in which case repeat sampling will not be required.

This visit will include:

- Collect blood samples for haematology and biochemistry.
- Collect urine for urinalysis.
- Collect NPA for SARS-CoV-2 testing.

The timing of these assessments is to ensure that results are available for review by the Investigator prior to inoculation on Day 0.

8.1.3 MALARIA CHALLENGE AGENT ADMINISTRATION (DAY 0)

Participants, including up to three reserve participants per inoculation day, will report to the clinical trial unit on the morning of the day of inoculation. The Investigator will review the participants' screening and eligibility confirmation visit results prior to their inoculation.

On admission to the clinical trial unit, participants will be required to undertake further procedures to determine whether they remain eligible to be enrolled. A reserve participant may be asked to replace a participant who does not continue to meet eligibility. The reserve participants will be compensated for the study visit even if not inoculated, as described in the Participant Information Sheet and Consent Form.

The procedures that will be undertaken prior to inoculation include:

- Verify that all applicable eligibility criteria have been met.
- Elicit information regarding any new medical conditions, illnesses and medication use since screening visit.
- Perform RAT for SARS-CoV-2 testing
- Perform alcohol breath test.
- Perform urine drug screen.
- Perform urine β-hCG pregnancy test for WOCBP.
- Perform abbreviated physical examination.
- Record vital signs
- Obtain a 12-lead ECG.
- Cannulate participants with an indwelling intravenous cannula (20 or 22 gauge) for the malaria parasite challenge agent, and record which arm is utilised.
- Collect blood samples for;
    - malaria 18S qPCR (baseline sample),
    - safety serum retention sample,

- malaria future research sample (if participant has consented)

Administration of the malaria challenge agent:

- Administer the malaria challenge agent of ~2,800 viable *P. falciparum* 3D7 infected human erythrocytes intravenously.
- Observe for a minimum of 60 minutes after inoculation to evaluate for immediate adverse reactions.
- Educate participants on signs and symptoms of malaria.

  a. Signs of malaria include fever (oral temperature above 38°C), chills/shivering/rigors, tachycardia, hypotension

  b. Symptoms of malaria include headache, myalgia, arthralgia, fatigue/lethargy, malaise, sweating/hot spells, loss of appetite, nausea, vomiting and abdominal discomfort.

- Provide participants with diary cards and thermometers to record any temperature readings during the study in the event of symptoms of fever. Participants will also record symptoms, details of self-administered take home antimalarial rescue medication and concomitant medications on the diary cards during the study.
- Record AEs and concomitant medications.
- Record vital signs prior to leaving the clinical trial unit (approximately 60 minutes after inoculation).

8.1.4 OUTPATIENT MONITORING PRE-IMP ADMINISTRATION (DAY 1 TO DAY 7)

Monitoring via phone (Days 1 to 3):
During this period, the participants are expected to be asymptomatic. A daily phone call or text message will be made to the participant by clinical trial unit staff to monitor participant well-being and to solicit any AEs and concomitant medications.

Daily visits to the clinical trial unit for monitoring (Days 4 to 7):
Follow-up from Day 4 to Day 7 will be undertaken through daily morning visits to the clinical trial unit.

The following procedures will occur during these visits:

- Perform symptom-directed physical examination if clinically indicated.
- Record vital signs.
- Collect blood sample for malaria 18S qPCR.
- Collect NPA for SARS-CoV-2 testing (Day 6 am)
- Collect blood samples for haematology and biochemistry (Day 7 am)
- Record AEs and use of concomitant medications.
- Check participant diary cards.

8.1.5 INPATIENT OBSERVATION AND IMP ADMINISTRATION (DAY 8 TO DAY 12)

Participants will be admitted to the clinical trial unit for single-dose administration of pyronaridine on Day 8 when parasitaemia for the majority of participants is expected to be above 5,000 parasites/mL.

The following procedures will occur at admission to the clinical trial unit (before pyronaridine administration):

- Perform RAT for SARS-CoV-2 testing
- Perform abbreviated physical examination.
- Obtain and review a 12-lead ECG.
- Record vital signs.
- Perform and review urine β-hCG pregnancy test for WOCBP.
- Record AEs and use of concomitant medications.
- Perform and review alcohol breath test and urine drug screen.
- Review haematology and biochemistry results from Day 7 am collection.
- Cannulate participants with an indwelling intravenous (18 or 20 gauge) cannula.
- Collect blood samples for
    - haematology and biochemistry,
    - malaria 18S qPCR,
    - parasite viability *ex vivo* growth,
    - drug concentration (baseline sample),
    - malaria future research sample (if participant consented)

The following procedures will occur after pyronaridine administration:

- Administer the relevant pyronaridine dose under direct observation.
- Follow up participants as in-patients for up to 96 hours to ensure tolerance of pyronaridine and adequate clinical response.
- Perform symptom-directed physical examination if clinically indicated.
- Record vital signs three times a day (approximately 6 – 8 hours apart) whilst confined.
- Obtain 12-lead ECG once daily while confined.
- Collect blood for haematology and biochemistry at 72 hours post-pyronaridine administration.
- Collect blood for malaria 18S qPCR following pyronaridine administration at 4, 8, 12, 16, 20, 24, 30, 36, 42, 48, 60, 72, 84 and 96 hours (see section 8.4 for time allowed time windows).
- Collect blood samples for drug concentration at 1, 2, 3, 4, 6, 8, 12, 16, 20, 24, 30, 36, 42, 48, 60, 72, 84 and 96 hours (see section 8.4 for time allowed time windows).
- Collect blood for parasite viability *ex vivo* growth at 4, 8, 12, 16, 20, 24, 48, 72 and 96 hours (see section 8.4 for time allowed time windows).
- Record AEs and use of concomitant medications.

Participants will be confined in the clinical unit for up to 96 hours to ensure tolerance of pyronaridine and adequate clinical response. In the event that parasitaemia is not reduced from peak parasitaemia value by approximately 10-fold during the confinement period, and/or parasite regrowth occurs (≥5000 parasites/mL and a 2-fold parasitaemia increase within 48 hours), rescue treatment with Riamet® will be initiated.

If participants are clinically well at the end of the 96 hour confinement, they will be discharged and monitored on an outpatient basis up to the EOS visit. Before exit from confinement, clinical trial unit

nursing staff must confirm with the Investigator if any participant requires symptom-directed physical examination prior to discharge.

The following procedures will occur prior to discharge from the clinical trial unit (96 hours):

- Perform symptom-directed physical examination if clinically indicated.
- Obtain a 12-lead ECG.
- Record vital signs.
- Collect blood samples for
    - malaria 18S qPCR,
    - drug concentration,
    - parasite viability *ex vivo* growth
- Record AEs and use of concomitant medications.

8.1.6 OUTPATIENT MONITORING POST-IMP ADMINISTRATION (DAY 13 TO DAY 50±2)

Follow up visits will be undertaken on an out-patient basis for clinical evaluation and blood sampling. Follow up visits will occur at least three times per week (may occur more frequently if clinically indicated) until Riamet® treatment.

The following procedures will take place during these visits:

- Obtain 12-lead ECG.
- Record vital signs.
- Record AEs and use of concomitant medications.
- Check participant diary cards.
- Perform symptom-directed physical examination if clinically indicated.
- Collect blood samples for drug concentration at 120 (Day 13±1), 168 (Day 15±1), 216 (Day 17±1), 288 (Day 20±1), 336 (Day 22±1), 384 (Day 24±1), 456 (Day 27±1), 504 (Day 29±1), 576 (Day 32±2), 744 (Day 39±2) and 864 (Day 44±2) hours (Days) post-dose
- Collect blood samples for malaria 18S qPCR at least 3 times per week until Riamet® treatment, timing will be at PI discretion based on previous 18S qPCR results and clinical symptoms. Where possible 18S qPCR blood sampling should coincide with drug concentration sampling time-points to avoid bringing participants back for outpatient visits unnecessarily.
- Collect blood samples for haematology and biochemistry on Days 15±1, 22±1, 29±1, 37±2 and 44±2.
- Collect blood samples for parasite lifecycle stage qRT-PCR at Investigator's discretion (if gametocytaemia is suspected based on low and stable parasitaemia). These samples should be aligned with the 18S qPCR to avoid bringing participants back for outpatient visits unnecessarily.
- Collect blood samples for malaria future research (if participant has consented) on Days 15±1 and 29±1.

8.1.7 ANTIMALARIAL RESCUE TREATMENTS

All participants will receive compulsory antimalarial rescue treatment with Riamet® on Day 47±2, or earlier in the following cases:

- Parasitaemia is not reduced by approximately 10-fold at the end of the 96 hour confinement period (Day 12) when compared with peak parasitaemia.
- Parasite regrowth occurs after an initial reduction in parasitaemia, with regrowth defined as ≥5000 parasites/mL and a 2-fold parasitaemia increase within 48 hours.
- Volunteer discontinuation/withdrawal from the study (see Section 7.2).
- Investigator's discretion in the interest of participant safety.

As qPCR results are not received in real-time, rescue may occur when these parasitaemia thresholds are presumed to have occurred.

If gametocytes are present as determined by qRT-PCR at any time point after the confinement period, a single dose of Primacin® will be administered at the Investigator's discretion. A participant's G6PD status will determine the dose of primaquine administered. If an allergy or contraindication to Riamet® or Primacin® develops, Malarone® will be administered. In the rare event that a participant cannot tolerate oral drugs, participants will be admitted to hospital and I.V. artesunate will be administered.

<u>The following procedures will be performed prior to antimalarial rescue treatment:</u>

- Perform symptom-directed physical examination if clinically indicated.
- Obtain a 12-lead ECG.
- Record vital signs.
- Record AEs and use of concomitant medications.
- Check participant diary cards.
- Collect blood samples for
    - haematology and biochemistry,
    - malaria 18S qPCR,
    - drug concentration

- Collect sample for parasite viability *ex vivo* growth. **NOTE only if antimalarial rescue treatment is administered prior to Day 47±2.**

Please note if a participant receives antimalarial rescue treatment after administration of pyronaridine but prior to Day 47±2, drug concentration, haematology, biochemistry and future malaria research blood collection will still be required for the full duration of the trial. Following antimalarial rescue treatment blood will be taken for 18S qPCR at the completion of Riamet and until at least one negative result is obtained.

8.1.8 END OF STUDY VISIT (DAY 50±2)

The EOS visit must occur at a minimum 3-days after commencement of compulsory antimalarial treatment.

The following procedures will be performed at the EOS visit:

- Perform a complete physical examination.
- Obtain a 12-lead ECG.
- Record vital signs.
- Record body weight.
- Record AEs and use of concomitant medications.
- Collect urine for urinalysis.
- Collect blood samples for
    - haematology and biochemistry,
    - serum β-hCG pregnancy test for WOCBP,
    - RBC alloantibodies,
    - serology,
    - malaria 18S qPCR,
    - drug concentration,
    - safety serum retention sample,
    - malaria future research sample (if participant consented)
- Collect diary card.

