QIMR Berghofer
Medical Research Institute

A single-centre Phase 1b study to assess the safety, tolerability, pharmacokinetic profile, and antimalarial activity of single doses of co-administered artefenomel (OZ439) and piperaquine phosphate (PQP) against early *Plasmodium falciparum* blood stage infection in healthy adult volunteers

Protocol Identifying Number: QP17C19

Principal Investigator: Prof. James McCarthy

Study Sponsor: Medicines for Malaria Venture

Local Sponsor: Clinical Network Services

Version Number: Protocol 4.0

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CONFIDENTIALITY STATEMENT

CONTENTS

LIST OF ABBREVIATION S

ACPR	Adjusted Adequate Clinical and Parasitological Response
ACT	Artemisinin Combination Therapy
AE	AE
AESI	AE of Special Interest
ALT	Alanine Aminotransferase
ANZCTR	Australia New Zealand Clinical Trial Registry
anti-HBc Ab	Anti-Hepatitis B Core Antibodies
anti-HCV	Anti-Hepatitis C Virus
anti-HIV1	Anti-Human Immunodeficiency Virus 1
anti-HIV2	Anti-HumanImmunodeficiency Virus 2
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
AUC_{inf}	AUC Curve to Infinite Time
AUC_{last}	AUC Curve to Last Quantifiable Concentration
BDI	Beck Depression Index
Blood Service	Australian Red Cross Blood Service
CRP	C-Reactive Protein
C_{max}	Maximum Plasma Concentration
CNS	Clinical Network Services
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for AEs
eCRF	Electronic Case Report Form
ECG	Electrocardiogram
EC_{50}	Half maximal effective concentration
EOS	End of Study
E_{max}	Maximum effective concentration
FDA	Food and Drug Administration
FSH	Follicle-Stimulating Hormone
FQ	Ferroquine
G6PD	Glucose-6-Phosphate Dehydrogenase
GCP	Good Clinical Practice
GI	Gastrointestinal
GMP	Good Manufacturing Practice
GPDI	General Pharmacodynamic Interaction
HAV	Hepatitis A Virus
HBs Ag	Hepatitis B surface antigen
HCV	Hepatitis C Virus
HEV	Hepatitis E Virus
HDL	High Density Lipoprotein
HIV	Human Immunodeficiency Virus
HPLC	High-Performance Liquid Chromatography System
HREC	Human Research Ethics Committee

IB	Investigator's Brochure
IBSM	Induced Blood Stage Malaria
ICH	International Conference on Harmonization
IMM	Independent Medical Monitor
IMP	Investigational Medicinal Product
IV	Intravenous
LC MS	Liquid Chromatography-Mass Spectrometry
LFT	Liver Function Test
LLOQ	Lower Limit of Quantification
MAP	Modelling Analysis Plan
MCB	Master Cell Bank
MedDRA	Medical Dictionary for Regulatory Activities
MFA	Membrane Feeding Assay
MIC	Minimum Inhibitory Concentration
MMV	Medicines for Malaria Venture
MPC	Minimal Parasiticidal Concentration
NHMRC	National Health and Medical Research Council
NLME	Non-linear mixed effect
NTF	Note To File
OZ439	Artefenomel
PD	Pharmacodynamic
PK	Pharmacokinetic
PK/PD	Pharmacokinetic/Pharmacodynamic
PQP	Piperaquine Phosphate
PRR	Parasite Reduction Ratio
$Pt_{½}$	Parasite clearance half-life
QIMR Berghofer	Queensland Institute of Medical Research Berghofer
qPCR	Quantitative Polymerase Chain Reaction
qRT-PCR	Quantitative Reverse Transcription Polymerase Chain Reaction
QTc	Corrected QT
RBC	Red Blood Cell
Rh	Rhesus Antibody
SAE	Serious AE
SAP	Statistical Analysis Plan
SDRT	Safety and Data Review Team
SERC	Single Exposure Radical Cure
SOP	Standard Operating Procedures
SUSAR	Suspected Unexpected Serious Adverse Reaction
$t_{1/2}$	Terminal Half-Life
TEAE	Treatment Emergent AE
TGA	Therapeutic Goods Administration
T_{max}	Time taken to reach C_{max}
TPGS	α-tocopherol polyethylene glycol 1000 succinate

TPP	Target Product Profile
ULN	Upper Limit of Normal
WHO	World Health Organization
WOCBP	Women ofChildbearing Potential

STATEMENT OF COMPLIA NCE

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 Subjects (Fortaleza, Brazil 2013)
- NHMRC National Statement on Ethical Conduct in Human Research, (2007 updated May 2015).
- Note for Guidance on Good Clinical Practice (GCP) – Annotated with Therapeutic Goods Administration (TGA) Comments (CPMP/ICH/135/95), as adopted by the Australian TGA (July 2000)
- Current ethics approved Clinical Trial Protocol

I agree to inform all subjects that the study drugs 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 GCP Section 4.8 and local requirements.

I agree to report AEs 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 Brochures, including the potential risks and side effects of the study drugs.

I agree to promptly report to the Human Research Ethics Committee (HREC) all changes in the research activity and all unanticipated problems involving risks to subjects. 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 subjects.

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 interests of the subjects.

_____ Date: _____

Professor James McCarthy, 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 Subjects (Fortaleza, Brazil 2013), the NHMRC National Statement on Ethical Conduct in Human Research (2007, updated May 2015) and the Note for Guidance on Good Clinical Practice – Annotated with TGA Comments (CPMP/ICH/135/95), as adopted by the Australian Therapeutic Goods Administration (July 2000).

Name	Signature	Date
Protocol Writer: Dr Rebecca Webster PhD Clinical Trials Project Manager QIMR Berghofer Medical Research Institute		

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

Name	Signature	Date
Sponsor Medical Director: Stephan Chalon, MD, PhD Medicines for Malaria Venture		
Sponsor Project Director: Jörg Möhrle PhD Medicines for Malaria Venture		

PROTOCOL SUMMARY

Title:	A single-centre Phase b1 study to assess the safety, tolerabil pharmacokinetic profile, and antimalarial activity of single doses o administeredartefenomel (OZ439) and piperaquine phosphate (P against early*Plasmodium falciparum* blood stage infection in health adultvolunteers.
Study Description:	This is a single-centre, open-label, adaptive, study using the*P. falciparum* induced blood stage malaria (BSM) inoculum as a model to characteris the pharmacodynamic (PD) activity of combined single dose administration of OZ439 and PQP The study will be conducted in a maximum of three cohorts (up subjects per cohort) using up to 4 different doses of OZ439 and PQP i each cohort. Subjects will be malaria naïve healthy males or females, aged between 18-55 years old, who meet all of the inclusion criteria and none of the exclusion criteria. The first cohort will be composed of 4 groups of 2 subjects each. Subjects will be randomised into one of 4 dose groups and administered single o doses of OZ439 and PQP in combination. The combined dose of OZ439 and PQP will be different for each of the 4 groups in this cohort as shov in Table 1. Table 1 OZ439 and PQP Cohort 1 Dose Drug / Dose group: 1A: OZ439 (mg) 200, PQP (mg) 480; 1B: OZ439 (mg) 200, PQP (mg) 640; 1C: OZ439 (mg) 400, PQP (mg) 480; 1D: OZ439 (mg) 400, PQP (mg) 640 The data captured from this first cohort will be used to determine relationship between OZ439 and PQP concentrations and parasit levels. Based on safety and tolerability data up to Day 42±2 and pharmacokinetic/pharmacodynamic (PK/PD) analysis outcomes (based PD data up to Day 42±2 and PK data up to Day 35±2), the dose(s) for the subsequent cohort will be determined.

After review of the PK/PD and safety data from Cohort 1 by Safety and Data Review Team (SDRT) it was determined that, the second cohort will be composed of 2 dose groups of 4 subjects each. Subjects will be randomised into one of 2 dose groups and administered single oral dose of OZ439 and PQP in combination. The combined dose of OZ439 and PQP will be different for each group in this cohort as shown in Table 2.

Table 2 OZ439 and PQP Cohort 2 Dose

Drug	Dose group	
	2A	2B
OZ439 (mg)	800	200
PQP (mg)	960	320

A similar analysis will be done at the end of cohort 2 combining cohort 1 and 2 data to decide the dose(s) to be tested in cohort 3. This will be decided by the funding sponsor and the Principal Investigator following review of the data by the SDRT and scientific evaluation.

The doses used in all cohorts will not exceed the maximum acceptable doses predefined for this study (800 mg for OZ439 and 1440 mg for PQP as determined in previous safety, pilot efficacy and phase 2 studies.

Each subject will be inoculated on Day 0 with approximately 2,800 viable parasites of *P. falciparum*-infected human erythrocytes administered intravenously. Subjects will be followed up daily via phone call or text message on Days 1 to 3 post-inoculation to solicit any AEs.

Subjects will then come to the clinical unit once daily from Day 4 until presence of asexual parasites is established by quantitative polymerase chain reaction (qPCR) targeting the 18S rRNA gene (referred to hereafter as malaria 18S qPCR). Once qPCR becomes positive and until OZ439 and PQP administration, subjects will come to the clinical unit twice daily, separated by approximately 12 hours, for clinical evaluation and blood sampling.

Subjects will be admitted to the clinical unit for single dose administration of OZ439 and PQP 6 days after malaria inoculation or earlier if a subject has a malaria clinical score >6 or at Investigator's discretion. Subjects will

	be followed up as inpatients for at least 72 hours to ensure tolerance investigational treatments and clinical response, then if clinically well c an outpatient basis for safety and clearance of malaria parasites via After discharge from the clinical unit, subjects will be followed up regularly for safety assessments, PK sampling, clinical evaluation, and malaria qPCR blood sampling until Day 42±2 (34 days after OZ439 an PQP administration). All subjects will receive a standard course of ther with Riamet® (artemether-lumefantrine) on Day 42±2, or earlier in the event of failure of clearance or recrudescence of parasitaemia at Investigators discretion based on subject safety. For guidance, the definitions for failure of parasite clearance and recrudescence are de – Failure of clearance defined as failure to clear parasitaemia by least 10-fold at 72 hours post-IMP administration – Recrudescence defined as ≥5 000 blood stage parasites/mL and a 2-fold parasitaemia increase within 48 hours, or re-occurrence of malaria symptoms with a malaria clinical score >6) The presence of gametocytes in subjects' blood will be determined by parasite lifecycle stage qRT-PCR or by the presence of stable low le parasitaemia. If gametocytes are present at the time of treatment wit Riamet®, Primacin™ (primaquine) will also be administered as a single oral dose AEs (AEs) will be monitored via telephone, within the clinical unit, a on outpatient review after malaria challenge inoculation and antima study drugs administration. Blood samples for safety evaluation, malar monitoring, and red blood cell antibodies will be drawn at screening a baseline and at nominated times after malaria challenge.

Objectives:	<u>Primary</u>: The primary study objectives are: a. To characterise the PK/PD relationship between OZ439 and PQP plasma concentrations and blood stage asexual parasitaemia in healthy subjects following *P. falciparum* IBSM infection. b. To evaluate the safety and tolerability of OZ439 and PQP when administered as single doses in healthy subjects following *P. falciparum* IBSM infection. <u>Secondary</u>: The secondary study objectives are: a. To describe the PK of OZ439 and PQP when co-administered as single doses in healthy volunteers under fasted conditions. b. To characterize the PD effect of co-administered single doses of OZ439 and PQP on clearance of *P. falciparum* asexual blood stage parasites from the blood of healthy subjects in the IBSM model. <u>Exploratory</u>: The exploratory study objectives are: a. To characterise specific cell subsets and immune signatures associated with control of parasite burden and pathogenesis following first exposure to *P. falciparum*, to identify specific cells, immunomodulatory molecules and immune pathways to target therapeutic intervention. b. To investigate the role of RBC complement regulatory proteins and anti-phosphatidylserine antibodies in malarial anaemia c. To investigate the association between serum complement activation, complement activating antibodies, and RBC complement regulatory protein expression

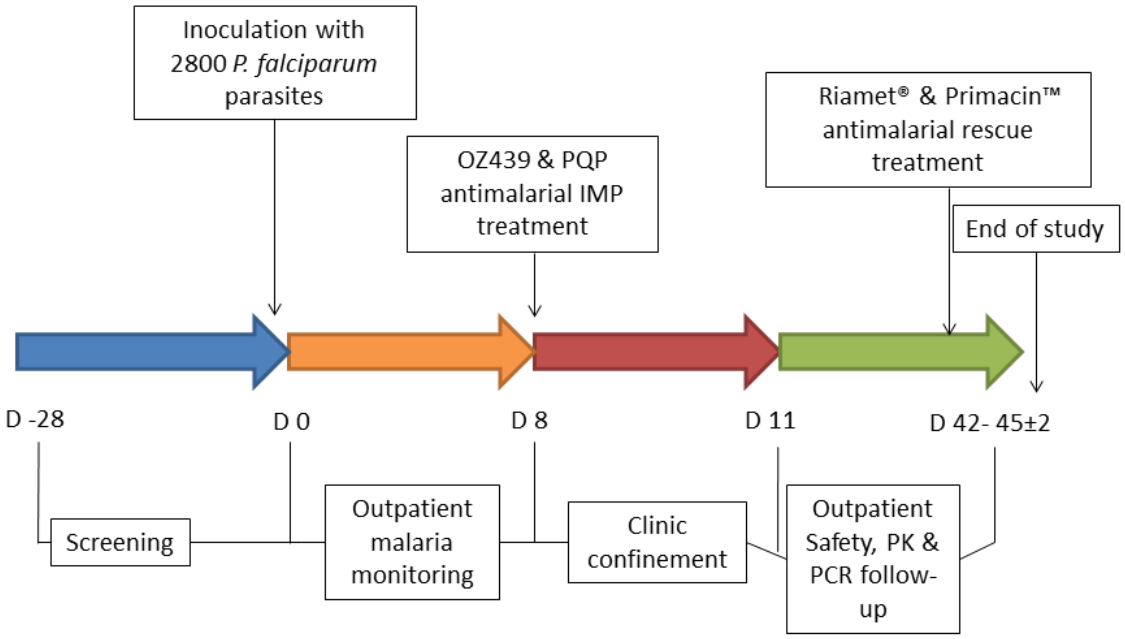
Endpoints:	Primary: Primary study endpoints: a. The PK/PD relationship between OZ439 and PQP pla concentrations and blood stage asexual parasitaemia wi determined by: • Effect of OZ439 on Emax and EC50 of PQP • Effect of PQP on Emax and EC50 of OZ439 b. The incidence, severity and relationship to OZ439 and PQ observed and self-reported AEs up to trial Day 42±2 after the co-administration of single doses of OZ439 and PQP in healthy subjects inoculated with IBSM Secondary: Secondary study endpoints: a. Estimation of OZ439 and PQP PK parameters up to trial Day 42±2 of single doses using non-compartmental methods: AUC0-168h, AUClast, AUC0-inf, Cmax, tmax, t1/2, tlag, C168h, CL/F, Vz/F and λinf. b. The effect of co-administered single oral doses of OZ439 and P on clearance of *P. falciparum* blood stage parasites from the blo of inoculated subjects as measured by qPCR up to trial Day 42±2. Parasite clearance will be assessed by the following paramet • Parasite clearance half-life (PCt½), • Parasite reduction ratio (PRR), • Percentage of subjects with recrudescence of parasita defined as ≥5 000 blood stage parasites/mL and a 2-fold increase within 48 hours, or re-occurrence of malari symptoms with a malaria clinical score >6)

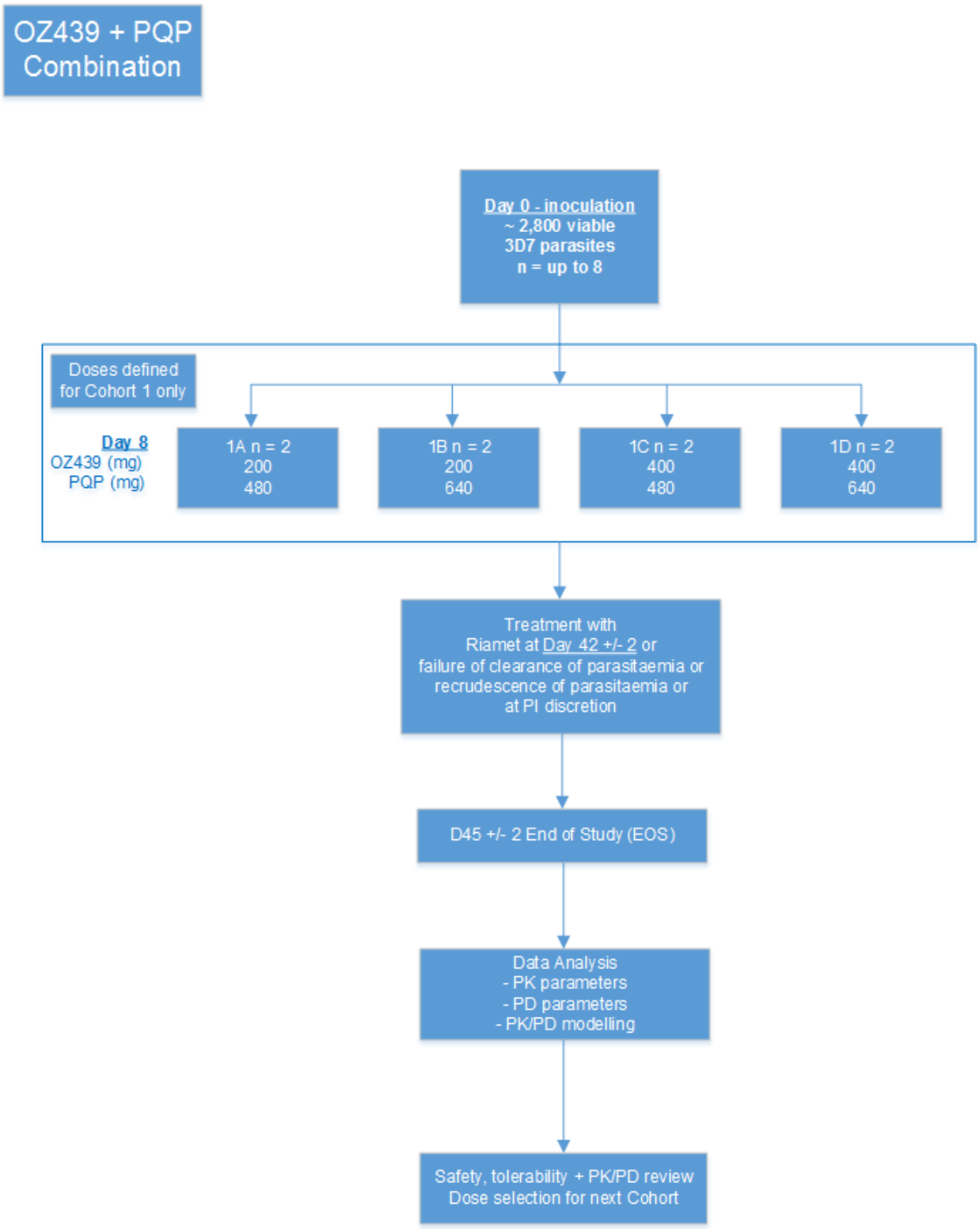
	Exploratory: a. Identification of immune checkpoint molecules b. The level of expression of complement regulatory proteins (CD35, CD55 and CD59) and phosphatidylserine on RBC. c. The level of complement activating antibodies and anti-phosphatidylserine antibodies
Population:	A total of up to 24 subjects will be enrolled in this study (up to 3 cohorts of 8 subjects each). Subjects will be malaria naïve healthy male and females, aged between 18-55 years old, who meet all of the inclusion criteria and none of the exclusion criteria. In each cohort, if more than 2 discontinuations due to non-safety related reasons occur; additional subjects may be recruited to replace discontinued subjects on agreement with the study sponsor. Subjects eligible for inclusion of the study will be further invited to participate in optional exploratory components that will require additional blood samples to be taken at scheduled time-points during the study. Details regarding these optional study components will be provided in a separate Participant Information Sheet and subjects agreeing to participate in these components will provide specific written consent for this. Refusal to participate in the optional study components will not jeopardise subjects' participation in the main study.
Phase:	Phase 1b
Number of sites enrolling subjects:	The study is planned to be performed in one investigational site: Q-Pharm Pty Ltd Level 5, 300C Herston Rd and Level 6, Block 8, Royal Brisbane and Women's Hospital Herston QLD 4006, Australia Additional sites in Australia may be added if necessary.
Description of study agents:	Malaria challenge agent: P. falciparum 3D7 blood stage challenge agent

	The P. falciparum 3D7 master cell bank (MCB) was produced from blo collected from a donor with clinical manifestation of malaria. Each 3D7 inoculum dose will be prepared aseptically from an aliquot of the P. falciparum 3D7 MCB. Each subject administered 3D7 will b inoculated intravenously with a dose of approximately 2,800 viable P. falciparum 3D7-infected RBCs in 2 mL of saline for injection. Investigational medicinal products: OZ439 OZ439 + α-tocopherol polyethylene glycol 1000 succinate (TPG granules for oral suspension, in 200 and 400 mg dosages and provid with sucrose PQP Piperaquine phosphate (PQP), 160 mg tablets. Antimalarial rescue medications: Artemether/lumefantrine Riamet® (20 mg artemether and 120 mg lumefantrine) will administered to all subjects beginning on approximately Day 2 (34 days after administration of OZ439 and PQP) or earlier in the event o failure of clearance or recrudescence of parasitaemia at Investigators discretion based on subject safety. A course of treatment comprises 6 do of 4 tablets administered orally over a period of 60 hours (total cour 24 tablets). Each dose of tablets should be taken with food or drink in fat (e.g., milk). Primaquine (if required) Subjects may receive a single oral dose of Primacin™ equivalent to 45 mg primaquine (6 tablets, each containing primaquine phosphate 13. equivalent to 7.5 mg of primaquine) to ensure complete clearanc gametocytes. Artesunate (if required) Treatment of subjects with intravenous artesunate will only occur in th event that subjects are unable to complete oral treatment with Riamet® (e.g. the subject is vomiting). Treatment with artesunate would be done at th recommended dose regimen of 2.4 mg/kg at approximately 0, 12, 24, hours and then daily for up to 7 days or until able to take oral drugs.

