# Statistical Analysis Plan

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**STATISTICAL ANALYSIS PLAN**

**A randomised, double-blind, placebo controlled, parallel group study in healthy adult volunteers to determine the tolerability and safety of pyronaridine (PYR) co-administered with piperaquine (PQP) under fasted conditions**

**FINAL VERSION 1.0, 17MAY2022**

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## LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

| Abbreviation or Specialist Term | Explanation |
|---|---|
| AE | Adverse Event |
| AUC | Area under the Concentration Time Curve |
| $AUC_{0-72}$ | Area under the Plasma Concentration Curve from Time Zero to 72 Hours |
| $AUC_{0-168}$ | Area under the Plasma Concentration Curve from Time Zero to 168 Hours – ATV only |
| $AUC_{0-inf}$ | Area under the Plasma Concentration Curve from Time Zero Extrapolated to Infinity |
| $AUC_{0-t}$ | Area under the Plasma Concentration Curve from Time Zero to the Last Quantifiable Concentration |
| BLQ | Below the Level of Quantification |
| CL/F | Apparent Total Plasma Clearance |
| $C_{max}$ | Maximum Observed Plasma Concentration |
| CSR | Clinical Study Report |
| CV | Coefficient of Variation |
| CYP | Cytochrome P450 |
| DRM | Data Review Meeting |
| ECG | Electrocardiogram |
| $F_{rel}$ | Relative Bioavailability |
| H | High |
| HR | Heart rate |
| ICF | Informed Consent Form |
| IMP | Investigational Medicinal Product |
| $\lambda_z$ | Terminal Elimination Rate Constant |
| L | Low |
| LLOQ | Lower Limit Of Quantification |
| LOQ | Limit Of Quantification |
| MedDRA | Medical Dictionary for Regulatory Activities |
| MMV | Medicines for Malaria Venture |
| PDF | Portable Document Format |
| PK | Pharmacokinetic(s) |
| PQP | Piperaquine tetraphosphate |
| PR | ECG PR Interval |
| PROC | Procedure in SAS |
| PYR | Pyronaridine tetraphosphate |
| QRS | ECG Ventricular Conductance Time |
| QT | ECG QT interval uncorrected |
| QTc | ECG QT interval corrected |
| QTcF | ECG QT interval corrected using Fridericia's formula |

| | |
|---|---|
| QTcI | ECG QT interval corrected using a subject specific ("individual") correction |
| QTcS | ECG QT interval corrected using a study specific correction |
| RPL | Richmond Pharmacology Ltd |
| RTF | Rich Text Format |
| SAP | Statistical Analysis Plan |
| SAS | Statistical Analysis Software |
| SD | Standard Deviation |
| SOC | System Organ Class |
| SOM | Study Operation Manual |
| TEAE | Treatment-Emergent Adverse Event |
| $t_{½}$ | Terminal Elimination Half-Life |
| TFLs | Tables, Figures and Listings |
| $t_{max}$ | Time to Maximum Observed Plasma Concentration |
| $V_z/F$ | Apparent Volume of Distribution during the Terminal Phase |

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# 1. INTRODUCTION

The purpose of this Statistical Analysis Plan (SAP) is to define the variables and analysis methodology to address the study objectives.

The protocol dated 07 February 2022, version 3.0, was used to prepare this SAP.

Pharmacokinetic parameters calculations and statistical analyses of PK data will be responsibility of PharmaKinetic (described in a separate PK analyses plan); PK TFLs and analyses based on Safety and ECG sets will be the responsibility of Richmond Pharmacology Ltd. Concentration-QTc analysis is optional and will be performed in future, as required.

# 2. STUDY OBJECTIVES AND ENDPOINTS

## 2.1 Study objectives

The objectives of this study are:

**Primary**

- To determine the safety and tolerability of the registered dose for treatment of acute uncomplicated malaria of PYR (once daily for three days) and the registered dose for the treatment of acute uncomplicated malaria of PQP (once daily for three days) when administered alone and in combination, in comparison with placebo.

**Secondary**

- To determine the pharmacokinetics (PK) of PYR and PQP when administered alone, and in combination, for three days.
- To determine the relationship between PQP and ECG parameters ($QT_C$, QRS and PR), between PYR and ECG parameters, and to evaluate any impact of the combination of PYR and PQP on ECG parameters.

**Exploratory**

- To investigate the exposures to PQP metabolites (e.g. N-oxidated metabolite, provided a future bioanalytical method is available).
- To determine if CYP genetic polymorphism influences drug PK and safety & tolerability.