If a participant is unwell with detectable parasitaemia or at the Investigator's discretion, the participant may be asked to return to the clinical trial unit after the EOS visit for safety follow up.

8.2 PHARMACOKINETIC AND PHARMACODYNAMIC ASSESSMENTS

8.2.1 PHARMACOKINETICS

Blood will be collected to quantify whole blood pyronaridine concentrations for PK analysis. PK data will be used to calculate the PK/PD parameters of pyronaridine using a population PK model (the primary endpoint). PK parameters of pyronaridine will also be calculated using non-compartmental methods (secondary endpoint).

Samples will be analysed for pyronaridine blood concentrations using a validated analytical method in compliance with the SOPs of the laboratory performing the analyses. Assays will be performed in a Sponsor approved laboratory.

Blood for pyronaridine concentrations will be collected pre-pyronaridine administration on Day 8, then at the following hours post-pyronaridine administration while confined in the clinical unit: 1, 2, 3, 4, 6, 8, 12, 16, 20, 24, 30, 36, 42, 48, 60, 72, 84 and 96 hours. As outpatients, participants will have blood collected

on the following days: 13, 15, 17, 20, 22, 24, 27, 29, 32, 39, 44, 47 and 50. Allowed time windows for sample collection are specified in Section 8.4.

8.2.2 PHARMACODYNAMICS

Malaria 18S qPCR

Blood will be collected to quantify total malaria parasitaemia by quantitative polymerase chain reaction targeting the gene encoding *P. falciparum* 18S rRNA (malaria 18S qPCR; reported as parasites/500uL packed RBCs). Malaria 18S qPCR data will be used to calculate the PK/PD parameters of pyronaridine (the primary endpoint) and to determine the kinetics of parasite clearance following pyronaridine dosing (secondary endpoint). Malaria 18S qPCR will also be used to monitor the parasitaemia of participants in real time in order to guide the timing of antimalarial rescue treatment initiation.

Samples for malaria 18S qPCR will be analysed using a validated analytical method in compliance with the SOPs of the laboratory performing the analyses. Assays will be performed in a Sponsor approved laboratory.

Blood for malaria 18S qPCR will be collected on Day 0 prior to inoculation with the malaria challenge agent, then once daily on Days 4, 5, 6 and 7. On Day 8, blood will be collected pre-pyronaridine administration, then at the following hours post-pyronaridine administration while confined in the clinical unit: 4, 8, 12, 16, 20, 24, 30, 36, 42, 48, 60, 72, 84 and 96 hours.

As outpatients, participants will have blood collected at least three times per week until antimalarial rescue treatment. Timing will be at PI discretion based on previous malaria 18S qPCR results and clinical symptoms. Where possible, malaria 18S qPCR blood sampling should coincide with drug concentration sampling time-points to avoid unnecessary outpatient visits. Following antimalarial rescue treatment, no further blood collection for malaria 18S qPCR is required for a participant once a minimum of two negative results are recorded.

Allowed time windows for sample collection are specified in Section 8.4.

Parasite lifecycle stage qRT-PCR

Additional blood samples may be collected to evaluate the presence of sexual parasite stages (gametocytes) and other parasite lifecycle stages using quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR). The qRT-PCR assays may target parasite transcripts including but not limited to: the female gametocyte-specific transcript *pfs25*, the male gametocyte-specific transcript *PfMGET*, and the ring-stage transcript *pfSBP-1* as appropriate. The samples may be taken at time points indicated by malaria 18S qPCR, or at the Investigator's discretion. The parasite lifecycle stage qRT-PCR data may be used to facilitate the calculation of the PK/PD parameters of pyronaridine (the primary endpoint). Further, the results may guide the requirement for Primacin® administration to participants prior to the end of study.

Parasite viability in *ex vivo* culture

Ex vivo growth is an indirect measure of parasite viability. While 18S qPCR quantifies all parasites (including those non-viable or dead in circulation), only viable parasites are able to replicate in *ex vivo* culture. Modelling of parasite growth observed *ex vivo* enables the fraction of viable parasites present in blood samples to be determined. The results of parasite *ex vivo* growth analyses will be supportive to malaria 18S qPCR data in determining the kinetics of parasite clearance following pyronaridine dosing (secondary endpoint).

Short term *ex vivo* cultures will be established from blood collected pre-pyronaridine administration (Day 8), then at the following hours post-pyronaridine administration while confined in the clinical unit: 4, 8, 12, 16, 20, 24, 48, 72 and 96. An additional blood sample will be collected prior to the initiation of Riamet® treatment in the event that treatment is initiated prior to Day 47±2.

Microscopic examination for evidence of parasitaemia or gametocytaemia may also be conducted at the Investigator's discretion.
Refer to the Laboratory Manual for sample preparation procedures and sample volumes.

8.3 SAFETY AND OTHER ASSESSMENTS

The schedule for all safety assessments is summarised in the SOA table (section 1.3) and in more detail in the study conduct schedule (Section 8.1). All safety assessments may be conducted at unscheduled visits or time points if required for the participant's safety at the discretion of the Investigator. In the case of elevated AST, ALT or total bilirubin, safety assessments will occur twice a week until resolution.

When several assessments are scheduled to take place at the same time point, the preferred order is: ECG, vital signs, blood sampling, urine sampling. When the malaria challenge agent, pyronaridine, and rescue medication are administered, procedures that should occur pre and post administration are detailed in Section 8.1.

Exact timing of blood sampling to measure pyronaridine blood concentrations and to monitor malaria parasitaemia is to be maintained as closely as possible and therefore safety assessments scheduled for these time-points should be conducted ahead of the time-point. Please refer to the allowed time windows (Section 8.4).

8.3.1 BECK DEPRESSION INVENTORY

Originally described by Beck et al (1961), the Beck Depression Inventory (BDI) is a validated objective tool for the assessment of depression. Updated in 1996, the BDI-II is a 21-item, self-report rating inventory that measures characteristic attitudes and symptoms of depression, and participants will be required to complete the BDI-II at Screening for eligibility. The BDI-II takes approximately 10 minutes to complete, although participants require a fifth – sixth grade reading level to adequately understand the questions.

A score of >20 at screening and/or a response of 1, 2 or 3 for item 9 indicating current suicidal ideation is exclusionary. A participant with a BDI-II score of 17 to 19 may be enrolled at the discretion an Investigator if they do not have a history of the psychiatric conditions mentioned in exclusion criterion 10-12 and their mental state is not considered to pose additional risk to the health of the participant or to the execution of the trial and interpretation of the data gathered. The results will be entered into the eCRF.

8.3.2 PHYSICAL EXAMINATION

A **full physical examination** includes the following:

Weight
Height **(*Screening only*)**
Review of systems excluding genitourinary examination and including the following:
Head, neck (including thyroid), ears, eyes, nose and throat
Heart/circulation
Chest
Lungs
Abdomen
Skin
Neurological exam

A Full physical examination will be performed at Screening and at EOS.

An **abbreviated physical examination** will include heart/circulation, chest, lungs, skin, and abdomen. An abbreviated physical examination will performed prior to inoculation with the malaria challenge agent and upon admission to the clinical trial unit on Day 8.

When the **symptom-directed physical examination** is performed, body systems will be reviewed only if clinically indicated.

All physical examinations after inoculation with the malaria challenge agent on Day 0, the following signs and symptoms of malaria infection should be considered:

- Signs of malaria include fever (oral temperature above 38°C), chills/shivering/rigors, tachycardia, hypotension
- Symptoms of malaria include headache, myalgia, arthralgia, fatigue/lethargy, malaise, sweating/hot spells, loss of appetite, nausea, vomiting and abdominal discomfort.

8.3.3 BODY MEASUREMENTS

Body weight (kg) and height (cm) will be measured at screening. Body weight (kg) will also be measured at the EOS visit.

8.3.4 VITAL SIGNS

Vital signs (temperature, heart rate, respiratory rate, and blood pressure) will be measured after the participant has rested in the supine position for at least 5 min and in the standing position within 3 min when changing from the supine to standing position (blood pressure and heart rate only) at Screening.

At all other time points, vital signs will be measured after the participant has rested in the seated position for at least 5 min.

The normal ranges for vital signs once on trial are:

Parameter	Range
Systolic blood pressure	90-140 mmHg
Diastolic blood pressure	50-90 mmHg
Heart rate	50-100 bpm
Temperature	35.0-37.5°C
Respiratory rate	10-25 breaths/min

8.3.5 12-LEAD ELECTROCARDIOGRAM (ECG)

A 12-lead ECG will be recorded after the participant has rested supine for at least 5 min. ECG tracings will be retained and labelled as per standard procedures at the clinical trial unit and will be recorded in the eCRF. Any clinically significant findings will be discussed with the Medical Monitor and Sponsor and documented as AEs. An Investigator will sign and date each ECG as evidence of their review.

The normal ECG ranges once on trial are:

Parameter	Range
PR interval	≤210 msec
QRS	50–120 msec
QTcF	Males: ≤450 msec Females: ≤470 msec

8.3.6 LABORATORY TESTS

The required pathology tube types and blood and urine volumes required for each procedure and laboratory processing procedures will be described in the Laboratory Manual.

Any significant deviations from results obtained during screening will be followed until resolution or investigated fully, or until the participant is referred to a general practitioner or medical specialist as appropriate. An Investigator will document the clinical significance of all results falling outside of the normal reference ranges.

Blood and urine samples for clinical laboratory assessments will be collected according to clinical trial unit SOPs. The parameters that will be measured are listed below. Additional reflex testing may be conducted by the local laboratory (as per their SOPs) if safety laboratory values for a participant fall outside of the normal range/parameters. Unscheduled testing may be performed at Investigator's discretion.

Blood will be collected for clinical laboratory evaluations including haematology, biochemistry, coagulation profile, serology, G6PD, pregnancy testing or FSH testing, and for red cell antibodies. All safety samples will be sent to national laboratories as named in this protocol (section 10.1.5).

