STATISTICAL ANALYSIS PLAN

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

Protocol No.: MMV_Pyronaridine_21_01

Product Code: Pyronaridine

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SAP APPROVAL

By my signature, I confirm that this SAP has been reviewed and has been approved for use on the MMV_Pyronaridine_21_01 study:

Name	Title / Company	Signature	Date
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1. ABBREVIATIONS

ADaM	Analysis Data Model
AE	Adverse Event
AESI	Adverse Event of Special Interest
ALP	Alkaline phosphatase
ALT	Alanine Aminotransferase
APR28	Adequate Parasitological Response Rate at Day 28
APTT	Activated partial thromboplastin time
AST	Aspartate Aminotransferase
ATC	Anatomical Therapeutic Chemical
AUC	Area Under the Blood Concentration Time Curve
AUC_{last}	Area under the curve from time 0 to last measurable concentration
AUC_{inf}	Area under the curve from time 0 to infinity
β-HCG	β-Human Chorionic Gonadotropin
BDI	Beck Depression Inventory
BIC	Bayesian Information Criteria
BLQ	Below the Limit of Quantification
BMI	Body Mass Index
CDISC	Clinical Data Interchange Standards Consortium
CI	Confidence Interval
CK	Creatine Kinase
CL/F	Apparent Oral Clearance
C_{max}	Maximum blood concentration observed
COVID-19	Corona virus disease 2019
CRA	Clinical Research Associate
CRF	Case Report Form
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
DBP	Diastolic Blood Pressure
DTS	Data Transfer Specification
EC50	Concentration at 50% of Maximum Effect
ECG	Electrocardiography
eCRF	Electronic Case Report Form
EDC	Electronic Data capture
EMAX	Maximum Effect
EOS	End of Study
FAS	Full Analysis Set
FSH	Follicular Stimulating Hormone
G6PD	Glucose-6-phosphatase
GGT	Gamma Glutamyl Transpeptidase
HBsAg	Hepatitis B Surface Antigen
HEENT	Head, Eyes, Ears, Nose, Throat
HIV	Human Immunodeficiency Virus
HR	Heart Rate
IBSM	Induced Blood Stage Malaria
IC50	Investigational Medicinal Product
IIV	Inter Individual Variability
IMP	Investigational Medicinal Product
INR	International Normalized Ratio
LDH	Lactate Dehydrogenase
LLOQ	Lower Limit of Quantification
LOQ	Limit of Quantification
MedDRA	Medical Dictionary for Regulatory Activities
MIC	Minimum Inhibitory Concentration
MMV	Medicine for Malaria Venture
MPC90	Minimal Parasiticidal Concentration
NCA	Non-Compartmental Analysis
ND	Non Detect
NLME	Nonlinear Mixed Effects

PC	Pharmacokinetics Concentration
PCSA	Potential Clinically Significant Abnormality
PD	Pharmacodynamics
Pf iRBC	P.falciparum Infected Red Blood Cell
PK	Pharmacokinetics
PKPD	Pharmacokinetic-pharmacodynamic
PN	Preferred Name
PRR_{48}	Parasite Reduction Ratio Over 48 hours
PT	Preferred Term
$PCt_{1/2}$	Parasite Clearance Half-Life
QIMRB	QIMR Berghofer Medical Research Institute
qPCR	Quantitative Polymerase Chain Reaction
qRT-PCR	Quantitative Reverse Transcriptase Polymerase Chain Reaction
QTcB	Corrected QT interval with Bazett's Formula
QTcF	Corrected QT interval with Fridericia's Formula
RAT	Rapid Antigen Test
SAE	Serious Adverse Event
SAEM	Stochastic Approximation Expectation Maximization
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SBP	Systolic Blood Pressure
SBQ	Swiss BioQuant Central
SD	Standard Deviation
SDTM	Study Data Tabulation Model
SE	Standard Error
SOC	System Organ Class
SOP	Standard Operating Procedure
SRC	Safety Review Committee
SSR	Southern Star Research
t1/2	Half-Life
TEAE	Treatment Emergent Adverse Event
Tmax	Time to Reach Maximum Blood Concentration
VIS	Volunteer Infection Study
Vss/F	Apparent Volume of Distribution at the Steady State After Single Dose
VPC	Visual Predictive Check
Vz/F	Apparent Volume of Distribution after Single Dose
WOCBP	Women of Child Bearing Potential
WHODrug	World Health Organization Drug Dictionary

2. INTRODUCTION

The following Statistical Analysis Plan (SAP) provides the outline for the statistical analysis of the data from the MMV_Pyronaridine_21_01 study. The SAP details the methods for statistical analysis of outcomes relating to safety, pharmacokinetics (PK), pharmacodynamics (PD), and population modelling of pharmacokinetic / pharmacodynamic (PK/PD) data arising from this clinical trial. This SAP has been developed and finalised prior to database lock and final analysis.

Planned analyses identified in this SAP will be included in the clinical study report (CSR), regulatory submissions, and future manuscripts. In addition, post hoc exploratory analyses not necessarily identified in this SAP may be performed to further examine study data. Any post hoc, or unplanned, exploratory analyses performed will be clearly identified as such in the final CSR.

Any significant changes from planned analyses will also be described in the final CSR.

3. PROJECT OVERVIEW

3.1 Study Design

This was a single-centre, open-label phase 1b study using the *P. falciparum* induced blood stage malaria (IBSM) model to characterise the PK/PD profile of pyronaridine.

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

Participants will attend the clinical unit once on Days 4, 5, 6 and 7 for clinical evaluation and blood sampling to monitor the progression of parasitaemia, using quantitative polymerase chain reaction (qPCR) targeting the gene encoding 18S rRNA (referred to hereafter as malaria 18S qPCR).

Participants will have a nasopharyngeal aspirate (NPA) for SAR-CoV-2 on Day 6 am, and blood collected on Day 7 am to monitor haematology and biochemistry.

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

Pyronaridine will be administered as a single oral dose. Different doses of pyronaridine will be administered across and within cohorts in order to effectively characterise the PK/PD relationship. Each cohort will comprise up to three dose groups, with participants randomised to a dose group on the day of dosing. The highest dose of pyronaridine administered will be no more than 720 mg; the lowest dose administered will be no less than 180 mg.

The proposed dosing regimen for the Cohort 1 assuming six participants are enrolled, is as below. If less than six Participants are enrolled the Safety Data Review Team (SDRT) will meet between Day 0 and Day 8, to decide on the dose/s to be administered.

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

The SDRT will review safety, tolerability, parasitaemia and PK/PD data to determine the dose to be administered to Cohort 2.

The study will conclude when sufficient data have been obtained to define the PK/PD parameters for pyronaridine (the primary endpoint).
A maximum sample size of 18 Participants administered pyronaridine is expected to be sufficient to achieve the primary endpoint.

Participants will be confined in the clinical unit for at least 96 h (Days 8 - 12) to monitor the safety and tolerability of pyronaridine dosing and to ensure adequate clinical response against *P. falciparum*. During confinement, regular safety assessments will be performed, and blood will be collected to monitor parasite clearance and pyronaridine concentration.

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

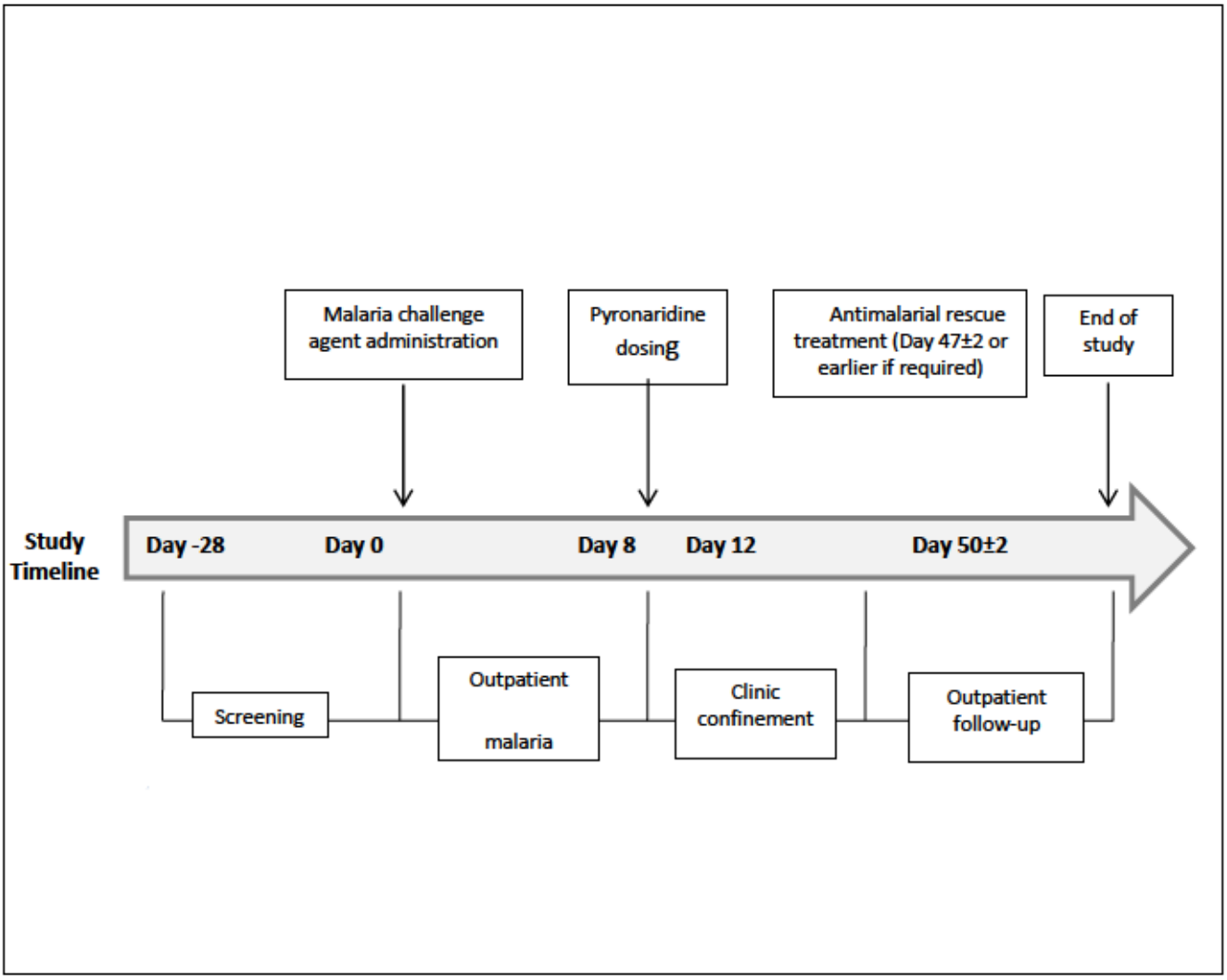
- Parasitaemia is not reduced by approximately 10-fold at the end of the 96-hour confinement period (Day 12) when compared with peak parasitaemia.
- Parasite regrowth occurs after an initial reduction in parasitaemia, with regrowth defined as ≥5000 parasites/mL and a two-fold parasitaemia increase within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