Duration of Study	It is estimated that the clinical portion of the study will be completed approximately 10 months
Duration subject participation:	Approximately 25 months (774 days) for each subject, including a screening period of up to 28 days, a period of observation follo inoculation of approximately 8 days, and a follow-up period after administration of OZ439 and PQP 37 days.

SCHEMATIC OF STUDY DESIGN




OZ439 + PQP Combination
Day 0 - inoculation
~ 2,800 viable
3D7 parasites
n = up to 8
Doses defined for Cohort 1 only
Day 8
OZ439 (mg)
PQP (mg)
1A n = 2
200
480
1B n = 2
200
640
1C n = 2
400
480
1D n = 2
400
640
Treatment with Riamet at Day 42 +/- 2 or failure of clearance of parasitaemia or recrudescence of parasitaemia or at PI discretion
D45 +/- 2 End of Study (EOS)
Data Analysis
- PK parameters
- PD parameters
- PK/PD modelling
Safety, tolerability + PK/PD review
Dose selection for next Cohort

1 KEY ROLES

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2 INTRODUCTION: BACKGR OUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 BACKGROUND INFORMATION

Malaria is the second most prevalent infectious disease in the world and threatens half of the world's population. In accordance with the latest estimates by the World Health Organization (WHO), there were an estimated 216 million new cases of malaria worldwide and 445,000 deaths in 2016 [1]. The majority of these deaths were due to infection with P. falciparum. The WHO has declared malaria control a global development priority and has changed their recommendation from control programs to eradication programs.

Malaria drug resistance is a major hurdle to achieving malaria eradication. Resistance has been developed not only to conventional monotherapies such as chloroquine, amodiaquine and sulfadoxine/pyrimethamine but also to the gold standard treatment for uncomplicated P. falciparum malaria, artemisinin combination therapy (ACT). Resistance to ACT's has been reported across the Greater Mekong Sub region in South-East Asia [2, 3] and the inevitable spread of artemisinin resistance to Africa is of great concern. Artemisinin resistance has been linked to mutations in the propeller domain of the P. falciparum Kelch13 gene [4]. Artemisinin-resistant isolates are not completely resistant to artemisinin, but instead have a slow parasite clearance phenotype compared to artemisinin-sensitive isolates. This can lead to reduced clearance of young ring-stage parasites, and therefore further maturation and sequestration of parasites leading to increased morbidity and mortality. As such, a continual generation of new compounds and drug combinations are needed to avoid a dangerous shortage of potential future treatment options.

To achieve eradication of malaria, long-lasting, single-dose treatments that completely clear malaria parasites from the body and provide a period of protection following the treatment are required. These ideal types of medicines are referred to as single-encounter radical cures (SERC). The WHO and Medicines for Malaria Venture (MMV) have identified the key properties that a SERC must have. These properties are defined by Burrows et al. (2017) in the antimalarial target product profile (TPP) [5]. A key requirement of a target product is the inclusion of two or more active molecules with different mechanisms of action. The aim of including two or more active compounds is to achieve an increased barrier to resistance by using drugs with different mechanisms of action, forcing the parasite to develop multiple simultaneous mutations in order to become resistant. Furthermore, in a combination therapy, one of the two active compounds should have a rapid onset of action, killing most of the parasite load. Both active compounds should maintain plasma concentrations levels above the minimal parasiticidal concentration (MPC) approximately the same time and ensure complete elimination of all parasites [5].

Artefenomel(OZ439),is a novel trioxolane,and a front-runner candidate for inclusion in a new antimalarial combination. It is a synthetic ozonide with potency comparable to artemisinin, with a rapid parasite clearance rate of approximately 3.6P. falciparum. Additionally, OZ439has a substantiallylonger half-life than artemisinin (46 - 62 h as opposed to 1-3 h for artemisinin[6]). For these reasons, clinical studies in which artefenomel is combined with a companion drug with a differentmechanismof action are being planned,or are currently in progress.

Piperaquine is a bis 4-aminoquinoline and was used mainly in China from the 1960's to the 1980's as an antimalarial monotherapy.In the 1980's, it became clear that parasite resistancehad developedto PQPmonotherapy.PQP is characterisedby slow absorption and a long elimination half-life (4–5 weeks) and is now widely used in combination with dihydroartemisinin as a fixed dosed ACT. The successful paring of PQP with an artemisinin, together with its long biological half-life, means PQP is potentially a good drug partner for OZ439.

The safety, tolerability and PK profiles for OZ439 and PQP are well established in healthy subjects and in patients.The antimalarial activity of the drugs individuallyhas been demonstrated in IBSM studies in healthy subjects and in patients with uncomplicated malaria.A Phase IIb study of a single-dose regimen of OZ439 800 mg in combination with 3 doses of PQP (640 mg, 960 mg, and 1440 mg) in adults and children with uncomplicated P. falciparummalariahas been performed in Africa and Asia.In thisstudy, none of the dose arms of the study met the protocol-defined efficacy threshold of ≥95% based on the primary endpoint of polymerase chain reaction (PCR) adjusted adequate clinical and parasitological response (ACPR) outcome on Day 28 in the per protocol analysisset.Therefore,an IBSM study assessing the antimalarial effect of OZ439 and PQP in a single dose combination could allow further understanding of the observed outcome of the OZ439 and PQP Phase IIb trial where treatment was also administered as a single dose combination.

Cumulatively, an estimated 1250 subjects have received OZ439 either alone or in combination with piperaquine phosphate(PQP), ferroquine (FQ), mefloquine, DSM265, or Cobicistat in clinical trials globally since the Development International Birth Date(until 29 March, 2018).

2.2 RATIONALE

This study aims toevaluatethe antimalarial activity of co-administeredsingle dosesof OZ439 and PQP in the IBSM model, and enable characterisation of the exposure-response relationship between OZ439 and PQP PK and blood stage asexual parasitaemia.Previous research has shown that the IBSM model is a good predictive model for real world antimalarial drug activity with single drug administration[7]. However, it is yet to be determined if the model is an effective predictive tool when two or more drugs are administered in combination.

We hypothesise that data obtained in a controlled disease-like setting (Phase 1 drug combination IBSM model) can be used to effectively predict the outcome of Phase 2 studies in patients with uncomplicated malaria. To evaluate this hypothesis, we will assess if the data of this trial can be used to predict what was observed in patients with uncomplicated malaria in the MMV_OZ439_13_003 Phase 2b combination trial [8].

The doses chosen for use in Cohort 1 are based on the PK/PD relationships from the IBSM monotherapy studies and potential PD interaction effects of the combination based on the phase IIb study. Several doses will be tested in Cohort 1, which should achieve parasite reduction but not complete cure, such that parasite regrowth can be observed, allowing an initial estimation of the PK/PD parameters of the 2 drugs when combined. The results of the first cohort will then be used to guide dose selection for Cohort 2 and 3, minimising the information on the PD interaction effect.

2.3 POTENTIAL RISKS AND BENEFITS

2.3.1 KNOWN POTENTIAL RISKS

Potential risks have been identified through review of previous clinical studies conducted to date using the IBSM model with P. falciparum isolates, as well as a review of the literature. P. falciparum 3D7 has been used to challenge 312 healthy subjects in 25 IBSM Phase 1 clinical trials, 18 of which were successfully undertaken at QIMR Berghofer PQ arm [9-34].

IBSM model risks

Risk management of blood borne infections

In this study, P. falciparum 3D7 inoculum will be used. This contains a very small amount of donor blood. However, the risk of infection from a possible blood borne virus from the blood transfused in this study is extremely low for a number of reasons. Firstly, the donors were screened and tested negative for the presence of active blood borne infections. Secondly, white blood cells were removed from the donor blood by the Australian Red Cross Blood Service (Blood Service), which lowers the risk of transfusion transmitted infections. Thirdly, the volume of blood used in the IBSM model for transmitting malaria is many thousands of times smaller than in a transfused unit, thus reducing the risk of infection. As part of the safety monitoring, all subjects will have serum stored for testing of blood borne viral infections before and after the study (Section 7.2.1). To date, no blood borne infections have been reported in any of the 312 subjects who have received the P. falciparum 3D7 inoculum in IBSM studies.

Risk management of reaction to the blood sample

The risk of developing red blood cell (RBC) alloantibodies in this study is considered extremely low since the donor blood used to produce the inoculant was blood group O RhD (D) Negative.

People with this blood group are generally considered "universal donors", as recipients of their blood have minimal risk of developing RBC alloantibodies when given much larger volumes of blood than is used in the IBSM model. However, it is possible that subjects could suffer a transfusion reaction after they receive the inoculum and develop alloantibodies to the donor RBCs that may make blood transfusion more difficult in the future. To date, one subject has developed an antibody response to a minor Rh antigen (E antibody) following inoculation with P. falciparum 3D7 [35]. However, there was no laboratory evidence to indicate that the specific R-E phenotype of the donor RBCs in the inoculum provoked production of this alloantibody. Subjects will be monitored for signs and symptoms in the period immediately after administration of the inoculum to further assess the risk of the inoculum causing a transfusion reaction. Subjects will also be tested for RBC alloantibodies at screening and at the end of the study as part of their safety monitoring (Section 7.2.1).

Women of childbearing potential (WOCBP) have a small additional risk of developing RBC alloantibodies that could cause problems during pregnancy. WOCBP have participated in several IBSM trials with P. falciparum isolate 3D7 with no known issues to date. Specific strict contraception requirements will be requested for this population during the study (Section 5.1). Including WOCBP in the trial enhances the generalisability of the study results.

Risk management of malaria infection

The number of viable blood stage parasites used to infect the subjects in this study (~2,800) is substantially lower than the parasitaemia reached after the bite of a single malaria-infected mosquito, where approximately 30,000 parasites are released into the blood when they break out of a single infected liver cell [36]. In this study, parasite growth and malaria symptoms will be closely monitored in subjects following administration of the challenge agent. The threshold for commencement of antimalarial drug treatment defined for this study (Day 8) has been selected since it is prior to the time point at which advanced clinical symptoms of malaria are likely to occur, as observed in the previous 25 clinical trials performed with the 3D7 challenge agent.

Risk management of liver function abnormalities

Transient, asymptomatic liver function test (LFT) abnormalities including rare cases of alanine aminotransferase (aspartate aminotransferase) elevations >10-fold the upper limit of normal (×ULN) have been reported in several subjects in IBSM studies [35, 37]. However, no changes in bilirubin were reported except for in one subject with unappreciated existing liver disease [35, 37]. The LFT derangements did not require treatment, and resolved by the end of the studies. A few cases of the LFT elevations were considered as serious AEs (SAEs) by two Pharma sponsors due to internal processes for SAE notifications. Following an independent review involving drug-induced liver injury experts, it was found that these LFT elevations are most likely a direct consequence of the malaria infection, rather than reflecting a direct drug-induced liver injury caused by an investigational antimalarial drug. As a precaution all subjects in this study will undergo regular

safety monitoring to assess for asymptomatic LFT abnormalities. Subjects are required to refrain from intake of possibly hepatotoxic substances such as alcohol and paracetamol during the course of the study (see Sections 5.2 & 7.6). Drugs of abuse are not permitted under any circumstance.

Risk management of cardiac AEs

To our knowledge, 3 cardiac SAEs have been reported in healthy subjects in the Netherlands participating in malaria challenge studies using sporozoites i.e. direct feeds by infected mosquitoes rather than IBSM infection. Refer to the *P. falciparum* 3D7 Investigator's Brochures for further details[35]. No cardiac SAEs caused by the inoculum have been reported in IBSM studies. 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 electrocardiogram (ECG) recordings will take place for all subjects.

<u>Investigational Medical Product (IMP) risks</u>

OZ439 risks

To date, OZ439 administered either as a monotherapy or in combination with a partner anti malarial, has been generally well tolerated in malaria patients and healthy subjects. In a monotherapy Phase IIa study of OZ439 doses of 200, 400, 800 and 1200 mg in malaria patients, increased blood creatine phosphokinase was the most frequently reported AE (4 AE); a dose relationship was not seen for this AE and no clinically relevant muscular AE were reported. Gastrointestinal (GI) AEs, including vomiting, abdominal pain, diarrhoea, and nervous system disorders, including dizziness and headache, were reported more frequently with 1200 mg OZ439 compared to the other dose cohorts. None of these AEs were SAEs.

One case of vasovagal syncope considered related to therapy was reported in association with sinus arrest and orthostatic hypotension. Two SAEs of atrial fibrillation were reported in healthy volunteers. One case occurred at day 35 post dose and was considered not related to the study drug. The second case was moderate atrial fibrillation, considered possibly related to the study drug in a subject who had received OZ439 and FQ. The event occurred in context of symptomatic orthostatic hypotension and resolved spontaneously without medical treatment.

In a Phase IIb study, where OZ439 was evaluated in combination with PQP, in patients with uncomplicated *P. falciparum* malaria, malaria and electrocardiogram (ECG) QTc prolongation were the most frequently reported AEs. Malaria was reported with a higher incidence in the OZ439 800 mg:PQP 960 mg and in the OZ439 800 mg:PQP 640 mg treatment arms than in the OZ439 800 mg:PQP 1440 mg treatment arm. The ECG QTc prolongation was reported with a higher incidence in the OZ439 800 mg:PQP 1440 mg and the OZ439 800 mg:PQP 960 mg treatment arms than in the OZ439 800 mg:PQP 640 mg treatment arm. Concentration-related QTc prolongation

has been widely documented with PQP. Diarrhea and vomiting were the most frequently reported AEs of the GI system in patients treated with both OZ439 and PQP. None of these AEs were SAEs.

The AE profile for OZ439 in healthy subjects was similar to the AE profile in malaria patients. Gastrointestinal AEs, including nausea, vomiting, and diarrhea, were the most frequently reported AEs in healthy subjects treated with OZ439 alone or OZ439 in combination with either PQP, MQ, or FQ. A tendency for a dose-response relationship was seen for GI events. These AEs were generally mild in intensity.

Eight subjects treated with OZ439, including 6 malaria patients and 2 healthy volunteers, have had SAEs, including *P. vivax* relapse, pyelonephritis, increased alanine aminotransferase (ALT), increased aspartate aminotransferase (AST), decreased neutrophil count, anemia, febrile convulsion and decreased hemoglobin in malaria patients; and atrial fibrillation and a gunshot wound in healthy subjects. Five SAEs reported in malaria patients (increased ALT, increased AST, decreased neutrophil count, anemia and decreased hemoglobin) were assessed as related to study drug by the Investigator.

An AE of vomiting led to study drug discontinuation in 1 malaria patient treated with OZ439. Ten healthy subjects treated with OZ439 discontinued a Phase Ia study due to an AE. Vomiting (5 subjects) was the most frequently reported AE leading to study discontinuation in the 10 healthy subjects. Except for a few cases observed with α-tocopherol polyethylene glycol 1000 succinate (TPGS) formulation, vomiting is not reported in healthy subjects at doses < 800 mg OZ439.

No Hy's law cases have been observed. However, mild to severe and reversible increases in ALT and AST were seen in OZ439-treated malaria patients; these increases did not appear to be dose-dependent. Decreases in hemoglobin, neutrophils, and platelets were also seen in OZ439-treated malaria patients; however, these decreases were consistent with those observed in acute malaria. Close monitoring of liver function is required in the clinical studies.

In the clinic, a significant effect on placebo-corrected change from baseline QTcF was not demonstrated for OZ439. In healthy subjects the effect of OZ439 800 mg alone on QTc was minimal, with a maximum mean QTcF increase from baseline and placebo of 8.5 ms (Study MMV_OZ439_12_002). In Study MMV_OZ439_12_003, 1 healthy subject with an undisclosed history of mitral and tricuspid regurgitation was discontinued from the study due to asymptomatic supraventricular/junctional tachycardia after dosing with 120 mg OZ439. QTc prolongations (both QTcB and QTcF) were seen when OZ439 was administered in combination with PQP to malaria patients and healthy subjects. Most of the prolongations were in the range of >30 ms but <60 ms; however, prolongations >60 ms were observed in malaria patients, with QTcF values that exceeded 500 ms in 2 patients (1 hypokalemic patient). Reversible right bundle branch block (3 patients), reversible first degree atrioventricular block (1 patient), and a mild reversible

sinus bradycardia (1 patient) were also observed in malaria patients treated with OZ439. Close monitoring of cardiac function is required in the clinical studies.

In healthy subjects treated with OZ439 alone or OZ439 in combination with PQP, gastrointestinal (GI) symptoms which include nausea, vomiting, and diarrhea, are the most commonly reported AEs and these AEs have been reported to be generally mild in intensity. OZ439 was clinically well tolerated in two previous IBSM studies (QP12C10 and QP14C12) where the highest dose tested was 500 mg. In a Phase IB IBSM study QP12C10 in healthy subjects infected with *P. falciparum*, most of the AEs reported were assessed as probably related to the inoculum (i. e., malaria). There were no events considered probably related to OZ439. In another Phase IB IBSM study QP14C12, healthy subjects infected with *P. falciparum* received OZ439 in combination with another investigational antimalarial, DSM265. No new clinically relevant safety signals were observed. OZ439 may be contraindicated in persons with known hypersensitivity to artemisinin-based compounds or to any components of the product formulation. Carcinogenicity studies have not been performed with OZ439. OZ439 has shown in vivo embryo-fetal toxicity in the rat. Therefore, OZ439 should not be administered to pregnant or breastfeeding women. However, OZ439 can be administered to women of child bearing potential (WOCBP) with use of a strict double method of contraception in well controlled clinical trials and under medical supervision. Healthy male and female subjects participating in studies of OZ439 must agree to use a double method of contraception for a duration defined in section 10.1 (Inclusion criteria) of this protocol.

No deaths have been reported in any of the clinical studies.

Additional information on non-clinical and clinical studies conducted with OZ439 is provided in the IB [38].

Piperaquine phosphate (PQP) risks

PQP as a combination antimalarial with dihydroartemisinin is well tolerated in both adults and children, with the main AEs reported being GI disturbances such as diarrhoea [39, 40]. Studies with dihydroartemisinin-PQP demonstrated corrected QT (QTc) interval prolongation during treatment [41, 42]. Very few individual patients have been observed to have a prolongation that could be regarded as clinically significant (>60 msec); of note, the QTc prolongation induced by PQP has not been reported to be associated with clinically relevant cardiovascular events, which would suggest a lack of pro-arrhythmic effect. Therefore, although statistically significant, the QTc prolongation observed following PQP therapy is unlikely to be of clinical concern. European regulatory authorities have advised that Eurartesim® (dihydroartemisinin-PQP) not be administered with food to reduce PQP peak concentrations, and caution that pre- and post-electrocardiographic monitoring be undertaken, and avoidance of concomitant recent exposure to drugs at risk of QTc prolongation [18, 42, 43]. Therefore, during this study, subjects will receive PQP under fasting conditions and will have ECGs recorded before and after treatment.

PQP has been clinically well tolerated in previous IBSM studies using this drug [34]. In these studies, PQP treatment demonstrated a robust safety profile in doses up to 960 mg when used for the treatment of uncomplicated P. falciparum malaria infection.