## 2.2 Endpoints

**Primary**

- The incidence, severity and relationship of Treatment-Emergent Adverse Events (TEAEs).
- Proportion of participants with clinically significant changes in laboratory safety tests (haematology, biochemistry, coagulation and urinalysis).

- Proportion of participants with morphological and/or rhythm abnormalities on electrocardiogram (ECG).
- Proportion of participants with clinically significant changes in ECG time intervals (PR, QRS, QT and $QT_c$ intervals) and clinically significant changes against baseline Holter.
- Proportion of participants with clinically significant changes in vital signs (systolic blood pressure, diastolic blood pressure and pulse rate).

**Secondary**

- PK parameters derived by non-compartmental methods including maximum observed plasma concentration ($C_{max}$), time to reach maximum plasma concentration ($t_{max}$), area under the plasma concentration-time curve from time zero to last detectable plasma concentration ($AUC_{0-t}$), area under the plasma concentration-time curve from time zero extrapolated to infinite ($AUC_{0-\infty}$), apparent total plasma clearance (CL/F), apparent volume of distribution during the terminal phase ($V_z$/F), terminal elimination rate constant ($\lambda_z$), and terminal elimination half-life ($t_{1/2}$).

  Difference between baseline corrected $QT_c$ following placebo and baseline corrected $QT_c$ following PQP alone, and with PYR.

**Exploratory**

- PK parameters of PQP metabolites (e.g. N-oxidated metabolite) and metabolites to parent drug ratios.
- The relationship between CYP genetic polymorphism, PK parameters, and safety & tolerability.

## 3. TRIAL DESIGN

### 3.1 Overall Trial Design

This will be a single-centre, randomised, double-blind, placebo-controlled, parallel group study to determine the safety, tolerability and PK of the registered dose to treat acute uncomplicated malaria of PYR + PQP, PYR + PQP placebo, PYR placebo + PQP, as well as PYR placebo + PQP placebo when administered orally once daily for three days to healthy adult male and female participants.
Forty (40) participants are planned to be enrolled and randomised to one of the four treatments in a 2:1:1:1 ratio as described below:

- Treatment 1 (N=16): PYR + PQP
- Treatment 2 (N=8): PYR + placebo for PQP
- Treatment 3 (N=8): placebo for PYR + PQP
- Treatment 4 (N=8): placebo for PYR + placebo for PQP

All participants will receive a single dose of PYR or placebo, as well as a single dose of PQP or placebo, on the morning of Day 1, Day 2 and Day 3. Participants will fast for at least three hours prior to dosing and four hours after dosing. Within

each treatment arm, fasted participants will take PYR/placebo tablets, followed by PQP/placebo tablets, at the following weight-dependent doses (Table 1).

**Table 1: Planned treatment arms and IMP administration**

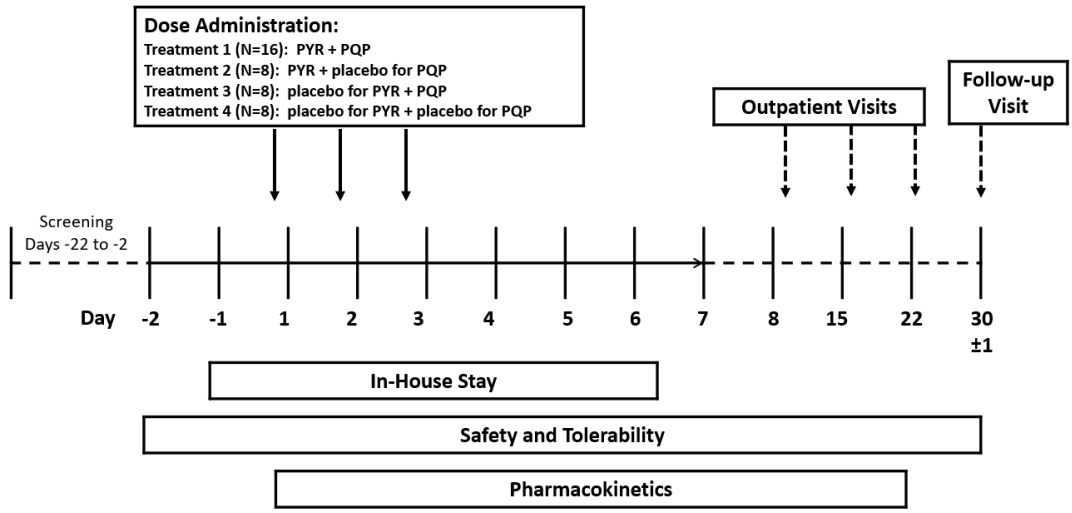
| Number of Participants | IMPs | Planned Dose (which depends on Body Weight [BW]) | Number of Doses/ Dose Interval |
|---|---|---|---|
| 16 | PYR | 540 mg (BW 50 kg - <65 kg); **OR** 720 mg (BW 65 kg or greater) | Single morning doses (fasted state) on D1, D2 and D3 |
| | PQP | 960 mg (BW 50 kg - <75 kg); **OR** 1280 mg (BW 75 kg or greater) | |
| 8 | PYR | 540 mg (BW 50 kg - <65 kg); **OR** 720 mg (BW 65 kg or greater) | |
| | Placebo for PQP | N/A | |
| 8 | Placebo for PYR | N/A | |
| | PQP | 960 mg (BW 50 kg - <75 kg); **OR** 1280 mg (BW 75 kg or greater) | |
| 8 | Placebo for PYR | N/A | |
| | Placebo for PQP | N/A | |