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

For Cohort 1, if gametocytes are present at or after the time of treatment with Riamet®, Primacin® (primaquine) will be administered as a single oral dose at the Investigator's discretion. From Cohort 2 onwards, a single dose of Primacin® will be administered at any time point after the confinement period when gametocytes are detected as determined by qRT-PCR, at the Investigator's discretion.

If an allergy or contraindication to Riamet® develops, Malarone® (atovaquone/proguanil) will be administered. If in the rare event participants are unable to complete oral antimalarial rescue treatment, they will be admitted to hospital to receive intravenous Artesunate.

3.2 Schema


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

3.3 Schedule of Activities

The below table summarises the activities and procedures to be conducted as per this protocol during screening, eligibility, malaria inoculation, out patient monitoring, confinement, and at the end of study.

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

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

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

[a] This visit is not required in the event that the screening visit is conducted within this period.
[b] Daily visits.
[c] See Section 8.1.5 for details of assessments to be performed prior to and after pyronaridine administration. Also note that there are multiple samples/assessments for some activities on the confinement days.
[d] Follow up visits will occur at least three times per week. See Section 8.1.6 for the specific timings of different activities.
[e] Symptom-directed physical exam will only be performed if clinically indicated.
[f] Supine & standing blood pressure and heart rate required at screening; seated measurements only at other time-points. On Day 0, record vital signs prior to inoculation and prior to leaving the clinical trial unit (approximately 60 minutes after inoculation). Record vital signs 3 times a day whilst confined.
[g] Haematology and biochemistry at screening includes lipids for cardiovascular risk factor.
[h] Riamet® will be administered to all participants on Day 47±2 or earlier as outlined in Section 8.1.7. Participants may be treated with Primacin® if gametocytaemia is suspected from parasite lifecycle stage qRT-PCR at the Investigator's discretion, to ensure clearance of gametocytes. If needed, participants will be administered a single oral dose of six Primacin® tablets (total dose 45 mg primaquine) with food, unless G6PD deficient in which case a low dose of 15 mg (two tablets) will be administered.
[i] Blood samples for malaria 18S qPCR will be collected following pyronaridine treatment at 4, 8, 12, 16, 20, 24, 30, 36, 42, 48, 60, 72, 84 and 96 hours. As outpatients, blood will be collected at least three times per week until antimalarial rescue treatment. Allowed time windows for sample collection are specified in Section 8.4.
[j] Blood samples for drug concentration measurements will be collected following pyronaridine treatment at 1, 2, 3, 4, 6, 8, 12, 16, 20, 24, 30, 36, 42, 48, 60, 72, 84 and 96 hours. As outpatients, blood will be collected on the following days: 13, 15, 17, 20, 22, 24, 27, 29, 32, 39, 44, 47 and 50. Allowed time windows for sample collection are specified in Section 8.4.
[k] Blood samples for parasite viability ex vivo growth will be collected following pyronaridine treatment at 4, 8, 12, 16, 20, 24, 48, 72 and 96 hours.
[l] Perform SARS-CoV-2 testing at the eligibility visit (NPA PCR), or at the screening visit if this visit is conducted two days prior to inoculation, Day 0 (RAT), Day 6am (NPA PCR) and Day 8 (RAT). Further details are specified in Section 8.3.7.
[m] Blood samples for future malaria research will be collected during the outpatient monitoring phase on Days 15±1 and 29±1

3.4 Objectives

3.4.1 Primary Objective

- To characterize the PK/PD relationship of pyronaridine in healthy participants experimentally infected with blood stage *P. falciparum.*

3.4.2 Secondary Objective(s)

- To evaluate the safety and tolerability of single oral doses of pyronaridine in healthy participants experimentally infected with blood stage *P. falciparum*.
- To characterize the parasite clearance kinetics following single doses of pyronaridine in healthy participants experimentally infected with blood-stage *P. falciparum*.
- To characterize the pharmacokinetics of pyronaridine following single oral dose administration in healthy participants experimentally infected with blood stage *P. falciparum*.
- To characterize the effect of pyronaridine on *P. falciparum* 3D7 parasite viability using phenotypic and molecular approaches.

3.5 Endpoints

3.5.1 Primary Endpoint(s)

- The PK/PD relationship between pyronaridine blood concentrations and blood stage asexual parasitaemia.

3.5.2 Secondary Endpoint (s)

3.5.2.1 Safety Parameters

The safety and tolerability of pyronaridine will be determined by recording the incidence, severity and relationship to pyronaridine of adverse events (as determined by clinical, laboratory and ECG examinations) from Day 8 up to Day 50±2.:

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

3.5.2.2 Parasite Clearance Kinetics

- The parasite clearance kinetics following dosing with pyronaridine will be determined by calculating the parasite reduction ratio over a 48-hour period (PRR48) and corresponding parasite clearance half-life ($PCt_{1/2}$)

3.5.2.3 PK Parameters

- The pharmacokinetics of pyronaridine will be determined by calculating the following parameters using non-compartmental methods:

Parameters:
C_{max}: Maximum blood concentration observed
t_{max}: Time to reach maximum blood concentration
AUC_{last}: Area under the curve from time 0 to last measurable concentration
AUC_{inf}: Area under the curve from time 0 to infinity
$t_{½}$: Half-life
CL/F: Apparent Oral Clearance
V_d/F: Apparent volume of distribution
λ_z: Terminal rate constant

3.6 Sample Size

A maximum sample size of 18 participants is expected to be sufficient to achieve the primary endpoint of the study, which is to determine the PK/PD parameters of pyronaridine. This sample size is not based on formal statistical calculations but rather is based on previous experience of characterizing the PK/PD relationship of antimalarials using the IBSM study trials (refer to the Blood stage Plasmodium falciparum challenge agent P. falciparum 3D7 Investigator's Brochure). The specific number of participants required will be dependent on the emerging results during the study; it is possible that less than 18 participants will be required.

3.7 Treatment Assignment and Randomisation

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

Replacement of Withdrawn/Discontinued Participants

Participants who sign the informed consent form, and are randomised and receive malaria inoculation, and subsequently withdraw, or are withdrawn or discontinued from the trial, may be replaced after mutual agreement between the Sponsor and the PI. The decision regarding the replacement of participants will be documented.

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

4. STATISTICAL CONSIDERATIONS

Data analysis will be performed according to the Sponsor's representative Standard Operating Procedures (SOPs).

The general analytical approach for all endpoints will be descriptive in nature. All summaries will present the data by dose group as well as by all participants combined.

Unless otherwise stated, the following statistical approaches will be taken:

Continuous variables:	Descriptive statistics will include the number of non-missing values, mean, standard deviation (SD), CV%, median, interquartile range, minimum, and maximum. The minimum and maximum values will be presented to the same number of decimal places as recorded in the raw data; mean and median will be presented to 1 decimal place more than raw data; and SD will be presented to two more decimal places than the raw data. The number of decimals for lab parameters may be reduced if the full presentation of the data includes more than 4 significant figures. In general, the mean and median will be reported with one decimal place more than the data, and the standard deviation will be reported with two decimal places more than the data.
Categorical variables:	Descriptive statistics will include frequency counts and percentages per category. Percentages will be rounded to one decimal place, with the denominator being the number of patients in the relevant population.
Imputation:	No imputation will be performed for missing data.
Confidence intervals (CIs):	CIs will be two-sided and will use 95% confidence levels. Any analyses requiring significance testing will use a two-sided test at the 5% significance level.
Unscheduled Visits	Unscheduled visits that do not have laboratory data on the same visit date will be excluded from summary tables.
Early termination visit	Assessments conducted at Early Termination will be excluded from visit-based summary tables.

4.1 Data Capture

4.1.1 Database

The primary method of data collection is via the study database, developed within the chosen Electronic Data Capture (EDC) platform, IBM Clinical Development. The database has been designed based on the final protocol, the system/core configuration, electronic Case Report Form (eCRF) specifications and/or mock eCRF and consistency check specifications.

Data will be entered directly into the EDC system. Site-collected data will be entered directly from source notes at the site and will be verified by Clinical Research Associates (CRAs) to ensure data integrity.

Refer to the Data Management Plan for further details.

4.1.2. Third Party Data

4.1.2.1 Safety Laboratory

Central safety laboratory data will be received from Sullivan Nicolaides (SNP) Pathology Central Laboratory as specified in the Data Transfer Specification. At least one transfer will be delivered prior to database lock and reconciled against CRF data. Following successful reconciliation and resolution of any data issues, the data will be incorporated into the End of Study analysis.