8.3.6.1 HAEMATOLOGY

Full blood count (FBC) with differential
White blood cell count (WBC)
WBC differential (diff)
A manual blood smear should be reviewed if there are immature/abnormal cells detected on the automated differential or if an automated differential was not able to be performed.
Neutrophils (NEUT)
Lymphocytes (LYM)
Monocytes (MON)
Eosinophils (EOS)
Basophils (BAS)
Mean corpuscular volume (MCV)
Red blood cell count (RBC)
Haemoglobin (HGB)
Haematocrit (HCT)
Platelet count (PLAT)
Red blood cell distribution width (RDW)
Reticulocyte count (RETI)
Blood Group and Rh(D) tests (***Screening only***)

8.3.6.2 BIOCHEMISTRY

Sodium (NA)
Potassium (K)
Chloride (CL)
Bicarbonate (BICARB)
Calcium (CA)
Corrected calcium (CCA)
Magnesium (MAG)
Glucose (GLUC)
Urea
Creatinine (CREAT)
Estimated glomerular filtration rate (eGFR) – CKD Epi calculation
Albumin (ALB)
Globulin

Total protein
Total bilirubin (BILI)
Direct (conjugated) bilirubin (BILDIR)
Alkaline phosphatase (ALP)
Alanine aminotransferase (ALT)
Aspartate aminotransferase (AST)
Gamma-glutamyl transferase (GGT)
Lactate dehydrogenase (LDH)
Phosphate (PHOS)
C-Reactive Protein (CRP)
Cholesterol (Chol)
Triglycerides (Trig)

8.3.6.3 LIPIDS

Total cholesterol
High density lipoprotein (HDL)
Low density lipoprotein (LDL)
Triglycerides

8.3.6.4 SEROLOGY

HIV 1/2 (anti-HIV1 and anti-HIV2 Ab)
Hepatitis B (HBsAg, anti-HBc [IgG + IgM if IgG is positive])
Hepatitis C (anti-HCV)

8.3.6.5 COAGULATION

Blood for Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT) and International Normalised Ratio (INR) testing will be collected at screening.

8.3.6.6 RBC ALLOANTIBODIES AND G6PD TESTING

Blood for red cell antibody testing will be collected for participants at screening and at the EOS visit.

Blood for G6PD testing will be collected at screening only.

8.3.6.7 PREGNANCY TESTING

At screening and at the EOS visit a serum β-hCG pregnancy test will be conducted for all female participants, and FSH levels will be tested in post-menopausal females (at least one year post-menopausal). On Days 0 and 8, a urine β-hCG pregnancy tests will be conducted in WOCBP. If a participant

is found to have a positive urine pregnancy test at any stage during the study, a confirmatory serum β-hCG will be performed. If confirmed positive, the participant will be discontinued from the study and rescued with Riamet®. Please refer to Section 8.5.9 for pregnancy-specific reporting procedures and follow-up.

8.3.6.8 URINALYSIS

Urine will be tested by dipstick at the clinical trial unit. If there are any abnormalities considered clinically significant in blood, leukocytes, or protein, the urine will be sent for formal laboratory urinalysis per the clinical trial unit standard procedure for microscopy, culture and sensitivity.

Glucose (GLUC)
Bilirubin (BILI)
Ketone (KETONES)
Specific gravity (SPGRAV)
Blood
pH
Protein (PROT)
Urobilinogen (UROBIL)
Nitrite
Leukocytes (WBC)
Microscopy, culture and sensitivity (***if required***)

8.3.6.9 URINE DRUG SCREEN AND ALCOHOL BREATH TESTS

All participants will be questioned about concomitant medications and use of recreational drugs. The urine drug screen may be repeated if the potential participant denies usage of any of these agents and the test result is believed to be a false positive.

Urine drug screen:	
Amphetamines	Opiates
Methamphetamines	Phencyclidine
Barbiturates	Tetrahydrocannabinol (cannabis)
Benzodiazepines	Tricyclic antidepressants
Cocaine	
Methadone	
Alcohol breath test	

If the results of the urine drug screens or alcohol breath tests are positive, participants will not be enrolled or administered pyronaridine on Day 8 unless there is an explanation acceptable to an Investigator (e.g., the participant has stated in advance that they consumed a prescription or over-the-counter product that contained the detected drug) and the participant has a negative urine drug screen on retest by the pathology laboratory.

8.3.7 SARS-COV-2 RISK MANAGEMENT:

A NPA will be conducted at the eligibility visit or screening visit if it falls within the eligibility window (SoA). A RAT may be conducted in place of PCR, if PCR is not available or if results are not expected back within the required timeframe. Participants who test positive for SARS-CoV-2 at this time-point will be excluded from the study, and will be managed as per Queensland Health guidelines for management of COVID-19 positive individuals.

All eligible participants will be required to have had a negative Rapid Antigen Test (RAT) immediately prior to (i.e., that morning) being admitted to the clinical trial site for inoculation with the malaria challenge agent (Day 0). If any participant tests positive for SARS-CoV-2 at this time-point they will be excluded from the study, and will be managed as per Queensland Health guidelines for management of COVID-19 positive individuals.

For participants who are inoculated with the malaria challenge agent, a repeat NPA for SARS-CoV-2 by PCR will be conducted on Day 6 (a RAT may be conducted in place of PCR, if PCR is not available or if results not expected back within the required timeframe). In addition, a RAT will be conducted prior to confinement on the morning of Day 8. If any participant tests positive at any time point before or after confinement they will be managed as per Queensland Health guidelines for management of COVID-19 positive individuals. Definitive antimalarial treatment will be delivered to the participants' home address, and a phone call will be made to ensure that the first dose is taken; additional daily phone contact will be made to ascertain adverse events and to ensure that all subsequent doses are taken. No further clinic visits will be conducted during the time that the participant is required to isolate; however, where possible an EOS visit will be conducted following release from isolation.

In the event that a participant tests negative by RAT on the morning of confinement (Day 8), but then tests positive by RAT during confinement, the participant will be released from the clinical trial site to isolate at home. Participants will leave the clinical site with the full course of definitive antimalarial medication. Daily phone contact will be made to ascertain adverse events and to ensure that all doses of antimalarial medication are taken. If a participant's condition was to deteriorate while isolating at home, the participant will be assessed over the phone by an Investigator and may be advised to present to a hospital Emergency Department if required, or clinical site staff may call 000 if appropriate. In the event that a participant is admitted to hospital, the PI will continue to liaise with treating clinicians. Study visits will resume where possible following the participant's release from isolation. All other participants still confined at the clinical trial site will undergo daily RAT testing for the duration of their confinement.

8.3.8 SAFETY SERUM RETENTION SAMPLE

A 5 mL blood sample will be collected on Day 0 prior to inoculation with the malaria challenge agent and at EOS.

Participants consent to this mandatory collection and storage and the use of the sample for safety assessments when they sign the Informed Consent Form for the study. Safety serum samples will be retained for at least 15 years from the completion of the study at the QIMR Berghofer Institute.

8.3.9 MALARIA FUTURE RESEARCH

Blood samples may be collected for storage and use in future malaria research under a separate protocol and consenting process. The future research will be under the local sponsorship of QIMR Berghofer Medical Research Institute and will involve a protocol and consent document approved by the QIMR Berghofer HREC.

If a participant consents to the collection of blood samples for future malaria research, up to six blood samples will be collected at time points that coincide with existing visits, including time points prior to and after administration of the malaria challenge agent.

Blood volume of each sample may be up to and not exceeding 30 mL, and will be appropriate for the day/time point. The total volume of blood taken including the main study samples will not exceed 470 mL per month.

A participant may change their decision to provide samples for future malaria research at any time prior to the EOS by notifying QIMR Berghofer, as local Sponsor, in writing. However, if a participant initially consents to future malaria research some of their blood has already been used for research purposes, the information from that research may still be used.

8.4 ALLOWED TIME WINDOWS

The following time windows will be allowed for vital signs recording, ECGs and blood sampling for clinical laboratory evaluations:

Time point	Allowed time window
Malaria inoculation (Day 0)	± 4 hours
Outpatient monitoring pre-IMP (Days 4-7)	± 12 hours
Inpatient observation pre-IMP (Day 8)	± 2 hours
Inpatient observation ≤24 hours post-IMP (Day 8-9)	± 30 minutes
Inpatient observation >24 hours post-IMP until discharge (Day 9-12)	± 60 minutes
Outpatient monitoring from discharge up to and including Day 30	± 24 hours
Outpatient monitoring from Day 31 until EOS visit	± 2 days

The following time windows will be allowed for blood sampling to quantify pyronaridine blood concentrations and to monitor malaria parasitaemia:

Time point	Allowed time window
Malaria inoculation (Day 0)	± 4 hours
Outpatient monitoring pre-IMP (Days 4-7)	± 12 hours
Inpatient observation pre-IMP (Day 8)	± 60 minutes
Inpatient observation ≤24 hours post-IMP (Day 8-9)	± 30 minutes
Inpatient observation >24 hours post-IMP until discharge (Day 9-12)	± 60 minutes
Outpatient monitoring from discharge up to and including Day 30	± 24 hours
Outpatient monitoring from Day 31 until EOS visit	± 2 days

8.5 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.5.1 DEFINITION OF ADVERSE EVENTS (AE)

An AE is any untoward medical occurrence, i.e., unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease that occurs in a participant during the course of the trial. An AE does not necessarily have a causal relationship with trial treatments or procedures.

AEs include, but are not limited to:

- A new symptom, sign or medical condition.
- A disease or medical condition detected or diagnosed during the course of the trial even though it may have been present prior to the start of the trial.
- An exacerbation of a pre-existing medical condition/disease.
- An increase in frequency or intensity of a pre-existing episodic disease or medical condition.
- Continuous persistent disease or symptoms present at trial start that worsen following the start of the trial.
- An abnormal assessment (e.g., change on physical examination, ECG finding) if it represents a clinically significant finding that was not present at trial start or worsened during the course of the trial.
- An abnormal laboratory test result if it represents a clinically significant finding (e.g., CTCAE grade 2 or above), symptomatic or not, which was not present at trial start or worsened during the course of the trial or led to dose reduction, interruption or permanent discontinuation of trial treatment.

Borderline abnormal laboratory findings and other objective assessments should NOT be routinely captured and reported as AEs. However, abnormal laboratory findings or other objective measurements that meet the following criteria should be captured and reported in the AE section of the eCRF:

- The result meets the criteria for reporting as an SAE.
- The test result is associated with accompanying symptoms, and/or
- It requires additional diagnostic testing or medical/surgical intervention, and/or
- It leads to a change in trial dosing, or discontinuation from the trial, significant additional concomitant drug treatment, or other therapy, and/or
- It is considered by an Investigator to be clinically significant or represent a clinically significant change from baseline.

Merely repeating an abnormal test, in the absence of any of the above conditions, does not constitute an AE. Any abnormal test result that is determined to be an error does not require reporting as an AE. Surgical procedures themselves are not AEs; they are therapeutic measures for conditions that may, or may not, be AEs. Malaria in itself is not considered an AE.