Details on the safety and efficacy of PQP as part of the combination product Eurartesim® can be found in the European Medicines Agency (EMA) European Public Assessment Report.

Rescue Medication risks

Riamet®, Primacin™ and Artesunate risks are detailed in their respective approved manufacturer's prescribing information (Appendix 1). Primacin™ may cause severe haemolytic anaemia in subjects with glucose-6-phosphate dehydrogenase (G6PD) deficiency. Subjects will be tested for G6PD deficiency at screening to ensure the safety of Primacin™. The G6PD status will determine how the subject is treated with Primacin™.

General risk management

The risk to subjects in this study will also be minimised as follows:

- Adherence to the inclusion/exclusion criteria to ensure that only subjects who do not have any perceived risk are enrolled in the study
- Close clinical and laboratory monitoring to ensure the safety and well-being of the subjects.
- Subjects will be prescribed curative therapy for malaria (Riamet® with the addition of Primacin™ if required) for final parasite clearance during or at the end of the study.
- The total volume of blood drawn from each subject enrolled into the study (including optional exploratory research sampling) will not exceed a standard unit of blood (approximately 450 mL) over any 30-day period.
- In the rare event that a subject requires hospitalisation at the request of the Principal Investigator or his representative, this will be done at the Infectious Diseases Unit, Royal Brisbane and Women's Hospital. Emergency procedures are in place at the Q-Pharm clinics for dealing with any unforeseen clinical emergencies which may arise.

With these safety provisions, the overall risk to the subjects in the study is considered to be minimal and acceptable, and the potential of future improved treatment for malaria is considered to outweigh these potential risks.

2.3.2 KNOWN POTENTIAL BENEFITS

There is no expected clinical benefit for the healthy subjects that will participate in this study. Overall, on the basis of the available non-clinical and clinical data, and prior knowledge, the risk-benefit profile of OZ439 and PQP are judged acceptable for the proposed clinical study.

3 OBJECTIVES AND PURPOSE

Primary:

a) To characterize the PK/PD relationship between OZ439 and PQP plasma concentrations and blood stage asexual parasitaemia in healthy subjects following *P. falciparum* IBSM infection.

b) To evaluate the safety and tolerability of OZ439 and PQP when co-administered as single doses in healthy subjects following *P. falciparum* IBSM infection.

Secondary:

a) To describe the PK of OZ439 and PQP when co-administered as single doses in healthy subjects under fasted conditions.

b) To characterize the PD effect of co-administered single doses of OZ439 and PQP on clearance of *P. falciparum* asexual blood stage parasites from the blood of healthy subjects in the IBSM model.

Exploratory:

The exploratory study objectives are:

a. To characterise specific cell subsets and immune signatures associated with control of parasite burden and pathogenicity following first exposure to P. falciparum, to identify specific cells, immunomodulatory molecules and immune pathways to target for therapeutic intervention.

b. To investigate the role of RBC complement regulatory proteins and anti-phosphatidylserine antibodies in malarial anaemia.

c. To investigate the association between serum complement activation, complement activating antibodies, and RBC complement regulatory protein expression.

4 STUDY DESIGN AND ENDPOINTS

4.1 DESCRIPTION OF THE STUDY DESIGN

This is a single-centre, open-label, adaptive study using the P. falciparum IBSM inoculum as a model to characterise the PD activity of combined administration of OZ439 and PQP.

The study will be conducted in a maximum of three cohorts (up to 8 subjects per cohort) using up to 4 different doses of OZ439 and PQP in each cohort. Subjects will be malaria-naïve healthy males or females, aged between 18-55 years old, who meet all of the inclusion criteria and none of the exclusion criteria. Additionally, the study includes optional, exploratory components, which require separate informed consent for subjects agreeing to participate in these.

The first cohort will comprise 4 groups of 2 subjects each. Subjects will be administered single oral doses of OZ439 and PQP in combination. The dose of OZ439 and PQP will be different for each group in Cohort 1, and subjects will be randomised into one of the dose groups listed in Table 1.

Table 1 OZ439 and PQP Cohort 1 Dose

Drug	Dose group			
	1A	1B	1C	1D
OZ439 (mg)	200	200	400	400
PQP (mg)	480	640	480	640

The data captured from this first cohort will be used to determine the relationship between OZ439 and PQP concentrations and parasitaemia levels. Based on safety and tolerability data up to Day 42±2 and PK/PD analysis outcomes (based on PD data up to Day 42±2 and PK data up to Day 35±2), of the drugs given in combination the dose(s) for the subsequent cohort will be determined.

After review of the PK/PD and safety data from Cohort 1 by SDRT it was determined that, the second cohort will be composed of 2 dose groups of 4 subjects each. Subjects will be randomised into one of 2 dose groups and administered single oral doses of OZ439 and PQP in combination. The combined dose of OZ439 and PQP will be different for each group in this cohort as shown in Table 2.

Table 2 OZ439 and PQP Cohort2 Dose

Drug	Dose group	
	2A	2B
OZ439(mg)	800	200
PQP (mg)	960	320

A similar analysis will be done at the end of cohort 2 combining cohort1 and 2 data to decide the dose(s) to be tested in cohort 3. This will be decided by the funding sponsor and the Principal Investigator following SDRT and scientific evaluation

The doses used in all Cohorts will not exceed 800 mg for OZ439 and 1440 mg for PQP as determined in previous safety, pilot efficacy and phase 2 studies. Each subject will be inoculated on Day 0 with approximately 2,800 viable parasites of *P. falciparum*-infected human erythrocytes administered intravenously. Subjects will be monitored daily via phone call or text message on Days 1 to 3 post inoculation to solicit any AEs.

Then, subjects will come to the clinical unit on a daily basis from Day 4 until the presence of asexual parasites is established by qPCR targeting the 18S rRNA gene (referred to as malaria 18S qPCR). Once qPCR becomes positive and until OZ439 and PQP administration, subjects will attend the clinical unit for twice-daily visits, separated by approximately 12 hours, for clinical evaluation and blood sampling.

Subjects will be admitted to the clinical unit for single dose administration of OZ439 and PQP 8 days after malaria inoculation or earlier if a subject has a malaria clinical score >6 or at Investigator's discretion. An intravenous cannula will be placed and preliminary blood samples collected. The participants will then be administered the IMP in a fasting state immediately. Subjects in the first cohort may all be dosed on the same day, once these exposures have been documented in previous studies and shown to be well tolerated.

Once single dose administration of OZ439 and PQP occurs, subjects will be followed up as inpatients for at least 72 hours to monitor for safety and tolerability of the treatment and to ensure adequate clinical and parasitological response. Blood samples will be collected pre-dose and following OZ439 and PQP treatment to measure plasma concentrations of OZ439 and PQP. Wherever possible, PK sampling will coincide with pre-dose blood collection for monitoring of parasitaemia.

After 72 hours, if clinically well, subjects will be discharged from the clinical unit and will be followed up regularly for safety assessments, PK sampling, clinical evaluation and malaria qPCR blood sampling until Day 42±2. All subjects will receive a standard course of therapy with

Riamet® (artemether-lumefantrine) on Day 42±2 or earlier in the event of failure of clearance of parasitaemia defined as failure to clear parasitaemia by at least 10-fold at 72 hours post-IMP administration. Riamet will also be administered in the event of recrudescence of parasitaemia (defined as ≥5 000 blood stage parasites/mL and a 2-fold increase within 48 hours, or a malaria clinical score >6) or at Investigator's discretion. If indicated by the presence of gametocytes at the time of treatment with Riamet®, Primacin™ 45 mg will also be administered as a single oral dose.

Subjects participating may optionally consent to be included in the exploratory components of the study which require additional blood samples to be collected at specified points throughout the study. These time-points coincide with those already scheduled as part of the main study assessments. If any of the main study time-points change the exploratory study time-points will change to match. Details of the exploratory components and analyses are provided in Section 7.2.2.

A review by the SDRT of data from each cohort will be conducted prior to dosing the subsequent cohort. Safety and tolerability data up to Day 42±2 and PK/PD analysis outcomes (based on PD data up to Day 42±2 and PK data up to Day 35±2), from all subjects who received treatment with OZ439 and PQP will be required for the review. A similar analysis will be done at the end of cohort 2 combining cohorts 1 and 2 data to decide the dose(s) to be tested in cohort 3. This will be decided by the funding sponsor and Principal Investigator following review of the data by the SDRT and scientific evaluation.

The doses used in all cohorts will not exceed the maximum acceptable doses predefined for this study (800 mg for OZ439 and 1440 mg for PQP) as determined in previous safety, efficacy and phase 2 studies.

4.2 STUDY ENDPOINTS

4.2.1 PRIMARY ENDPOINT

- The PK/PD relationship between OZ439 and PQP plasma concentrations and blood stage asexual parasitaemia will be determined by:
  - Effect of OZ439 on E_{max} and EC_{50} of PQP
  - Effect of PQP on E_{max} and EC_{50} of OZ439

- The safety and tolerability of a single combined dose of OZ439 and PQP will be evaluated by the incidence, severity and relationship of observed and elicited AEs up to trial Day 42±2 after the co-administration of single doses of OZ439 and PQP to subjects inoculated with IBSM.

4.2.2 SECONDARY ENDPOINTS

- Estimation of OZ439 and PQP PK parameters up to trial Day 42 ±2 after co-administration of single doses using non-compartmental methods: AUC_{0-168h}, AUC_{last}, AUC_{0-inf}, C_{max}, t_{max}, $t_{1/2}$, t_{lag}, C_{168h}, CL/F, Vz/F and λ_{inf}.

- The effect of co-administered single oral doses of OZ439 and PQP on clearance of *P. falciparum* blood stage parasites from the blood of inoculated subjects as measured by qPCR up to trial Day 42 ±2 after co-administration of OZ439 and PQP. Parasite clearance will be assessed by the following parameters:
    - Parasite clearance half-life ($Pt_{½}$).
    - Parasite reduction ratio (PRR).
    - Percentage of subjects with recrudescence of parasitaemia defined as ≥5 000 blood stage parasites/mL and a 2-fold increase within 48 hours, or re-occurrence of malaria symptoms with a malaria clinical score >6)

4.2.3 EXPLORATORY ENDPOINTS

- Identification of immune checkpoint molecules.
- The level of expression of complement regulatory proteins (CD35, CD55 and CD59) and phosphatidylserine on RBC.
- The level of complement activating antibodies and anti-phosphatidylserine antibodies.

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 SUBJECT INCLUSION CRITERIA

Subjects eligible for inclusion in this study must fulfil all of the following criteria:

Demography

1. Adult (male and female) subjects between 18 and 55 years of age inclusive, who do not live alone (from inoculation day until at least the end of Riamet® treatment) and will be contactable and available for the duration of the trial and contactable up to 2 weeks following the End of Study visit (approximately 9.5 weeks).

2. Body weight minimum 50 kg, body mass index between 18 and 32 kg/m^2, inclusive.

Health status

3. Certified as healthy by a comprehensive clinical assessment (detailed medical history, complete physical examination and special investigations).

4. Vital signs after 5 minutes resting in supine position:

   - 90 mmHg ≤ systolic blood pressure (SBP) ≤ 140 mmHg,
   - 40 mmHg ≤ diastolic blood pressure (DBP) ≤ 90 mmHg,
   - 40 bpm ≤ heart rate (HR) ≤ 100 bpm.

5. Must have QTcF ≤450 ms, QTcB ≤450 ms for male subjects, QTcF ≤470 ms, QTcB ≤470 ms for female subjects and PR interval ≤210 ms at screening and at pre-inoculation or inoculation day.

6. Heterosexual women of childbearing potential should be surgically sterile or using an insertable, injectable, transdermal or combination oral contraceptive approved by the TGA combined with a barrier contraceptive for the duration of the study, and have negative results on a urine pregnancy test done before inoculation. Abstinent, heterosexual female subjects must agree to start a double method if they start a sexual relationship during the study. Adequate contraception does not apply to subjects of childbearing potential with same sex partners (abstinence from penile-vaginal intercourse), when this is their preferred and usual lifestyle. Female subjects with same sex partners must not be planning in vitro fertilisation within the required contraception period.

   Women of non-childbearing potential who will not require contraception during the study are defined as: post-menopausal (spontaneous amenorrhoea for ≥ 12 months, or spontaneous

amenorrhoea for ≥12 months and follicle-stimulating hormone (FSH) ≥ 40 IU/mL; either should be together with the absence of oral contraceptive use for > 12 months).

Male subjects participating must agree to use a double barrier method of contraception including condom plus diaphragm or condom plus intrauterine device or condom plus stable oral/transdermal/injectable hormonal contraceptive by the female partner from the time of informed consent through to 90 days after the last dose of OZ439 and PQP. Abstinent male subjects must agree to start a double barrier method if they begin sexual relationships during the study and up to 90 days after the last dose of study drug.

Male subjects with female partners that are surgically sterile, or male subjects who have undergone sterilisation and have had testing to confirm the success of the operation may also be included.

Regulations

7. Having given written informed consent prior to undertaking any study-related procedure.
8. Must be willing and able to communicate and participate in the whole study.

5.2 SUBJECT EXCLUSION CRITERIA

Subjects fulfilling any of the following criteria will not be eligible for inclusion in this study:

Medical history and clinical status

1. Haematology, clinical chemistry, coagulation or urinalysis results at screening or on admission prior to inoculation or IMP administration that are outside of Sponsor-approved clinically acceptable laboratory ranges documented in the laboratory manual or are considered clinically relevant.
2. Any history of malaria or participation in a previous malaria challenge study.
3. Must not have 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 study (for endemic regions see https://map.ox.ac.uk/country-profiles/#!/). Bali is not considered a malaria-endemic region.
4. Participation in any investigational product study within the 12 weeks preceding IMP administration.
5. 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.

6. Symptomatic postural hypotension at screening or 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 minutes when changing from supine to standing position.

7. History of splenectomy.

8. History or presence of undiagnosed (by an allergist/immunologist) or treated (by a physician) food or known drug allergies (including but not limited to allergy to any of the antimalarial rescue medications to be used in the study), or history of anaphylaxis or other severe allergic reactions. Note. Subjects with seasonal allergies/hay fever, house dust mite or allergy to animals that are untreated and asymptomatic at the time of dosing can be enrolled in the study

9. History of convulsion (including intravenous drug or vaccine induced episodes) Note. A medical history of a single febrile convulsion during childhood is not an exclusion criterion.

10. Presence of current or suspected serious chronic diseases such as cardiac or autoimmune disease (HIV or other immunodeficiencies), insulin-dependent and non-insulin dependent diabetes (excluding glucose intolerance if exclusion criterion 4 is met), progressive neurological disease, severe malnutrition, acute or progressive hepatic disease, acute or progressive renal disease, porphyria, psoriasis, rheumatoid arthritis, asthma, epilepsy, or obsessive-compulsive disorder.

11. 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 5 years of screening, regardless of whether there is evidence of local recurrence or metastases.

12. Subjects with history of schizophrenia, bipolar disease, or other severe (disabling) chronic psychiatric diagnosis including depression or receiving psychiatric drugs or who have been hospitalised within the past 5 years prior to enrolment for psychiatric illness, history of suicide attempt, or confinement for danger to self or others.

13. History of serious psychiatric condition that may affect participation in the study or preclude compliance with the protocol, including but not limited to past or present psychoses, disorders requiring lithium, a history of attempted or planned suicide, more than one previous episode of major depression, any previous single episode of major depression lasting for or requiring treatment for more than 6 months, or any episode of major depression during the 5 years preceding screening.

The Beck Depression Inventory (Appendix 4) will be used as an objective tool for the assessment of depression at screening. In addition to the conditions listed above, subjects with a score of 20 or more on the Beck Depression Inventory 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 subjects will be referred to a general practitioner or medical specialist as appropriate. Subjects with a Beck score of 17 to 19 may be enrolled at the discretion of the 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 subject or to the execution of the study and interpretation of the data gathered.

14. History of recurrent headache (e.g. tension type, cluster or migraine) with a frequency of ≥2 episodes per month on average and/or severe enough to require medical therapy.

15. Presence of acute infectious disease or fever (e.g. sublingual temperature ≥38.5°C) within the 5 days prior to inoculation with malaria parasites.

16. Evidence of acute illness within the 4 weeks prior to screening that the Investigator deems may compromise subject safety.

17. Significant intercurrent disease of any type, in particular liver, renal, cardiac, pulmonary, neurologic, rheumatologic, or autoimmune disease by history, physical examination, and/or laboratory studies including urinalysis.

18. Subject has a clinically significant disease or any condition or disease that might affect drug absorption, distribution or excretion (e.g. gastrectomy, diarrhoea)

19. Blood donation of any volume within 1 month before inclusion, or participation in any research study involving blood sampling (more than 450 mL/unit of blood), or blood donation to Australian Red Cross Blood Service (Blood Service) or other blood bank during the 8 weeks prior to the treatment drug dose in the study.

20. Subject unwilling to defer blood donations to the Blood Service for at least 6 months.

21. Medical requirement for intravenous immunoglobulin or blood transfusions.

22. Subject who has ever received blood transfusion.

23. History or presence of alcohol abuse (alcohol consumption more than 40 g/4 standard drinks per day) or drug habituation, or any prior intravenous usage of an illicit substance.

24. Tobacco use of more than 5 cigarettes or equivalent per day, and unable to stop smoking for the duration of the clinical unit confinement.

25. Female subject who is breastfeeding.

Interfering substances

26. Any vaccination within the last 28 days.

27. Any corticosteroids, anti-inflammatory drugs, immunomodulators or anticoagulants. Any subject currently receiving or having previously received immunosuppressive therapy (including systemic steroids, adrenocorticotrophic hormone or inhaled steroids) at a dose or duration associated with hypothalamic-pituitary-adrenal axis suppression (e.g. 1 mg/kg/day prednisone, chronic use of inhaled high potency corticosteroids such as budesonide 800 µg/day or fluticasone 750 µg, or equivalent).

28. Any recent (<6 weeks) or current systemic therapy with an antibiotic or drug with potential antimalarial activity (e.g. chloroquine, piperaquine phosphate, benzodiazepine, flunarizine, fluoxetine, tetracycline, azithromycin, clindamycin, doxycycline etc.).

29. Ingestion of any poppy seeds within the 24 hours prior to the screening blood tests (subject will be advised by phone not to consume any poppy seeds in this time period).

30. 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).

31. Unwillingness to abstain from consumption of grapefruit or Seville oranges from inoculation day until end of the study

32. Unwillingness to abstain from consumption of quinine containing foods/beverages such as tonic water and lemon bitter, from inoculation day until end of Riamet® treatment.

33. Use of prescription drugs or non-prescription drugs or herbal supplements (such as St John's Wort), within 14 days or 5 half-lives (whichever is longer) prior to the malaria inoculation. As an exception, ibuprofen (preferred) may be used at doses of up to 1.2 g/day or paracetamol at doses of up to 4 g/day after discussion with the Investigator. Limited use of other non-prescription medications or dietary supplements, not believed to affect subject safety or the overall results of the study, may be permitted on a case-by-case basis following approval by the Sponsor in consultation with the Investigator. Subjects are requested to refrain from taking non-approved concomitant medications from recruitment until the conclusion of the study.

General conditions

34. Any subject who, in the judgment of the Investigator, is likely to be non-compliant during the study, or is unable to cooperate because of a language problem or poor mental development.

35. Any subject in the exclusion period of a previous study according to applicable regulations.

36. Any subject who is the Principal Investigator or any sub-Investigator, research assistant, pharmacist, study coordinator, or other staff thereof, directly involved in conducting the study.

37. Any subject without a good peripheral venous access.

Biological status

38. 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).

39. Positive urine drug test. Any drug listed in Section 7.2.1 in the urine drug screen unless there is an explanation acceptable to the Investigator (e.g., the subject has stated in advance that they consumed a prescription or over-the-counter product which contained the detected drug) and/or the subject has a negative urine drug screen on retest by the pathology laboratory. Any subject testing positive for acetaminophen (paracetamol) at screening may still be eligible for study participation, at the Investigator's discretion.

40. Positive alcohol breath test.

Specific to the study

41. 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.
- Electrolyte disturbances, particularly hypokalaemia, hypocalcaemia, or hypomagnesaemia.
- ECG abnormalities in the standard-lead 12-lead ECG (at screening or at pre-inoculation on inoculation day) which in the opinion of the Investigator is clinically relevant or will interfere with the ECG analyses.

42. Known hypersensitivity to artesunate or any of its excipients, artemether or other artemisinin derivatives, piperaquine phosphate, proguanil/atovaquone, primaquine, or 4-aminoquinolines.