No unit conversion of laboratory data will be performed.

4.1.2.2 PK Laboratory

PK samples will be analysed by Swiss BioQuant Central (SBQ) and PK parameters will be derived by PharmaKinetic. Final PK assay data will be transferred to SSR, as specified in the SBQ DTS, for incorporation into the PC SDTM.

4.1.2.3 PD Laboratory

All parasitaemia data will be generated by the Queensland Paediatric Infectious Diseases (QPID) laboratory and captured electronically using MARS (18S qPCR parasitaemia data). All parasite life-stage data (i.e. gametocytemia and ring-stage parasites) will be generated by the QPID laboratory and transferred electronically to the Queensland Institute of Medical Research (QIMR) Berghofer and this data will be used for PK/PD modelling. QIMR will provide the 'raw' PCR data 18s and RT-PCR to SSR as per DTS, and final PD parameters will be in the PRR report.

4.1.2.4 PK/PD Laboratory

PK/PD analysis will be performed by MMV, Pharmacometrics. SSR will be required to provide the PK concentration and parasitemia data, as well as any CRF datasets required to perform the PK/PD analyses (e.g. demographics, drug administration data).

4.2 Statistical Programming

4.2.1 Programming Specifications

4.2.1.1 Safety Analysis

Programming specifications will be prepared to detail the SAS programming of CDISC (SDTM and ADaM) datasets and listings, tables and figures.

4.2.1.2 PD Analysis

Data manipulation and data analyses for all PD data will be performed using R (highest version available).

4.2.1.3 PK Analysis

The estimation of pharmacokinetic parameters by non-compartmental analysis (NCA) methods will be performed using Phoenix WinNonlin software (v8.3 or a more recent version, Certara USA, Inc., USA).

4.2.1.4 PKPD Analysis

All data processing, analysis, model setup and modeling result analysis including goodness-of-fit plots will be performed in R 4.1.3 and IQRtool package 1.10.0 [IQRtools].
NLME modelling will be performed with Monolix 2019R1 [MLX19] using Stochastic Approximation Expectation Maximization (SAEM) for parameter estimation.

4.2.2 Baseline

Baseline will be defined as:

Safety

- The last scheduled observation prior to the administration.of the malaria challenge inoculum [Inoculation Baseline],
- The last scheduled observation prior to the first administration of IMP [Treatment Baseline].

PD

- The last observation prior to the administration of the IMP for PD data.

PK

- The last observation prior to the administration of the IMP for PK data.

4.2.3 Change from Baseline

Change from Baseline will be calculated as:

$$Change\ from\ baseline = (postbaseline\ value) - baseline\ value$$

4.2.3.1 Days relative to Inoculation

$$Days = (Assessment\ Date - Inoculation\ Administration\ Date) + 1$$

4.2.3.2 Days relative to IMP administration

$$Days = (Assessment\ Date - First\ Study\ Drug\ Administration\ Date) + 1$$

4.2.4 IMP Treatment Groups

Tabulations will summarise data by the following IMP groups and these may vary for each cohort:

Pyr 360 mg
Pyr 540 mg
Pyr 720 mg
Overall

5. ANALYSIS SETS

The analysis sets to be used for the analyses will be: Full Analyses set (FAS), IMP set, PK set, PD set and PK/PD set.
The number of participants in each analysis set will be summarised, with a corresponding listing.

5.1 Analysis Set Descriptions

5.1.1 Full Analysis Set

The Full Analysis Set (FAS) will consist of all enrolled participants, i.e. participants that received the malaria challenge agent.

5.1.2 IMP Set

The IMP Set will include all enrolled participants administered with the IMP.

5.1.3 PK Analysis Set

The PK analysis set will include all participants with at least one available valid (i.e. not flagged for exclusion) PK concentration measurement, who received any study treatment and experienced no protocol deviations with relevant impact on PK data.

5.1.4 PD Analysis Set

The PD analysis set will include all participants inoculated with the malaria challenge agent and who develop parasitaemia detectable by qPCR, received a dose of pyronaridine, and have no protocol deviations or other circumstances that would exclude them from the PD analysis.

5.1.5 PK/PD Analysis Set

The PK/PD analysis set will include all participants from the PK set and all participants from the PD set.

6. PROTOCOL DEVIATIONS

Analysis Set: FAS

All protocol deviations will be listed, grouped by participant and dose group

The protocol deviation summary table will capture all deviations as minor deviations and all violations as important deviations. A footnote will be added to the summary table documenting the change.

The protocol deviation summary table will include:

- The total number of minor protocol deviations
- The total number of important protocol deviations
- The number of Participants who reported at least one minor protocol deviation
- The number of Participants who reported at least one important protocol deviations

7. PARTICIPANT DISPOSITION

Analysis Set: FAS

Disposition

A listing of participant disposition will present:

- Date of informed consent
- Date of inoculation
- Date of IMP administration
- Date of rescue medication administration including Primacin
- Did the participant complete the study?
- Date of completion / early withdrawal
- Primary reason for early withdrawal (including instances where early termination was related to COVID-19)

Detailed early withdrawal information will also be listed in a separate listing.
If there are any deaths reported, a separate death listing will be prepared.
The number and percentage of Participants entering and discontinuing the study will be summarised by dose group and overall along with the reason for discontinuation. The participant disposition summary table will include:

- Number of participants signed informed consent
- Number of participants who received IMP
- Number of participants who completed the full study
- Number of participants withdrawn from the study early
- Reason for early withdrawal

8. DEMOGRAPHIC AND BASELINE INFORMATION

Analysis Set: FAS

8.1 Demographics

Demographic data will be listed for all enrolled participants and summarised by dose group and overall. Data includes:

Age
Sex
Women of Child-bearing potential
Post-menopausal
Race
Ethnicity
Weight (kg)
Height (m)
BMI (kg/m2)

8.2 Medical History

All medical history data will be listed, grouped by participant.

Medical history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA version 24.1) and summarised by system organ class (SOC) and preferred term (PT).

8.3 Prior Medications

Prior medications are defined as any medication that is started before first inoculation with the malaria challenge agent, regardless of when it ended. If a medication has a missing or partial

missing start/end date or time and it cannot be determined whether it was taken before IMP or concomitantly, it will be considered as prior and concomitant. Medications will be coded using WHODrug Dictionary September 2021 release.

Prior medications will be summarised by Anatomical Therapeutic Class (ATC) and preferred name (PN) and will also be listed. The summary tables will show the number and percentage of participants taking each medication by ATC and PN.

8.4 Drug Screen

Drug screen data will be listed for all participants.

8.5 Alcohol Screen

Alcohol screening data will be listed for all participants.

8.6 SARS-CoV-2 Screening

Any positive SARS-CoV2 screening results will be listed.

8.7 Follicle Stimulating Hormone (FSH)

FSH results will be listed for Post-menopausal women only.

8.8 Beck Depression Inventory

Beck Depression Inventory (BDI-II) data will be listed for all participants, including answers to the individual questions as well as the total scores, which are auto-calculated within the CRF.

9. TREATMENT EXPOSURE

Analysis Set: FAS Set

Participant exposure to the protocol-specified treatments (IMP, malaria challenge agent and rescue medications Riamet, Malarone, IV Artesunate and Primacin) will be listed and summarised by dose of IMP and overall.

10. PRIMARY ENDPOINT ANALYSIS (PK/PD ANALYSIS)

Analysis Set: PK/PD Analysis Set

10.1 Overview of methodology for PK/ PD Modelling

A population PKPD model will be derived from the concentrations and parasitemia observed in the challenge participants. It is achieved by sequentially developing a population PK model to describe the observed individual PK profiles and a population PD model for quantifying the relationship of blood concentrations and the parasite clearance.
Then, the PD model is combined with a PK model in malaria patients to predict the parasitemia profiles this population, assuming the PKPD relationship will be the same in patients as in the challenge participants.

10.2 Sources of Data

Data from study MMV_Pyronaridine_21_01 will contain information on the individual Pyronaridine tetraphosphate doses, Pyronaridine* PK concentrations, the triplicated parasitemia levels, gametocyte data and typical covariates such as age, height, weight, sex and race

10.3 Pharmacokinetics

Blood for Pyronaridine concentrations will be collected pre-Pyronaridine tetraphosphate administration on Day 8, then at the following hours post-Pyronaridine tetraphosphate administration while confined in the clinical unit: 1, 2, 3, 4, 6, 8, 12, 16, 20, 24, 30, 36, 42, 48, 60, 72, 84 and 96 hours. As outpatients, participants will have blood collected on the following days: 13, 15, 17, 20, 22, 24, 27, 29, 32, 39, 44, 47 and 50.

10.4 Pharmacodynamics

10.4.1 Malaria 18S qPCR

Blood will be collected to quantify total malaria parasitemia by quantitative polymerase chain reaction targeting the gene encoding P. falciparum 18S rRNA (malaria 18S qPCR; reported as parasites/500uL packed RBCs). Malaria 18S qPCR data will be used to calculate the total parasite counts. Blood for malaria 18S qPCR will be collected on Day 0 prior to inoculation with the malaria challenge agent, then once daily on Days 4, 5, 6 and 7. On Day 8, blood will be collected pre-Pyronaridine tetraphosphate administration, then at the following hours post-Pyronaridine tetraphosphate administration while confined in the clinical unit: 4, 8, 12, 16, 20, 24, 30, 36, 42, 48, 60, 72, 84 and 96 hours. As outpatients, participants will have blood collected at least three times per week until antimalarial rescue treatment.

10.4.2 Parasite lifecycle stage qRT-PCR

Additional blood samples may be collected to evaluate the presence of sexual parasite stages (gametocytes) and other parasite lifecycle stages using quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR). The qRT-PCR assays will be done for the following targets: the female gametocyte-specific transcript pfs25, the male gametocyte-specific transcript PfMGET, and the ring-stage transcript pfSBP-1.