8.5.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An SAE is defined as an event that fulfils at least one of the following criteria:

- Results in death.
- Is life-threatening

  - The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at immediate risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe.
- Requires inpatient hospitalisation or prolongs existing hospitalisation, unless this is for:
  - Elective or pre-planned treatment for a pre-existing condition that is unrelated to the trial and has not worsened since the start of the trial.
  - Cosmetic surgery, or for social reasons, or respite care in the absence of any deterioration in the participant's general condition.
- Results in persistent or significant disability/incapacity.
- Is a congenital abnormality/birth defect.
- Is considered medically important
  - Medical and scientific judgement should be exercised in deciding whether other AEs are to be considered serious, such as important medical events that may not be immediately life-threatening but may jeopardise the participant or may require intervention to prevent one of the other outcomes listed in the definition above. Examples of such events are: intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias; convulsions that do not result in hospitalisation; development of drug dependency or drug abuse.

A **Suspected Unexpected Serious Adverse Reaction (SUSAR)** is any SAE where a causal relationship with a trial intervention (malaria challenge agent, pyronaridine, antimalarial rescue medication) is at least a reasonable possibility, and the event is not listed in the IB.

8.5.3 DEFINITION OF EVENTS OF SPECIAL INTEREST

An AE of special interest (AESI), whether serious or non-serious, is one of scientific and medical concern specific to the Sponsor's product or program, for which ongoing monitoring and rapid communication by an Investigator to the Sponsor and medical monitor could be appropriate. Such an event might require further investigation in order to characterise and understand it. Depending on the nature of the event, rapid communication by the trial Sponsor to other parties (e.g., regulators) might also be warranted (CIOMS VI, ICH E2F, 2010). Any abnormalities listed below should be reported as an AESI.

Hepatic:

- Any ALT or AST value above 5×ULN.
- An elevation in bilirubin 2×ULN.
- Any AST or ALT value above 2×ULN and (total bilirubin >1.5×ULN).
- Any AST or ALT value above 2×ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia (eosinophil percent or count above the ULN).
- Constitutes a possible Hy's Law case
  - Hy's Law case is defined as a participant with any value of alanine or aspartate aminotransferase greater than 3×ULN together with an increase in total bilirubin to a value greater than 2×ULN and

not associated to an alkaline phosphatase value greater than 2×ULN (FDA Guidance on Drug Induced Liver Injury: Premarketing Clinical Evaluation [2009]).

Cardiac:

- QTcF at any time >480 msec.
- Bundle branch block (except right bundle branch block that was present prior to pyronaridine administration)
- Any arrhythmia, except:
  - sinus bradycardia that is clinically asymptomatic and not associated with any other relevant ECG abnormalities
  - sinus tachycardia that is clinically asymptomatic, is associated with a body temperature >38.0°C, and not associated with any other relevant ECG abnormalities
  - respiratory sinus arrhythmia
  - wandering atrial pacemaker, or
  - isolated single premature atrial/ventricular complex (i.e., no bigeminy, trigeminy, couplets, triplets or salvos) that does not occur more than once in a particular ECG tracing

Haematological:

- Haemoglobin drop >20.0 g/L from baseline (at eligibility visit)
- Absolute neutrophil count (ANC) $<0.5 \times 10^9$/L.
- Platelet count $<75 \times 10^9$/L.

Dermatological: *†

Clinical signs of possible cutaneous adverse reactions (clearly related to malaria challenge agent inoculation and/or IMP administration; this does not include irritation from dressings and/or ECG dots) such as:

- Dermatitis
- Rash including
  - erythematous
  - macular
  - papular
  - maculopapular
  - pruritic
  - pustular
  - vesicular

*If one of these cutaneous reactions is observed, pictures of the lesions should be obtained when feasible.
†Dermatological AEs need not be reported as AESIs if clearly unrelated to the malaria challenge agent or IMPs (e.g. rash from cannula dressing or ECG dots).

All AESIs, including those that do not meet the definition of an SAE, must be reported as per section 8.5.7

8.5.4 CLASSIFICATION OF AN ADVERSE EVENT

8.5.4.1 SEVERITY OF EVENT

In addition to determining whether an AE fulfils the criteria for an SAE or not, the severity of AEs experienced by participants will be recorded in accordance with the Common Terminology Criteria for Adverse Events (CTCAE) v5.0, published 27 November 2017.

The severity of AEs will be graded as follows:

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2: Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living.
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalisation or prolongation of hospitalisation indicated; disabling; limiting self-care activities of daily living.
- Grade 4: Life-threatening consequences; urgent intervention indicated.
- Grade 5: Death related to AE.

A mild, moderate, or severe AE may or may not be serious. These terms are used to describe the intensity of a specific event. Medical judgement should be used on a case-by-case basis.

Seriousness, rather than severity assessment, determines the regulatory reporting obligations.

8.5.4.2 RELATIONSHIP TO STUDY INTERVENTION

The PI or delegate must assess the relationship of each event to the malaria challenge agent, pyronaridine, and the antimalarial rescue medications (separately) and decide whether, in his or her medical judgement, there is a reasonable possibility that the event may have been caused by any of the trial interventions. Where possible, a distinction should be made between events considered related to the malaria challenge agent, pyronaridine, and the antimalarial rescue medications.

If there is no valid reason for suggesting a relationship, then the AE should be classified as “not related”. Alternatively, if there is any valid reason for suspecting a cause-and-effect relationship between the malaria challenge agent, pyronaridine, or the antimalarial rescue medications and the occurrence of the AE (even if undetermined or untested), then the AE should be considered as “related” to whichever product is relevant. This should be documented in the participant’s clinical file (source data) and eCRF.

The following may guide this assessment:

Related/suspected:

The temporal relationship between the event and the administration of the malaria challenge agent and/or pyronaridine and/or the antimalarial rescue medications is compelling and/or follows a known or suspected response pattern to that product, and the event cannot be explained by the participant’s medical condition, other therapies or accident.

Not related/not suspected:

The event can be readily explained by other factors such as the participant's underlying medical condition, concomitant therapy or accident and no plausible temporal or biologic relationship exists between the challenge agent and/or pyronaridine and/or the antimalarial rescue medications and the event.

In addition to the assessments of relationship to the trial intervention/s, the Investigator should comment on the AE record in the eCRF whether an AE is related to the trial participation of the participant (trial procedures etc.).

Of note, a sign or symptom associated with malaria infection (confirmed by a positive malaria 18S qPCR at the onset of the event) that is of expected intensity, frequency and duration for the individual participant in the context of this trial is considered to be a malaria challenge agent-related event.

8.5.4.3 EXPECTEDNESS

A 'Suspected Unexpected Serious Adverse Reaction (SUSAR)' is a Serious AR that is unexpected. The 'expectedness' of a serious AR is assessed in the light of the Reference Safety Information (RSI) of the investigator brochures.

8.5.5 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

All AEs must be documented and followed up by an Investigator until:

- the event is resolved, or
- no further medically relevant information in relation to the event can be expected, and
- an Investigator considers it justifiable to terminate the follow-up

Events that are unresolved at the time of the participant's last follow-up visit should continue to be followed up by an Investigator for as long as medically indicated or until the participant is referred to a general practitioner or medical specialist as appropriate. The Sponsor retains the right to request additional information for any participant with ongoing AE(s)/SAE(s) at the end of the trial, if judged necessary.

All AEs should be treated appropriately. An Investigator will decide upon the appropriate action to be taken in response to an AE, which may include one or more of the following:

- no action taken (i.e., further observation only), or
- administration of a concomitant medication, or
- hospitalisation or prolongation of current hospitalisation (event to be reported as an SAE), or
- other

In a case of occurrence of an SAE, regardless of whether or not it is judged to be related to any of the trial treatments or procedures, the participant will receive appropriate care under clinical supervision until all the symptoms of the SAE have diminished or resolved and the participant's condition has improved.

For ongoing AEs, care will be provided for a period of time as specified in the clinical trial unit work instruction protocols. However, if the nature of the ongoing AE is determined by an Investigator as not being associated with any of the trial treatments or procedures, the participant will be advised to visit his/her general practitioner for further clinical care that might be required.

8.5.6 ADVERSE EVENT REPORTING

It is Investigator's responsibility to document and report all AEs occurring in the clinical trial whether spontaneously reported by the participant, observed by an Investigator (either directly or by laboratory or other assessments), or elicited by general questioning. The period of observation for collection of AEs extends from the time of inoculation with the malaria challenge agent to EOS. These AEs must be recorded on specific AE pages of the eCRF. Events reported prior to this will be recorded as medical history, unless the symptoms worsen during the trial.

The following information should be recorded for all AEs:

- Description
- Dates and times of onset and resolution
- Duration in hours
- Time of onset relative to IMP administration, inoculation with the malaria challenge agent, and/or administration of antimalarial rescue medications
- Seriousness (SAE or not)
- Severity
    - If severity of an AE changes, only one AE will be reported, with the highest severity recorded in the eCRF and listings and tables. In the clinical file (source data), the description of the AE will report the various severities observed over time.
    - If the AE resolves and then reoccurs, then two AEs will be reported.
- Action taken in response to the AE (including treatment given).
- Outcome.
- Relationship to the trial treatments or procedures (causality assessment), including malaria challenge agent, antimalarial medication, or any other treatment or procedure conducted during the trial.

8.5.7 SERIOUS ADVERSE EVENT AND EVENT OF SPECIAL INTEREST REPORTING

An Investigator will take immediate appropriate action in response to SAEs and AESIs to ensure participant safety and in an attempt to identify the cause of the event. The Investigator must notify the SAE or AESI to the pharmacovigilance provider (Prime Vigilance) by email within 24 hours of becoming aware of the event.

All reports must be signed by the Principal Investigator or delegate and notified to Prime Vigilance preferably by email or fax to:

Email: MMV@primevigilance.com

Back-up fax number: +44 800 471 5694

Prime Vigilance Contact:

Head Office: +44 1483 307920

Any copies of participant's medical records provided for SAE or AESI reporting must have all participant identifiers redacted before submission.

The report forms will always be completed as thoroughly as possible with all available details of the event and signed by the Principal Investigator or delegate. If the Principal Investigator or delegate does not have all information regarding an event, he/she will not wait to receive additional information before reporting the event. A follow-up SAE or AESI report should be completed within 14 days, or if there is no new information the report form should be updated when additional information is received.

The Principal Investigator or delegate will always provide an assessment of causality at the time of the initial report.

Email transmission of the report forms is the preferred method to transmit this information to Prime Vigilance. In rare circumstances notification by telephone is acceptable, with a copy of the report form sent by overnight mail.

The Principal Investigator or delegate, or responsible person according to local requirements, will comply with the applicable local regulatory requirements related to the reporting of SAEs to regulatory authorities and the HREC. Any SAE that meets the criteria of a SUSAR will be reported to the TGA by the Sponsor in accordance with the Sponsor's reporting procedures.