Abbreviations: BW: body weight; D: day; IMP: investigational medicinal product; kg: kilograms; mg: milligrams; PQP: piperaquine; PYR: pyronaridine.

Participant screening will take place between Day -22 and Day -2. Each participant will receive verbal and written information, then sign the Informed Consent Form (ICF), before any screening procedures take place. Participants will be admitted on Day -1 and dosed once in the mornings of Day 1, Day 2 and Day 3. Participants in the sentinel group will be discharged on Day 7 and will return to the unit for outpatient assessments on Days 8, 15, 22. Non-sentinel group participants may be discharged on Day 5 and return for outpatient assessments on Days 6, 8, 15 and 22, provided there are no clinically significant safety and tolerability signals and the SRC are in agreement.

Final assessments will be carried out at the follow-up visit on Day 30 (+/-1 day). The trial Schedule of Assessments in protocol details all assessments that will take place during the trial. The study design features as well as the number of participants may be adapted according to the Adaptive Features. This trial will use a sentinel dosing strategy.

**Figure 1: Trial flow chart**




Abbreviations: N: number of participants in corresponding treatment arm; PQP: Piperaquine; PYR: Pyronaridine.
Participants in the sentinel group and the main group remained in-house until Day 7.

## 3.2 Sample Size

This is an exploratory trial and the sample-size is not based on formal statistical considerations. The numbers assigned to each treatment group are considered adequate to describe the tolerability, safety and pharmacokinetics.
Assuming that an event occurs in 10% of the cases, the probability to see at least one case in 16 subjects on combination treatment is 81%. If the event occurs in 20% of the cases, this probability increases to 97%.
Participants recruitment has stopped at 37.

## 3.3 Randomisation and Blinding

Subject Randomisation

All subjects in this trial will be assigned to a treatment regimen according to a randomisation schedule generated by a statistician using PROC Plan. Details regarding the unique screening and subject number will be included in the SOM.

Eligible subjects will be randomly assigned in a 2:1:1:1 ratio to one of four treatment arms on Day 1, at the point of dosing. Treatment arms are outlined below:

- Treatment 1 (N=16): PYR + PQP
- Treatment 2 (N=8): PYR + placebo for PQP
- Treatment 3 (N=8): placebo for PYR + PQP
- Treatment 4 (N=8): placebo for PYR + placebo for PQP.

Sentinel dosing (N=10) will occur: four subjects for treatment arm 1, and two subjects from each of treatment arms 2, 3 and 4, will be dosed before the remaining subjects of each treatment arm are dosed together (see protocol Section **Error! Reference source not found.**).

Methods for ensuring blinding

The trial will take place in a double-blind fashion whereby subjects and clinical trial site staff are blinded to the active or placebo trial drug assignment.

PQP is uncoated and bitter to taste, the effect may be persistent for some time after administration. This effect cannot be masked and may provide an indication of the treatment received. As the key safety and tolerability endpoints are objective assessments and parameters, it is anticipated that there will be no impact on the primary trial objective to determine the safety and tolerability of the registered doses of IMP.

The pharmacy staff preparing the investigational products will not be blinded to trial drug assignment. During the trial, the individual randomisation codes will be kept in the site's clinical trials pharmacy, accessible to the pharmacy personnel only. Upon completion of the trial, after the database lock and after the blind is revealed, the randomisation list will be filed in the Trial Master File (TMF).

Sponsor staff involved in clinical decision-making (such as those involved in SRC decisions) will be blinded to trial drug assignment.

# 4. STATISTICAL ANALYSES

## 4.1 General Notes for Statistical Analyses

In general, descriptive statistics for continuous variables will include number of non-missing values (n), arithmetic mean, standard deviation (SD), median, minimum, and maximum.