10.5 Data Assembly

First a master dataset will be assembled formatted according to the MMV dataset standard. (cf., "MS_IQdataset_V4_20210219.docx"). Blood concentration will be in ug/mL, total parasite levels in counts/mL, and doses in mg. Gametocyte data is in copies/uL.
Parasite data after the first administration of rescue medication is flagged by setting the IGNORE column to "Rescued". The time in hours is relative to the (first) Pyronaridine tetraphosphate administration time.
A general analysis dataset is derived from the master dataset. Gametocyte data will be converted to parasite counts following the instructions in "Analyzing RNA data by K.Collins Nov2017.docx". Total counts of parasites were flagged to be ignored (by setting the column IGNORE to "gametocytes") if counts of gametocytes exceeded 10% of total counts.
The viable parasites counts will be derived by multiplication of the viable fraction values with the mean parasite count at treatment administration.
A general analysis dataset is derived from the master dataset. The general analysis dataset will contain the following observations: Pyronaridine blood concentrations, total counts of parasites, as well as counts of gametocytes and viable parasite counts. In addition, dosing records for P. falciparum inoculation, Pyronaridine tetraphosphate administration and rescue medication administration will be defined. It is advised to define additional columns DOSELEVEL and DOSEMULT to indicate the dose amount and number of doses a participant received as covariate columns.
NLME datasets for the PK and PD modeling will be derived from the general dataset.

10.5.1 PK modeling data set

Observations below the lower limit of quantification (LLOQ) will be censored by setting the CENS column for these observation records to 1 and setting the corresponding dependent variable (DV) column to the LLOQ (→ M3 method). Observation records with missing values and records with missing times will be ignored. Records flagged to be ignored will be removed from the dataset. Observations not analysed in the PK modelling (i.e., all parasite count records) will be removed. As a result, the PK modelling dataset only contains Pyronaridine tetraphosphate dosing records and Pyronaridine blood concentration records.

10.5.2 PD modeling data set

Observations below the LLOQ will be censored by setting the CENS column for these observation records to 1 and the corresponding DV value to the LLOQ, i.e., 10 p/mL (→ M3 method). Total counts of parasites for which counts of gametocytes exceeded 10% of total counts are flagged to be ignored (by setting the column IGNORE to "gametocytes"). Observation records with missing values and records with missing times will be ignored. Records flagged to be ignored will be removed from the dataset. Observations not analysed in the PD modelling analysis (i.e., concentration records) will be removed. As a result, the PD modelling dataset only contains Pyronaridine tetraphosphate dosing records, total parasite counts (i.e., triplicates).
The model baseline parasitemia value is added to the dataset as geometric mean of parasite counts at time of first Pyronaridine tetraphosphate dose.

The total parasite counts will be log-transformed for the PD modelling and estimated individual PK model parameters will be added to the dataset as regressors.

10.6. Model building

10.6.1. General PKPD model building approach

Population models of the PK and PD will be developed in step-wise manner.

1. A population PK model will be developed to obtain individual PK parameter estimates based on which the individual PK profiles are described well.
2. A parasite growth model (parasite growth rate and deviation of parasitemia at inoculation from parasitemia at treatment administration) will be estimated from the total parasite counts before treatment administration. Estimation of typical and individual PD parameters will be performed with IQRtools SysFit. Other appropriate growth models, e.g. an oscillatory model, will be tested if advisable.
3. The PKPD model will be build using the individual PK parameter estimates and individual growth model parameter estimates as regression parameters. Estimation of typical and individual PD parameters will first be performed with IQRtools SysFit to investigate model identifiability and second with Monolix to additionally estimate IIV.

Estimations will be done using gradient-based SysFit algorithm in IQRtools or SAEM algorithm in Monolix. Other appropriate methods may be used if advisable.
Inter-individual variability (IIV) is implemented for normally distributed parameters using the following equation with θ_0 as population average parameter, θ_i as individual parameter, and η_i as random effect that is distributed normally around zero ($\eta_i \sim N(0, \omega^2)$).

$$\theta_i = \theta_0 + \eta_i$$

The following equation is used for log-normally distributed parameters.

$$\ln(\theta_i) = \ln(\theta_0) + \eta_i$$

Continuous covariates on log-normally distributed parameters will be implemented as follows.

$$\theta_i = \theta_0 \cdot \frac{COV_i}{COV_{median}}^{\beta} \cdot \eta_i$$

θ_i represents the individual parameter, θ_0 the population mean value, COV_i the individual covariate value, COV_{median}, the population median covariate value, β the covariate coefficient, and η_i the individual random effect.
Categorical covariates on log-normally distributed parameters will be implemented as follows.

$$\theta_i = \theta_0 \cdot \Sigma_j\left(e^{\beta_j \cdot COV_{ij}}\right) \cdot \eta_i$$

The individual covariate values COV_ij are 1 if participant i belongs to the j_{th} category of the covatiate and 0 otherwise. $beta_j$ is the covariate value for the j_{th} category. For one category, i.e. the reference, $beta_j$ is 0.
For the NLME modeling with Monolix, the log-likelihood and the Fisher information matrix will be approximated by linearization. The number of iterations in the burn-in and the accumulation phase will be 500 and 200 respectively, but might be adjusted if required based on inspection of parameter estimate and objective function traces along estimation iterations. Individual parameters will be determined as conditional modes.
For PD modelling with IQRtools SysFit implementation a non-linear fixed-effects modelling approach is employed. Typical and individual parameters are both treated as fixed effects. The parameterization of individual parameters and covariates is fully consistent with the NLME parameterization above. The variances ω^2 of NLME random effects are accounted for by quadratic priors, $l_{prior}(\eta) = \frac{1\eta^2}{2\omega^2}$, added to the log-likelihood function, where the values for ω^2 are fixed and the η parameters are estimated. The profile-likelihood method [RAU09,

KAS19] is used to compute confidence intervals of the parameters estimated with the SysFit approach.

10.6.1.1 Model evaluation

Assessment of model adequacy and decisions about increasing model complexity will be driven by the data and guided by goodness-of-fit criteria, including

1. visual inspection of diagnostic scatter plots (observed vs. predicted concentration, residual/weighted residual vs. predicted concentration or time and histograms of individual random effects, for example),
2. successful convergence of the minimization routine with at least 2 significant digits in parameter estimates,
3. plausibility of parameter estimates,
4. precision of parameter estimates,
5. correlation between model parameter estimation errors<0.95, and
6. the Bayesian information criterion (BIC), given the minimum objective function value and number of estimated parameters.

For the nonlinear fixed effects models, additional diagnostic plots will be produced:

7. objective function values across multiple fits from randomised initial guesses, and
8. profile likelihood plots

All parameter estimates will be reported with a measure of estimation uncertainty, such as the relative standard error of the estimates. The individual PK fits will be used to evaluate the adequacy to use the individual PK parameter estimates as regression parameters for PD modeling.

The adequacy of simulation with the selected models will be evaluated by visual predictive checks (VPCs). VPCs for each dose level will be performed by simulation of the study 200 times taking parameter estimation uncertainty into account. 95%-confidence intervals for the 5th percentile, the median, and the 95th percentile will be derived and compared to the corresponding values based on the data.

10.6.2. PK modeling

The PK of Pyronaridine will be modeled using compartmental models describing the absorption after oral administration, distribution between central and peripheral compartments, and the elimination from the central compartment. The visual data analysis will guide the selection of models that will be tested (e.g., with respect to number of compartments, linear or saturable elimination, absorption kinetics and error model). Covariates will be included if appropriate. Estimations will be done using SAEM algorithm in Monolix. Other appropriate methods may be used if advisable.

10.6.3. PD modeling

PD model development will be done using gradient based algorithms in IQRTools SysFit. The selected model will then be estimated with SAEM in Monolix to allow for estimation of IIV. Other appropriate methods may be used if advisable. As a general concept the changes in living parasite P are modeled as the effect of a net exponential growth rate GR and a killing or clearance rate $Kill$ due to Pyronaridine. The initial parasitemia P_{base} at time t_0 will be derived from the mean parasitemia which was observed at treatment administration and a parameter $PLerr$ to allow for population and individual deviations. The equations are expressed in the log-scale such as:

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

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

Three different models describing the relationship of Pyronaridine blood concentration on the killing rate will be tested:

(1) The "E_{max}-model" assumes a direct effect of concentrations on parasite killing/clearance, such as:

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

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

(2) The "Turnover model" implements an indirect response model reflecting the idea that the kinetics of drug-receptor binding lead to a lagged concentration-effect relationship, such as:

$$\begin{aligned} Kill &= E_{max} \cdot R \\ \frac{dR}{dt} &= k_{in}\left(\frac{C_c^{Hill}}{C_c^{Hill} + EC_{50}^{Hill}} - R\right) \end{aligned}$$

where k_{in} represents the binding rate of the drug to a "receptor", which results in a lag-time in the response. Furthermore, EC_{50} represents the concentration that results in a 50% occupancy of the receptor which leads to 50% of the maximum effect given enough time to reach it.

(3) The "Clearance model" assumes that the observed parasites are a mixture of living and dead parasites. The drug has a direct effect on the killing rate of the parasites, and then the parasites are cleared, but still observed. Therefore, the equations, in the normal scale, are:

$$\begin{aligned} \frac{dP_{Alive}}{dt} &= P_{Alive} \cdot (GR - Kill) \\ \frac{dP_{Dead}}{dt} &= P_{Alive} \cdot Kill - CLpara \cdot P_{Dead} \\ Kill &= E_{max} \cdot \frac{C_c^{Hill}}{C_c^{Hill} + EC_{50}^{Hill}} \\ P_{Obs} &= P_{Alive} + P_{Dead} \\ P_{Dead}(t_0) &= 0 \end{aligned}$$

where $CLpara$ is the clearance rate of dead parasites, and P_{obs} is the count of measured parasite. If viable parasitemia is available then it is possibility to differentiate between the killing rate and the clearance rate. Otherwise, both population are measured indistinguishably, and E_{max} is fixed to the *in vitro* E_{max} value for this model.