8.5.8 REPORTING EVENTS TO PARTICIPANTS

Not applicable

8.5.9 REPORTING OF PREGNANCY

Pregnancy itself is not defined as an AE/SAE. Any complication or termination of pregnancy for medical reasons are to be reported as an AE/SAE. Spontaneous abortion, still birth or congenital anomaly must be reported as an SAE. Any WOCBP enrolled in the study who becomes pregnant during the study and the following 100 days after pyronaridine administration should be followed through delivery or termination of the pregnancy. The Investigator will collect pregnancy information and report to Primevigilance within 24 hours of becoming aware of a participant's pregnancy using the pregnancy form and following the same process as described in section 8.5.7 for SAEs and AESIs. Pregnancy follow-up should be recorded on the same form including pregnancy outcomes. Once pregnancy is confirmed, pregnant female participants will be immediately withdrawn from the study and will be managed as per section 7.2. Where possible, the Investigator will also attempt to collect and report information regarding pregnancy outcomes of female partners of any male participants who were administered pyronaridine in this study and the following 70 days after pyronaridine administration. Appropriate signed informed consent will be required directly from the pregnant female partner to obtain and report this information. Any

participant's female partner who becomes pregnant during the study should be followed through delivery or termination of the pregnancy.

9 STATISTICAL CONSIDERATIONS

The following sections describe the statistical analysis as it is foreseen when the study is being planned. A detailed Statistical Analysis Plan (SAP) and Modelling Analysis Plan (MAP) will be finalised and approved prior to database lock. The SAP and MAP will provide details of all analyses to be performed as well as the format of listings and tables to be provided for completion of the Clinical Study Report (CSR). Any deviations from the SAP will be described and justified in the final CSR.

9.1 STATISTICAL HYPOTHESES

The primary objective of this study is to characterise the PK/PD relationship of pyronaridine in healthy participants experimentally infected with blood stage *P. falciparum*. There is no pre-specified statistical hypothesis for testing this objective.

9.2 SAMPLE SIZE DETERMINATION

A maximum sample size of 18 participants is expected to be sufficient to achieve the primary endpoint of the study, which is to determine the PK/PD parameters of pyronaridine. This sample size is not based on formal statistical calculations but rather is based on previous experience of characterising the PK/PD relationship of antimalarials using the IBSM study. The specific number of participants required will be dependent on the emerging results during the study; it is possible that less than 18 participants will be required. The SDRT will review the data after the completion of each cohort and decide when sufficient data has been obtained to complete the primary endpoint. No more than 18 participants will be enrolled in this study.

9.3 POPULATIONS FOR ANALYSES

In the first instance, two (2) analysis datasets will be used for study analyses: Full Analysis Set (FAS) and Safety Set. Additional analysis populations like the ones to be used for calculation of PK/PD parameters, will be defined in the SAP. The number of participants in each analysis set will be summarised, with a corresponding listing.

The FAS will consist of all enrolled participants. The FAS will be used to assess all participant disposition, baseline, demographic and protocol deviation data. The safety analysis dataset will include all participants who receive the malaria challenge agent on Day 0.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

Continuous variables will be summarised with the number of observations (non-missing values), mean, standard deviation, median, quartiles, minimum and maximum. The minimum and maximum values will be presented to the same number of decimal places as recorded in the raw data. The mean, median and SD will be presented to one more decimal place than the raw data. Categorical variables will be summarised with the number of observations and the numbers and percent from each category. Percentages will be rounded to one decimal place, with the denominator being the number of participants in the relevant population with non-missing data. No missing data will be imputed. If required, CIs will be two sided and will use 95% confidence levels. Any analyses requiring significance testing will use a two-sided test at the 5% significance level.

The inoculation baseline will be defined as the last available assessment prior to the inoculation. The IMP baseline will be defined as the last available assessment prior to the administration of the IMP. Unscheduled visits will be excluded from summary tables. Assessments conducted at Early Termination will be excluded from summary tables.

9.4.2 ANALYSIS OF THE PRIMARY ENDPOINT

The primary endpoint is the PK/PD relationship of pyronaridine. The basic PK/PD relationship will link the drug killing rate to its blood concentration with a sigmoidal E_{max} model characterized by the E_{max}, EC_{50} and Hill coefficient parameters. If needed alternative models will be applied, for instance to account for a lag in the drug response, which will contain additional parameters. The parameters will be estimated using a non-linear mixed-effect modelling of pyronaridine concentration-time and parasitaemia-time profiles. First, a population PK model will be constructed with the measured concentrations; it will allow to obtain the individual PK parameters and calculate the full PK profile for each individual. Second, the PD parameters will be estimated with the measured parasitaemia and the predicted individual PK profiles.

As a general concept the changes in living parasite P are modelled as the effect of a net exponential growth rate GR and a killing or clearance rate $Kill$ due to pyronaridine with an initial parasitaemia P_{base} at time t_0 (time of first observation). The equations are expressed in the log-scale such as:

$$\begin{aligned} \frac{dPL}{dt} &= GR - Kill \\ PL(t_0) &= PL_{base} \end{aligned}$$

where $PL = \ln(P)$ is the log-transformed parasite counts.

The effect of concentrations on parasite killing/clearance will be described as:

$$Kill = E_{max} \cdot \frac{C_c^{Hill}}{C_c^{Hill} + EC_{50}^{Hill}}$$

where C_c is the concentration in the central compartment, E_{max} is the maximum effect of the drug, EC_{50} is the concentration that results in 50% of the maximum effect and $Hill$ is the Hill coefficient.

Alternative models may be tested if the exploration of the data indicate a different behaviour, e.g. if a lag time is observed before the parasites are cleared.

Other key parameters such as the minimum inhibitory concentration (MIC), the minimal parasiticidal concentration (MPC_{90}), and the parasite reduction rate in 48 h ($log_{10}PRR_{48}$) will be derived from the PD model. The MIC is defined as the concentration when parasite clearance by the drug equals the parasite growth, i.e., the time at which the minimum parasite concentration is observed. It is calculated with the following equation in case of the E_{max} model.

$$MIC = EC_{50}\left(\frac{GR}{E_{max} + GR}\right)^{1/Hill}$$

The MPC_{90} is defined as the concentration at which the clearance effect is at 90% of the maximum. It is calculated as follows:

$$MPC_{90} = EC_{50} \cdot 9^{1/Hill}$$

The PRR_{48} is defined as the parasite clearance achieved within 48 hours, usually given as the reduction of values on log_{10} transformed scale. The maximum capacity of parasite clearance in 48 hours is determined as follows assuming that concentrations are maintained well above the MPC_{90} for this time span.

$$PRR_{48} = 48h\frac{E_{max} - GR}{\ln(10)}$$

Full details of the modelling analysis will be described in the SAP.

PK and PD parameters will be tabulated as population estimates and relative standard error (RSE).

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINTS

This study has three secondary endpoints.

Safety parameters:
The safety and tolerability of pyronaridine will be assessed by clinical review of the following parameters:

- AEs (including SAEs and AESIs)
- Vital signs
- 12-lead ECG
- Haematology, Biochemistry, urinalysis
- Physical examination

The analysis of this endpoint is discussed in Section 9.4.4 below.

Parasite clearance kinetics:
The parasite clearance kinetics following dosing with of pyronaridine will be determined by calculating the parasite reduction ratio over a 48 hour period (PRR_{48}) and corresponding parasite clearance half-life ($PCt_{1/2}$); the incidence of parasite regrowth (n and % per dose group) will be recorded.

The PRR_{48} will be estimated using the slope of the optimal fit of the log-linear relationship of the parasitaemia decay. The optimal fit will be derived using summarised replicate parasitaemia data, which have been cleaned by dealing with potential outliers, values below the limit of detection and non-detectable values (ND). The optimal fit of the log-linear parasitaemia-by-time relationship is determined by using left and right censoring to systematically remove the potential lag phase and tail phase of the parasitaemia decay. The decay rate, estimated by the slope coefficient from the log-linear decay regression, will be calculated for each participant. The overall dose-specific PRR_{48} will be estimated with its 95% CI by calculating the weighted average slope estimate and corresponding standard error (SE) using an inverse-variance method. Only participants who have optimal regression models with appropriate fit contribute toward the dose-specific PRR_{48}. Details will be presented in the SAP.

The parasite clearance half-life (Pt ½) will be derived from the optimal decay rate. $PCt_{1/2}$ is a transformation of PRR_{48} into a per hour clearance rate. Details regarding the calculation of this parameter will be presented in the SAP.

PK parameters:
The following PK parameters will be determined using non-compartmental methods from whole blood pyronaridine concentration-time profiles: AUC_{last}, AUC_{inf}, C_{max}, t_{max}, $t_{1/2}$, CL/F, Vd/F and λ. All PK variables (normally and log-normally distributed) will be summarized using arithmetic and geometric means, minimum, median, maximum, SD and coefficient of variation (CV %) of arithmetic and geometric means. Summary tables showing the median and range for t_{max} and geometric mean and CV% for all other PK variables will be provided by day and dose.

Whole blood concentration, values below the lower limit of quantification (LLOQ) will be set to zero. All mentioned parameters will be expressed as mean ± SD, unless otherwise stated. Details regarding the calculation of this parameter will be presented in the SAP.

9.4.4 SAFETY ANALYSES

All descriptive statistics for safety parameters will be evaluated using the Safety Set.

All AE data will be listed for each volunteer, including severity, relationship to IMP (pyronaridine and malaria challenge agent), relationship to non-IMP protocol-specific treatments, outcome and actions taken. In addition, listings of AEs leading to discontinuation of the study, AESIs, SAEs and deaths, will be provided as applicable. Adverse events (AE) will be coded according to MedDRA and grouped by SOC and PT.

The main AE summaries will be restricted to Treatment Emergent Adverse Events (TEAEs) only, where a TEAE is defined as an AE that commences on, or after, the first administration of IMP up to the end-of-study (EOS) visit (inclusive). TEAEs without an onset date or time will be defined as treatment emergent except if an incomplete date (e.g., month and year) clearly indicates that the event started prior to first administration of IMP, or if the AE stop date indicates that the event stopped prior to the first administration of IMP.

At a minimum, the following AE summary tables will be provided:

- Overall summary of AEs
- All TEAEs
- TEAEs by severity
- TEAEs by relationship to the IMP
- TEAEs by relationship to rescue medications
- Serious TEAEs
- TEAEs leading to study withdrawal
- AESIs
- All Inoculation Period AEs
- Inoculation Period AEs by relationship to the malaria challenge agent

Lab parameters, physical examination, Body measurements, vital signs, Beck Depression inventory score and 12-lead ECG will be listed for all participants and visits. Summary tables for each scheduled visit, including observed values, change from each baseline and clinical significance of each abnormal value, will be presented.