Descriptive statistics for PK parameters will include N - the number of participants, n – the number of samples, n(LLOQ) - the number of samples <LLOQ (lower limit of quantification), arithmetic mean, SD - standard deviation, CV - coefficient of variation, geometric mean, median, minimum, maximum.

Categorical variables will be summarized using frequency counts and percentages.

For all tables, except PK parameter tables, descriptive statistics for minimum and maximum will be presented with the same decimal digits as the original data, and with 1 more decimal place than the original data for mean and median; SD and SE will be reported with 2 more decimal places than the original data. Degrees of freedom based on the Kenward Roger approximation will be presented with one decimal.

PK parameters will be presented as follows in the listing: $C_{max}$ and $t_{max}$ will be presented as given in the raw data; other PK parameters will be presented with 3 decimal places. Descriptive statistics for PK parameters will be presented with decimal places as appropriate for the particular parameter and treatment group.

The analyses will be presented by treatment group (2 monotherapies, combination therapy and placebo), overall as well as stratified by body weight group:

- < 65 kg
- 65 - < 75 kg
- ≥ 75 kg.

- Treatment arm 1:
  - PYR 540 mg + PQP 960 mg
  - PYR 720 mg + PQP 960 mg
  - PYR 720 mg + PQP 1280 mg
- Treatment arm 2
  - PYR 540 mg
  - PYR 720 mg
- Treatment arm 3
  - PQP 960 mg
  - PQP 1280 mg
- Overall (for safety set)
- Placebo

All collected data will be presented in by-subject listings. Listings will be ordered by treatment group, body-weight group and subject number and will include all randomized subjects.

Unless otherwise stated, baseline will be defined as the last non-missing value prior to first administration of study drug. Changes from baseline values will be calculated as the post-baseline assessment value minus the baseline value. Only observed values from scheduled time points will be used to create summary tables.

Deviations from the planned analyses will be described in the final clinical study report (CSR).

Page layout of the TFLs will be in landscape mode and will be provided as individual RTF format. Further details of page layout will be provided in the TFL shell document.

If repeated measurements are made at a time point, the first scheduled value will be used for summary analysis, unless otherwise stated in relevant section of this SAP.

### 4.2 Interim Analysis

No interim analysis is planned for this study.

### 4.3 Analysis Sets

The analysis of data will be based different analysis sets according to the purpose of analysis. Subject eligibility for each analysis set will be finalised before the

database hard lock. A subject who withdraws prior to the last planned observation in a tail period will be included in analyses up to the time of discontinuation.

The following data sets will be used for analysis and presentation of the study data:

Safety Set

The safety set will consist of all randomised subjects who received at least one dose of the IMP. The safety set will be used for the safety analyses.

PK Set

See PKAP.

ECG Set

The ECG set will consist of those subjects in the safety set that have at least one valid pre-dose ECG assessment and one valid post-dose assessment. An ECG assessment will be considered valid if it is based on at least two evaluable replicates with measurable QT and RR.

The analysis set for intensive cardiac assessment will be based on the intersection of the PK set and the ECG set. In addition, subjects on placebo will be included with plasma concentrations of PYR and PQP set to zero. Individual $QT_c$/concentration pairs will be excluded from this set if the time of ECG and the time of blood sampling are too far apart: > 15min.

## 4.4 Subject Disposition

All subjects will be included in the summary of subject disposition. This will present the overall number of subjects, the frequency and percentage of subjects randomized and treated, and who completed or discontinued from the study, along with reason for discontinuation.

Furthermore, the number and percentage of subjects in each analysis set will be tabulated. Discontinued subjects will be listed. Subject assignment to analysis sets will be listed. Screen Failures will not be listed or included in summary tables.

## 4.5 Demographic Characteristics

Individual subject demographics (including age, sex, race and ethnicity) and body measurement data (height, body weight and body mass index and body weight group as defined above) at screening will be listed and summarized for the safety set. Height will be measured in centimetres and weight in kilograms, body mass index will be given in $kg/m^2$.

Other baseline characteristics with Beck depression inventory questionnaire will be listed only.

### 4.6 Inclusion and Exclusion Criteria

The inclusion and exclusion criteria text will be listed, as well as failed eligibility criteria for each randomised subject, if any.

### 4.7 Protocol Deviations

The final review of protocol deviations will be performed at the DRM prior to database lock. The protocol deviations will be listed.

### 4.8 Medical and Surgical History

Medical and Surgical history data will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) dictionary Version 24.0 (or higher) and listed individually. Surgical history data will be listed separately. Medical and Surgical history data will be summarised using frequency and percentage by SOC and preferred term.