During base model building, the Hill coefficient may not be identifiable. Then the best value for the Hill coefficient will be determined by estimating models with fixed Hill coefficients and selecting the best model based on the Bayesian information criterion (BIC). Considered Hill coefficients are 1, 2, 3, 5, 7, and 10. If covariate modeling is performed, the Hill coefficient is fixed in the value determine in the base model building.

IIV will be assumed on all parameters but the Hill coefficient. Parameters are assumed to be log-normally distributed besides the log-transformed deviation from the baseline parasitemia value $PLerr$ for which normal distribution will be assumed. The standard deviation of the IIV will be fixed to 0.2 for the SysFit approach. When parameters are estimated with Monolix, standard deviations of the IIV will be estimated.
Using the SysFit approach for PD modelling, parameter estimation will be started from 24 initial guesses. Identifiability of the best fit will be analysed using the profile likelihood method.

10.7. Derivation of secondary efficacy parameters

The minimum inhibitory concentration (MIC), the minimal parasiticidal concentration (MPC90), and the PRR48 are derived from the PD models. The MIC is defined as the concentration when parasite clearance by the drug equals the parasite growth, i.e., the time at which the minimum parasite concentration is observed. It is calculated with the following equation in case of the Emax model.

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

For the other models, the calculation is only valid at steady-state. However, an apparent MIC is determined by simulations as the concentration C_c at the time when the predicted parasite counts are at the minimum.
The MPC90 is defined as the concentration at which the clearance effect is at 90% of the maximum. It is calculated as follows for the Emax model.

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

For the other models, the calculation is only valid at steady-state. However, an apparent MPC90 is determined by simulations as the blood concentration C_c at the time when the predicted clearance effect is at 90% of the maximum during the drug elimination phase.
The PRR48 is defined as the parasite clearance achieved within 48 hours, usually given as the reduction of values on log10 transformed scale. The maximum capacity of parasite clearance in 48 hours is determined as follows assuming that concentrations are maintained well above the MPC90 for this time span.

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

The actual PRR48 for a given dose is determined as the difference of predicted parasite levels between the time when the dose is given and 48 hours later.
Key efficacy parameters will be derived using the simulations of viable parasites in case the clearance model is selected as final model.

10.8. Post-hoc Analysis

Simulations of clinical efficacy parameters and endpoints in African children

Simulations will be performed using the already available PK model established and patients Simulations will be performed for 1, 2 and 3 daily dose regimens at the adult equivalent 720mg Pyronaridine tetraphosphate. For each dose level, 200 trials with 625 participants will be performed. Dose levels, number of trials and number of participants may be adjusted if advisable. For each trial a new set of population parameters will be sampled from the uncertainty distribution and individual parameters were sampled from the IIV. The covariates age, body weight and baseline parasitemia will be sampled from a clinical dataset of real patients.
The baseline parasitemia in patients are typical 1000 fold higher than in challenge participants and in the range of 10^4 p/uL
The following parameters will be calculated (median and 95%CI):

- Cmax: maximum Pyronaridine blood concentration
- AUC: Area under the Pyronaridine blood concentration curve

- PRR48: Parasite reduction ratio after 48h
- Parasite clearance time: time to reach the microscopic LOD (10 p/uL)
- ETF CSoff: Early treatment failure clinical symptoms off; Parasitemia on day 2 higher than on day 0, irrespective of axillary temperature, or, parasitemia on day 3, or, parasitemia on day 3 >= 25% of cound on day 0.
- ETF CSon: Early treatment failure clinical symptoms on; Parasitemia on day 2 higher than on day 0, irrespective of axillary temperature, or, parasitemia on day 3 >= 25% of cound on day 0.
- LCF28: Late clinical failure at day 28: Parasitemia above LLOQ on any day between day 4 and day 28 in patients who did not previously meet any of the criteria of ETC CSon.
- LPF28 CSoff: Late parasitological failure at day 28 clinical symptoms off: Parasitemia above LLOQ on any day between day 7 and day 28 with axillary temperature < 37.5°C in patients who did not previously meet any of the criteria of ETF CSoff.
- LPF28 CSon: Late parasitological failure at day 28 clinical symptoms on: Parasitemia above LLOQ on any day between day 7 and day 28 with axillary temperature < 37.5°C in patients who did not previously meet any of the criteria of ETF CSon or LCF28.
- APR28 CSoff: Adequate parasitological response at day 28 clinical symptoms off; Absence of parasitemia on day 28 irrespective of axillary temperature, in patients who did not previously meet any of the criteria of ETF CSoff or LPF28 CSoff.
- APR28 CSon: Adequate parasitological response at day 28 clinical symptoms on; Absence of parasitemia on day 28 irrespective of axillary temperature, in patients who did not previously meet any of the criteria of ETF CSon or LCF28 or LPF28 CSon.

10.9. Presentation of Results

A statistical and graphical analysis of the data (based on the general analysis dataset will include tables and figures listed in Appendix, Table A.

The analysis results will be reported in the main body of the CSR and technical details will be reported as an appendix (referred to here as the 'technical appendix'). The technical appendix will include the clinical data used in the analysis, data assembly, modelling methods, all results, discussions, and conclusions.

Key results will include description and interpretation of the selected model, parameter estimates, and diagnostic plots of goodness-of-fit and simulations and will be included in the main text. Limitations of the model prediction will be discussed in the Discussion section of the technical appendix and mentioned in the main text of the CSR.

11. SECONDARY ENDPOINTS ANALYSIS

11.1 PD Analysis

Analysis Set: PD Analysis Set

11.1.1 Data Handling

Handling of replicates

The data will be recorded as triplicate parasitaemia and duplicate gametocytaemia values for each subject at each timepoint. The replicate data will be summarised by calculating the geometric mean of the parasitaemia and parasite life-stage data (i.e. gametocytemia and ring-stage parasites) values per subject and timepoint, and will be log10 transformed for statistical analyses.

For any replicates that were non-detects (ND), the value will be substituted with 1 parasite/mL prior to calculating the geometric mean for parasitemia quantified from qPCR 18s, or as the LOD/2 of the assay for parasite life-stage data (i.e. gametocytemia and ring-stage parasites).

11.1.2 Parasite clearance kinetics

The Parasite Reduction Ratio (PRR) and corresponding parasite clearance half-life ($PCt_{1/2}$) of asexual parasites is derived from the clearance rate of parasitaemia after administration of pyronaridine treatment. The analysis of PD response to investigational antimalarial therapy consists of:

- calculating the optimal parasite clearance rate (slope coefficient from the log-linear decay regression of qPCR data) for each individual, then,
- estimating dose specific parasite clearance rate and 95% confidence interval (CI) by calculating the weighted average slope estimate and corresponding standard error (SE) using an inverse-variance method.

The data used for all model selection and fitting calculations is comprised of the $\log_{10}$ transformed geometric mean of parasitaemia per timepoint per participant up to the first timepoint all parasitaemia replicates are ND. If during initial parasite clearance there are no samples ND for a participant, the last timepoint included in the analyses will be three timepoints after the minimum observed parasitemia value. All subsequent timepoints are set to 'missing' regardless of whether parasitaemia values increased afterwards due to potential recrudescence. Sensitivity analyses may be performed using other thresholds to determine parasite clearance, for example, but not limited to, the lower limit of quantification (LOQ) (i.e. the lower limit of the reportable range) of 32 parasites/mL or limit of detection of 111 parasites/mL [1], to assess robustness of PRR estimates.

The PRR per 48 hours, PRR_{48}, for asexual parasite will be estimated using the slope of the optimal fit of the log-linear relationship of the parasitaemia decay over time from pyronaridine administration for each individual, as detailed in CTM QIMR SOP 41 and Marquart et al. [8].

Table 1: Symbols and definitions of terms used in parasite clearance analysis (Part 1)

Symbol	Definition
$PRR_{48,i}$	Individual specific Parasite Reduction Ratio per 48 h
$PRR_{48,D}$	Dose specific Parasite Reduction Ratio per 48 h
$\beta_{1,i}$	Slope coefficient of log10 parasitaemia vs. time profile for individual i
$SE_{\beta_{1,i}}$	SE corresponding to $\beta_{1,i}$ for individual i
$\bar{\beta}_{1,D}$	Dose specific average slope estimate
$SE(\bar{\beta}_{1,D})$	Weighted SE of dose specific average slope estimate
PCt½	Parasite clearance half-life

Regression Modelling to Determine Optimal Fit

A regression modelling approach to remove potential lag and/or tail phases of the parasitaemia decay profile will be used to determine the optimal log-linear decay regression. The algorithm considers removing parasitaemia data points in an iterative process from both ends of the parasitaemia curve, i.e. a combination of right censoring (removing values from the tail phase) and left censoring (removing values from the lag phase), and uses model selection techniques to find the optimal log-linear regression.

The algorithm to obtain the log-linear decay for each participant is based on the log-linear regression detailed in Equation (1), where Time is the number of hours since administration of antimalarial treatment $(Time = 1, \dots, m)$, and β_0 and β_1 are the intercept and slope estimates, respectively.

$$\log_{10} Parasitemia = \beta_0 + \beta_1 Time \quad (1)$$

Based on the parasitaemia data for each participant, the iterative algorithm to determine the optimal log-linear decay for each participant is summarised in Table 2. The iterative algorithm is continued until a minimum of four observations are available.

The optimal log-linear regression model for a participant is deemed an appropriate fit if the overall model p-value < 0.001.

Table 1: Iteration process to determine the optimal log-linear decay curve.