Healthy subjects who do not fulfil all the inclusion criteria, and/or fulfil any of the exclusion criteria should not be enrolled into the study without exception. In case of doubt, the Investigator is to confer with the medical monitor for agreement. Waivers for inclusion of volunteers that are not meeting all eligibility criteria will not be granted.

Subjectswho are excluded from participation on study days for any of the abovereasonsmay be eligible to participate ona postponed schedule, if the Investigatorconsiders this appropriate.

5.3 CONTRACEPTION

Male and femalesubjects who are sexually active must use, with their partner, 2 approved methods of highly effective contraception from the time of informed consent until 90 daysafter the last dose ofOZ439 and PQP.
Two or more of the following methods are acceptable and must include at least 1 barrier method:

- Surgical sterilisation (vasectomy(male), tubal ligation (female))
- Placement of an intrauterine device or intrauterine system
- Hormonal contraception (implantable, patch, oral, injectable)
- Barrier methods (this must be a condom or their partner's use of an occlusive cap [diaphragm or cervical/vault caps] with spermicidal foam/gel/film/cream/suppository)

Alternatively, true abstinence is acceptable when it is in line with the subject's preferred and usual lifestyle. If a subject is usually not sexually active but becomes active, they, with their partner, must comply with the contraceptive requirements detailed above.

Adequate contraception does not apply to subjects of childbearing potential with sexpartners (abstinence from penile-vaginal intercourse), when this is their preferred and usual lifestyle. Female subjects with same sex partners must not be planning in vitro fertilisation within the required contraception period.

Women of non-childbearing potential who will not require contraception during the study are defined as: postmenopausal (spontaneous amenorrhoea for ≥ 12 months, or spontaneous amenorrhoeafor 6-12 months and follicle-stimulating hormone (FSH) ≥ 40 IU/mL; either should be together with the absence of oral contraceptive use for > 12 months) or permanently sterilised (e.g. hysterectomy, bilateral salpingectomy).

Adequate contraceptionis also not required formale subjectswith female partners that are permanently sterilised(or male subjects who have undergone sterilisation and have had testing to confirm the success of the sterilisation.

5.4 STRATEGIES FOR RECRUITMENT AND RETENTION

Up to 24 subjects are planned to be enrolled in the study. It is estimated that approximately 75 subjects may need to be screened to complete enrolment. Four reserve subjects will be required at each inoculation day to ensure 8 subjects are dosed for each cohort.

Subjects will be recruited from the QIMR Berghofer Human Research Ethics Committee (QIMR Berghofer HREC) approved database of healthy subjects maintained by PharmQ, or by a general or study specific advertisement via print, radio or poster media to students of Queensland universities or to the general community, as approved by the QIMR Berghofer HREC. No restrictions will apply for ethnic or racial categories; the expected population may include all Australian racial categories.

Subjects who complete the study up to Day 45 V2/EOS will be paid $3,865 compensation for their participation. Subjects who withdraw or are withdrawn from the study will be compensated on a fractional basis for their involvement unless they are withdrawn as a consequence of their misconduct. Reserve subjects who do not participate in the study will be paid $150 compensation (per inoculation day) for the inconvenience associated with their attendance for screening and for their attendance on the inoculation day, in case they are required to participate. Volunteers who fail screening due to an underlying medical condition previously unknown to them will be reimbursed $75 for their time, and provided with the appropriate referrals for guidance and counselling for their condition.

5.5 SUBJECT WITHDRAWAL OR TERMINATION

5.5.1 REASONS FOR WITHDRAWAL OR TERMINATION

Subjects are free to withdraw from the study at any time. A subject may be considered withdrawn if he/she states an intention to withdraw, fails to return for scheduled protocol visits for any reason, or becomes lost to follow-up. Subjects may also be withdrawn by the Investigator. Possible reasons for withdrawal by the Investigator include the occurrence of a SAE, failure by the subject to comply with the requirements of the protocol, or for any other reason at the Investigator's discretion.

5.5.2 HANDLING OF SUBJECT WITHDRAWALS OR TERMINATION

If a subject is withdrawn from the study, the funding Sponsor will be informed immediately. If there is a medical reason for withdrawal, the subject will remain under the supervision of the Principal Investigator until satisfactory health has returned.

The Investigator will make every effort to determine the primary reason for a subject's withdrawal from the study and record this information in the electronic case report form (eCRF). For subjects who are lost to follow-up, the Investigator will demonstrate due diligence by documenting all steps taken to contact the subject (e.g. dates of phone calls, registered letter, home visit, etc.) in the source documents. If earlier withdrawal from further study procedures occurs, the subject will be asked to complete the antimalarial rescue treatment. The subjects will also be asked to complete the early termination evaluation as described in Section 7.3.5.

If the subject is withdrawn from the study procedures or follow-up for any reason, with the subject's permission, medical care will be provided for any SAEs that occurred during participation in the study until the symptoms of any SAEs are resolved and the subject's condition becomes stable. Follow-up for AEs is described in Section 8.3.

If a subject is withdrawn due to a study drug-related AE or due to termination of the study, the early-termination subject will not be replaced. If a subject does not complete the study for reasons other than safety, the early termination subject may be replaced after mutual agreement between the funding Sponsor and the Investigator. The decision regarding the replacement of subjects will be documented.

5.6 PREMATURE TERMINATION OR SUSPENSION OF STUDY

The Sponsor, Principal Investigator, QIMR Berghofer HREC and Regulatory Authorities independently reserve the right to discontinue the study 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 abovementioned 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 Principal Investigatorwill ensure that adequate consideration is given to the protection of the subjects' interests and safety. The Investigatormust review all subjects as soon as practical and complete all required records.

In addition to the classic assessment of SAEs and the occurrence/severity of other AEs by the Sponsor and the Investigator, after exploring potential confounding factors, the following criteria should be considered as guidance for the decision to stop inoculation of further subjects:

- A subject experiences an SAE that is related to the inoculum.
- There is insufficient response to OZ439 and PQP.
- The Investigator and Sponsor may decide to stop inoculation based on other safety signals not described in the above criteria.

6 STUDY AGENT

6.1 STUDY AGENT(S) AND CONTROL DESCRIPTION

6.1.1 ACQUISITION

Challenge Agent (hereafter referred to as inoculum)

P. falciparum 3D7

The P. falciparum 3D7 master cell bank (MCB) was produced from a person with O Rh(D) Negative blood who was infected with the parasite by mosquito bite. The MCB 3D7 was cryopreserved, aliquoted into cryovials and stored in liquid nitrogen under controlled conditions. Refer to the P. falciparum 3D7 Investigator's Brochure for more details [35]. A 3D7 MCB cryovialswill be retrieved from storage, thawed, and used to aseptically prepare the 3D7 inoculum at Q-Gen.

Study Drug

OZ439

OZ439 granules + α-tocopherol polyethylene glycol 1000 succinate (TPGS) granules for oral suspension (200 mg and 400 mg strengths of OZ439) will be provided in aluminium sachets. Excipient grade sucrose will be provided by Q-Pharm pharmacy. The drug product will be shipped either directly to Q-Pharm, or via Pharmaceutical Packaging Professionals Pty Ltd (Victoria, Australia). Import will be facilitated by MMV and the TGA will be notified using the Clinical Trial Notification (CTN) scheme.

Piperaquine phosphate (PQP)

Piperaquine phosphate, 160 mg will be provided from a stock at Pharmaceutical Packaging Professionals Pty Ltd and shipped directly to Q-Pharm.

Antimalarial Rescue Medications

Riamet® and Primacin™ (if required)

Riamet® and Primacin™ will be acquired by Q-Pharm, labelled according to identity, brand or source, and batch number. The supplies will be held in appropriate locked storage conditions at Q Pharm until required. The contents of the label for the drug to be administered to the subjects will be in accordance with all applicable regulatory requirements. The inoculum strain (P. falciparum 3D7) used in the challenge model has been proven to be sensitive to the rescue medications.

Artesunate (if required)

If a subject vomits or cannot tolerate oral drugs, then artesunate will be administered intravenously as described in Section 6.1.5. This drug is the recommended parenteral treatment for malaria in Australia. Currently, it is a Special Access Scheme drug and has been sourced from Guilin Pharmaceutical (Shanghai) Co., Ltd. Import was facilitated by MMV. The manufacture of intravenous (IV) artesunate is undertaken in a WHO-Prequalified GMP facility (http://www.mmv.org/access/access-portfolio/artesunate-injectable-artesunate).

The rescue drugs, i.e., Riamet®, Primacin™, and artesunate will be inventoried prior to the beginning of trial enrolment on trial accountability logs in regards to condition upon receipt, including lot numbers. The Investigator or qualified designee will ensure that the received drugs are the specified formulation. The site pharmacist or qualified designee is responsible for maintaining an accurate inventory and accountability record of drug supplies for this trial.

6.1.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

Inoculum

P. falciparum 3D7

Each 3D7 inoculum dose will contain parasitised and unparasitised RBCs, 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 the GCP guidelines and the Access to Unapproved Therapeutic Goods-Clinical Trials in Australia [44].

Study Drug

OZ439

OZ439 is available as OZ439 + α-tocopherol polyethylene glycol 1000 succinate (TPGS) granules for oral suspension dosage form. OZ439 granules and TPGS granules are co-packaged in a 2-compartment sealed sachet. The OZ439 granules and TPGS granules are physically separated in the sachet packaging. Dose strengths of 200 mg and 400 mg OZ439 are available. The OZ439 and TPGS granules for oral suspension are mixed with water to form an oral suspension, and sucrose is added prior to administration to the subject. The sucrose is added to make the oral suspension palatable. Reconstitution steps will be described in the Pharmacy Manual. The contents of the label for drug to be administered to the subjects will be in accordance with all applicable regulatory requirements.

Piperaquine phosphate (PQP)

PQP is formulated as tablets (160 mg per tablet). PQP will be labelled according to identity, brand or source, and batch number. The contents of the label for drug to be administered to the subjects will be in accordance with all applicable regulatory requirements.

Antimalarial Rescue Medications

Riamet® and Primacin™ and Artesunate

Riamet® tablets (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 (primaquine phosphate) are round, flat, orange uncoated tablets. Primacin™ tablets are available in bottles of 28 or 56 tablets. Primacin™ will be labelled according to identity, brand or source, and batch number. The contents of the label for drug to be administered to the subjects will be in accordance with all applicable regulatory requirements.

Artesunate for I/V administration is presented as a powder for reconstitution (60 mg artesunic acid) in a vial.

6.1.3 PRODUCT STORAGE AND STABILITY

Inoculum

P. falciparum 3D7

The inoculum is prepared at QC on inoculation day (Day 0). The time between preparation of the 3D7 inoculum and administration to each subject will be a maximum of 2 hours, during which

time all inocula will be stored on ice. The Pharm pharmacist will document receipt conditions and time restrictions of use.

Study Drug

OZ439

OZ439 + TPGS granules for oral suspension drug products should be stored between 15-30°C. Food grade sucrose is to be stored at room temperature.

Piperaquine phosphate (PQP)

PQP tablets are to be stored at 15 to 25°C.

Antimalarial Rescue Medications

Riamet® and Primacin™

- Riamet® is to be stored below 30°C and protected from moisture (dispersible tablets).
- Primacin™ is to be stored below 25°C.
- Artesunate: store in tightly closed containers, protected from light.

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

6.1.4 PREPARATION

Inoculum

P. falciparum 3D7

The inoculum will be prepared aseptically in Q-Gen (QIMR Berghofer), from a frozen cryovial of the P. falciparum 3D7 MCB, by nominated QIMR Berghofer staff members under the guidance of the Investigator. The infected RBCs will be thawed, washed, resuspended in saline, diluted in a final volume of 2 mL of clinical grade saline, and dispensed into syringes [35]. Any remaining unused infected RBCs will be discarded as per approved standard operating procedures.

Study Drug

OZ439

The OZ439 and TPGS granules for oral suspension are mixed with water to form an oral suspension and sucrose is added prior to administration to the subject. The sucrose is added to make the oral suspension palatable. The 4.39 will be drunk and then the cup rinse water will be used to swallow the PQP tablets. The reconstitution procedure will be described in the Pharmacy Manual.

Piperaquine phosphate (PQP)

PQPis available as tablets and no preparation is required. The tablets will be swallowed with the water that is used to rinse the OZ439suspension cup with.

OZ439 and PQP will be administered at the same time. Subjects will be fasted for 6 hours pre- and post-dose.

Antimalarial Rescue Medications

Riamet®andPrimacin™

Riamet®andPrimacin™ are available as tablets and no preparation is required.

6.1.5 DOSING AND ADMINISTRATION

Inoculum

P. falciparum3D7

An inoculum dose, containing an estimated 800 viable P. falciparum3D7 parasite-infected RBCs in a volume of 2 mL, will be administrated intravenously to each subject on Day 0. The actual number of parasites inoculated will take into account the loss of viability resulting from cryopreservation, storage and thawing. On inoculation day, subjects may have food until at least half an hour prior to inoculation. Subjects will undergo intravenous cannulation with an appropriate 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. All subjects will be inoculated intravenously within 60 minutes of each other. See Section 6.1.3 for stability information. An extra syringe will be prepared to quantify the parasite count of the inocula by malaria 18S qPCR.

Study Drug

OZ439and piperaquine phosphate (PQP)

Subjects will be admitted to the clinical unit for single dose administration of OZ439 and PQP, 8 days after malaria inoculation or earlier if a subject has a malaria clinical score >6 or at Investigator's discretion. The doses to be administered to subjects in Cohort 1 and 2 are shown in Tables 1 and 2 respectively (Section 4.1). Subsequent doses for subsequent cohorts will be determined by the SRT following safety, tolerability and PK/PD review. Subjects will be fasted for ≥6 hours pre and post OZ439 and PQP administration. The OZ439-TPGS granules will be mixed with water to form a suspension. Sucrose will be added to the suspension to assist with palatability. PQP tablets will be administered immediately after the suspension and swallowed

with the OZ439 cup rinse water. The reconstitution procedure will be described in the Pharmacy Manual.

Antimalarial Rescue Medications

Riamet® and Primacin™

All subjects will receive compulsory treatment with Riamet® on trial Day 42 ± 2, or at failure of OZ439 and PQP to clear parasitaemia, or at evidence of recrudescence (defined as ≥ 5 000 blood stage parasites/mL and a 2-fold increase within 48 hours, or a malaria clinical score > 6) at the Investigator's discretion. Riamet® tablets containing 20 mg artemether and 120 mg lumefantrine will be administered as 6 doses of 4 tablets (total course of 24 tablets) given over a period of 60 hours (total dose of 480 mg artemether and 2.88 g lumefantrine). Each dose of tablets administered orally should be taken with food or drinks rich in fat (e.g., milk). Subjects will be reminded of the potential side effects of Riamet® and given the Consumer Medicine Information for Riamet® (Appendix 1).

Subjects may also be treated with Primacin™ at the time of Riamet® treatment if gametocytaemia is suspected from parasite lifecycle stage or PCR or by the presence of stable low level parasitaemia, or at the Investigator's discretion, to ensure complete clearance of gametocytes. If needed, subjects will take 6 Primacin™ tablets, each containing 13.2 mg primaquine phosphate equivalent to 7.5 mg primaquine (the total primaquine dose will be 45 mg). Primacin™ for oral use will be taken as a single dose with food. Subjects will be reminded of the potential side effects of Primacin™ and given the Consumer Medicine Information for Primacin™ (Appendix 1).

IV artesunate may be used as a rescue medication if a subject vomits or cannot tolerate oral drugs. See artesunate section above.

6.1.6 ROUTE OF ADMINISTRATION

Inoculum

P. falciparum 3D7

Intravenous

Study Drug

Artefenomel (OZ439)

Oral administration

Piperaquine phosphate (PQP)

Oral administration

Antimalarial Rescue Medications

Riamet®andPrimacin™

Oral administration

Artesunate

If a subject vomits or cannot tolerate oral drugs, then artesunate will be administered intravenously as described in Section 6.1.5.

6.1.7 STARTING DOSE AND DOSE ESCALATION SCHEDULE

Dosing with the malaria inoculumand rescue drugs is presented in Section 6.1.5. No dose escalation will be performed.

Study Drug

Dosing with OZ439 and PQP will be performed as described in section 4.1. The doses of OZ439 and PQP for subjects enrolled in cohort 1 are shown in Table 1 of the Protocol Summary. Subjects will be randomised to one of the four groups that comprise Cohort 1. The doses of OZ439 and PQP for subjects enrolled in cohort 2 are shown in Table 2 of the Protocol Summary. Subjects will be randomised to one of the two groups that comprise Cohort 2. The OZ439 and PQP doses to be evaluated in subsequent cohorts will be determined at the SDRT meeting based on the safety andtolerability data up to Day 42±2, and PK/PD analysis outcomes (based on PD data up to Day 42±2 and PK data up to Day 35±2) of the drugs given in combination in previous Cohort(s).

The doses used in all Cohorts will not exceed 800 mg for OZ439 and 1440 mg for PQP as determined in previous safety, pilot efficacy and phase 2 studies.

6.1.8 DOSE ADJUSTMENTS/MODIFICATIONS/DELAYS

Not applicable.

6.1.9 DURATION OF THERAPY

See Section 6.1.5for dose information.

6.1.10 TRACKING OF DOSE

The P. falciparum challenge agent, OZ439 and PQP, and if required Primacin™ will be administered at the clinical unit in the presence of clinical unit staff. Subjects may be administered

Riamet® on site for initial dosing followed by monitoring, either in the clinic or by phone for 3 days to ensure adherence to Riamet® therapy.

6.2 STUDY AGENT ACCOUNTABILITY PROCEDURES

The Q-Pharm pharmacist or delegate, as nominated by the Principal Investigator, is responsible for maintaining accurate study agent accountability records throughout the study. Study agents include the challenge agents (OZ439 and PQP), and the rescue medications. Dispensing, accountability and documentation will be in accordance with Q-Pharm standard procedures. All products will be inventoried upon receipt by the Q-Pharm 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 agents and the time restrictions of use for the oral suspension of OZ439 and TPGS. The lot numbers and expiry dates of the inoculum and antimalarial drugs will be documented. The Q-Pharm 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 subjects and all dose changes during the study must be recorded in the eCRFs. 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 site once drug accountability 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 site with the rest of the study records.

7 STUDY PROCEDURES AND SCHEDULE

7.1 STUDY PROCEDURES/EVALUATIONS

7.1.1 STUDY SPECIFIC PROCEDURES

Medical history

Medical history will be elicited at screening as described below.

Past Medical/Surgical History Includes:
History of all known allergies
Current medications, including over-the-counter and herbal preparations

History of substance abuse and recreational drug use
History of depression, anxiety, mental illness, emotional problems, use of psychiatric medicati
Surgical procedures and results

Physical examination

Complete Physical Examination Includes:

Weight (Screening only)
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

Abbreviated Physical Examination: an abbreviated physical examination will be performed at Day 0 (inoculation day) and Day 8 (OZ439/PQP dosing day). The examination will include; heart/circulation, chest, lungs and abdomen.

Symptom-Directed Physical Examination: physical examinations will be symptom-directed at specified time points. Body systems will be reviewed only if clinically indicated at the discretion of the Investigator (see Appendix 3 for a list of symptoms and signs of malaria).

Beck Depression Inventory

All subjects will be required to complete the Beck Depression Inventory at screening. This is a validated questionnaire used as an objective tool for the assessment of depression (See Appendix 4).

Vital signs/12-lead ECG and Safety Laboratory Sample Allowed Time Windows

Time point	Tolerance window
Vital Signs/ 12-Lead Electrocardiogram/Safety Laboratory Sample	
In confinement	
Pre-dose	- 90 min to 0 h
0-4 hours inclusive after IMP	± 10 min
4-12 hours inclusive after IMP	± 10 min
16-72 hours inclusive after IMP	± 40 min
Out patient	

72-120 hours inclusive	± 120 min
168-EOSinclusive	± 48 hours

Vital signs

Vital signs (temperature, heart rate, respiratory rate and blood pressure) will be measured at screening after the subject has rested in the supine position for at least 5 minutes and in the standing position within 2-3 minutes when changing from the supine to standing position (blood pressure and heart rate only). At all other time-points, vital signs will be measured after the subject has rested in the seated position for at least 5 minutes. Tympanic temperature will be taken at the clinical unit.

The normal ranges for vital signs once on study 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
Peripheral O2 Saturation	>=95%

Electrocardiograms

Triplicate 12-lead ECG will be recorded after resting in supine for at least 5 minutes. The same body position will be assumed by the subject for each recording. ECG tracings will be retained and labelled as per standard procedures at the clinical unit. The mean of the three values will be recorded in the eCRF. Any clinically, significant findings will be discussed with the Medical Monitor and Sponsor and documented as adverse events. The Investigator will sign and date each ECG as evidence of their review.