### 4.9 Study Drug Administration

Study drug administration data including treatment received, dose (unit), date and time of administration will be listed by subject.

### 4.10 Prior and Concomitant Medications

All prior and concomitant medications will be coded using the World Health Organization Drug Dictionary version 2022 (or higher) and will be listed individually. The frequency and percentage of prior and concomitant medications will be summarized by Anatomical Therapeutic Chemical and Preferred Name. Separate tables will be given for prior and concomitant medications. Prior medications are defined as those for which the end date and time is prior to the date, and time of first study drug administration. Concomitant medications are defined as those with start date and time on or after the date and time of first study drug administration and those with start date and time prior to the first study drug administration but with end date and time on or after the date and time of first study drug administration.

If medication dates are incomplete and it is not clear whether the medication was concomitant, it will be assumed to be concomitant.

### 4.11 Safety Analysis

Safety analyses will be performed on the safety set.

Safety analyses will include an analysis of all AEs, ECGs, clinical laboratory data, vital sign measurements and physical examination results and will be presented using descriptive statistics. No formal statistical analysis will be performed.

*4.11.1 Adverse Events*

A Treatment Emergent Adverse Event (TEAE) is any adverse event that commences after the start of administration of study drug.

AEs with unknown start date/time will be assumed to be treatment-emergent unless the end date/time is known to be before the first administration of study drug. Otherwise missing or partial dates will be listed as such.

The incidence of TEAEs (after dosing) will be summarized using the safety set. The MedDRA dictionary Version 24.0 (or higher) will be used to classify all AEs reported during the study by System Organ Class (SOC) and Preferred Term. A summary of TEAEs including the incidence of subjects who experienced TEAEs (number and percentage of subjects) and incidence of TEAEs (number of events) will be presented for each treatment group and overall, by severity and by relationship to study drug.

Serious TEAEs (after dosing) and AEs leading to withdrawal will be summarized by SOC and Preferred Term for each treatment group and overall, and by relationship to study drug.

Subjects having multiple AEs within a category (e.g., overall, SOC and Preferred Term) will be counted once in that category. In each table, SOC and Preferred Term will be presented in descending order of overall incidence rate (alphabetical order will be used in case of equal rates).

All adverse events will be listed.

*4.11.2 Laboratory Data*

Clinical laboratory parameters (including blood chemistry, haematology, coagulation, urinalysis and other laboratory results) will be listed and abnormal parameters will be flagged and graded as high (H/x) or low (L/x) (where defined) according to reference ranges and CTCAE grade. Absolute (observed) values and changes from baseline (continuous variables) will be summarized for each parameter and scheduled time point by treatment group. The number of volunteers presenting out-of-range and clinically relevant values will be summarised by treatment group. The scheduled lab value will be used for summary analysis if repeated measurements are made at any time point.

For summary statistics, a lab value with "<" will be replaced with a numeric value by removing the "<" sign. In the listings, the values will be displayed as originally reported by the laboratory.

The mean and individual change from baseline value of ALT/AST/Bilirubin will be plotted by treatment group and by time-point. The number of subjects presenting out-of-range and clinically relevant values will be summarised.

The qualitative urinalysis data will be listed only.

| Test Code | Test Unit | Range Low | Range High | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
|---|---|---|---|---|---|---|---|
| ALT | IU/L | 10 | 50 | >ULN - 3.0 x ULN | >3.0 - 5.0 x ULN | >5.0 - 20.0 x ULN | >20.0 x ULN |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| AST | IU/L | 0 | 37 | >ULN - 3.0 x ULN | >3.0 - 5.0 x ULN | >5.0 - 20.0 x ULN | >20.0 x ULN |
| Total bilirubin | UMOL/L | 0 | 20 | >ULN - 1.5 x ULN | >1.5 - 3.0 x ULN | >3.0 - 10.0 x ULN | >10.0 x ULN |

*4.11.3 Vital Signs*

Vital signs data (SBP, DBP, Pulse rate, temperature) will be listed for individual subjects. Summary statistics of absolute (observed) values and changes from baseline will be calculated for each parameter and scheduled timepoint by treatment group.