Step 1: For each participant, fit the full model - fit a linear regression (as defined by Equation 1) to all m parasitaemia values of participant i Step 2: Fit two models: (a) Fit linear regression model to $m-1$ parasitaemia values, removing the first observation. (b) Fit linear regression model to $m-1$ parasitaemia values, removing the last observation. Step 3: Determine the best model of Step 2(a) and Step 2(b), defined as the model corresponding to the minimum overall model p-value. Step 4: Of the best model defined in Step 3, repeat Step 2 and Step 3 in an iterative process until a minimum of four observations. Step 5: Of the $m-3$ best models selected per iteration (including the full model (Step 1)), the optimal model was defined by the minimum overall model p-value.

Estimating Participant Specific PRR

The slope and corresponding SE estimate of the optimal linear regression model is used to calculate the participant specific PRR_{48} estimate and corresponding 95% confidence interval (95% CI), as shown in Equation (2) and (3), respectively.

$$PRR_{48,i} = 10^{-48 \times \beta_{1,i}} \quad (2)$$

$$95\%\ CI:\ 10^{-48\left(\beta_{1,i} \pm 1.96 \times SE(\beta_{1,i})\right)} \quad (3)$$

where $\beta_{1,i}$ and $SE_{\beta(1,i)}$ are the slope and corresponding standard error of the slope parameter of the optimal linear regression model, respectively.

Estimating Dose Specific PRR ($PRR_{48,D}$)

Of the s participants with appropriate overall fit (p<0.001), the average PRR_{48} and corresponding 95% CI for each cohort is estimated by using the inverse variance method to calculate the weighted average linear regression slope $(\overline{\beta_1})$ and corresponding SE. The weighted average slope for s participants in the dose with appropriate overall fit is given by Equation (4):

$$\overline{\beta_1} = \frac{\sum_{i=1}^{s}(w_i \times \beta_{1,i})}{\sum_{i=1}^{n} w_i},\ i = 1, \dots, s \quad (4)$$

where the weight is the inverse of the squared standard error, $w_i = \frac{1}{SE(\beta_{1,i})^2}$. The standard error of $\overline{\beta_1}$ is estimated as, $SE(\overline{\beta_1}) = \sqrt{\frac{1}{\sum_{i=1}^{s} w_i}}$.

Therefore the dose specific PRR ($PRR_{48,D}$) and corresponding 95% confidence interval is estimated as shown in Equation (5) and (6), respectively:

$$PRR_{48,D} = 10^{-48\times\overline{\beta_1}} \qquad (5)$$

$$95\%\ CI:\ 10^{-48\times\left(\overline{\beta_1} \pm 1.96\times SE(\overline{\beta_1})\right)} \qquad (6)$$

Parasite Clearance Half Life

The parasite clearance half–life (PCt½) will be derived from the optimal decay rate. The relationship between PRR_{48} and parasite clearance half-life (*PCt½*) is a simple transformation of the PRR_{48} as shown in Equation (7):

$$PCt_{½} = log_{10}(2) \times \left(\frac{48\ hours}{log_{10}(PRR_{48,i})}\right) = \frac{\log_{10}(2)}{-\beta_{1,i}} \qquad (7)$$

where $PRR_{48,i}$ is the parasitaemia ratio estimated over a 48-hour interval that is subsequently transformed into a per hour clearance rate.

Comparison of Dose Specific PRR_{48}

To determine whether there are differences between dose specific PRRs, an omnibus test for between group differences is used. The test is used to assess whether there are differences in the weighted mean slope of the J doses, using the test statistic shown in Equation (8),

$$Q_B = \sum_{j=1}^{J} w_{j\cdot}\left(\bar{\beta}_{j\cdot} - \bar{\beta}_{\cdot\cdot}\right)^2 \sim \chi^2_{J-1} \qquad j = 1, \ldots, J \qquad (8)$$

The weight for the j^{th} dose is denoted by $w_{j\cdot} = \sum_{i=1}^{s_j} w_{ij}$ for participant i with appropriate overall fit in dose j. The $\bar{\beta}_{j\cdot}$ is the weighted average slope for dose j as defined in Equation (9), and $\bar{\beta}_{\cdot\cdot}$ is the weighted grand mean given by:

$$\bar{\beta}_{\cdot\cdot} = \frac{\sum_{j=1}^{J} w_{j\cdot}\bar{\beta}_{j\cdot}}{\sum_{j=1}^{J} w_{j\cdot}} \qquad (9)$$

Post-hoc pair-wise comparisons can be calculated using the test statistic $Z_G = \frac{G}{\sqrt{v_G}}$, where G is the contrast ($G = c_1\bar{\beta}_{1\cdot} + \cdots + c_J\bar{\beta}_{J\cdot}$) and v_G is the variance of the contrast ($v_G = \frac{c_1^2}{w_{1\cdot}} + \ldots + \frac{c_J^2}{w_{J\cdot}}$). The p-value of the L pair-wise comparisons can be calculated using the Scheffe method, by comparing Z_G^2 to a chi-squared distribution with $L - 1$ degrees of freedom.

Parasite Regrowth

Incidence of parasite regrowth will be reported as the number and percentage of participants in each dose group, with regrowth defined as ≥5000 parasites/mL and a two-fold parasitaemia increase within 48 hours after initial reduction in parasitaemia.

11.1.3 Presentation of Results

Parasitaemia data will be listed for all enrolled subjects and summarised for the PD population. PD parameters will be presented for all subjects included in the PD population.

Parasite life-stage data (pfs25, pfMGET and sbp1 qRT-PCR) will be listed by participant and timepoint.

A full report on the pharmacodynamic data analysis will be included in the appendices of the CSR. This report will include a listing of PD endpoints (PRR_{48}, $PC_{t1/2}$), a table for Summary of PD endpoints by dose group and a figure for individual log-parasitaemia regression fit

List of planned tables, listings and figures are in Appendix, Table A.

11.2 PK Analysis

Analysis Set: PK Analysis Set

11.2.1 Source of data

Blood concentration of Pyronaridine, treatment and blood sampling information will be recorded and merged to produce PK concentration data for analysis.

11.2.2 Imputation of Non-Numerical or Negative Values

The imputation of non-numerical or negative values reported in the input data set will be performed as follows:

- Pre-dose sample times will be entered as zero
- Values that are below the limit of quantification (BLQ) obtained prior to the C_{max} will be entered as zero
- Values that are BLQ after the C_{max} will be treated as missing
- Pharmacokinetically plausible concentration value(s) below LLOQ at time points between two measurable concentration values are replaced by the LLOQ/2 value, flagged and included in the PK evaluation. In the event of an anomalous below LLOQ result mid profile, the data will be interrogated by the pharmacokineticist and raised with the client before being altered.
- Should partial AUCs be required then values that are BLQ after C_{max} may be imputed as zero for these partial areas if lambda-z cannot be determined
- Values that are quantifiable after at least 2 consecutive BLQ values after C_{max} will be treated as missing for the calculation of PK parameters
- Actual time from dose administration will be used for parameter estimation except for the pre-(first) dose data point which will be set to the nominal time of 0 hour. Data with missing time information will be discarded.
- Values that are reported as “No Result” or “No Sample” etc. will be treated as missing

11.2.3 Rules for Pharmacokinetic Parameter Estimation using WinNonlin

Blood concentration vs time profiles of pyronaridine will be generated for each subject. Pharmacokinetic parameters will be estimated using standard Phoenix WinNonlin methods, details of which may be found in the documentation accompanying the WinNonlin software package. The following constraints will apply:

Parameter Estimation	Constraint
Trapezoidal Method	Linear trapezoidal linear/log interpolation method
Number of Points used for Lambda-z	At least 3, not including C_{max}
Minimum Requirements for AUC	At least 3 consecutive quantifiable concentrations
Dose	As supplied
Sampling Times	Actual sampling times (for final analysis)

Where possible, the elimination rate constant (lambda-z) will be calculated for all subjects. The value of lambda-z will be determined by the slope of the regression line of the natural log transformed concentrations vs time.

The choice of data points for determination of lambda-z will be applied by the Phoenix software as a default method, the pharmacokineticist who may adjust the selection to provide a more appropriate fit and records of this will be documented in the software data.

11.2.4 Data Quality

The following flags/footnotes may be applied to the pharmacokinetic parameters:

Flag	Footnote
a	Rsq of regression was <0.8
b	Period used for regression analysis was less than 2-fold the calculated half-life
c	Extrapolated portion of AUC_{0-inf} >20%
d	Insufficient post-C_{max} data points for estimation of lambda-z
e	Entire profile BLQ, no pharmacokinetic parameters could be calculated
f	Regression could not be fitted

11.2.5 PK parameters

The following parameters will be estimated:

CDISC Term	Parameter	Definition	DP or SF	No. of DP/SF
TMAX	T_{max}	Time of maximum observed concentration	DP	2
TLAG	T_{lag}	The delay between time of dosing and the appearance of concentration	DP	2
CMAX	C_{max}	Maximum observed concentration	SF	3
AUCLST	AUC_{last}	Area under the curve from 0 time to the last measurable concentration	SF	3
AUCIFO	AUC_{0-inf}	Area under the curve from 0 time extrapolated to infinity	SF	3
LAMZHL	$t_{1/2}$	Apparent elimination half-life	DP	2
LAMZ	Lambda-z	Slope of the apparent elimination phase	DP	4

CDISC Term	Parameter	Definition	DP or SF	No. of DP/SF
CLFO	CL/F	Total body clearance after extravascular administration	SF	3
VZFO	V_z/F	Apparent volume of distribution based on the terminal phase after extravascular administration	SF	3

DP=decimal places
SF=significant figures

11.2.6 Presentation of Results

All bioanalytical data will be listed and summarised according to the nominal sampling timepoint.

All pharmacokinetic parameters generated will be listed and summarised. Where pharmacokinetic data fails to meet the defined criteria in Section 11.2.4. (Data Quality) the affected results will be excluded from the descriptive statistics will be flagged as per exclusion reason.

