PHARMACOKINETIC DATA ANALYSIS PLAN

A randomized, open label, two-part, parallel-group, phase I study to evaluate the pharmacokinetics of Piperaquine oral dispersible Granules Formulation compared to Piperaquine hard Tablets administered as a single dose in fasting condition (Part 1) and of Piperaquine oral dispersible Granules Formulation administered as single dose in various fed states (Part 2) in healthy adult participants

PharmaKinetic Study Number:	PHK2020-174
Sponsor Study Number:	MMV_SMC_22_01
Sponsor:	Medicines for Malaria Venture (MMV) ICC – Block G, 3rd floor 20, Route de Pré-Bois PO Box 1826 1215 Geneva 15 Switzerland
Date of Analysis Plan:	02nd October 2023
Version Number:	Version 2.0

Approval Signatures
This PK analysis plan has been approved by.

On behalf of Sponsor,

On behalf of PharmaKinetic,

1 Document Version History

Version Number	Reason for Update	Updated By:	Date
Version 1.0	First version issue	Rachael White	8th August 2023
Version 2.0	Version updated to include AUC(0-24) as a required PK parameter	Rachael White	2nd October 2023

2 Table of Contents

3 Study Design

3.1 Outline Design and Pharmacokinetic Objectives

This is a randomized, open label, parallel-design study to evaluate the PK profile and relative bioavailability of single dose of a PQP oral granule formulation dispersed in water (test) as compared to the PQP hard tablet formulation (reference) in fasting conditions (Part 1) and to assess the BA/FE of the PQP dispersible granules formulation when administered with different food restrictions (Part 2) among African participants residing in Tanzania, in Bagamoyo Town and surrounding areas. A total of sixty (60) healthy adult participants (male and female) will be enrolled in Part 1 and Part 2.

Part 1: PQP tablet / dispersible granule administered in fasting condition of at least 10 hours

- Group 1: PQP hard tablet, 320 mg (N=12)
- Group 2: PQP dispersible granule, 320 mg (N=12)

Transition to Part 2:

- To minimize the risk to healthy participants, a decision on transitioning from Part 1 to Part 2, will be taken by the Safety Review Committee (SRC), based on the safety and preliminary PK interim report of the granule formulation, compared to the PQP hard tablet, with safety data obtained up to Day 15 and PK data obtained up to Day 8. If necessary, doses may be readjusted for the Part 2 in order to assess the food effect, to ensure that the predicted exposure of PQP from the granule formulation in the fed state doesn't exceed the exposure achieved in adults administered a dose of 960 mg PQP in a tablet of Eurartesim® in fasted conditions. Note, the observed food effect when film-coated tablets (Eurartesim®) are administered with a high fat/high calorie meal is a 3-fold increase in exposure.

Part 2: PQP dispersible granule administered in fed conditions (planned as 320 mg)

- Group 3: High-fat meal (N=12)
- Group 4: Low-fat meal representative of African diet (N=12)
- Group 5: Whole milk 250 ml (N=12)

3.1.1 Pharmacokinetic Objectives

Primary:

- To determine the relative bioavailability of a single oral dose of PQP dispersible granule formulation (Test) as compared to PQP hard tablet formulation (Reference) in the fasted state.

Secondary:

- To assess the effect of different types of meal composition on the PK of single doses of PQP dispersible granule formulation in healthy adult participants.

- To further evaluate the PK of a single oral dose of PQP granule formulation in the fasted state and different fed states in healthy adult participants.

3.1.2 Pharmacokinetic Endpoints

Primary:

- Relative bioavailability (F_{rel}) using the ratio of the geometric means for the dispersible granule (test) over the hard tablet (reference) in fasted state for area under the plasma concentration-time curve from time zero to 72 hours (AUC_{0-72h}), 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 to 168 hours (AUC_{0-168h}), area under the plasma concentration-time curve from time zero extrapolated to infinity ($AUC_{0-\infty}$), and Maximum plasma concentration (C_{max}).