Out-of-reference-range values will be flagged as high (H) or low (L) and as being clinically relevant or not: the number of subjects presenting out-of-range and clinically relevant values will be summarised.

| Parameter | Normal Range |
|---|---|
| Temperature (tympanic) | 35-37.5°C |
| Systolic Blood Pressure (Supine) | 90–140 mmHg |
| Diastolic Blood Pressure (Supine) | 40–90 mmHg |
| Postural Drop (systolic, symptomatic) | ≤20 mmHg drop |
| Pulse Rate (supine) | 40–100 bpm |
| Respiratory Rate | 8-20 breaths per min |

*4.11.4 Telemetry and Holter*

Cardiac telemetry and Holter data (Start / End Date time and evaluation) will be listed by subject.

*4.11.5 Physical Examination*

The physical examination performed at screening and at the post-trial visit will be listed only and include an assessment of the following: general appearance, skin, eyes/ears/nose, neck, lymph nodes, throat, heart, lungs, abdomen, musculoskeletal system and extremities.

*4.11.6 Electrocardiograms*

ECG analyses will be performed on two sets of ECGs: all ECGs prior to adjudication by a cardiologist and selected triplicates from each time-point after adjudication. The latter analyses will be performed as contracted.

All un-adjudicated ECG data (PR, QRS, QT, $QT_cB$, $QT_cF$ and HR) including H/L flags and overall ECG evaluation will be listed. Out-of-reference-range values will be flagged as clinically relevant or not. Reference ranges for ECGs are as follows:

| Parameter | Normal Range |
|---|---|
| Heart Rate (Supine) | 40-100 bpm |
| PR Interval | 120–220 ms |
| QRS duration | ≤120 ms |
| QTcF | ≤450 ms (Males & Females) |

For summary statistics an arithmetic mean value, of the last 3 evaluable ECGs of each timepoint, will be used at each time-point. All below analyses will be performed using this set.
ECG data and changes from baseline will be summarised using descriptive statistics.
The change from baseline will be derived using the arithmetic mean value of each time-point triplicate minus baseline value, where baseline is the arithmetic mean of the pre-dose QTcF values of Day 1.

Furthermore, categorical analysis of QTcF data will be presented as follows:

- Absolute QTcF interval prolongation
    - QTcF interval > 450 ms to ≤ 480 ms
    - QTcF interval > 480 ms to ≤ 500 ms
    - QTcF interval > 500 ms
- Change from baseline in QTcF interval
    - QTcF interval increases from baseline > 30 msec to ≤ 60 msec
    - QTcF interval increases from baseline > 60 ms

The mean change from baseline value of $QT_cF$ will be plotted by treatment group and by time-point. The number of subjects presenting out-of-range and clinically relevant values will be summarised.

Additional categorical analysis will be presented as follows:

- increase of PR from baseline >25% resulting in PR > 200 ms
- increase of QRS from baseline >25% resulting in QRS > 120 ms
- decrease of HR from baseline >25% resulting in HR < 50 bpm
- increase of HR from baseline >25% resulting in HR > 100 bpm.

### 4.12 ECGs analyses (adjudicated)

Analysis of drug related QT/$QT_c$ interval changes relative to plasma PK concentrations is optional and will be conducted as required. This analysis will focus on the known PQP-induced QTc prolongation and possible impact of PYR on this effect. The principles of this analysis follow the statistical methods described by Garnett et al., 2018 [1]. This analysis will be performed on the analysis set for intensive cardiac assessment.

In addition, a per timepoint analysis will be performed. While this analysis is based on minimal assumptions on the nature of the data, it has limited power to show the absence of a prolonging effect. On the other hand, the concentration-$QT_c$ analysis has been shown to have this power, but it is based on more restrictive assumptions. Concentration/ $QT_c$ modelling may be performed after completion of the Clinical Study Report and will be delivered in a separate report.

Baseline

For all quantitative parameters, baseline will be the average over the values obtained at the three pre-dose timepoints of Day 1.

Heart rate correction

$QT_cF$ will be used for ECG analyses unless there is an effect of one of the drugs on HR exceeding 10 bpm. Evidence of the absence of such an effect will be considered given, if either the absolute mean time matched difference to placebo is below 10 bpm for all timepoints and for both monotherapies and the combination therapy or a concentration-effect model similar to the one described for QTc below excludes an effect exceeding 10 bpm at relevant concentrations. If an effect of the therapy on HR cannot be excluded following the criteria above, $QT_cI$ and QTcS will be determined based on data extracted from the 24h Holter data and the primary correction method will be selected based on the mean squared slope criteria.
More specifically, around 30 ECG snapshots will be extracted from Holters spread across the whole recording of Day 1, aiming to capture wide variety of HR values and RR and QT values determined. For each subject, these RR/QT pairs will be ranked by heart rate and the pairs with odd rank assigned to the training set and the remaining ones to the validation set. Based on the data in the training set, a regression of the form