Bioanalytical and PK Parameters Listings

All bioanalytical and pharmacokinetic NCA parameters generated will be listed in individual tables according to the analyte and treatment. The data listings will be generated as defined by the PK Concentration population.

Bioanalytical and Pharmacokinetic Summary Tables

Bioanalytical and PK parameters summary tables will be performed by SSR.

Summary statistics (i.e., mean, median, SD, CV%, minimum, maximum, n, geometric mean, geometric SD and geometric CV%) will be calculated for PK results for each time point, parameter and treatment.

All summary statistics (i.e., mean, median, SD, CV%, minimum, maximum and n) will be presented for all PK parameters for blood by treatment. Also, geometric mean, geometric SD and geometric CV% will be presented for all PK parameters (except T_{max}) by treatment. The T_{max} summary statistics will be provided as n, minimum, median, and maximum only.

All the data summary tables will be generated as defined by the PK Analysis Set. Additional summary tables might be produced if required and requested.

Bioanalytical and Pharmacokinetic Figures

All arithmetical mean blood concentration vs. time curves will be produced by treatment on both linear/linear and log_{10}/linear scales.

All spaghetti plots of individual blood concentrations against nominal sampling times after dosing for each treatment will be produced on both a linear/linear and log_{10}/linear scale. Each volunteer's concentration profile will be represented on these plots with a different symbol and a legend will be included on the plots to define the symbols used.

List of summary tables, listings and figures are in Appendix, Table A.

11.3 Safety

Analysis Set: Safety data will use both FAS and IMP Sets

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

- Adverse events (AEs)
- Vital signs
- 12-lead ECG
- Haematology, biochemistry, urinalysis
- Physical examination

11.3.1 Adverse Events

AEs will be separated into two categories.

- Inoculum-emergent AEs (IEAEs)= AEs beginning any time from inoculation with the challenge agent until the end of study.

- Treatment-emergent AEs (TEAEs)= AEs beginning any time from administration of the IMP until the end of study.

11.3.1.1 AE Listings

All AE data will be listed for each participant and will include:

- IEAEs
- TEAEs
- IEAEs related to malaria
- TEAEs related to IMP
- IE SAEs (Serious adverse events)
- TE SAEs
- IEAEs leading to discontinuation or withdrawal
- TEAEs leading to discontinuation or withdrawal
- IE AESIs (Adverse Events of Special Interest)
- TE AESIs

Parameters to be included in the listings will be:

- Event Term
- Dates, times and study days of onset and resolution
- Severity (CTCAE Grades 1 to 5)
- Outcome (Not Recovered / Not Resolved; Recovered / Resolved; Recovered / Resolved with Sequelae; Recovering / Resolving; Fatal; Unknown)
- Relationship of AE to IMP (Not Related, Related)
- Relationship of AE to rescue medication (Not Related, Related)
- Relationship of AE to malaria challenge agent (Not Related, Related)
- Relationship of AE to study procedures (Not Related, Related)
- Action taken with IMP (No action taken; Rescue medication administered instead of IMP)
- Other actions taken (including withdrawal from the study)
- Seriousness (and Serious Adverse Event (SAE) criteria)

- AESI status (Yes/No)
- Relatedness to COVID-19 (Yes/No)

Derived parameters:

- Duration in hours
- Time in days of onset relative to first IMP administration

11.3.1.2 AE Tables

The number of events, as well as the number and percentage of participants experiencing an AE, will be summarised by, dose group and overall as follows:

Overall summary of IEAEs (FA set)
Overall summary of TEAEs (IMP set)

Summary of IEAEs by System Organ Class and Preferred Term (FA set)
Summary of TEAEs by System Organ Class and Preferred Term (IMP set)

Summary of IEAEs by System Organ Class, Preferred Term and Maximum Severity (FA set)
Summary of TEAEs by System Organ Class, Preferred Term and Maximum Severity (IMP set)

Summary of Malaria-Related IEAEs by System Organ Class and Preferred Term (FA set)
Summary of IMP-Related TEAEs by System Organ Class and Preferred Term (IMP set)

Summary of Malaria-Related IEAEs by System Organ Class, Preferred Term and Maximum Severity (FA set)
Summary of IMP-Related TEAEs by System Organ Class, Preferred Term and Maximum Severity (IMP set)

Summary of IE SAEs by System Organ Class and Preferred Term (FA set)
Summary of TE SAEs by System Organ Class and Preferred Term (IMP set)

Overall Summary of IE AESIs (FA set)
Overall Summary of TE AESIs (IMP set)

Summary of IE AESIs by System Organ Class and Preferred Term (FA set)
Summary of TE AESIs by System Organ Class and Preferred Term (IMP set

For the summary tables, participants who experience the same AE (in terms of the MedDRA preferred term) more than once will only be counted once.
Additional summary tables of AEs related to rescue medications will be included if requested by MMV.

11.3.2 Concomitant Medication

Medications used in this study will be coded using WHODrug Global version B3 Sep 2021 – Added Context.

Concomitant medications are defined as medications continued or newly received at or after administration of the malaria challenge agent through to the End of Study visit.

If a medication has a missing or partial missing start/end date or time and it cannot be determined whether it was taken before initial treatment or concomitantly, it will be considered as prior and concomitant.

Concomitant medications will be summarised by ATC and PN. The summary tables will show the number and percentage of participants taking each medication by ATC and PN.

For the summaries of concomitant medications, participants who take the same medication (in terms of the ATC and PN) more than once will only be counted once for that medication.

Use of rescue medications will be analysed as part of the treatment exposure analysis (Section 8).

11.3.3 Laboratory

Parameters

Hematology

- Basophils (absolute)
- Eosinophils (absolute)
- Haematocrit
- Haemoglobin
- Lymphocytes (absolute)
- Monocytes (absolute)
- Neutrophils (absolute)
- Platelet count
- Red blood cell count
- White blood cell count

Biochemistry

- Alanine Aminotransferase (ALT)
- Albumin
- Alkaline phosphatase (ALP)
- Aspartate Aminotransferase (AST)
- Bicarbonate
- Bilirubin (direct)
- Bilirubin (total)
- Calcium
- Calculated eGFR (CKD-EPI)
- C-Reactive protein
- Chloride
- Cholesterol Total
- Creatinine
- Creatine kinase
- Gamma Glutamyl Transpeptidase (GGT)
- Glucose (random and fasting)
- Glucose-6-phosphatase (G6PD)
- Lactate dehydrogenase (LDH)
- LDL
- HDL
- Phosphate
- Potassium
- Protein Total
- Sodium
- Triglyceride
- Troponin T/high sensitivity
- Urea
- Uric Acid

Pregnancy Test and FSH

β-Human Chorionic Gonadotropin (β-HCG) (women of child bearing potential only)
Follicular Stimulating Hormone (FSH) (post-menopausal women only)

Urinalysis

Dipstick Testing	***Quantitative Assessments***	***Urine Chemistry***
Red blood cells	Red blood cells	Glucose
Glucose	White blood cells	Protein
Ketone		
White blood cells		
Protein		
pH		
Red blood cells		
Glucose		
Ketone		

Coagulation

- International Normalized Ratio (INR)
- Activated Partial Thromboplastin Time (APTT)

Serology

- Hepatitis B surface antigen (HB sAG)
- Hepatitis C antibodies
- Red blood cell antibodies
- Anti-hepatitis B core antibodies (anti-HBc Ab)
- Human Immunodeficiency Virus (HIV) Ag/Ab 1/2 (anti-HIV1 and anti-HIV2 Ab)

Biostatistical methods

Listings

All laboratory parameters and change from treatment baseline values will be presented in data listings. Values outside the laboratory reference range will be listed with flags if considered low or high for values outside the reference ranges (where applicable) and if values considered to be clinically significant by the investigator.

Pregnancy test data will be listed only, for all women. FSH data will be listed only, for all post-menopausal women.

Coagulation data as well as qualitative and quantitative urinalysis data will also be listed only.

Tables

Haematology, biochemistry and continuous dipstick urinalysis laboratory data will be summarised for each scheduled visit, including observed values, absolute change from treatment baseline

Categorical dipstick urinalysis results will be summarised for each scheduled visit using frequency tabulations.

Laboratory abnormalities will be presented as cross-tabulations of the abnormality at each post-treatment baseline analysis visit versus the treatment baseline. Numbers of participants with treatment-emergent abnormalities will also be shown.

In addition, the liver function tests, namely AST, ALT, and total bilirubin, are used to assess possible drug-induced liver toxicity. The proportion of patients with Potential Clinically Significant Abnormality (PCSA) values at any post base line visit will summarized. The highest value of PCSA and time to the highest PCSA reading will also be summarized. Any of the following event will be considered as a PCSA:

- Any ALT or AST value above 5×ULN.
- An elevation in bilirubin 2×ULN.
- Any AST or ALT value above 2×ULN and (total bilirubin >1.5×ULN).
- Any AST or ALT value above 2×ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia (eosinophil percent or count above the ULN).
- Constitutes a possible Hy's Law case
  - Hy's Law case is defined as a Participant with any value of alanine or aspartate aminotransferase greater than 3×ULN together with an increase in total bilirubin to a value greater than 2×ULN and not associated to an alkaline phosphatase value greater than 2×ULN (FDA Guidance on Drug Induced Liver Injury: Premarketing Clinical Evaluation [2009]).

11.4 Vital Signs

Parameters

- Systolic Blood Pressure (SBP) (mmHg)
- Diastolic Blood Pressure (DBP) (mmHg)
- Heart Rate (beats/minute)
- Temperature (°C)
- Respiratory rate (beats/min)

Biostatistical methods

All vital signs data will be listed for all participants. Any values outside of the protocol defined normal ranges will be flagged, with clinical significance status presented for out-of-range results.