Secondary

- The F_{rel} using the ratio of the geometric means for the dispersible granules formulation between fed [high-fat meal, low-fat African meal, and milk] over the dispersible granule formulation fasted [reference] for AUC_{0-72h}, AUC_{0-t}, AUC_{0-168h}, $AUC_{0-\infty,}$ and C_{max}. If the dose is adjusted in Part 2 of the study the F_{rel} calculations will be dose adjusted.

- PK parameters for the hard tablet (fasted) and the dispersible granule formulations (fasted and fed); C_{max}, AUC_{0-24h}, AUC_{0-72h}, AUC_{0-t}, AUC_{0-168h}, $AUC_{0-\infty}$, time to maximum plasma concentration (T_{max}), terminal elimination rate constant (λz), terminal elimination half-life ($t_{1/2}$), apparent volume of distribution during the terminal phase (Vz/F), apparent total plasma clearance (Cl/F), and percentage of AUC that is due to extrapolation from t_{last} to infinity ($\%AUC_{extrap}$).

4 Populations for Analysis

4.1 Pharmacokinetic Population

The PK Population will include all subjects who have received at least one dose of IMP and who have a minimum of 1 valid post-dose analytical result for pharmacokinetic parameter estimation. The pharmacokinetic population will be confirmed once all PQP plasma concentration data have been received. The PK population will be used to list and to summarise PK data.

The following PK subsets may also be defined for statistical comparisons;

PK subset 1: If any subjects vomit within 4 hours after dosing, their PK data will be assessed, and a decision may be taken to remove these subjects from the statistical comparisons. This would be defined as PK subset 2. This subset will only be deemed necessary if vomiting appears to impact the resulting PK parameters. The generation of this subset will be justified and will be documented formally where needed.

5 Pharmacokinetic Data Analysis

5.1 Pharmacokinetic Parameter Estimation

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

5.1.1 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
- 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
- Values that are reported as "No Result" or "No Sample" etc. will be treated as missing

5.1.2 Rules for Pharmacokinetic Parameter Estimation using WinNonlin

Plasma concentration vs time profiles of PQP (and metabolite) 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/log trapezoidal rule
Number of Points used for Lambda-z	At least 3 post C_{max}, not including C_{max}
Minimum Requirements for AUC	At least 3 consecutive quantifiable concentrations
Sampling Times used in analysis	Actual sampling times; when no actual sampling time is available the nominal sampling time will be used
Rounded Dose Level	3 Significant Figures or 2 decimal places

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 in order to provide a more appropriate fit and records of this will be documented in the software data.

5.1.3 Data Quality

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

Flag	Footnote
a	Rsq of regression was <0.8
b	Extrapolated portion of $AUC_{(0\text{-inf})}$ >20%
c	Insufficient post-C_{max} data points for estimation of lambda-z
d	Entire profile BLQ, no pharmacokinetic parameters could be calculated

In the event that a reliable lambda-z cannot be determined, or the extrapolated portion of $AUC_{(0\text{-inf})}$ is >20%, then the parameter estimates derived using lambda-z and/or $AUC_{(0\text{-inf})}$ may be deemed unreliable and excluded from the summary statistics. Additional flags may be applied based on emerging data.

5.1.4 Definition of Pharmacokinetic Parameters

The following pharmacokinetic parameters for PQP (and metabolite) in plasma will be estimated where possible and appropriate for each subject and treatment.