$$\log(QT) \sim \log(RR) + 1$$

will be performed. Let $\beta_i$ be the regression coefficient ("slope") thus obtained for subject i, then the individual correction for this subject will be

$$QTcI_i = \frac{QT}{RR^{\beta_i}}$$

The exponent for the study-specific correction (QTcS) will be the median of the individual $\beta_i$ across subjects and the correction will be calcualted along the same lines.
For each subject i and each of the three correcton methods QTcF, QTcI and QTcS, a linear regression on the pairs of RR and QTc in the validation set of that subject will be fitted and the slope $b_{ik}$ for the regression of the validation data of subject i with method k be obtained. For each method, the mean squared slope across

subjects will be determined and the method with the smallest mean slope will be used as primary correction method.

Per timepoint analysis

For all quantitative ECG parameters, descriptive statistics will be given for the change from baseline. In addition, for $QT_c$, a linear model with the two treatments (PYR and PQP) and their interaction as factors and baseline as covariate will be fitted for each timepoint; the difference between each of the active treatments and placebo will be estimated based on this model; and two-sided 90% confidence intervals (CI) will be given.

Hysteresis

Hysteresis between the effect of each of the monotherapies and its concentration will be investigated based on the results of the per timepoint analysis and the mean concentrations under these therapies. These time courses will be displayed in two or three panels respectively using the same time axis and, in addition, as hysteresis loops, separately for Day 1 and Day 3. Finally the enGRI [4] may also be calculated.

Concentration-$QT_c$ analysis

Concentration-QTc analysis is optional and will be performed in future, as required. It might be based on a separate analysis plan and reported in an addendum to the final CSR or a separate report, as appropriate.

This analysis will be based on the change of $QT_c$ from baseline and will use baseline $QT_c$ (centred on the mean across subjects) and the concentrations of PYR and PQP as covariates. Concentrations of relevant metabolites may also be included. Interaction between concentrations of PYR and PQP will also be included. Interaction terms with metabolites, if any, will not be used.

Concentrations BLQ will be set to nought. Likewise, concentrations for participants under placebo will be set to nought, as well as those for the drug not given to a participant.

Treatment with levels Placebo, PYR, PQP and combination (referred to as "treatment intercept" in the sequel) and time will be used as discrete fixed effects.(Note that the same timepoint at different days will be considered distinct.)

A series of linear models including one or more of the concentrations will be fitted. The model including both moieties will have the form (in the notation of lmer in R):

$$dQTc \sim TP + trt + CPYR*CPQP + Cbl -1 + (1 + CPYR + CPQP|Subj)$$

where

- dQTc is the change from baseline of QTc
- TP is the factor describing timepoints
- trt is the treatment intercept
- CPYR is the concentration of PYR
- CPQP is the concentration of PQP
- Cbl is the centered baseline
- Subj is the factor describing participants (as unit of observation).

From this model simplified models will be derived by removing one of the concentrations variables.

The most appropriate one will be selected based on the Akaike Information Criterion (AIC) and the size of the fixed treatment effect. A significant treatment effect (based

on an F-test, $p < 0.05$ two sided) will be considered an indication for model misfit. For the best fitting linear model, predictions of the effect on $QT_c$ will be made. These predictions will be made separately for each treatment group and will be based on the mean concentrations of all moieties involved in the respective model.
For the predictions, the $T_{max}$ of every moiety included in the model will be determined per participant and the mean concentration of all moieties involved at these timepoints will be used. Since a geometric mean will be nought if at least one of its components is BLQ, geometric means will only be used for the moiety for which $T_{max}$ was determined, for concentrations of moiety j at $T_{max}$ of moiety i with $i \neq j$, arithmetic means will be used.

Linearity of the concentration-ΔQTc relationship will be explored based on quantile plots as described in Garnett et al (2018) [1]. In addition, a significant treatment intercept can be interpreted as sign of either hysteresis or nonlinearity. If the best fitting linear model has a significant treatment effect, nonlinear e-max models will also be considered, and predictions will be made based on the best fitting model.
In this case, hysteresis between $QT_c$ and the concentrations of the relevant analytes will be investigated graphically.
If the above models fail to converge even after rescaling of the variables included to a range between [-30, 30] and [-100, 100], they will be simplified using these steps: Removal of random slopes and, if convergence is still not reached, simplifying of the covariance structure (originally unrestrained) to compound symmetry.

Absence of an effect of concern of the IMP on $QT_c$ will be concluded if the predictions based on the best-fitting model at the concentrations described above exclude an effect exceeding 10 ms, i.e. the upper limit of the two-sided confidence interval for these predictions is below 10 ms.