The normal ECG ranges once on study are:

Parameter	Range
PR interval	<= to 210 ms
QRS	>50 to < = to120 ms
QT interval	>200 to < 500
QTcB/QTcF	Males: ≤ 450 msec
QTcB/QTcF	Females ≤470 msec

Blood sampling

Main Study - Blood will be collected for clinical laboratory evaluations including haematology, biochemistry, serology, pregnancy testing and/or FSH testing (see Section 7.2.1). Blood samples will also be collected to monitor malaria parasitaemia and to quantify plasma drug concentrations for PK analysis (see Section 7.2.2).

Optional exploratory components - Blood will be collected to characterise specific cell subsets with an aim to identify immune-regulatory pathways initiated during malaria infection that can be targeted for clinical advantage. Blood samples will also be collected to investigate early changes to RBC that leads to malaria induced anaemia.

The estimated blood volume required for both the main study and optional exploratory components is listed in Appendix 2. The total volume of blood drawn from each subject will not exceed 450 mL in any given 30-day period. The total blood volume at day 30, for both the main study and optional exploratory components, will be approximately 404 mL and by EOS at day 45, for both the main study and optional exploratory components, will be approximately 485 mL.

This volume provides an allowance for unscheduled safety laboratory assessments that may be required at the discretion of the Principal Investigator or the Sponsor to ensure subject safety.

Urine sample collection

Urine will be collected for urinalysis and drug screening (see Section 7.2.1).

AE recording

AEs will be recorded as described in Section 8.

Malaria Clinical Score

The following 14 signs/symptoms frequently associated with malaria will be graded by the Study nurse or doctor using a 4-point scale (absent: 0; mild: 1; moderate: 2; severe: 3) and summed to generate a total Malaria Clinical Score (maximum total score possible is 42). Individual scores for each symptom as well as the total score will be recorded.

Headache	Anorexia
Myalgia (muscle ache)	Nausea
Arthralgia (joint ache)	Vomiting
Fatigue/lethargy	Abdominal discomfort
Malaise (general discomfort/uneasiness)	Fever
Chills/Shivering/Rigors	Tachycardia
Sweating/hot spells	Hypotension

Medical Diary

Provide subjects with diary cards and instructions for subjects to record symptoms and concomitant medications during the study.

Temperature Self Recording

Provide subjects with thermometers to record any temperature readings during the study in the event of symptoms of fever, sublingual temperature will be taken by subjects at home for practical reasons.

7.1.2 STANDARD OF CARE STUDY PROCEDURES

Not applicable.

7.2 LABORATORY PROCEDURES/EVALUATIONS

7.2.1 CLINICAL LABORATORY EVALUATIONS

Any significant deviations from results obtained during screening period will be followed until resolution or investigated fully, or until the subject is referred to a general practitioner. The Investigator will document the clinical significance of all results falling outside of the normal reference ranges. All abnormal laboratory test results judged as being clinically significant will be recorded as AEs.

The estimated blood volume required for these tests is listed in Appendix 2.

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 automated differential or if an automated differential was not able to be performed.
Neutrophils (NEUT)
Lymphocytes (LYM)
Monocytes (MON)
Eosinophils (EOS)
Basophils (BAS)
Red blood cell count (RBC)
Mean corpuscular volume (MCV)
Haemoglobin (HGB)
Haematocrit (HCT)
Platelet count (PLAT)
Reticulocyte count (RETIC) (Screening and EOS or early termination visit only)
Blood Group and Rh(D) tests (Screening only)

RBC alloantibodies testing

Blood for RBC alloantibodies testing will be collected at screening Day 1 and Day 45±2 or early termination visit.

G6PD testing

Blood for G6PD testing will be collected at screening only. G6PD deficiency is not an exclusion criterion but will be determined at screening to ensure the safety of Primacin™.

Coagulation testing

Blood for PT, APTT and INR will be collected at screening visit.

Biochemistry and CRP biomarker

Sodium (SODIUM)	Alkaline phosphatase (ALP)
Potassium (K)	Alanine aminotransferase (ALT, SGPT)
Chloride (CL)	Aspartate aminotransferase (AST, SGOT)
Bicarbonate (BICARB)	Calcium corrected
Glucose (GLUC)	Phosphate (PHOS)
Urea	Lactate dehydrogenase (LDH)
Creatinine (CREAT)	Magnesium (Screening only)
Urate	Cholesterol (Screening only)
Albumin (ALB)	Triglycerides (Screening only)
Globulin	HDL (Screening only)
Total protein	Estimated glomerular filtration rate (eGFR)
Total bilirubin (BILI)	C-Reactive Protein (CRP) (Not included at Screening)
Direct (conjugated) bilirubin (BILDIR)	

Urinalysis

Urine will be tested by dipstick at the clinical unit. If there are any abnormalities considered clinically significant in blood, leucocytes or protein, the urine will be sent for formal laboratory urinalysis per the clinical unit's standard procedure.

Glucose (GLUC)
Bilirubin (BILI)
Ketone (KETONES)
Specific gravity (SPGRAV)
Blood
pH
Protein (PROT)

Urobilinogen (UROBIL)
Nitrite
Leukocytes (WBC)
Formal laboratory urinalysis (if required)

Urine drug screens and alcohol breath tests

If the results of the urine drug screens or alcohol breath tests are positive, subjects will not be enrolled in the clinical study

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

Subjects testing positive for paracetamol at screening and/or inoculation day may still be eligible for study participation, at the investigator's discretion. Subjects requiring paracetamol on a daily basis would not be eligible to enrol in the study, however use of any over-the-counter medication during the study is restricted and potential subjects should not discontinue their usual medications in order to participate in the study.

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

Serology

HIV 1/2 (anti-HIV1 and anti-HIV2 Ab)
Hepatitis B (HBs Ag, anti-HBc (IgG + IgM if IgG is positive))
Hepatitis C (anti-HCV)
Hepatitis A (anti-HAV) (IgM) - performed from stored sample for testing, at investigator's ***discretion***
Hepatitis E (anti-HEV) (IgM) - performed from stored sample for testing, at investigator's ***discretion***
Epstein-Barr virus - performed from stored sample for testing, at investigator's ***discretion***
Cytomegalovirus - performed from stored sample for testing, at investigator's ***discretion***

Safety serum storage

Blood for serum storage as safety retention samples will be collected at times outlined in Section 7.3.

7.2.2 OTHER ASSAYS OR PROCEDURES

The estimated blood volume required for these tests is listed in Appendix 2.

PK/PD Allowed Time Windows

Time point	Tolerance window
Pharmacokinetic/pharmacodynamic	
In confinement	
Pre-dose	- 60 min to 0 h
0-4 hours inclusive after IMP	± 2 min
4-12 hours inclusive after IMP	± 5 min
16-72 hours inclusive after IMP	± 30 min
Out patient	
72-120 hours inclusive	± 120 min
168-EOS	± 48 hours

Malaria monitoring

Blood will be collected to monitor parasitaemia using qPCR targeting the 18S rRNA gene. Additional blood (up to approximately 2.5 mL per time-point) may be collected for parasite lifecycle stage qRT-PCR at the Investigator's discretion. This is to evaluate for the presence of sexual parasite stages (gametocytes) and other parasite lifecycle stages in the blood. This blood may also be used for research into various aspects of parasite biology e.g. gametocytes, parasite lifecycle stages, recrudescence, commitment etc. The qPCR may target genes 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.

Microscopic examination for evidence of parasitaemia or gametocytaemia may be conducted at the Investigator's discretion.

Malaria monitoring will continue until a minimum of one negative 18S qPCR is detected post rescue therapy

PK blood collection and processing

Blood sampling for PK analysis will be performed at scheduled time points from immediately before dosing with OZ439 and PQP until 816 hours post-dose, (allowed time windows as described above).

Blood samples will be collected either by direct venepuncture or via an indwelling cannula inserted in a forearm vein. Details of the volume of blood collected are listed in Appendix 2.

A description of the required lab procedures will be described in the Laboratory Manual.

The actual sample collection date and time will be entered in the PK blood collection section of the eCRF. Any sampling problems will be documented in the eCRFs.

Pharmacokinetic analytical methods

Plasma concentrations of OZ439 and PQP will be quantified by LC MS/MS in the selected reaction monitoring mode using heated electrospray ionization in positive ion mode.

OZ439: Plasma samples will be precipitated with five volume equivalents of a mixture of acetonitrile/methanol (4/1, v/v) containing the internal standard. After centrifugation, the samples will be diluted with a mixture of water/acetonitrile (1/1, v/v). An aliquot of 2 µL of the sample will be injected onto the high-performance liquid chromatography system (HPLC).

PQP: Plasma samples will be precipitated with ten volume equivalents of acetonitrile containing the internal standard. After centrifugation and dilution with one equivalent of water, an aliquot of 25.0 µL of the supernatant will be injected onto the HPLC system.

Optional exploratory components

Blood sampling for immune cell characterisation and complement regulatory protein analysis will be performed at scheduled time points as detailed in Section 7.3. The time-points for the exploratory components are scheduled to coincide with blood collection time-points of the main study. If timepoints of the main study change the exploratory study-time-points will change to match to ensure subject comfort and convenience.

Blood samples will be collected either by direct venepuncture or via an indwelling cannula inserted in a forearm vein. Details of the volume of blood collected are listed in Appendix 2.

Optional exploratory components analytical methods

Specific cell subsets will be sorted from blood collected on Days 0 (prior to malaria inoculation), 4, 8 (prior to OZ439 and PQP administration), 15 and at EOS. The host immune response generated by these sorted cells will be investigated at the DNA, RNA and protein levels with an integrative high dimensional multi-omics strategy. DNA, RNAs, including mRNA, microRNA and long non-coding RNA (lncRNA), and proteins will be extracted from the same sample and samples will be assayed in a high dimensional biology approach. mRNA, miRNAs, and lncRNA will be profiled using NanoString, RNAseq, and/or whole transcriptome analysis. Comprehensive panels of molecules involved in T cell activation, function, and exhaustion will be evaluated using multi parametric flow cytometry. Proteogenomics will also be investigated, whereby data from high resolution mass-spectrometers (e.g. QTRAP) will generate fingerprints for peptides which are mapped against NGS transcriptomic and genomic data

Expression of complement regulatory proteins (CD35, CD55, and CD59) and phosphatidylserine on RBC will be assessed by flow cytometry and will be quantified by commercial ELISA from blood samples collected on Day 0 (prior to malaria inoculation), Day 8 (prior to OZ439 and PQP administration), Days 10, 12, 13, 15, 18 and at EOS.

7.2.3 SPECIMEN PREPARATION, HANDLING, AND STORAGE

Q-Pharm's standard work instructions will apply to the allowed time windows for study procedures and sample collection. If the observation time and blood sampling time coincide, for precision of timing, blood collection will take precedence over other procedures scheduled at the same time.

Blood will be collected into tubes containing the appropriate anticoagulant. Samples will be processed according to the laboratory requirements.

Biological samples will be retained for the time required for assessment and analysis, and may then be discarded. Safety serum samples will be stored indefinitely with the permission of the subjects for any retrospective safety assessments.

7.2.4 SPECIMEN SHIPMENT

Samples collected will be shipped to nominated local or international laboratories for assessment (Section 1). The site staff will be responsible for shipment of samples to analytical laboratories for testing. Samples must be packed securely together with completed shipment forms in shipping containers together with sufficient dry ice as per Shipper procedures.

7.3 STUDY SCHEDULE

7.3.1 SCREENING

A screening visit will be scheduled after an initial phone interview conducted by clinical unit staff has occurred to review background information. For the screening visit, potential subjects will be asked to come to the clinical unit after an overnight fast of ≥8 hours. During this initial screening visit, an Investigator will discuss the details of the study with the potential subject, and the potential subject will read the Participant Information Sheet and be encouraged to ask questions.

Subjects will be fully informed of the nature of the study at this time, and advised of the requirement to repeat some screening tests during the 3 day Day-1 safety visit (if required) and/or on inoculation day to determine their continuing eligibility. Subjects must confirm that they will not be living alone from inoculation day until the end of the Riamet® treatment.

Individuals willing to be considered for inclusion may sign the Consent Form during the screening visit, or return to the clinical unit after further consideration. The subject will be given a copy of the Participant Information Sheet and signed Consent Form for their records. The signed and dated

originals will be held on file by the clinical unit. Participation consent must be obtained from all subjects prior to screening tests.

The consenting process will take place with one of the PHRQ trained medical staff or Investigators and will include the following:

1. Provide the Participation Information Sheet and Informed Consent Form and give the subject sufficient time to review the contents.
2. Explain the study via the Participation Information Sheet and gain informed consent from the subject, if they are willing to consent.
3. Ensure the subject and member of staff taking consent have signed and dated the Informed Consent Form and received a signed copy.

Once the potential subject has provided written consent to participate, the pre-study screening may be initiated. The screening will be conducted within 4 weeks prior to the inoculation day and will include:

1. A screening number will be assigned to each subject.
2. Elicit a complete medical history and use of medications.
3. Elicit a social history including recreational drug, alcohol and tobacco use.
4. Perform alcohol breath test.
5. Perform a drugs of abuse screen.
6. Perform complete physical examination.
7. Ask subject to complete the Beck Depression Inventory.
8. Assess the cardiovascular disease risk as per exclusion criterion 4.
9. Record vital signs.
10. Obtain a 12-lead ECG in triplicate.
11. Collect urine for urinalysis.
12. Collect blood samples for haematology, biochemistry, RBC alloantibodies, G6PD testing, and serology (viral hepatitis B and C and HIV).
13. Collect blood for coagulation profile
14. Perform serum β-hCG pregnancy test for all female subjects and follicle stimulating hormone test (FSH) for post-menopausal females.
15. Verify subject meets inclusion/exclusion criteria.

Subjects who complete all screening procedures and satisfy all entry criteria will be considered eligible to participate in this study. To be eligible for study entry, laboratory values at screening must not be outside the range of the normal values at a level deemed to be clinically significant according to Sponsor approved criteria. For eligibility parameters, a repeat may be requested to exclude laboratory error. Re-screening will not be allowed unless the Investigator considers the cause of the initial pre-screening failure to be of an acute and completely reversible nature.

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

7.3.2 ELIGIBILITY CONFIRMATION VISIT

Day -3 to Day -1 safety visit

Subjects (including approximately 4 reserve subjects as mentioned in Section 5.4) will report to the clinical 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:

1. Collect blood samples for haematology, biochemistry (including CRP biomarker) and serum β-hCG pregnancy test for all female subjects
2. Collect urine for urinalysis.

The timing of these assessments is to ensure that results are available for review by the Investigator prior to inoculation on inoculation day. Subjects with clinically significant laboratory findings at this stage will not be eligible for inoculation.

Malaria inoculation day (Day 0)

Each subject (and approximately 4 reserve subjects) will report to the clinical unit on the morning of Day 0. The Investigator will review the subjects' screening and eligibility confirmation visit results prior to their enrolment into the study and subsequent inoculation. The Investigator will emphasise the requirement to return to the clinical unit for malaria drug treatment after the malaria inoculation and confirm that they will not be living alone from Day 0 until the end of the study by checking housemates contact details recorded at screening visit.

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

The procedures that will be undertaken prior to inoculation on Day 0 include:

1. Verify that all applicable eligibility criteria have been met.
2. Elicit information regarding any new medical conditions, illnesses and medication use since screening.
3. Perform alcohol breath test and urine drug screen
4. Perform urine β-hCG pregnancy test for WOCBP subjects

5. Conductabbreviatedphysical examination.
6. Record vital signs.
7. Obtain 12lead ECGin triplicate.
8. Cannulate subjects with an indwelling intravenous cannula for the malaria inoculum, and record which arm is utilised.
9. Collect blood samples for malaria qPCR and safety serum storage.
10. Collect optional blood samples for immune cell characterisation and complement regulatory proteins (if consented separately).
11. Perform Malaria Clinical Score.

Administration of the malaria inoculum:

1. Administer the malaria inoculum of ~2200 viableP. falciparum3D7 infected human RBCsintravenously in the morning (approximately 9:00 AM).
2. Observe for a minimum of 60 minutes after inoculation to evaluate for immediate adverse reactions.
3. Educate subjects on signs and symptoms of malaria (Appendix 3).
4. Emphasise to subjects the importance of returning on the nominated day (approximately Day 8), or as advised by the clinical unit staff, if IMP antimalarial treatment.
5. Provide subjects with diary cards and thermometers to record any temperature readings during the study in the event of symptoms of fever. Subjects will also record symptoms and concomitant medications on the diary cards during the study.
6. RecordAEs and concomitant medications.
7. Record vital signs prior to leaving the clinical unit (approximately 60 minutes after inoculation).
8. Record malaria clinical score prior to leaving the clinical unit (malaria clinical score baseline sample; see Section 7.1.1).

7.3.3 FOLLOW-UP

Malaria monitoring via phone (Days 1-3)

During this period, subjects are expected to be asymptomatic. A daily phone call or text message will be made to the subjects by clinical unit staff to monitor subject well-being and to solicit any AEs.

Daily visits to the clinical unit for malaria monitoring (Day 4 AM until qPCR positive for malaria)

Follow-up from Day 4 until qPCR becomes positive will be undertaken through daily visits (approximately 8:00 AM) to the clinical site.

The following procedures will occur during these visits:

1. Performsymptom-directed physical examination(when signs or symptoms of malaria are identified and it is clinically indicated (Appendix 3).
2. Elicit information regarding any new medical conditions,illnessesand medication use since screening
3. Record vital signs.
4. Collect blood sample for malaria 18S qPCR.
5. Collect optional blood samples for immune cell characterisation, Day 4 (if consented separately).
6. Record malaria clinical score.
7. RecordAEs and use of concomitant medications.
8. Check subject diaries.

Day when qPCR positive until treatment day (Day 8)

Follow-up from the day that malaria 18S qPCR becomes positive until treatment day will be undertaken through twice daily (AM & PM) visits to the clinical site separated by approximately 12 hours (i.e. 06:00 – 11:00 and 18:00 – 23:00). The following procedures will occur during these visits:

1. Performsymptom-directed physical examination
2. Elicit information regarding any new medical conditions,illnessesand medication use since screening
3. Record vital signs.
4. Collect blood sample for malaria 18S qPCR.
5. Record malaria clinical score.
6. RecordAEs and use of concomitant medications.
7. Check subject diaries.

In-patient observation and IMP antimalarial treatment phase (Day 8-Day 11) Subjects will be admitted to the clinical unit for 72 hours on the morning of Day 8 (or earlier if a subject has a malaria clinical score >6 or at Investigator discretion) for OZ439 and PQP treatment and monitoring of clinical symptoms of malaria.

Admission

The following procedures will occur at admission to the clinical unit:

1. Performabbreviated physical examination
2. Perform alcohol breath test and drug screen
3. Elicit information regarding any new medical conditions or illnesses.

4. Record vital signs.
5. Obtain12-lead ECGin triplicate.
6. Collect urine for urinalysis.
7. Perform urine β-hCG pregnancy test forWOCBPsubjects
8. Cannulate subjects with an indwelling intravenous cannula.
9. Collect blood samples forhaematology, biochemistry(including CRPbiomarker), malaria 18S qPCR (parasite clearance baseline sample), and PK analysis (measureOZ439 and PQPlevels; PK baseline sample).

NOTE: As these test results may not be available before the drug administrations, the results of this time point will be used for future interpretation of study results.

10. Collect optional blood samplesfor immune cell characterisation and complement regulatory proteins(if consented separately)
11. Record malaria clinical score.
12. Recordand AEs and use of concomitant medications.

Treatment and observation

The following procedures will occur during treatment and observation:

1. Administer the appropriate doses of OZ439 and PQP to fasted(≥6 hours) subjects under direct observation, asdescribed in (Section 6.1.5)
2. Follow up subjects as in-patients for 72 hours to monitor safety and tolerability of the treatment and adequate clinical response.
3. Performsymptom-directed physical examinationwhen signs or symptoms of malaria are identified and it is clinically indicated.
4. Obtain12-lead ECG intriplicate at 4, 6, 8, 12, 24 and72 (D4) hours post-OZ439 and PQP administration.
5. Record vital signs 3 times a day whilst confined.
6. Record malaria clinical score 3 times a day whilst confined.
7. Collect blood samples for malaria 18S qPCR following treatment at 2, 4, 8, 12, 16, 20, 24, 30, 36, 42, 48, 60, and 72 hours postOZ439 and PQP administrationas per allowed time windows.
8. Collect blood samples for PK analysis ofOZ439 andPQPat 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 16, 24, 48, and 72 hours postOZ439 and PQPadministration as per allowed time windows
9. Collect optional blood samplesfor complement regulatory proteins(if consented separately)at approximately 48 hourspost-OZ439 and PQP treatment.
10. Recordand AEs and use of concomitant medications.