Table 2: Vital Signs Normal Ranges

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

Vital sign parameters will be summarised by presenting summary statistics for observed values and change from treatment baseline values for each scheduled visit.

11.5 Body Measurements

Definition of variables

- Height (Screening Only)
- Weight

- Body mass index (BMI)

Biostatistical methods

Body measurement data will be listed for all participants and visits including height, weight and Body Mass Index (BMI) which is auto-calculated within the CRF.

Observed values, as well as changes from treatment baseline, will be summarised descriptively for weight and BMI by visit.

Physical Examination

Parameters

- Weight
- HEENT (Head, Eyes, Ears, Nose, Throat)
- Height (screening only)
- Chest / Lungs
- Abdomen
- Skin
- Neurological / Reflexes
- Heart / circulation
- Neck

Biostatistical methods

Physical examination findings will be listed for all participants and visits

11.6 12-lead ECG

Parameters

-
- PR interval (msec)
- QRS interval (msec)
- QTcF interval (msec)
- Overall ECG assessment (Normal, Abnormal, Not Evaluable)
- ECG abnormality (as appropriate)
- Clinical Significance

Biostatistical methods

ECG parameters will be listed for all participants and visits. Triplicate ECGs will be presented in listings for individual readings as well as the mean for each triplicate. The triplicate means will be used for the summary table. Any values outside of the protocol defined normal ranges (Table XX) will be flagged.

Table 3: ECG Normal Ranges

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

Observed values, as well as changes from treatment baseline, will be summarised descriptively for all ECG parameters by visit. An addition table will present the frequencies of participants who fulfill the following prolongation (change from baseline) criteria, considering all scheduled and non-scheduled visit data:

- QTcF prolongation >30
- QTcF prolongation >60
- QTcF >450 msec for males
- QTcF >470 msec for females
-

11.7 Presentation of Safety Results

The table summary and listings of AEs and lab parameters will be presented for the CSR, and are provided in Appendix, Table B.

12. EXPLORATORY PARAMETERS

Not Applicable

13. CHANGES TO THE PLANNED ANALYSIS

There is no change to the planned analysis by the time when this SAP was written.

14. INTERIM AND FINAL ANALYSIS

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

15. SOFTWARE

SAS® Version 9.4 or higher (SAS Institute, Cary, North Carolina, USA) for Safety, demographic and baseline characteristics, volunteer disposition, protocol deviations, prior medications and treatment exposure analyses
All PK/PD analyses will be performed in R 4.1.3 and IQRtool package 1.10.0 [IQRtools]. NLME modelling will be performed with Monolix 2019R1 [MLX19] using Stochastic Approximation Expectation Maximization (SAEM) for parameter estimation. For PD modelling where typically the variance of random-effects cannot be estimated from the data, the IQRtools SysFit approach [KAS19] will be used to estimate typical and individual PD parameters.
Data manipulation and data analyses for all pharmacodynamic data will be performed using R (highest version available).

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1) Wang CYT, Ballard EL, Pava Z Marquart L, Gaydon J, Murphy SC, Whiley D, O'Rourke P, McCarthy JS (2021) Analytical validation of a real-time hydrolysis probe PCR assay for quantifying Plasmodium falciparum parasites in experimentally infected human adults, *Malaria Journal*, 20(181).

2) Marquart L, Baker M, O'Rourke P, McCarthy JS (2015) Evaluating the pharmacodynamic effect of antimalarial drugs in clinical trials by quantitative PCR, *Antimicrobrial Agents and Chemotherapy,*59, 4249–59.

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4) **KAS19** Kaschek D, Mader W, Fehling-Kaschek M, Rosenblatt M, Timmer J. Dynamic Modeling, Parameter Estimation, and Uncertainty Analysis in R. J. Stat. Soft. 2019 Apr 30;88(10):1-32.

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6) **MLX19** http://lixoft.com/

7) **RAU09** Raue A, Kreutz C, Maiwald T, Bachmann J, Schilling M, Klingmüller U, Timmer J. Structural and practical identifiability analysis of partially observed dynamical models by exploiting the profile likelihood. Bioinformatics. 2009 Jun 8;25(15):1923-9.

8) MMV_Pyronaridine_VIS_Version 4.0_27July2022_SIGNED

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17.

18.

19. APPENDIX

Table A. PD and PK Planned Tables, Listings and Figures

Title	Analysis Set
PD Listings	
Pharmacodynamic –Parasitemia	PD
Pharmacodynamic –Parasite Life Cycle Stages	PD
PK Listings	
Pharmacokinetic Concentrations	PK
Pharmacokinetic Parameters	PK
PD Tables	
Summary of Pharmacodynamic in qPCR 18s	PD
Summary of Pharmacodynamic in Parasite Life Cycle Stages	PD
PK Tables	
Summary of Pharmacokinetic Concentration	PK
Summary of Pharmacokinetic Parameters	PK
PD Figures	
Individual qPCR 18s-time Profiles	PD
Individual RT-PCR pfs25-time Profiles	PD
Individual RT-PCR pfMGET-time Profiles	PD
Individual RT-PCR SBP-1-time Profiles	
PK Figures	
Arithmetic Mean (+SD) Pharmacokinetic Concentration-time Profiles	PK
Individual Pharmacokinetic Concentration-time Profiles	PK

Table B. Baseline Characteristics and Safety Analysis Tables and Listings

Title	Analysis Set
Listings	
Participant Disposition	FAS
Treatment Group Assignment	FAS
Protocol Deviations	FAS
Analysis Population Assignment	FAS
Demographics	FAS
Medical History	FAS
Beck Depression Index (BDI-2)	FAS
Pregnancy Test	FAS
Follicile Stimulating Hormone (FSH)	FAS
Serology Screening	FAS
Urine Drug Screening	FAS
Alcohol Breath Test	FAS
Prior and Concomitant Medications	FAS
Malaria Challenge Agent Administration	FAS
Study Drug Administration	FAS
Rescue Medication Administration	FAS
Inoculum-Emergent Adverse Events	FAS
Treatment-Emergent Adverse Events	IMP
Malaria Challenge Agent related Inoculum-Emergent Adverse Events	FAS
IMP related Treatment-Emergent Adverse Events	IMP
Inoculum Emergent Serious Adverse Events	FAS
Treatment Emergent Serious Adverse Events	IMP
Inoculum-Emergent Adverse Events Leading to Discontinuation	FAS
Treatment-Emergent Adverse Events Leading to Discontinuation	IMP

Inoculum-Emergent Adverse Events of Special Interest	FAS
Treatment-Emergent Adverse Events of Special Interest	IMP
Deaths	FAS
Individual Haematology Results	FAS
Individual Biochemistry Results	FAS
Coagulation Screening	FAS
Individual Abnormal Coagulation Results	FAS
Individual Urinalysis Results (dipstick)	FAS
Individual Urine Microscopy Results	FAS
Individual Vital Signs Results	FAS
Individual ECG Parameters Results	FAS
Physical Examination	FAS
Tables	
Analysis Populations	FAS
Participant Disposition	FAS
Demographics and Baseline Characteristics	FAS
Summary of Prior Medications	FAS
Protocol Deviations	FAS
Treatment Exposure	FAS
Overall Summary of Inoculum Emergent Adverse Events	FAS
Overall Summary of Treatment Emergent Adverse Events	IMP
Summary of Inoculum Emergent Adverse Events by MedDRA System Organ Class and Preferred Term	FAS
Summary of Treatment Emergent Adverse Events by MedDRA System Organ Class and Preferred Term	IMP
Summary of Inoculum Emergent Adverse Events by Maximum Severity	FAS
Summary of Treatment Emergent Adverse Events by Maximum Severity	IMP
Summary of Inoculum related Treatment Emergent Adverse Events by MedDRA System Organ Class, Preferred Term	FAS
Summary of IMP Related Treatment Emergent Adverse Events by MedDRA System Organ Class, Preferred Term	IMP
Summary of Inoculum Related IEAEs by System Organ Class, Preferred Term and Maximum Severity	FAS
Summary of IMP Related TEAEs by System Organ Class, Preferred Term and Maximum Severity	IMP

Inoculum Emergent Serious Adverse Events by MedDRA System Organ Class and Preferred Term	FAS
Treatment Emergent Serious Adverse Events by MedDRA System Organ Class and Preferred Term	IMP
Overall Summary of Inoculum Emergent Adverse Events of Special Interest (AESI)	FAS
Overall Summary of Treatment Emergent Adverse Events of Special Interest (AESI)	IMP
Inoculum Emergent Adverse Events of Special Interest (AESI) by MedDRA System Organ Class and Preferred Term	FAS
Treatment Emergent Adverse Events of Special Interest (AESI) by MedDRA System Organ Class and Preferred Term	IMP
Summary of Concomitant Medications (FAS)	FAS
Summary of Concomitant Medications (IMP)	IMP
Haematology (Summary and Change from IMP Baseline)	FAS
Haematology (Categorical)	FAS
Cross-Tabulation of Haematology Abnormalities Versus IMP Baseline	FAS
Biochemistry (Summary and Change from IMP Baseline)	FAS
Biochemistry (Categorical)	FAS
Cross-Tabulation of Biochemistry Abnormalities Versus IMP Baseline	FAS
Liver Function: Summary of Participant Potentially Clinically Significant Abnormality	FAS
Liver Function: Summary of Peak Readings Since IMP Administration	FAS
Liver Function: Summary of Time to Peak Readings Since IMP Administration in Days	FAS
Urinalysis (Categorical)	FAS
Vital Signs Results and Change from IMP Baseline	FAS
Abnormal Vital Signs Results	FAS
ECG Results and Change from IMP Baseline	FAS
ECG QTc Categorical Analyses	FAS
ECG Clinical Assessment	FAS

MMV_PYR_21_01 SAP_V1.1_06Feb23

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