9.4.5 BASELINE DESCRIPTIVE STATISTICS

Demographic data will be summarised by descriptive statistics and listed for all enrolled participants. This will include total number of observations, mean, standard deviation, and range for continuous variables that are normally distributed for medians and interquartile ranges for non-parametric continuous variables, and number and percentages for categorical variables.
Volunteer disposition will be summarised and presented in a flow diagram. Trial completion, trial withdrawals, exclusions, and violations will be summarised and the reasons for withdrawal, exclusions, and violations will be listed.

Medical history, current medical conditions, prior and concomitant medications, results of laboratory screening tests, drug and alcohol screening tests, and any other relevant baseline information will be listed by participant and treatment group. Medical history will be coded using MedDRA and summarised by SOC and PT.

9.4.6 PLANNED INTERIM ANALYSES

This study has no formal interim analyses other than review of safety, tolerability, parasitaemia and PK parameters by the SDRT between cohorts.

9.4.7 SUB-GROUP ANALYSES

Not applicable.

9.4.8 TABULATION OF INDIVIDUAL PARTICIPANT DATA

All individual participant data will be listed by measure and time-point.

9.4.9 EXPLORATORY ANALYSES

Not applicable.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 ETHICAL STANDARD

The trial will be conducted in accordance with the protocol approved by the HREC(s), the principles of the Declaration of Helsinki (Recommendations guiding Medical Doctors in Biomedical Research Involving Human Participants, Fortaleza, Brazil 2013), the NHMRC National Statement on Ethical Conduct in Human Research (2007, updated 2018) and the Integrated Addendum to ICH E6 (R1): Guideline for Good Clinical Practice E6 (R2) (November 2016) — with introductory comments of the Australian TGA.

The PI will minimise any discomfort experienced by participants during the trial. The only invasive procedures will be the IV inoculation of the malaria challenge agent and the blood collection by cannulation/venepuncture. The total volume of blood drawn from each participant will not exceed 470 mL in any 30-day period during trial participation.

10.1.2 ETHICAL REVIEW

The protocol, Participant Information Sheets, and Informed Consent Forms will be reviewed by the approving HREC(s) and no trial activities will be initiated prior to approval. All amendments and addenda to the protocol and consent forms will similarly be submitted to the approving HREC(s) for approval prior to their implementation.

Changes to the final trial protocol can only be made with the prior consent of the PI, the Sponsor and the approving HREC(s). All such changes must be attached to (or incorporated into) the final protocol and communicated to all relevant members of the clinical trial unit staff and, if appropriate, to trial participants. All deviations from this trial protocol will be included in the trial master file and included in the CSR. The types of amendments are discussed below.

Administrative or minor changes

Administrative or minor changes include but are not limited to changes in trial staff or contact details (e.g., Sponsor instead of contract research organisation monitors). Amendments for administrative or minor changes may be suitable for executive review (expedited) by the approving HREC(s).

Substantial amendment

Significant changes require a substantial amendment. Significant changes include but are not limited to: new data affecting the safety of participants, change of the objectives/endpoints of the trial, eligibility criteria, dose regimen, trial assessments or procedures, treatment or trial duration with or without the need to modify the Participant Information Sheet and Informed Consent Form. Substantial amendments are to be approved by the HREC(s). The implementation of a substantial amendment can only occur after formal approval from the Sponsor, HREC(s), and PI.

Urgent amendment

An urgent amendment might become necessary to preserve the safety of the participants included in the trial. The requirements for approval should in no way prevent any immediate action being taken by the PI or the Sponsor in the best interests of the participants. Therefore, if deemed necessary, the Investigator can implement an immediate change to the protocol for safety reasons with notification to the medical monitor as soon as practicably possible. This means that, exceptionally, the implementation of urgent amendments will occur before submission to, and approval by, the HREC(s). In such cases, the PI must notify the Sponsor within 24 hours. A related substantial amendment will be written and submitted to HREC(s) as soon as practicable but no later than 7 working days, together with a description of the steps that have already been taken in regard to implementation of this amendment.

HREC approval of future research

In the event that the PI or the Sponsor want to perform testing on the samples that is not described in the protocol, additional approval will be sought from the approving HREC(s). This may be done if a participant consents to blood storage for use in future research.

10.1.3 INFORMED CONSENT PROCESS

10.1.3.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Each potential participant will be given the trial Participant Information Sheet and Informed Consent Form that describes in detail the trial interventions, trial procedures, and risks.

All participants will receive an Informed Consent for Malaria Future Research and an option to grant permission to be contacted about involvement in future trials. If a potential participant chooses not to consent to these options this will not impact their eligibility for the trial.

10.1.3.2 CONSENT PROCEDURES AND DOCUMENTATION

During the initial screening visit/recruitment, potential participants will read the Participant Information Sheet. An Investigator will explain the trial via the Participant Information Sheet and the candidate participants will be encouraged to ask questions. Potential participants will be informed that participation is voluntary and that they may withdraw from the trial at any time, without prejudice. Potential participants will have the opportunity to discuss the trial with their family or think about it prior to agreeing to participate.

Individuals willing to be considered for inclusion in the trial will sign and date the Informed Consent Form in the presence of an Investigator. Participants will be given a copy of their signed Informed Consent Form. The conduct of the informed consent process will be documented in the source document (including the date) and the form will be signed before the participant undergoes any trial-specific procedures; only once the participant has consented to the trial may trial-specific screening activities commence.

10.1.4 STUDY DISCONTINUATION AND CLOSURE

The Sponsor, PI, approving HREC(s), and regulatory authorities independently reserve the right to discontinue the trial at any time for safety or other reasons. Circumstances that may warrant termination or suspension include, but are not limited to:

- determination of unexpected, significant, or unacceptable risk to participants
- insufficient compliance with protocol requirements
- data that are not sufficiently complete and/or evaluable
- determination that the primary endpoint has been met

This will be done in consultation with the Sponsor where practical. In the event of premature trial termination or suspension, the above-mentioned parties will be notified in writing by the terminator/suspender stating the reasons for early termination or suspension (with the exception of the Sponsor's responsibility for notifying the regulatory authorities). After such a decision, the Sponsor and the Investigator will ensure that adequate consideration is given to the protection of the participants' interest and safety. The Investigator must review all participants as soon as practical and complete all required records.

10.1.5 CONFIDENTIALITY AND PRIVACY

Participants will be informed that their data will be held on file by the clinical trial unit and that these data may be viewed by staff of the clinical trial unit (including, where necessary, staff of the clinical trial unit other than the named Investigators).

In the event of a notifiable disease being discovered during the trial, the appropriate Public Health authorities will be notified in accordance with the Queensland Public Health Regulation 2018.

Upon request, the Investigator(s)/institution(s) will permit direct access to source data and documents for trial-related monitoring, audits, HREC review, and regulatory inspection(s) by the Sponsor (or their appropriately qualified delegates) and regulatory authorities.

Participants will also be informed that a report (CSR) of the trial will be submitted to the Sponsor and may also be submitted to regulatory authorities and perhaps for publication, but that they will only be identified in such reports by their trial identification number, their sex and age. The Investigators will undertake to hold all personal information in confidence.

Participants will be informed that samples collected for the purposes described in the protocol will be sent to the Sponsor's nominated national or international laboratory for assessment.

10.1.6 FUTURE USE OF STORED SPECIMENS AND DATA

As part of the trial, safety serum retention samples will be stored for 15 years at QIMR Berghofer for retrospective safety assessments that may later be indicated. Participants consent to this storage and the use of the sample for safety assessments when they sign the Informed Consent Form for the trial.

For all other samples, consent must be obtained from the participants to store and use their samples for malaria future research.

Under a separate protocol and consenting process, blood samples may be collected for storage and use in future malaria research. The separate study will be under the local sponsorship of QIMR Berghofer Institute and will involve a protocol and consent document approved by the QIMR HREC. All samples will be stored at QIMR Berghofer in accordance with the laboratory SOPs. The Investigator will ensure that confidentiality will be maintained continuously in all future research that involves use of these samples. No genetic testing will be performed on the stored samples without obtaining consent from the participants. The stored samples will not be sold or used directly for production of any commercial product. There are no benefits to participants in the collection, storage, and subsequent research use of their samples. Reports about future research done with participant samples will NOT be kept in participant health records, but a participant's samples may be kept with the trial records or in other secure areas.

10.1.7 KEY ROLES AND STUDY GOVERNANCE

Principal Investigator	A/Prof. Bridget Barber MBBS, DTM&H, MPH, FRACP, PhD QIMR Berghofer Medical Research Institute Level 5, 300C Herston Road Herston QLD 4006, Australia Tel: +61 424 737 153 Email: Bridget.Barber@qimrberghofer.edu.au
Sponsor Medical Director	Anne Claire Marrast, MD Medicines for Malaria Venture Route de Pré-Bois 20 1215 Geneva 15

	Switzerland Email: marrasta@mmv.org
Sponsor Project Director	Jörg Möhrle, PhD Medicines for Malaria Venture Route de Prés-Bois 20 1215 Geneva 15 Switzerland Tel: +41 22 555 0330 Email: moehrlej@mmv.org
Local Study Sponsor	Southern Star Research Pty Ltd (SSR) 1 Merriwa St Gordon, NSW 2072 Australia Tel: +61 (0)2 9011 6266
Study Monitor	Southern Star Research Pty Ltd (SSR) 1 Merriwa St Gordon, NSW 2072 Australia Tel: +61 (0)2 9011 6266
Institutional Ethics Committee	QIMR Berghofer Medical Research Institute Human Research Ethics Committee (QIMR Berghofer HREC; EC00278) Locked Bag 2000, Royal Brisbane and Women's Hospital, Brisbane QLD 4029, Australia Tel: +61 (0)7 3362 0117
Biostatistician (parasitaemia)	Stacey Llewellyn QIMR Berghofer Medical Research Institute 300C Herston Rd Herston QLD 4006, Australia Tel: +61 (0)7 3845 3579 Email: Stacey.Llewellyn@qimrberghofer.edu.au
Biostatistician	Southern Star Research Pty Ltd (SSR) 1 Merriwa St Gordon, NSW 2072 Australia Tel: +61 (0)2 9011 6266
Pharmacometrician	Nathalie Gobeau Medicines for Malaria Venture Route de Pré-Bois 20 1215 Geneva 15 Switzerland Email: gobeaun@mmv.org
Independent Medical Monitor	Dr Joanne Marjason Southern Star Research Pty Ltd 1 Merriwa St Gordon, NSW 2072, Australia Tel: +61 (0)427 959 156 Email: jmarjason@southernstarresearch.com

Clinical Study Centre	USC Clinical Trials Unit <u>Moreton Bay</u> Health Hub Morayfield 19-31 Dickson Road Morayfield QLD 4506 Australia Tel: +61 (0)7 54563965 Email: ctchhm@usc.edu.au <u>South Bank</u> Building A2, SW1 Complex 52 Merivale Street South Brisbane QLD 4101 Tel: +61 (0)7 5409 8630 Email: ctcsouthbank@usc.edu.au
Laboratories	<u>Clinical laboratory measurements:</u> Sullivan Nicolaides Pathology Central Laboratory (SNP) 24 Hurworth Street Bowen Hills, QLD 4006, Australia Tel: +61 (0)7 3377 8782 <u>Parasite quantification in blood samples:</u> Ms. Claire Wang Queensland Paediatric Infectious Diseases Laboratory (Q-PID), SASVRC, Level 8, Centre for Children's Health Research 62 Graham Street South Brisbane, QLD 4101, Australia Tel: +61 (0)7 3069 7462 Email: claire.wang@uq.edu.au <u>Investigational drug quantification in blood samples:</u> Dr. Christoph Siethoff CEO Swiss BioQuant AG, Kägenstrasse 18 4153 Reinach, Switzerland Tel: +41 (0) 617 1698 12 Email: Christoph.Siethoff@swissbioquant.com, mail@swissbioquant.com
Serious AE and AESI reporting	Prime vigilance Tel: +44 (0) 800 471 5694 Email: mmv@primevigilance.com

10.1.8 SAFETY OVERSIGHT

The SDRT will be responsible for decisions related to the safety of participants and the continuation of the trial. The SDRT will meet to assess any event/s that trigger the discontinuation rules or as needed to provide a recommendation and findings to the approving HREC(s) and the PI.