Prior to exit from the clinical unit

Subjects will be allowed to leave the clinical unit 72 hours post-OZ439 and PQP treatment at the Investigator's discretion.

The following procedures will occur prior to discharge from the clinical unit:

1. Perform symptom-directed physical examination at Investigators discretion
2. Record vital signs.
3. Collect blood samples for haematology, biochemistry (including CRP biomarker), PK analysis and malaria 18S qPCR (~72 hours post-OZ439 and PQP dosing).
4. Obtain 12-lead ECG in triplicate
5. Record AEs and use of concomitant medications.

Out-patient monitoring post-OZ439 and PQP treatment (Day 12 up to Day 42±2)

Follow-up at either AM (approximately 08:00) or AM and PM (if necessary, approximately 12 hours apart) will be undertaken on an out-patient basis through visits post-discharge for clinical evaluation and blood sampling.

The following procedures will take place during these visits:

1. Elicit information regarding any new medical conditions or illnesses.
2. Collect a blood sample for malaria 18S qPCR in the morning and in the evening on days 12 and 13 and then three times weekly or at Investigator's discretion based on parasitaemia until Day 42±2.
3. Collect blood samples for parasite lifecycle stage-qPCR at the Investigator's discretion (Section 7.2.2).
4. Collect blood samples for PK analysis of OZ439 and PQP at 72, 96±2, 120±48, 168±48, 240±48, 336±48, 504±48, 672±48 and 840±48 hours after OZ439 and PQP administration.
5. Collect blood samples for haematology and biochemistry (including CRP biomarker) and urine for urinalysis at 336±48, 504±48 and 672±48 hours post-OZ439 and PQP administration or at the Investigator's discretion.
6. Collect optional blood samples for complement regulatory proteins (if consented separately) at 96±2, 120±48, 168±48, 240±48 hours post-OZ439 and PQP
7. Collect optional blood samples for immune cell characterisation (if consented separately) at 168±48 hours post-OZ439 and PQP.
8. Perform symptom-directed physical examination when signs or symptoms of malaria are identified and it is clinically indicated.
9. Record vital signs.
10. Record malaria clinical score if vital signs are abnormal or at the Investigator's discretion.
11. Record AEs and use of concomitant medications.
12. Check subject diaries.

Rescue treatment with Riamet®

All subjects will receive a standard course of therapy with Riamet® (artemether-lumefantrine) on trial Day 42±2 or earlier in the event of failure of clearance or recrudescence of parasitaemia at Investigators discretion based on subject safety.

- Failure of clearance defined as failure to clear parasitaemia by at least 10-fold at 72 hours post-IMP administration
- Recrudescence: defined as ≥5 000 blood stage parasites/mL and a 2-fold parasitaemia increase within 48 hours, or re-occurrence of malaria symptoms with a malaria clinical score >6.)

Subjects may take the doses at the clinical unit or at home, as determined by the Investigator. Subjects will receive a phone call or text message from clinical unit staff to check on symptoms and ensure compliance and completion of treatment following the doses taken at home. Primaquine™ may also be administered at the time of Riamet® treatment (Section 6.1.5).

The following procedures will be performed prior to Riamet® treatment

1. Elicit information regarding any new medical conditions or illnesses.
2. Perform symptom-directed physical examination.
3. Record vital signs.
4. Collect blood samples for haematology, biochemistry (including CRP biomarker).
5. Collect blood samples for malaria 18S qPCR, and parasite lifecycle stage qRT-PCR (if required).
6. Collect blood samples for PK analysis of OZ439 and PQP if Riamet® treatment occurs on trial Day 42±2.
7. Record malaria clinical score if vital signs are abnormal or at the Investigator's discretion.
8. Record AEs and use of concomitant medications.
9. Obtain 12-lead ECG in triplicate.
10. Check subject diaries.

Follow-up post rescue treatment if Riamet® is administered prior to Day 42±2

For all subjects a follow-up visit will occur 3 days (+2 days) after initiation of Riamet treatment. The following procedures will be performed at this visit.

1. Perform symptom-directed physical examination.
2. Record vital signs.
3. Collect blood samples for haematology, biochemistry (including CRP biomarker).
4. Collect blood for malaria 18S qPCR (if the result is positive the subject will be followed up until a minimum of one negative qPCR is detected).

7.3.4 END OF STUDY VISIT (EOS)

The following procedures will be performed at the EOS visit after completion of Riamet® treatment (Day 45±2):

1. Elicit information regarding any new medical conditions or illnesses.
2. Perform full physical examination.
3. Record vital signs.
4. Collect blood samples for haematology, biochemistry (including CRP biomarker),
5. Collect blood for malaria 18S qPCR (if the result is positive the subject will be followed up until a minimum of one negative qPCR result is detected).
6. Collect blood for parasite lifecycle stage qRT-PCR (if required).
7. Collect urine for urinalysis.
8. Record malaria clinical score
9. Collect blood samples for RBC alloantibodies, serology and safety serum storage (2 serum samples).
10. Collect optional blood samples for immune cell characterisation and complement regulatory proteins (if consented separately).
11. Perform serum β-hCG pregnancy test for WOCBP subjects.
12. Record AEs and use of concomitant medications.
13. Obtain 12-lead ECG in triplicate.

See Section 8.3 for follow-up procedures for ongoing AEs/SAEs.

7.3.5 EARLY TERMINATION VISIT

If withdrawal occurs at any stage of the study, the subject will be asked to complete an EOS evaluation. In addition, subjects are informed of the essential requirement to complete the antimalarial drug treatment for their safety, via the Participant Information Sheet.

Participation in an early termination evaluation by each subject is voluntary. Procedures during the early termination visit are the same as for the Day 45±2/EOS visit (Section 7.3.4).

7.3.6 UNSCHEDULED VISIT

Unscheduled visits for malaria 18S qPCR or safety monitoring may be required at the Investigator's discretion based on parasitaemia, clinical symptoms or laboratory results. Subjects will be contacted by phone to arrange these visits. Where possible, visits will be arranged at a time that is both convenient for the subject and meets any clinical urgency as decided by the Investigator. Unscheduled visits will be documented in the source documents and eCRF.

7.3.7 SCHEDULE OF EVENTS TABLE

The Schedule of Events Table summarises the procedures to be conducted as per this protocol during screening, confinement and post-confinement. Section 7.1 and 7.2 provide detailed information on the procedures.

An experienced nurse will be in attendance at the clinical unit when subjects are on site and the Investigator will be available within approximately 30 minutes call back if required.

In the schedule of events, evaluation may be in the morning, between 6:00 AM to 11:00 AM, and in the afternoon, between 6:00 PM to 11:00 PM, therefore separated by approximately 12 hours.

Some safety and laboratory evaluation days may vary ±2 days based on qPCR counts and clinical unit visits at the Investigator's discretion.

Table 2: Schedule of Events

Procedure	Screening D-28 to D-1	Eligibility visit[a] D-3 to D-1	Malaria inoculation day D0	Post-inoculation phone contact D1 to D3	Malaria monitoring[b] D4 to D7	OZ439 and PQP treatment and clinical unit confinement D8 to D11	Out-patient monitoring D12 to D42±2	Rescue treatment with Riamet® D42±2 or earlier if required	EOS visit D45±2
Informed consent & BDI	X								
Medical history, eligibility & prior medications	X		X						
Drug & alcohol screen	X		X			X			
Full physical examination	X								X
Abbreviated physical examinatic			X			X[r]			
Symptom-directed physical examination[n]					X	X	X	X	
Vital sign assessment	X		X		X	X	X	X	X
ECG	X		X			X[j]		X	X
Urinalysis	X	X				X[k]	X		X
Haematology & biochemistry	X	X				X	X	X	X
Coagulation profile[e]	X								
G6PD testing	X								
RBC alloantibody	X								X
Serology	X								X
Serum β-hCG pregnancy test	X[d]	X							X
Urine β-hCG pregnancy test			X			X			
Safety serum storage			X						X
AEs & concomitant medications			X	X	X	X	X	X	X
Malaria clinical score			X		X	X	X[l]	X	X
Malaria 18S qPCR blood sampling			X		X	X	X[m]	X	X
Parasite lifecycle stage qRT-PCR blood sampling[f]							X	X	X
OZ439 and PQP concentration blood sampling						X	X	X[s]	
Phone call or text message								X[n]	
Malaria inoculum			X[g]						
OZ439 and PQP treatment						X			
Riamet® treatment[h]								X	
Primacin™ treatment[i]								X	

Immune cell characterisation (optional)			X[o]		X[p]	X[q]	X		X
Complement regulatory proteins (optional)			X[o]			X[q]	X		X

PQP: piperaquine phosphate, EOS: End of Study, BDI: Beck Depression Inventory, ECG: electrocardiogram, G6PD: glucose-6-phosphate dehydrogenase, RBC: red blood cell, PK: pharmacokinetic, PD: pharmacodynamic, qPCR: quantitative polymerase chain reaction, qRT-PCR: reverse transcription qPCR

[a] An additional safety visit will occur between Day 3 and Day -1 to collect samples for haematology, biochemistry and urinalysis, unless screening laboratory assessments were conducted within this period.

[b] Daily visits until qPCR positive, and then twice daily visits until OZ439 and PQP treatment.

[c] Symptom-directed physical examinations will be performed only if clinically indicated at the discretion of the Investigator (see Appendix 3 for a list of symptoms and signs of malaria).

[d] Perform follicle stimulating hormone test (post-menopausal females) at screening.

[e] To be performed at screening visit.

[f] May be performed at the Investigator's discretion, most probably pre and post administration of rescue medication to measure the level of gametocytaemia.

[g] Cannulate subjects with an indwelling intravenous cannula for malaria inoculum, and record which arm is utilised. Cannulate subjects with an indwelling intravenous cannula for the malaria inoculum, and record which arm is utilised. Administer the malaria inoculum of ~2 800 viable P. falciparum 3D7 infected human RBCs intravenously in the morning (approximately 9:00 AM). Observe for a minimum of 60 minutes after inoculation to evaluate for immediate adverse reactions. Educate subjects on signs and symptoms of malaria (Appendix 3). Emphasise to subjects the importance of returning on the nominated day (approximately Day 8), or as advised by the clinical unit staff, for IMP antimalarial treatment. Provide subjects with diary cards and thermometers to record temperature readings during the study in the event of symptoms of fever. Subjects will also record symptoms and concomitant medications on the diary cards during the study.

[h] Riamet® treatment will occur on Day 42 ± 2, or earlier if there is failure of clearance defined as failure to clear parasitaemia by at least 10-fold at 72 hours post-IMP administration or recrudescence of parasitaemia (defined as ≥5 000 blood stage parasites/mL and a 2-fold increase within 48 hours, or a malaria clinical score ≥ 6), or at the Investigator's discretion.

[i] Primacin™ treatment if required (Section 6.1.5)

[j] ECG to be performed in triplicate at 4, 6, 8, 12, 24 and 72 hours post-OZ439 and PQP dosing, and prior to Riamet treatment or at the Investigator's discretion

[k] At the time of admission to clinical unit only.
[l] Only if vital signs are abnormal, or at the investigator's discretion.
[m] 18s malaria to be performed Day 12 (am & pm), Day 13 (am & pm) then 3x per week until EOS or Investigators discretion
[n] Phone call for 3 days to ensure adherence to Riamet® treatment
[o] Blood collection pre-inoculation
[p] Blood collection Day 4 visit
[q] Blood collection pre-OZ439 and PQP dosing
[r] Abbreviated physical examination pre-OZ439 and PQP dosing
[s] Blood collection for OZ439 and PQP PK at Day 4 +/- 2. If Riamet® treatment occurs prior to Day 4 -2 the PK collection will continue as per scheduled time-points

7.4 JUSTIFICATION FOR SENSITIVE PROCEDURES

Not applicable.

7.5 CONCOMITANT MEDICATIONS, TREATMENTS, ANDPROCEDURES

Concomitant medications, treatments and procedures are those occurring from malaria inoculation until the end of the study (last visit). Those occurring prior to inoculation are classified as prior medications, treatments and procedures. Medications taken in the 28 days before the malaria inoculation will be recorded as prior medication. Prior and concomitant medications, treatments and procedures permitted in this study are outlined in the inclusion/exclusion criteria (Section 5.1 and 5.2).

On inoculation day, subjects will be questioned in relation to relevant aspects of compliance with the study protocol, including drug intake since their screening visit. Details of all other drugs taken (prescription and over-the-counter, systemic and topical administration) will be recorded at this time and appropriate action taken. The investigator may permit the use of ibuprofen up to 1.2 g/day or paracetamol up to 4 g/day, 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 subjects and as such is not a prohibited substance. Any medication taken from inoculation day to the end of the study (last visit), for treatment of a medical condition or AE is to be recorded in the concomitant medication pages in the eCRF. The exact dose and timing of each dose should be recorded.

7.5.1 PRECAUTIONARY MEDICATIONS, TREATMENTS, AND PROCEDURES

Not applicable.

7.6 PROHIBITED MEDICATIONS, TREATMENTS, ANDPROCEDURES

Subjects will be informed and reminded of the following restrictions during recruitment, the informed consent process, and during screening and other assessments:

- Subjects should not consume grapefruit or Seville oranges from inoculation day until the end of the study.
- Subjects should not consume quinine containing foods/beverages such as tonic water, lemon bitter, from inoculation day until the end of the Riamet® treatment.
- Subjects should not eat any poppy seeds in the 24 hours before the following points: screening, inoculation day, and day of admission for OZ439 and PQP treatment.

- Subjects should not eat or drink any food or beverages that contain alcohol (e.g. beer, wine, and mixed drinks) during confinement at the clinical unit and also 24 hours prior to each alcohol breath test. Subjects should not drink more than 2 standard drinks per day from 24 hours before inoculation until the end of the Riamet® treatment.
- Subjects should not consume beverages that contain xanthine bases (e.g. Red Bull, coffee) during confinement at the clinical unit. Subjects should not consume more than 400 mg caffeine per day, equivalent to more than 4 cups of coffee, from inoculation until the end of the Riamet® treatment.
- Subjects should not use tobacco during confinement at the clinical unit. Subjects should not smoke more than 5 cigarettes or equivalent per day from inoculation until the end of the Riamet® treatment.

7.7 PROPHYLACTIC MEDICATIONS, TREATMENTS, AND PROCEDURES

Not applicable.

7.8 RESCUE MEDICATIONS,TREATMENTS, AND PROCEDURES

The rescue medications used in this study are Riamet® and Primacin™ (if required). See details in Section 6.1. If a subject vomits or cannot tolerate oral drugs, then artesunate will be administered intravenously as described in Section 6.1.5.

7.9 SUBJECT ACCESS TO STUDY AGENT AT STUDY CLOSURE

Not applicable.

8 ASSESSMENT OF SAFETY

8.1 SPECIFICATION OF SAFETY PARAMETERS

Safety of a single combined dose of OZ439 and PQP will be evaluated by the incidence, severity and relationship of observed and self-reported AE's, AESI's and SAE's. Other safety parameters monitored during this study include physical examination, vital signs, clinical biochemistry, haematology, urinalysis, triplicate ECG, serology, RBC alloantibody testing, and malaria clinical score. See Sections 7.1 and 7.2 for details of these procedures. All safety parameters will be recorded in the eCRF. Safety monitoring will be specified in a safety management plan.

8.1.1 DEFINITION OF AES (AE)

An AE is defined as any untoward medical occurrence, i.e., any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease that occurs in a subject during the

course of the study and which does not necessarily have a causal relationship with this treatment (i.e., whether or not considered drug-related).

A treatment-emergent AE (TEAE) is an event that emerges following treatment with the investigational medicinal products (IMPs, OZ439 and PQP), having been absent pre-treatment, or worsens relative to the pre-treatment state.

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

Borderline abnormal laboratory findings and other objective assessments should NOT be routinely captured and reported as AEs, as they will be collected and analysed separately. 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 the Investigator or Sponsor 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 which may, or may not, be AEs.

Certain information, although not considered an AE, must be recorded, reported, and followed up as indicated for an SAE (see Section 8.4.2 Serious AE Reporting). This includes:

- Pregnancy exposure to an IMP. If a pregnancy is confirmed, use of the IMP must be discontinued immediately. Information about pregnancy exposure includes the entire course of pregnancy and delivery, and perinatal and neonatal outcomes, even if there are no abnormal findings. Both maternal and paternal exposures are considered other reportable information. For exposure involving the female partner of a male subject, the necessary information must be collected from the subject, while respecting the confidentiality of the partner.
- Lactation exposure to an IMP with or without an AE.
- Overdose of an IMP as specified in this protocol with or without an AE.
- Inadvertent or accidental exposure to an IMP with or without an AE

8.1.2 DEFINITION OF SERIOUS AES (SAE)

A seriousAE (SAE) is defined as an AE which fulfils at least one of the following criteria:

Results in death.
Is life-threatening

- The term "lifethreatening" in the definition of "serious" refers to an event in which the subject 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 preplanned treatment or standard monitoring for a pre-existing condition that is unrelated to the study and has not worsened since the start of the study.
- Cosmetic surgery, or for social reasons, or respite care in the absence of any deterioration in the subject's general condition.

Results in persistent or significant disability/incapacity.
Is a congenital abnormality or 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 lifethreatening but may jeopardise the subject 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.

Constitutes a possible Hy's Law case

- Hy's Law case is defined as a subject 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]).

A Suspected Unexpected Serious Adverse Reaction (SUSAR) is any SAE where a causal relationship with the malaria inoculum (3D7) or the IMPs (OZ439 and PQP) is at least a reasonable possibility, and the event is not listed in the IB(s) and/or Summary of Product Characteristics.

8.1.3 DEFINITION OF AES OF SPECIAL INTEREST (AESI)

An AESI (serious or non-serious) is one of scientific and medical concern specific to the sponsor's product or programme, for which ongoing monitoring and rapid communication by the Investigator to the sponsor 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 AESI:

Hepatic:

- any ALT or AST above 5xULN
- an elevation in bilirubin 2xULN
- any AST or ALT above 2xULN and (TBL > 1.5x ULN or INR > 1.4)
- any AST or ALT above 2xULN 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)

Cardiac:

- QTcB or QTcF at any time >480 msec,
- bundle branch block (except right bundle branch block that was present prior to IMP 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, and
        - associated with a body temperature >38.0°C, and
        - not associated with any other relevant ECG abnormalities
    - respiratory sinus arrhythmia,

- wandering atrial pacemaker,
- 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:
- HB drop >2.0 g/dL from baseline prior to inoculation
- Absoluteneutrophil count ≤500/µl.
- Platelet count ≤75,000 /mm3

Dermatological:*
Clinical signs of possible cutaneous adverse reactions such as:
- dermatitis,
- rash,
- erythematous rash,
- macular rash,
- papular rash,
- maculopapular rash,
- pruritic rash,
- pustular rash,
- vesicular rash

* if one of these cutaneous reaction is observed and when feasible, pictures of the lesions should be obtained

8.2 CLASSIFICATION OF AN AE

8.2.1 SEVERITY OF EVENT

In addition to determining whether an AE fulfils the criteria for a SAE or not, the severity of AEs experienced by study subjects will be graded according to the Common Terminology Criteria for AEs v4.03published 14 June 2010 (CTCAE v4.03). This guidance provides a common language to describe levels of severity, to analyse and interpret data, to scale the aggregate AE score, and to articulate the clinical significance of all AEs.

The severity of AEs willbe 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 (see Section 8.1.2). These terms are used to describe the intensity of a specific event. Medical judgment should be used on a case-by-case basis.

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

For guidance for assigning severity of the malaria, the purpose-designed malaria clinical score will be used (Section 7.1.1).

8.2.2 RELATIONSHIP TO STUDY AGENT

The Investigator will decide if AEs are related to any of the study agents or procedures. Where possible, a distinction should be made between events considered related to the malaria challenge agent, the IMPs, rescue treatments, or other protocol-mandated procedures. The assessment of causality will be made using the following definitions:

Unrelated

This category is applicable to those AEs which are judged to be clearly and incontrovertibly due to extraneous causes (disease, environment, etc.) and do not meet the criteria for the relationship listed under unlikely, possible or probable.