Assay sensitivity

In a study analysing the effect of a combination of OZ439 and PQP, Darpö et al 2015 [2] found an effect of PQP concentration on QTc of 0.0475 ms/(ng/mL) with a 90 % confidence interval of (0.038, 0.057). If the effect of PQP in the primary linear model of this analysis is similar to this value, assay sensitivity will be deemed shown.

*Presentation of results*

For each model the parameter estimates with SE, t-value, df and two sided 90 % CI will be tabulated. The df and CI will be estimated based on the Kenward-Roger approximation. In addition, the following values will be tabulated for each model: AIC, residual variance, variance of each of the random effects, F-value for the treatment intercept with its corresponding p-value.

dQTc will be plotted against the concentrations of both moieties, with treatment group shown by different symbols. For models based on one concentration only, a regression line shifted by the average of the time effects will be added.

For each model, one or more quantile plots [1] will be presented using concentration of each concentration involved as abscissa and the partial residuals with respect to this concentration as ordinate. The predicted effect of the respective moiety will be given as a regression line with two-sided 90 % confidence range. Note that this

effect can be obtained from the original dQTc values by subtracting the predictions had the same participant taken placebo and the concentrations of all moieties set to 0.

### 4.13 Pharmacokinetic Data Reporting

PK parameters and report will be generated by Pharmakinetic, PK TFLs will be generated by RPL.

#### 4.13.1 Pharmacokinetic Listings

PK plasma concentration data will be listed for each individual subject by treatment and timepoint. In addition, the sum of the moiety data (proguanil and cycloguanil) will be listed individually according to treatment.

PK parameters will be listed for each individual subject by treatment and timepoint.

The data listings will be generated as defined by the PK population in PKAP. The data will be reported to the original decimal points as per source data.

#### 4.13.2 Pharmacokinetic Summary Tables

Summary statistics for plasma concentrations (N - the number of participants, n – the number of samples, n(LLOQ) - the number of samples <LLOQ, arithmetic mean, SD - standard deviation, CV - coefficient of variation, geometric mean, median, minimum, maximum) will be calculated at each time point for each treatment.

Summary statistics for the PK parameters (N - the number of participants, arithmetic mean, SD - standard deviation, CV - coefficient of variation, geometric mean, median, minimum, maximum) will be presented for each treatment group (except Tlag and Tmax). The Tmax and Tlag summary statistics will be provided as n, minimum, median and maximum only.

#### 4.13.3 Pharmacokinetic Figures

Arithmetical mean plasma concentration vs. time curves for each analyte which includes all treatments will be produced on both linear/linear and log10/linear scales.

Spaghetti plots of individual plasma concentrations against actual sampling times after dosing for each analyte and treatment will be produced on both a linear/linear and log10/linear scale. Each subject'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.

#### 4.13.4 Values Below the Limit of Quantification and Missing Values

When calculating the figures and summary statistics for a concentration at a given time point, all BLQ values will be set to zero except when an individual BLQ falls between 2 quantifiable values, in which case it will be set to missing. Samples with no reportable value due to a bioanalytical issue or missing samples will be set to missing.

### 4.14 Exploratory Analysis

Blood samples for CYP polymorphism will be collected and analysis will be optional and may be performed retrospectively. SAP addendum will be issued as appropriate.

## 3. REFERENCES

1. Garnett C, Bonate P, Dang Q, Ferber G, Huang D, Liu J, Mehortra D, Riley P, Sager P, Tornoe C, Wang Y. Scientific white paper on concentration-$QT_c$ modelling. Journal of Pharmacokinetics and Pharmacodynamics. 2018;45, 383-397. https://link.springer.com/article/10.1007%2Fs10928-017-9558-5. Accessed on 15 October 2021.
2. Tornøe CW et al (2011) Creation of a knowledge management system for QT analyses. J Clin Pharmacol 51(7):1035–1042
3. Darpö B, Ferber G, Siegl P, Laurijssens B, Macintyre F, Toovey S, Duparc S. Evaluation of the QT effect of a combination of piperaquine and a novel anti-malarial drug candidate OZ439, for the treatment of uncomplicated malaria. British Journal of Clinical Pharmacology. 2015;80(4):706-15. Accessed 29 November 2021.
4. Ferber, G., Darpo, B., Garnett, C. et al. Detection and impact of hysteresis when evaluating a drug's QTc effect using concentration-QTc analysis. J Pharmacokinet Pharmacodyn 48 (2021) 187 - 202. https://doi.org/10.1007/s10928-020-09725-w

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