The SDRT will meet at the end of each cohort to review safety data (AEs, AESIs, SAEs, and laboratory data), parasitaemia data, and the available PK and PK/PD modelling data. Based on this review, the SDRT will decide on whether to progress to the next cohort and will decide on the doses of pyronaridine to be administered.

The SDRT may also decide to modify the frequency and timing of blood sample collection for malaria 18S PCR and/or pyronaridine blood concentration measurements as long as blood volume is not greater than 470 mL in any 30-day period

The role and composition of the SDRT is outlined in the study specific SDRT Charter, but at a minimum the SDRT will be comprised of;

- MMV Medical Director or delegate
- MMV Vice President, Head of Translational Medicine or delegate
- Principal Investigator (PI) or delegate or delegate
- Local Sponsor Medical Monitor or delegate
- MMV Pharmacometrician or delegate (ad hoc as required)

10.1.9 CLINICAL MONITORING

It will be the Sponsor's responsibility to ensure that the trial is monitored in accordance with the requirements of GCP. The conduct of the trial will be reviewed internally by the clinical trial unit in accordance with their SOPs and work instructions, and GCP guidelines. The trial will be monitored according to the SOPs of the Sponsor and all serious breaches, suspected breaches and protocol deviations will be reported to the Sponsor. Serious breaches that impact participant safety or data integrity will also be reported to the approving HREC.

During the trial, trial monitor(s) (on behalf of the Sponsor) will visit the clinical trial unit regularly (or, under exceptional circumstances, access remotely the clinical unit files) to check the completeness of participant records, accuracy of eCRF entries, adherence to the protocol and to GCP, the progress of enrolment, and to ensure that trial interventions are being stored, dispensed, and accounted for according to specifications. Key trial personnel will be required to be available to assist the monitor during these visits (or provide the Monitor with an access to the required documents, if the visit is performed remotely).

The Investigator will be required to give the monitor access to all relevant source documents to confirm their consistency with the eCRF entries. At a minimum, the Sponsor will require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, and documentation of SAEs, AESI and AEs, and the recording of data that was used for all primary and safety variables. Additional checks of the consistency of the source data with the eCRFs will be performed according to the trial-specific monitoring plan. No information captured in the source documents about the identity of the participants will be disclosed.

10.1.10 QUALITY ASSURANCE AND QUALITY CONTROL

The clinical trial unit will perform internal quality management of trial conduct, data and biological specimen collection, documentation and completion.

Quality control procedures will be implemented beginning with the data entry system and data quality control checks that will be run on the database that will be generated. Any missing data or data anomalies will be communicated to the site for clarification and resolution.

The clinical trial unit will provide direct access to source data and documents, and reports for the purpose of monitoring and auditing by the Sponsor, and inspection by regulatory authorities.

Data management, including the development and management of a secure database, will be performed in accordance with regulatory requirements. The designated data management vendor will review the data entered into the eCRFs by clinical trial unit staff for completeness and accuracy. A formal querying process will be followed whereby the data management team will request the site personnel to clarify any apparent erroneous entries or inconsistencies and will request additional information from the site as required.

Medical history/current medical conditions and AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) terminology (version 20.1 or higher). Prior and concomitant medications will be coded using the WHO Drug Dictionary Enhanced (WHO Global; March 2014 or later).

After all data have been captured and reviewed, all queries have been resolved with the site, and any protocol deviations that were identified during the data management processes have been confirmed by the site, the database will be declared to be complete and accurate. The database will be locked and made available for data analysis. Any changes to the database after that time may only be made, in consultation with the Sponsor and in accordance with documented database unlock and relock procedures.

10.1.11 DATA HANDLING AND RECORD KEEPING

10.1.11.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Participants will be assigned a unique identifier when participating in the study, and any participant datasets or records that are transferred to the Sponsor or CRO will only contain this identifier. Any other identifiable information about the participant will not be transferred. The PI will ensure procedures are in place to appropriately protect the confidentiality of the participant records and data, including adequate safe guards for digital/computer access. The participants will be informed that their personal study-related data will be used by the Sponsor and that their medical records may be examined by auditors and regulatory agencies.

Each participant will have a clinical file (source data) and eCRF (for protocol specific data) into which relevant data will be recorded. All recording of source data and documents will be done in RealTime, the

clinical trial unit's eSource system. Pathology reports, ECGs and any other paper source will be uploaded to RealTime.

Guidelines for eCRF completion including correcting data and responding to data queries will be provided by the Sponsor or CRO. A log of names, signatures, and initials of all staff authorised to enter data into a participant's clinical file and eCRF will be kept. Corrections to source document (whether paper or electronic) must have an audit trail (i.e., must not obscure or delete the original entry, and the date/time of correction and identity of the person making the correction must be clearly indicated).

10.1.11.2 STUDY RECORDS RETENTION

In compliance with the ICH/GCP guidelines, the Investigator/Institution will maintain all eSource and all paper source documents that support the data collected from each participant, as well as all study documents as specified in ICH/GCP Section 8, Essential Documents for the Conduct of a Clinical Trial, and all study documents as specified by the applicable regulatory requirement(s). The Investigator/Institution will take measures to prevent accidental or premature destruction of these documents. Essential documents must be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the IMP. These documents will be retained for a longer period if required according to the applicable regulatory requirements or per agreement with the Sponsor. It is the responsibility of the Sponsor to inform the Investigator/Institution as to when these documents no longer need to be retained. If the responsible Investigator retires, relocates, or for any other reasons withdraws from his responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility. The Sponsor must be notified in writing of the name and address of the new custodian. Under no circumstance shall the Investigator relocate or dispose of any study documents without having obtained written approval from the Sponsor. If it becomes necessary for the Sponsor or the appropriate regulatory authority to review any documentation related to the study, the Investigator must permit access to such reports.

10.1.12 PROTOCOL DEVIATIONS

Protocol deviation: any departure, change, and/or addition from the trial design or procedures defined in the protocol that has received approval by the competent authorities and favourable opinion from the approving HREC.

Suspected serious breach: a report that is judged by the reporter as a possible serious breach, but has yet to be formally confirmed as a serious breach by the Sponsor.

Serious breach: a breach of GCP or the protocol that is likely to affect to a significant degree: a) the safety or rights of a trial participant, and/or b) the reliability and robustness of the data generated in the clinical trial.

Note to File (NTF): a record that documents in detail actions taken, important decisions made, or explains a sequence of events where no other detailed record exists to enable the conduct of the trial to be reconstructed.

Reporting requirements:

- All protocol deviations, suspected serious breaches, and serious breaches will be documented in the trial master file and included in the CSR.
- All NTFs, protocol deviations, suspected serious breaches, and serious breaches will be viewed by the PI's delegate and signed by the PI.
- All protocol deviations, suspected serious breaches and serious breaches will be reported and assessed at each SDRT meeting.
- All serious breaches will be reported by the clinical trial unit to the Sponsor and the approving HREC(s) as early as possible, but within 7 days.
- All participant-specific protocol deviations will be captured in a log by the clinical site and reported by the clinical trial unit to the PI's delegate and signed by the PI at the end of each cohort and shared with the Sponsor for review and sign off before database lock.
- Protocol deviation logs will be submitted by the clinical trial unit to the Sponsor and approving HREC via inclusion with the annual report.

10.1.13 PUBLICATION AND DATA SHARING POLICY

The data management, statistical, and medical writing team appointed by the Sponsor will collaborate to provide a detailed CSR upon conclusion of the trial. The CSR will include appendices of all tables and listings generated during the analyses of data. The tables, figures and listings will be provided by the Sponsor. The Sponsor will undertake to ensure that all safety observations made during the conduct of the trial are documented in the CSR.

Publication and reporting of results and outcomes of this trial will be accurate and honest, and undertaken with integrity and transparency. The Sponsor recognises that the PI has a responsibility to ensure that results of scientific interest arising from the trial are appropriately published and disseminated. Publication of results will be volunteered to fair peer-review. Authorship will be given to all persons providing significant input into the conception, design, and execution or reporting of the research. No person who is an author, consistent with this definition, will be excluded as an author without his/her permission in writing. Authorship will be discussed between researchers prior to trial commencement (or as soon as possible thereafter) and reviewed whenever there are changes in participation. Acknowledgement will be given to collaborating institutions and hospitals and other individuals and organisations providing finance or facilities.

Neither the complete nor partial results of the study achieved under this protocol, nor any of the information provided by the Sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study Sponsor. Any Investigator involved with this study is obligated to provide the Sponsor with complete study results and all data derived from the study.

The Sponsor will ensure that the key design elements of this protocol are posted in a publicly accessible database such as Australian New Zealand Clinical Trials Registry (ANZCTR) or Clinicaltrials.gov. In addition, upon trial completion and finalisation of the CSR, the results of this trial will be submitted for publication in an open access journal and/or posted in a publicly accessible database of clinical trial results.

10.1.14 CONFLICT OF INTEREST POLICY

Potential conflicts of interest will be identified, assessed, managed and documented, with any action taken and reasons for taking action recorded during the ongoing risk management process.

10.2 LIABILITY/INDEMNITY/INSURANCE

The Local Sponsor will ensure sufficient insurance is available to enable it to indemnify and hold the Investigators and relevant staff as well as any hospital, institution, ethics committee or the like, harmless from any claims for damages for unexpected injuries, including death, that may be caused by the participant's participation in the trial but only to the extent that the claim is not caused by the fault or negligence of the participant(s) or Investigators. The Sponsor adheres to the guidelines of Medicines Australia for injury resulting from participation in a company sponsored trial, including the provision of 'No-fault clinical trial insurance'.