Unlikely

In general, this category is applicable to an AE which meets the following criteria (must have the first two):

1. It does not follow a reasonable temporal sequence from administration of any of the study agents.
2. It may readily have been produced by the subject's clinical state, environment or toxic factors, or other modes of therapy administered to the subject.
3. It does not follow a known pattern of response to the study agents.
4. It does not reappear or worsen when any of the study agents are re-administered.

Possible

This category applies to those AEs in which the connection with any of the study agents appears unlikely but cannot be ruled out with certainty. An AE may be considered possible if or when (must have the first two):

1. It follows a reasonable temporal sequence from administration of any of the study agents.
2. It may have been produced by the subject's clinical state, environment or toxic factors, or other modes of therapy administered to the subject.
3. It follows a known pattern of response to any of the study agents.

Probable

This category applies to those AEs which are considered, with a high degree of certainty, to be related to the study agents. An AE may be considered probable if (must have the first three):

1. It follows a reasonable temporal sequence from administration of any of the study agents.
2. It cannot be reasonably explained by the known characteristics of the subject's clinical state, environment or toxic factors, or other modes of therapy administered to the subject.
3. It disappears or decreases on cessation or reduction in dose.
4. It follows a known pattern of response to any of the study agents.
5. It reappears on re-administration.

8.2.3 EXPECTEDNESS

An AE is regarded as an unexpected event if its nature or severity is not consistent with the applicable reference safety information (IB or approved manufacturer's prescribing information for marketed drugs). Events that add significant information on the specificity, severity or frequency of previously described reactions are also regarded as unexpected.

Expected AEs from the malaria infection are listed in Appendix 3 and the IB for the 3D7 inoculum [35]. Expected AEs from the antimalarial drugs used are listed in OZ439 IB [38] and the EMA European Public Assessment Report for PQP, Riamet® Consumer Medicine Information, Primacin™ Consumer Medicine Information, and artesunate WHO Public Assessment Report 2011 (see Appendix 1).

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

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

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

Events that are unresolved at the time of the subject's last follow-up visit should continue to be followed up by the Investigator for as long as medically indicated. The Sponsor retains the right to request additional information for any subject with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

All AEs should be treated appropriately. The 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).
- Dosing with the IMP is withheld and the subject withdrawn from the study.
- Administration of a concomitant medication.
- Hospitalisation or prolongation of current hospitalisation (event to be reported as an SAE).
- Other.

In a case of occurrence of SAEs, regardless of whether or not it is judged to be challenge agent or antimalarial drug-related, the subject will receive appropriate care under clinical supervision until all the symptoms of the SAEs have diminished or resolved and the subject's condition improved.

For ongoing AEs, care will be provided for a period of time as specified in the clinical site work instruction protocols. However, if the nature of the ongoing AE is determined by the Investigator as not being inoculum- or antimalarial drug-associated, the subject will be advised to visit his/her own general practitioner for further clinical care that he might require.

8.4 REPORTING PROCEDURES

8.4.1 AE REPORTING

It is the Investigator's responsibility to document and report all AEs occurring in the study whether spontaneously reported by the subject, observed by the 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 up to the end of the study; 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 study.

The following information should be recorded for all AEs:

- Description of the AE.
- Dates and times of onset and resolution of the event.
- Duration of the event in hours.
- The time of onset relative to inoculum, or the OZ439 and PQP treatment and/or the antimalarial rescue drugs
- Seriousness of the AE (SAE or not).
- Severity of the event.
- Action taken in response to the event (including treatment required).
- Outcome of the event.
- Relationship of the event to the study agents or procedures (causality assessment), including inoculum, IMPs, rescue medication, or any other treatment or procedure conducted during the study.
- Changes in the severity of an AE will be documented to allow assessment of the duration of the event at each level of severity.
- AEs worsening in severity will be considered unresolved and those reducing in severity will be considered resolving.
- AEs characterised as intermittent require documentation of onset and duration at each episode.
- Only one AE is reported if there is a variation in intensity (with highest intensity for final CSR Tables): the description will also report the various severities over time,
- If the AE resolves and then reoccur at a later date: then two AEs are reported
- All malaria-specific AEs will be tabulated and results graded according to a purpose-designed malaria clinical score table (Section 7.1.1).

8.4.2 SERIOUSAE REPORTING

The Investigatorwill take immediate appropriate action in response to SAEs to ensure subject safety and in an attempt to identify the causes of the event. The Investigatorwill notify Prime Vigilance, of any SAE within24 hours of becoming aware of the event. The notification should be in writing by email or fax, and documented on a standard SAE reporting form.

SeriousAE Reporting:
Prime Vigilance
Email: MMV@primevigilance.com
Fax: +44 800 471 5694

Medical Director
Stephan Chalon, MD, PhD
Medicines for Malaria Venture
Route de Pré-Bois 20
1215 Geneva 15
Switzerland
Email: chalons@mmv.org Representative

Medical Monitor:
Dr. med Michael Marx MD
Medical Director
ICON Clinical Research
Internal Tel: 7123 1568
ExternalTel : +49 6103 904 1568
Mobile : (+49) 172 677 7401

Email:STUDY-MA-DL-3037-0010-Blinded@iconplc.com

The Investigatorwill complete a follow-up SAE report within 14 days of the SAE, unless no further information is available in which case the follow-up report will be provided as soon as new information becomes available. The follow-up SAE report will be sent to the same recipients as the initial report as described above. Other supporting documents may be requested by these parties and will be provided by the Investigator or a delegate as soon as possible.

Any SAE that meets the criteria of a SUSAR (Section 8.1.2) will be reported to the TGA by the local sponsor CNS in accordance with the Sponsor's reporting procedures.

8.4.3 UNANTICIPATED PROBLEM REPORTING

Not applicable.

8.4.4 AES OF SPECIAL INTEREST (AESI)

All AESIs, including those that do not meet the definition of an SAE, must be notified to the Pharmacovigilance provider (Prime vigilance) within 24 hours of the investigator becoming aware of the occurrence of the AESI. The notification should be in writing by email or fax (Prime vigilance contact details mentioned above in 8.4.2 section) and documented on a standard AESI reporting form. Within 1 business day of receipt of any safety reports (initial or follow-up reports), Prime vigilance will notify the medical monitor (with the Sponsor medical monitor in copy), the sponsor and the DSRT of the event and its follow-up and will include all available information.
The notification of follow-up information will follow the same procedure and timelines as the initial report.

8.4.5 REPORTING OF PREGNANCY

Pregnancy in a female subject or in a male subject's female partner during the study should be reported and followed as described beneath. Pregnancy does not constitute an AE as such and the pregnancy outcome will not be recorded in the eCRF unless it is considered to be an AE.

The Investigator must notify Prime Vigilance, in an expedited manner of any pregnancy occurring from the date of informed consent signature until 90 days after administration of OZ439 and PQP. The same process as described for AEs and AESI as described in Section 8.4.2 should be followed.
In all cases, the pregnancy must be followed until birth of the child, and the outcome of the pregnancy and birth reported as above by completing the appropriate section of the Pregnancy Report Form used for the initial notification. The timelines of the outcome reporting vary as follows:

- Normal outcomes should be reported within 45 days of birth/delivery
- Abnormal outcomes should be reported in an expedited manner as described in Section 8.4.2.

An additional SAE Report form must be completed if the subject or subject's partner sustains a serious event. A Parent-Child/Foetus Report (PCFR) must be completed if the child/foetus sustain an event.

8.5 STUDY HALTING RULES

See Section 5.5.

8.6 SAFETY OVERSIGHT

Safety oversight will be undertaken by the Principal Investigator and the Medical Monitor who will serve as an independent expert to advise on clinical safety specifically in the situation where expert external advice is required regarding the need for administration of alternative/rescue antimalarial treatment in the circumstance of sub-optimal response.

The SDRT will be responsible for decisions related to the safety of subjects and the continuation of the study. The role and composition of the DRT is outlined in the study specific DRT Charter. The SDRT will be composed of the Principal Investigator, Medical Monitor, and a physician with expertise in clinical trials or infectious diseases. The DRT will review the clinical and laboratory safety data as well as the recorded AEs and SAEs. The DRT makes recommendations to the Sponsor. These recommendations are approved by the DRT Chair who signs a letter of recommendation that is sent to the Principal Investigator and the Sponsor.

A review by the SDRT of data from each cohort will be conducted prior to dosing the subsequent cohort. Safety and tolerability data up to Day 42±2, and PK/PD analysis outcomes (based on PD data up to Day 42±2 and PK data up to Day 35±2) from all subjects who received treatment with OZ439 and PQP will be required for the review. A similar analysis will be done at the end of cohort 2 combining cohorts 1 and 2 data to decide the dose(s) to be tested in cohort 3. This will be decided by the funding sponsor and the Principal Investigator following review of the data by the SDRT and scientific evaluation.

Additionally, the SDRT may meet to assess any events that trigger the stopping rules or as needed to provide a recommendation and findings to QIMR Berghofer HREC and the Principal Investigator, in accordance with the approved DRT Charter.

Whether at a scheduled or unscheduled meeting, the DRT will consider safety signals to determine whether or not they can recommend that the study continue.

The SDRT will also review the safety and tolerability data from the study after completion of the last cohort.

9 CLINICAL MONITORING

It will be the Sponsor's responsibility to ensure that the study is monitored in accordance with the requirements of GCP. The conduct of the study will be reviewed internally by the clinical unit (Q-Pharm) in accordance with their standard procedures and work instructions, and GCP guidelines. The study will be monitored according to the SOPs of the monitoring CRO appointed this task by the Sponsor and all protocol deviations will be reported to the Sponsor. Protocol deviations that impact subject safety or data integrity will also be reported to the QIMR Berghofer HREC.

During the study, appointed study monitor(s) (on behalf of the Sponsor) will visit the site to check completeness of subject records, accuracy of eCRF entries, adherence to the protocol and to GCP, progress of enrolment, and to ensure that study agents were stored, dispensed, and accounted for according to specifications. Key study personnel are required to be available to assist the study monitor during these visits.

The Investigator will be required to give the monitor access to all relevant source documents to confirm their consistency with the eCRF entries. The Sponsor will require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of SAEs, and the recording of data that is 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 study specific monitoring plan. No information in source documents about the identity of the subjects will be disclosed.

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL ANALYSIS PLAN

The following sections describe the statistical analysis as it is foreseen when the study is being planned. A detailed Statistical Analysis Plan (SAP) will be finalised and approved prior to database lock and 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.

For the safety primary endpoint, we hypothesise that inoculation with ~2 800 viable *P. falciparum* 3D7 parasite-infected RBCs and treating with OZ439 and PQP on trial Day 8 will be safe and will result in no SAEs, and will not cause severe malaria symptoms.

This study is not powered to compare IMP dosages but rather to answer the primary objectives.

10.2 ANALYSIS DATASETS

The safety analysis dataset will include all subjects who receive the malaria inoculum. This population will be used to analyse all safety data as well as demographic and baseline data.

The population(s) used for analysis of PK, PD and PK/PD analysis will be defined in the SAP.

10.3 DESCRIPTION OF STATISTICAL METHODS

10.3.1 GENERAL APPROACH

This is an adaptive dose finding study using the IBSM model to characterise the PK/PD relationship between OZ439 and PQP for the treatment of early *P. falciparum* blood stage infection.

Continuous data will be summarised using descriptive statistics (mean and standard deviation, or median and interquartile range). Categorical data will be presented using the number (N) and percentage (%) (using the number of subjects without missing data in the calculation).

10.3.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

A model-based analysis is foreseen to characterise the PK/PD relationship between QZ439 and PQP plasma concentrations and blood stage asexual parasitaemia. For assessing the contribution of each compound and their interaction effect, data of this combination therapy study, will be pooled with data from previous human challenge monotherapy studies for each compound, i.e. QP12C01 (OZ439) and QP13C05 (Piperaquine).

The final PK/PD model will describe the time courses of plasma concentrations and of the parasite counts by a nonlinear mixed effects (NLME) modelling approach. The change in parasite count will be modelled as the joint effects of parasite growth and drug concentration.

The model will be developed in step wise manner. First, a joint PK model for OZ439 and piperaquine will be established potentially taking PK drug-drug interaction into account. Second, the PD model will be developed using individual PK parameters as regressors. The effect of each compound on parasite clearance is described by a sigmoidal E_{max} model or related models to take into account time delays of the drug action if appropriate. PD interactions between OZ439 and PQP will be described using the General PD Interaction (GPDI) model. PD interaction scenarios to be tested with the GLDI model include a change in each drug's EC_{50}, E_{max} or EC_{50} and E_{max} caused by the other drug.

Graphical displays will be given, where appropriate. Details of the modelling analysis will be described in the modelling analysis plan (MAP).

10.3.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

The secondary endpoints are PD and PK parameters.

PK parameters

Estimation of OZ439 and PQP PK parameters over 28 days after administration of single doses using non-compartmental methods:

The following pharmacokinetic parameters will be determined using non-compartmental methods from plasma concentration-time data from all cohorts: $AUC_{0\text{-}168h}$, AUC_{last}, $AUC_{0\text{-}inf}$, C_{max}, t_{max}, $t_{1/2}$, t_{lag}, C_{168h}, CL/F, Vz/F and λ_{inf}. These data will be summarized descriptively by treatment and dose.

For calculation of descriptive statistics of blood concentrations, values below the LLOQ will be set to zero.

Pharmacokinetic parameters will be determined using STATA® (version 14.0 or higher).

PD parameters

Parasite Reduction Ratio (PRR). The PRR of asexual parasites will be based on the decay of parasitaemia after drug treatment determined by malaria 18S qPCR. The PRR for asexual parasites will be estimated using the slope of the optimal fit of the log-linear relationship of the parasitaemia decay[45]. The optimal fit can 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 of qPCR data, will be calculated for each subject. The overall cohort dose specific PRR 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 subjects who have optimal regression models with appropriate fit contribute toward the dose-specific PRR. Details are presented in the SAP.

Parasite clearance half-life ($t_{1/2}$). The $PT_{1/2}$ will be derived from the optimal decay rate. Details regarding the calculation will be in the SAP.

Percentage of subjects with recrudescence of parasitaemia. The percentage of subjects with recrudescence of parasitaemia following treatment with OZ439 and PQP. This will be determined by the number of subjects who experience recrudescence which is defined as ≥5 000 blood stage parasites/mL and a 2-fold parasitaemia increase within 48 hours, or recurrence of malaria symptoms with a malaria clinical score >6. This will be determined by parasite lifecycle qRT-PCR.

10.3.4 SAFETY ANALYSES

The overall number and percentage of subjects with at least one AE (and SAE) will be tabulated over the entire study period. All AE data will be summarised by pooled treatment group and study period, i.e. Day 0 to Day 8 (inoculum), Day 8 after dosing or earlier to Day 42 ±2 (OZ439 and PQP) and Day 42 ±2 after dosing (Rescue treatment) to (EOS visit).

Treatment emergent AE's will also be summarised with frequency counts by MedDRA system organ class (SOC; i.e. body system) and preferred term (PT), for each pooled treatment group and by Study period.

Vital signs, routine safety laboratory data and ECG parameters will be presented in data listing and will be summarised descriptively by treatment group and by protocol specified time point.

Where applicable, both absolute values and change from baseline (inoculation and IMPs administration) will be presented and listings of clinically relevant abnormal laboratory results will be generated.

The safety and tolerability of a single combined dose of OZ439 and PQP will be evaluated by the incidence, severity and relationship of observed and reported AEs up to trial Day 42±2.

10.3.5 ADHERENCE AND RETENTION ANALYSES

Not applicable.

10.3.6 BASELINE DESCRIPTIVE STATISTICS

Demographic data will be summarised by descriptive statistics and will include total number of observations (n), mean, standard deviation (SD) and range for continuous variables and number and percentages with characteristics for dichotomous variables.

The subject disposition will be summarised. Study completion, study withdrawals, exclusions and violations will be summarised and the reasons for withdrawal, exclusions and violations will be listed.

Medical history, current medical conditions, previous and concomitant medications, results of laboratory screening tests, drug and alcohol screening tests and any other relevant baseline information will be listed by subject and cohort.

10.3.7 PLANNED INTERIM ANALYSES

10.3.7.1 SAFETY REVIEW

A review of the safety and tolerability data from the preceding cohort will be conducted by the SDRT prior to inoculation of the next cohort. All safety and tolerability data up to and including Day 35±2 will be required for each review.

See Section 5.5 for study stopping rules.

10.3.7.2 EFFICACY AND PK REVIEW

A review of the parasitaemia data and PK data analysis from the preceding cohort will be conducted by the SDRT prior to inoculation of the next cohort. All data up to and including Day 42±2 will be required for each review. A review of the PK/PD analysis based on PD data up to Day 42±2 and PK data up to Day 35± 2 post-dose from the preceding cohort(s) will be conducted by the SDRT prior to inoculation of the next cohort.

10.3.8 ADDITIONAL SUB-GROUP ANALYSES

Not applicable.

10.3.9 MULTIPLE COMPARISON/MULTIPLICITY

Not applicable.

10.3.10 TABULATION OF INDIVIDUAL RESPONSE DATA

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

10.3.11 EXPLORATORY ANALYSES

No formal statistical analyses are planned for exploratory endpoints.

10.4 SAMPLE SIZE

A total of up to24 healthy subjects will be enrolled in the study (up to 3 cohorts of 8 subjects each). Subjects will be malaria naïve healthy male or female adults, aged between 18 and 55 years old, who meet all of the inclusion criteria and none of the exclusion criteria.

In each cohort, if more than 2 discontinuations due to safety related reasons occur; additional subjects may be recruited to replace the discontinued subjects on agreement with the study sponsor.

Historically, 8 participants in a dose has proven to be sufficient to characterise the effect of a drug on malaria parasite kinetics following induction of IBSM of healthy subjects.

10.5 MEASURES TO MINIMIZE BIAS

10.5.1 ENROLLMENT/ RANDOMIZATION/ MASKING PROCEDURES

Randomisation will be performed for subjects in all cohorts. The 8 subjects of all cohorts will be randomised to one of the dose groups for each cohort (see Table 1 and 2 Section 4.1) after inoculation day but prior to Day 8.

The randomisation schedule will be generated by a statistician using a validated system. A copy of the randomisation schedule will be sent to the clinical unit pharmacist and clinical unit project manager.

10.5.2 EVALUATION OF SUCCESS OF BLINDING

Not applicable.

10.5.3 BREAKING THE STUDY BLIND/SUBJECT CODE

Not applicable.

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The Investigator will maintain source documents for each subject in the study. Information entered into eCRFs will be traceable to these source documents in the subject's file at the site. The Investigator must certify that the data entered into the eCRFs are complete and accurate. After database lock, the Investigator will retain copies of the subject data for archiving at the investigational site.

Upon request, the Investigator(s)/institution(s) will permit direct access to source data/documents for trial-related monitoring, audits, Ethics Committee review, and regulatory inspection(s) by the Sponsor (or their appropriately qualified delegate) and Regulatory Authorities. Direct access includes examination, analysis, verification and reproduction of records and reports that are important to the evaluation of the trial.

12 QUALITY ASSURANCE AN D QUALITY CONTROL

Data management, including the development and management of a secure database, will be performed in accordance with regulatory requirements. CNS will review the data entered into the eCRFs by clinical unit staff for completeness and accuracy. A formal querying process will be followed whereby the data management team will request the clinical site staff to clarify any apparent erroneous entries or inconsistencies and will request additional information from the clinical 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 are to be coded using the WHO-DDE dictionary (March 2014 or later).

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

Clinical monitoring will be conducted as described in Section 9.

Audits may be carried out by Sponsor quality assurance representatives, local authorities or authorities to whom information on this study has been submitted. All documents pertinent to this study must be made available for such inspections after adequate notice of intention to audit.

13 ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 ETHICAL STANDARD

The study will be conducted in accordance with the protocol approved by the QIMR Berghofer HREC, 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 May 2015) [48] and the Note for Guidance on Good Clinical Practice – Annotated with TGA Comments (CPMP/ICH/135/95), as adopted by the Australian Therapeutic Goods Administration (July 2000) [47].

The Investigator will minimise any discomfort experienced by subjects during the study. The only invasive procedures will be the intravenous inoculation of the malaria inoculum and the blood collection by cannulation/venepuncture. The maximum amount of blood to be collected from an individual in the study would be up to approximately 495 mL (Appendix 2)

Blood Volume	Time-frame	mL (approximately)
Main study	Screening to Day 30	239
Optional components	Screening to Day 30	173
Total	Screening to Day 30	412
Main study	Screening to Day 45 (EOS)	297
Optional components	Screening to Day 45 (EOS)	201
Total	Screening to Day 45 (EOS)	497

The total volume of blood drawn from each subject will not exceed 450 mL in any 30-day period. This volume includes allowance for unscheduled safety and qPCR assessments that may be required at the discretion of the Principal Investigator or the Sponsor to ensure subject safety.