10.3 ABBREVIATIONS

λ	Elimination Rate Constant
ACT	Artemisinin Combination Therapy
AE	Adverse Event
AESI	Adverse Event of Special Interest
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ANZCTR	Australia New Zealand Clinical Trial Registry
anti-HBc Ab	Anti-Hepatitis B Core Antibodies
anti-HCV Ab	Anti-Hepatitis C Virus Antibodies
anti-HIV1 Ab	Anti-Human Immunodeficiency Virus 1Antibodies
anti-HIV2 Ab	Anti-Human Immunodeficiency Virus 2 Antibodies
AR	Adverse Reaction
AUC	Area Under the Curve
$AUC_{0-\infty}$	AUC to Infinite Time
AUC_{0-last}	AUC to Last Quantifiable Concentration
AUC_{0-t}	AUC to Last Measurable at t time
B-hCG	Beta Human Chorionic Gonadotropin
Blood Service	Australian Red Cross Blood Service
BMI	Body Mass Index
C_{max}	Maximum Plasma Concentration
CL/F	Apparent Oral Clearance
CMI	Consumer Medicine Information
CRO	Clinical Research Organisation
CRU	Clinical Research Unit
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for AEs
DBP	Diastolic Blood Pressure
eCRF	Electronic Case Report Form
ECG	Electrocardiographs
EC_{50}	Half Maximal Effective Concentration
EOS	End of Study
E_{max}	Maximum effective concentration
FBC	Full Blood Count
FDA	Food and Drug Administration
FSH	Follicle-Stimulating Hormone
G6PD	Glucose-6-Phosphate Dehydrogenase
GCP	Good Clinical Practice
HBs Ag	Hepatitis B Surface Antigen
HIV	Human Immunodeficiency Virus
HR	Heart Rate
HREC	Human Research Ethics Committee
IB	Investigator's Brochure
IBSM	Induced Blood Stage Malaria

ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product
IV	Intravenous
LFT	Liver Function Test
MAP	Modelling Analysis Plan
MCB	Master Cell Bank
MedDRA	Medical Dictionary for Regulatory Activities
MIC	Minimum Inhibitory Concentration
MMV	Medicines for Malaria Venture
MPC_{90}	Minimal Parasiticidal Concentration
NHMRC	National Health and Medical Research Council
NPA	Nasopharyngeal Aspirate
NTF	Note To File
PD	Pharmacodynamics
PICF	Patient Information Consent Form
PK	Pharmacokinetics
PK/PD	Pharmacokinetics/Pharmacodynamics
PRR	Parasite Reduction Ratio
PRR_{48}	Parasite Reduction Ratio in 48 hours
PT	Preferred Term
$Pt_{½}$	Parasite Clearance Half-Life
QIMR Berghofer	Queensland Institute of Medical Research Berghofer
qPCR	Quantitative Polymerase Chain Reaction
qRT-PCR	Quantitative Reverse-Transcriptase Polymerase Chain Reaction
RAT	Rapid Antigen Test
RBCs	Red Blood Cells
RDW	Red Blood Cell Distribution Width
Rh	Rhesus Antibody
RSI	Reference Study Information
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SBP	Systolic Blood Pressure
SDRT	Safety and Data Review Team
SE	Standard Error
SOC	System Organ Class
SOP	Standard Operating Procedure(s)
SUSAR	Suspected Unexpected Serious Adverse Reaction
$t_{½}$	Terminal Half-Life
TGA	Therapeutic Goods Administration
T_{max}	Time taken to reach C_{max}
ULN	Upper Limit of Normal
Vd/F	Apparent Volume Distribution
WBC	White Blood Cell
WOCBP	Women of Childbearing Potential
WHO	World Health Organisation

11 REFERENCES

1. World Health Organization. World Malaria Report 2020. Geneva.

2. Conrad MD, Rosenthal PJ. Antimalarial drug resistance in Africa: the calm before the storm? Lancet Infect Dis. 2019;19:e338-e51.

3. Croft SL, Duparc S, Arbe-Barnes SJ, Craft JC, Shin CS, Fleckenstein L, et al. Review of pyronaridine anti-malarial properties and product characteristics. Malar J. 2012;11:270.

4. Bailly C. Pyronaridine: An update of its pharmacological activities and mechanisms of action. Biopolymers. 2021;112:e23398.

5. Burrows JN, Duparc S, Gutteridge WE, Hooft van Huijsduijnen R, Kaszubska W, Macintyre F, et al. New developments in anti-malarial target candidate and product profiles. Malar J. 2017;16:26.

6. McCarthy JS, Baker M, O'Rourke P, Marquart L, Griffin P, Hooft van Huijsduijnen R, et al. Efficacy of OZ439 (artefenomel) against early *Plasmodium falciparum* blood-stage malaria infection in healthy volunteers. J Antimicrob Chemother. 2016;71:2620-7.

7. McCarthy JS, Lotharius J, Ruckle T, Chalon S, Phillips MA, Elliott S, et al. Safety, tolerability, pharmacokinetics, and activity of the novel long-acting antimalarial DSM265: a two-part first-in-human phase 1a/1b randomised study. Lancet Infect Dis. 2017;17:626-35.

8. McCarthy JS, Marquart L, Sekuloski S, Trenholme K, Elliott S, Griffin P, et al. Linking murine and human *Plasmodium falciparum* challenge models in a translational path for antimalarial drug development. Antimicrob Agents Chemother. 2016;60:3669-75.

9. McCarthy JS, Ruckle T, Djeriou E, Cantalloube C, Ter-Minassian D, Baker M, et al. A Phase II pilot trial to evaluate safety and efficacy of ferroquine against early *Plasmodium falciparum* in an induced blood-stage malaria infection study. Malar J. 2016;15:469.

10. Llanos-Cuentas A, Casapia M, Chuquiyauri R, Hinojosa JC, Kerr N, Rosario M, et al. Antimalarial activity of single-dose DSM265, a novel plasmodium dihydroorotate dehydrogenase inhibitor, in patients with uncomplicated *Plasmodium falciparum* or *Plasmodium vivax* malaria infection: a proof-of-concept, open-label, phase 2a study. Lancet Infect Dis. 2018;18:874-83.

11. Phyo AP, Jittamala P, Nosten FH, Pukrittayakamee S, Imwong M, White NJ, et al. Antimalarial activity of artefenomel (OZ439), a novel synthetic antimalarial endoperoxide, in patients with *Plasmodium falciparum* and *Plasmodium vivax* malaria: an open-label phase 2 trial. Lancet Infect Dis. 2016;16:61-9.

12. Investigator's Brochure: Pyronaridine Tetraphosphate Tablets. 2.0 ed: Shin Poong Pharm Co., Ltd.

13. Pyramax 180 mg/60mg fim-coasted tablet: Summary of Product Characteristics. Seoul, South Korea: Shin Poong Pharmacweutical Co., Ltd.

14. Ratcliff A, Siswantoro H, Kenangalem E, Maristela R, Wuwung RM, Laihad F, et al. Two fixed-dose artemisinin combinations for drug-resistant falciparum and vivax malaria in Papua, Indonesia: an open-label randomised comparison. Lancet. 2007;369:757-65.

15. Fu S, Xiao SH. Pyronaridine: A new antimalarial drug. Parasitol Today. 1991;7:310-3.

16. Chang C, Lin-Hua T, Jantanavivat C. Studies on a new antimalarial compound: pyronaridine. Trans R Soc Trop Med Hyg. 1992;86:7-10.

17. Shao BR. A review of antimalarial drug pyronaridine. Chin Med J (Engl). 1990;103:428-34.

18. Ringwald P, Bickii J, Basco L. Randomised trial of pyronaridine versus chloroquine for acute uncomplicated falciparum malaria in Africa. Lancet. 1996;347:24-8.

19. Looareesuwan S, Kyle DE, Viravan C, Vanijanonta S, Wilairatana P, Wernsdorfer WH. Clinical study of pyronaridine for the treatment of acute uncomplicated falciparum malaria in Thailand. Am J Trop Med Hyg. 1996;54:205-9.

20. Nieman AE, de Mast Q, Roestenberg M, Wiersma J, Pop G, Stalenhoef A, et al. Cardiac complication after experimental human malaria infection: a case report. Malar J. 2009;8:277.

21. van Meer MP, Bastiaens GJ, Boulaksil M, de Mast Q, Gunasekera A, Hoffman SL, et al. Idiopathic acute myocarditis during treatment for controlled human malaria infection: a case report. Malar J. 2014;13:38.

22. Watts RE, Odedra A, Marquart L, Webb L, Abd-Rahman AN, Cascales L, et al. Safety and parasite clearance of artemisinin-resistant *Plasmodium falciparum* infection: A pilot and a randomised volunteer infection study in Australia. PLoS Med. 2020;17:e1003203.

12 APPENDIX

Sponsor approved inclusion ranges

Test	Unit	Low	High
Sodium	mmol/L	130	150
Potassium	mmol/L	3.0	5.5
Chloride	mmol/L	0.90 x LLN	1.10 x ULN
Calcium (Corr)	mmol/L	0.90 x LLN	1.10 x ULN
Urea	mmol/L	N/A	1.75 x ULN
Urate	mmol/L	N/A	1.75 x ULN
Creatinine	umol/L	N/A	1.0 x ULN
Creatine kinase	U/L	N/A	< 2.5 x ULN
eGFR	mL/min/1.7 3m^2	60	N/A
Glucose Fasted	mmol/L	N/A	1.0 x ULN
Total Bilirubin	umol/L	N/A	1.0 x ULN
ALP	U/L	N/A	1.5 x ULN
AST	U/L	N/A	1.0 x ULN
ALT	U/L	N/A	1.0 x ULN
GGT	U/L	N/A	1.0 x ULN
Cholesterol	mmol/L	N/A	1.2 x ULN
HDL Cholesterol	mmol/L	0.9x LLN	N/A
LDL Cholesterol	mmol/L	N/A	1.25 x ULN
Hb	g/L	0.9x LLN	1.1.x ULN
Plats	x10^9/L	0.9x LLN	1.1 x ULN
WCC	x10^9/L	0.9x LLN	1.1 x ULN
Neuts	x10^9/L	1.0 x LLN	1.1 x ULN
Lymphs	x10^9/L	1.0 x LLN	1.1 x ULN
Protein (dipstick)		N/A	1+ or 30mg/dL
Ketones (dipstick)		N/A	<3+ or <80mg/dL
Red Blood Cells (MCS)		N/A	<20* *A result ≥ 20 is acceptable for female participants currently menstruating.
White Blood Cells (MCS)		N/A	<10
Casts (MCS)		N/A	<2/high power field