13.2 ETHICAL REVIEW

The protocol, Participant Information Sheets and informed consent forms will be reviewed by the QIMR Berghofer HREC, and no study activities will be initiated prior to approval from the QIMR Berghofer HREC. All amendments and addenda to the protocol and consent forms will similarly be submitted to the QIMR Berghofer HREC for approval prior to their implementation.

Changes to the final study protocol can only be made with the prior consent of the Principal Investigator, the Sponsor and the QIMR Berghofer HREC. All such changes must be attached to, or incorporated into, the final protocol, and communicated to all relevant members of the QIMR staff and, if appropriate, to study subjects. All deviations from this study protocol will be included in the trial master file and included in the CSR. An assessment of the significance of each protocol deviation will be given in the CSR. All deviations/amendments will be reported to Sponsor and the QIMR Berghofer Project Manager. The different types of amendments are discussed below.

Non-substantial amendment

Administrative or logistical minor changes require a non-substantial amendment. Such changes include but are not limited to changes in study staff or contact details (e.g., Sponsor instead of CRO monitors) or minor changes in the packaging or labelling of study drug. An amendment deemed to be non-substantial must have no ethical implications.

The implementation of a non-substantial amendment may be done without notification to the QIMR Berghofer HREC. It does not require their approval or to be signed by the Investigator. The QIMR Berghofer HREC will be notified of these non-substantial changes in the next submission round, with the annual study report or study close out report, which ever will be submitted first to QIMR Berghofer HREC.

Substantial amendment

Significant changes require a substantial amendment. Significant changes include but are not limited to: new data affecting the safety of subjects, change of the objectives/endpoints of the study, eligibility criteria, dose regimen, study assessments/procedures, treatment or study duration, with or without the need to modify the Participant Information Sheet and Informed Consent.

Substantial amendments are to be approved by the QIMR Berghofer HREC. The implementation of a substantial amendment can only occur after formal approval by the QIMR Berghofer HREC and must be signed by the Investigator.

Urgent amendment

An urgent amendment might become necessary to preserve the safety of the subjects included in the study. The requirements for approval should in no way prevent any immediate action being

taken by the Investigator or the Sponsor in the best interests of the subjects. Therefore, if deemed necessary, an Investigator can implement an immediate change to the protocol for safety reasons. This means that, exceptionally, the implementation of urgent amendments will occur before submission to and approval by the QIMR Berghofer HREC.

In such cases, the Investigator must notify the Sponsor within 24 hours. A related substantial amendment will be written within 10 working days and submitted to the QIMR Berghofer HREC, 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 Principal Investigator or the Sponsor want to perform testing on the samples that is not described in the protocol, additional QIMR Berghofer HREC approval will be sought. This may be done if a subject has consented to blood storage for use in future research (Section 13.3.1)

13.3 INFORMED CONSENT PROCESS

13.3.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO SUBJECTS

The Participant Information Sheet and informed Consent Form describes in detail the study agents, study procedures, and risks. Subjects will also receive an Informed Consent for Blood Storage and an option to grant permission to be contacted about future study involvement.

Details regarding the optional study components will be provided in a separate Participant Information Sheet and subjects agreeing to participate in these components will provide specific written consent for this. Subjects will also receive an Informed Consent for Blood Storage and an option to grant permission to be contacted about future study involvement. Refusal to participate in the optional study components will not jeopardise a subjects' participation in the main study.

Subjects will also receive the product insert for artesunate, and the Consumer Medicine Information for Riamet® and Primaquine™ (if required; Appendix 1). Subjects may also receive the Consumer Medicine Information for any other registered antimalarial agents in the trial if these are required.

13.3.2 CONSENT PROCEDURES AND DOCUMENTATION

During the initial screening visit/recruitment, potential subjects will read the Participant Information Sheet. The Investigator or clinical unit staff will explain the study via the Participant Information Sheet and the potential subjects will be encouraged to ask questions. Individuals

willing to be considered for inclusion in the study will sign and date the informed Consent Form in the presence of an Investigator. Subjects will be given a copy of their signed informed Consent Form. Once the subject has consented to the study, the specific screening activities may commence. See Section 7.3.1 for further details.

13.4 SUBJECT AND DATA CONFIDENTIALITY

Subjects will be informed that their data will be held on file by PQPharm and that these data may be viewed by staff of QPPharm (including, where necessary, staff of PQPharm other than the named Investigator(s)).

Upon request, the Investigator(s)/institution(s) will permit direct access to data and documents for trial-related monitoring, audits, Ethics Committee review, and regulatory inspection(s) by the Sponsor (or their appropriately qualified delegates) and Regulatory Authorities (see Section 11).

Subjects will also be informed that a report of the study will be submitted to the Sponsor and may also be submitted to government agencies and perhaps for publication, but that they will only be identified in such reports by their study identification number, and their gender and age. The Investigator undertakes to hold all personal information in confidence.

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

13.4.1 RESEARCH USE OF STORED HUMAN SAMPLES, SPECIMENS OR DATA

Samples and data collected during this study will be used to achieve the study objectives.

Samples and data will be stored according to PHQ Pharm and QIMR Berghofer SOPs, and access will be limited to authorised personnel. Biological samples will be retained for the time required to complete analysis, and may then be discarded.

13.5 FUTURE USE OF STORED SPECIMENS

As part of the study, safety serum samples will be stored indefinitely at PQPharm/QIMR Berghofer for retrospective safety assessments that may later be indicated. Subjects consent to this storage and the use of the sample for safety assessments, when they sign the informed Consent Form for the study.

For all other samples, consent must be obtained from the subjects to store and use their samples for future research. Consent will be obtained via the Informed Consent for Blood Storage that subjects receive during recruitment/screening. Subjects can decide if they want their samples to be

used for future research or have their samples destroyed at the EOS. A subject's decision can be changed at any time prior to the EOS by notifying the study doctors or nurses in writing. However, if a subject has consented to future use and some of their blood has already been used for research purposes, the information from that research may still be used.

Any future research using the stored samples that is beyond the current study will be reviewed by the QIMR Berghofer HREC (Section 13.2). 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. The vials containing the samples of the consented subjects will be coded and the identifying information will not be released to any unauthorised third party. The subjects can also choose (via the Informed Consent for Blood Storage Form) for the samples to be labelled with only the study number, malaria strain and visit. No genetic testing will be performed on the stored samples and no use without obtaining consent from the subjects. The stored samples will not be sold or used directly for production of any commercial product. There are no benefits to subjects in the collection, storage and subsequent research use of their samples. There is about future research done with subject samples will NOT be kept in their health records, but a subject's samples may be kept with the study records or in other secure areas.

14 DATA HANDLING AND RECORD KEEPING

14.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Each subject will have a clinical file (source data) and case report form (CRF, for protocol specific data) into which relevant data will be recorded. All recording will be done only in black ink. Corrections will only be made by drawing a single line through the incorrect entry, writing the correction in the nearest practicable space, and initialling and dating the correction. Correction fluids are not allowed.

A log of names, signatures and initials of all staff authorised to enter data into a subject's Clinic File and CRF will be kept. Upon completion of each study visit, all CRFs will be reviewed internally by the clinic for omissions or apparent errors so that these can be corrected without delay. Any corrections made after the review and signature of the Principal Investigator will be noted in the audit trail and will required reauthorisation (electronic sign off) by the Principal Investigator

14.2 STUDY RECORDS RETENTION

All source data, clinical records and laboratory data relating to the study will be retained in the archive of the clinical unit (IQ-Pharm) for a minimum of 15 years after the completion of the study. Data will be available for retrospective review or audit by arrangement with the Chief Executive

Officer of the clinical unit. Written agreement from the Sponsor must precede destruction of the same.

14.3 PROTOCOL DEVIATIONS

Protocol Deviation: a protocol deviation is any departure or change from, and addition to, the study design or procedures defined in the protocol that has received approval by the competent authorities and favourable opinion from the ethics committees.

Important Protocol Deviation: an important protocol deviation (sometimes referred to as a violation) is a protocol deviation that has or has the potential to affect the rights, safety or well being of the trial subjects and may impact the integrity (completeness, accuracy and reliability) of the data to a degree that the data are not usable.

NTF: a note to file is 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.

All protocol deviations will be documented in the trial master file and included in the CSR. An assessment of the significance of each protocol deviation will be discussed in the CSR.

All NTFs, protocol deviations and important protocol deviations are to be viewed by the Principal Investigator or delegate (QIMR Berghofer Project Manager) and signed by the Principal Investigator.

All NTFs, protocol deviations and important protocol deviations will be assessed and significance assigned at the end of each cohort by the SDRT team.

All important protocol deviations will be reported by the clinical site to the Sponsor and at the Sponsors request to the QIMR Berghofer HREC as early as possible, but within 7 days. A protocol violation report form provided by QIMR Berghofer will be used for this purpose.

All protocol deviations will be reported by the clinical site to the QIMR Berghofer PM as early as possible but within 7 days, and to the Sponsor at the end of each cohort. A protocol deviation report form provided by QIMR Berghofer will be used for this purpose.

Protocol deviation logs will be submitted by the clinical site to the Sponsor and QIMR Berghofer HREC via inclusion with the annual report.

14.4 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 study. This will include appendices of all tables and listings generated during the analyses of data. The tables and listings will be

provided by CNS. The Sponsor undertakes to ensure that all safety observations made during the conduct of the trial are documented in this report.

Publication and reporting of results and outcomes of this trial will be accurate and honest, undertaken with integrity and transparency and in accordance with the relevant clauses outlined in the QIMR Berghofer Policy on Criteria for Authorship [48]. QIMR Berghofer and the Principal Investigator have a responsibility to ensure that results of scientific interest arising from the clinical trials are appropriately published and disseminated. Publication of results will be subjected 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 according to the QIMR Berghofer Policy on Criteria of Authorship. 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 study commencement (or as soon as possible thereafter) and reviewed whenever there are changes in participation. Acknowledgment will be given to collaborating institutions and hospitals and other individuals and organisations providing finance or facilities. All conflicts arising from disputes about authorship will be reviewed by the QIMR Berghofer Director.

In any press releases, publications or presentations, MMV's financial contribution to the study and its participation in the collaboration shall be expressly acknowledged. QIMR Berghofer agrees that MMV will be entitled to access all the de-identified clinical trial data upon completion of the trial. Data will not be released publicly until the manuscript is accepted for publication. In the case of no publication, information will only be released to the public and media in accordance with the QIMR Berghofer Corporate Media Strategy Policy [49]. However, the Investigator undertakes not to make any publication or release pertaining to the study and/or results of the study without the Sponsor's prior written consent, being understood that the Sponsor will not unreasonably withhold its approval. The Sponsor has the right to publish the results of the study at any time.

The Investigator shall not use the name(s) of the Sponsor and/or of its employees in advertising or promotional material or publication without the prior written consent of the Sponsor. The Sponsor shall not use the name(s) of the Investigator and/or the collaborators in advertising or promotional material or publication without having received his/her and/or their prior written consent(s).

MMV or the local 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 study completion and finalisation of the study, the report results of this trial will be either submitted for publication in an open access journal and/or posted in a publicly accessible database of clinical trial results.

15 STUDY ADMINISTRATION

15.1 STUDY LEADERSHIP

See Section 1 for key roles.

15.2 LIABILITY/INDEMNITY/ INSURANCE

The study Sponsor will ensure sufficient insurance is available to enable it to indemnify and hold the Investigator(s) 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 subject's participation in the study but only to the extent that the claim is not caused by the fault or negligence of the subject's Investigator(s). 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'.

16 CONFLICT OF INTEREST POLICY

No conflicts of interest are applicable in this study.

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APPENDIX

Appendix 1: Product information and consumer medicine information

Riamet®

- Product Information (TGA, updated August 2016)
- Consumer Medicine Information (TGA, updated December 2015)

Primacin™

- Product Information (TGA, updated February 2017)
- Consumer Medicine Information (TGA, updated February 2017)

Artesunate

- WHO Public Assessment Report (2011)

Appendix 2: Total blood volume

Procedure	Sample	Volume per sample (mL)	No. samples per subject	Total volume per subject (mL)
Laboratory Safety Assessment	Haematology (including G6PD)	2	7	14
		4	2	8
	Biochemistry /biomarker(may include Serology, CRP,)	5	6	30
		8.5	3	25.5
	Coagulation profile	2.7	1	2.7
	Safetyserum storage	5	2	10
	RBC alloantibody	4	2	8
Bioanalysis	PK analysis	4	21	84
Cannulation	Discard	2	19	38
Malaria Monitoring	Malaria 18S qPCR	2	36	72
	Parasite lifecycle stage qRT-PCR	2	2	4
Main study total (mL)				~297
Exploratory (optional)	ImmuneCell Characterisation	53	2	106
		26	3	78
	Complement Regulatory Proteins	2	8	16
Additional optional exploratory total (mL)				~203
Main study and exploratory components (mL)				~497

This table is indicative and may vary based on qPCR levels and safety-follow-up samples. Additional blood samples may be taken for unscheduled safety and qPCR assessments as required by the Investigator, provided the total volume taken during the study does not exceed 450 mL during any period of 30 consecutive days.

Appendix 3: Symptoms and signs of malaria

Following challenge via the intravenous malaria parasite inoculation and during the post-challenge period, the following signs and symptoms of malaria will be monitored:

Signs of malaria

- Fever (oral temperature of ≥38°C)
- Chills/shivering/rigors
- Tachycardia
- Hypotension

Symptoms of malaria

- Headache
- Myalgia (muscle ache)
- Arthralgia (joint ache)
- Fatigue/lethargy
- Malaise (general discomfort/uneasiness)
- Sweating/hot spells
- Anorexia
- Nausea
- Vomiting
- Abdominal discomfort

Appendix 4: Beck Depression Inventory

Appendix 5: Version History

Version	Date	Author(s)/Reviewer(s)	Revisions
1.0-1.1	29.1.18	Rebecca Webster	Minor typographical updates
1.1-2.0	19.3.18	Rebecca Webster	See table below
2.0-3.0	30.4.18	Rebecca Webster	See table below
3.0-4.0	20.8.18	Rebecca Webster	See table below

Summary of Changes to QP17C19 (Version 1.1 to 2.0)

Protocol Section(s)	Change	Rationale
Abbreviations	• Added 2 additional abbreviations	• Missing from initial protocol
Protocol Summary and Objectives and Purpose	• Added optional exploratory objectives and endpoints	• Additional scientific exploratory objectives have been added to the protocol. These are optional studies and participants that choose to participate will sign a separate consent form
Key Roles	• An additional independent medical monitor has been added to the trial key roles • Data management, site monitoring and regulatory function personnel has been added to the trial key roles	• The sponsor requested an additional medical monitor • A specific person from the local sponsor has been identified to perform this role
4.1	• Added additional text regarding the exploratory study components	• Additional scientific exploratory objectives have been added to the protocol. These are optional studies and participants that choose to participate will sign a separate consent form
6.1.1	• Change of sucrose provider from MMV to Q-Pharm	• Updated to highlight that sucrose will be provided by the phase 1 site pharmacy
7.1.1	• Added additional text to further explain;	• Clarification and completeness of protocol

	– ECG procedural requirement – exploratory study blood sampling – medical diary cards and temperature self-recording	
7.2.2	• Added details about; – PK blood collection and processing – optional exploratory components – optional exploratory components	• Clarification and completeness of protocol and updated information regarding the additional exploratory objectives
7.3 Study Schedule	• Added blood collection for optional exploratory components at each relevant time-point • Added a subject diary card check at all relevant time-points	• Updated with information related to the additional exploratory objectives
7.3.7	• Updated schedule of events	• Clarification of schedule of participant activities in line with above described text
13.1	• Updated study total blood volume to include exploratory components blood volume	• Updated with information related to the additional exploratory objectives
14.3	• Added text about reporting of protocol deviations and violations	• Clarification of reporting obligations
Appendix 2	• Updated total blood volume	• Updated due to additional blood volume being required for exploratory optional components

Summary of Changes to QP17C19 (Version 2.0 to 3.0)

Protocol Section(s)	Change	Rationale
Protocol Summary, 4.1, 6.1.5 7.3.3	• Added criteria for IMP administration (malaria clinical score >6) and definition of recrudescence a a criterion for rescue medication administration (defined as ≥5 000 blood stage parasites/mL and a 2 fold increase within 48 hours or a malaria clinical score >6	• Criteria for IMP and rescue medication administration based on a clinical/biological response was deemed necessary rather than just a defined time-point for these activities
Key Roles	• An additional co-investigator has been added to the trial k roles	
2.3 IMP risks	• Added vasovagal, orthostatic hypotension and atrial fibrillation to the risks section	• Additional risks were identified
5.1 Inclusion criteria	• Change from 50 to 40 mmHg ≤ diastolic blood pressure • Added male subjects with female partners that are surgically sterile, or male subjects who have undergon sterilisation and have had testing to confirm the succes of the sterilisation may also be included.	• More appropriate criteria
5.2 Exclusion criteria	• Change Participation in any investigational product study within the 12 weeks precedin the study to Participation in any investigational product study within the 12 weeks preceding IMP administration. • Clarification that Symptomatic postural hypotension at screening, irrespective of the decrease i blood pressure, or asymptomatic postural	• More appropriate criteria

	hypotension defined as a decrease in systolic blood pressure ≥20 mmHg within 2-3 minutes when changing from supine to standing position, could be repeated if abnormal • Clarification and separation o severe allergic reaction, anaphylaxis and convulsions	
7.1.1	• Stated that the mean of the three ECGs would be recorded	• Clarification of protocol
7.2.1	• Added MCV to haematology • Added coagulation testing	• Missed in previous protoco
7.3.2	• Updated schedule of events • CRP will be included in all biochemistry except screenin • Urine for urinalysis will be collected at EOS • Coagulation added to table o events	• Clarification of schedule of participant activities
7.3.3	• An additional malaria PCR time point added	• Required to collect blood for PRR every 6 hours afte initial administration of IMP
8.1.3	• Changed ALT or AST above 3x ULN to 5x ULN • QTcB or QTcF prolongation from baseline clarification of parameter • Clarification of haematological AESI	• Clarification and specification of appropriate AESI's for malaria challenge studies
13.1 Appendix 2	• Updated blood volume	• Updated since added coagulation profile

Summary of Changes to QP17C19 (Version 3.0 to 4.0)

Protocol Section(s)	Change	Rationale
Protocol Summary, 4.1,	• Added planned dose regime for Cohort 2 post SDRT meeting and scientific data evaluation	• Cohort 2 doses selected after SDRT review.
Protocol Summary, 4.1, 7.3.3 8.6, 10.3	• Updated trial day naming convention	• Study Day numbers were updated as per study design scheme and study flowchart for consistency.
7.3.3	• Added ECG assessment to time of rescue treatment administration	• Provide additional safety data prior to rescue treatment
5.2	• Updated citrus consumption till the EOS rather than end of Riamet treatment	• Consumption of these substances could interfere with PK analysis. PK samples may still be collected post Riamet administration
7.6	• Updated alcohol grams into units and standard drinks (Australian)	• Updated to be consistent with the eCRF
8.4.5	• Updated pregnancy wording	• WOCBP can be included in this study as per inclusion criteria under the condition of usage of highly effective method of birth control and therefore this section needed to be revised
10.5	• Changed randomisation to occur after inoculation but prior to Day 8.	• Logistically this provides more time for the clinical site to prepare for dosing.
7.1.1	• Added 'on study IBSM specific ranges' for ECGs and vital signs	• Provide additional information and alignment with eCRF guidelines
7.3.2 7.3.3	• Changed symptom directed physical examination to a compulsory abbreviated	• Provide additional safety assessment of participants

	physical examination prior to inoculum and drug dosing	prior to inoculum and drug dosing
7.3.3	• Added text regarding assessments to be performe if participants are administered antimalarial rescue treatment prior to the (Day 42) scheduled time point.	• To ensure safety assessments which are required to be performed a the end of rescue treatmen are captured in the protoco if participants are provided antimalarial rescue therapy prior to the scheduled time point.
7.2.1 7.3	• Defined CRP as a biomarker	• CRP is a biomarker and no a biochemistry safety assessment
7.2.2 7.3.3	• Added the following wording to applicable protocol sections "Malaria monitoring will continue until a minimum of one negative 18S qPCR is detected post rescue therapy"	• Provide clarification on number of negative qPCR required after rescue therapy to consider a subject successfully rescued
7.3.4	• Changed capturing malarial clinical score to be mandatory at EOS	• To provide additional safety information
7.3.7	• Updated SoE table Added abbreviated physical exa Added rescue treatment column	• To be consistent with text in the protocol