Table 1 Pharmacokinetic Parameters and Reporting Specifications

Data transfer Term	Parameter	Definition	DP or SF	No. of DP/SF
TMAX	T_{max}	Time at which the maximum observed plasma concentration occurs	DP	2
CMAX	C_{max}	Maximum observed plasma concentration	SF	3
AUC024	$AUC_{(0-24)}$	Area under the curve from time zero to 24 h	SF	3
AUC072	$AUC_{(0-72)}$	Area under the curve from time zero to 72 h	SF	3
AUC0168	$AUC_{(0-168)}$	Area under the plasma concentration curve from time zero to 168 hours	SF	3
AUCLST	$AUC_{(0-t)}$	Area under the plasma concentration curve from time zero up to the last quantifiable concentration	SF	3
AUCIFO	$AUC_{(0-inf)}$	Area under the curve from time zero extrapolated to infinite time	SF	3
AUCPEO	$\%AUC_{extrap}$	Percentage of AUC that is due to extrapolation from tlast to infinity	DP	2
LAMZHL	$t_{1/2}$	Apparent terminal elimination half-life	DP	2
LAMZ	Lambda-z	Terminal Rate constant	DP	4
CLFO	CL/F	Apparent total plasma clearance after extravascular administration	SF	3
VZFO	V_z/F	Apparent volume of distribution during the terminal phase after extravascular administration	SF	3
FRELCMAX*	Frel C_{max}	Relative bioavailability calculated as the ratio of the C_{max} test/C_{max} reference	DP	3
FRELAUC72*	Frel $AUC_{(0-72)}$	Relative bioavailability calculated as the ratio of the $AUC_{(0-72)}$ test/ $AUC_{(0-72)}$ reference	DP	3
FRELAUC168*	Frel $AUC_{(0-168)}$	Relative bioavailability calculated as the ratio of the $AUC_{(0-168)}$ test/ $AUC_{(0-168)}$ reference	DP	3
FRELAUCLST*	Frel $AUC_{(0-t)}$	Relative bioavailability calculated as the ratio of the $AUC_{(0-t)}$ test/ $AUC_{(0-t)}$ reference	DP	3
FRELAUCINF*	Frel $AUC_{(0-inf)}$	Relative bioavailability calculated as the ratio of the $AUC_{(0-inf)}$ test/ $AUC_{(0-inf)}$ reference	DP	3

DP=decimal places
SF=significant figures

* The Frel Values will be generated for the comparisons listed in section 4.2. For Part 2 of the study, the calculations may be done on dose corrected parameters if the dose is adjusted in this part of the study.

5.2 Statistical Analysis

The relative bioavailability will be assessed for PQP using the ratio of the geometric means for $AUC_{(0-72)}$, $AUC_{(0-t)}$, $AUC_{(0-168)}$, $AUC_{(0-inf)}$, and C_{max} as follows:

- For Part 1 the comparisons will be Group 2 PQP dispersible granule (320 mg, fasted) [test] over the Group 1 PQP hard tablet (320 mg, fasted) [reference].

- For Part 2 the data from Part 1 Group 2 PQP dispersible granule (320 mg, fasted) will be used as the reference against the following fed test conditions (and dose adjusted where relevant):
  - High-fat meal (Group 3)
  - Low-fat meal representative of African diet (Group 4)
  - Whole milk (Group 5)

A linear mixed effect model will be used to obtain the geometric means ratios, with the logarithm of the PK parameter as response variable, the sequence, treatment and the period as fixed effects, and the subject within sequence as random effect. Least square mean differences (test – reference) will be extracted from the model with two-sided 90% confidence intervals. Mean ratios will be reported with 90% confidence limits after back transformation from the log-scale. The assumption of normality of the distributions will be made when running exploratory bioequivalence analysis.

Bioequivalence analysis will be performed using the Bioequivalence module contained within Phoenix WinNonlin. The following will be selected when analysing the study data

In the Bioequivalence Model tab the following will be selected

- Type of study will be assigned as a Parallel Study
- Type of bioequivalence will be set to Average
- The reference formulation will be set depending on the study part as described above

In the Bioequivalence Fixed effects tab the following will be selected

- Sequence, Formulation and Period are added to the Model specification
- Ln(x) is added to the dependent variables

Normally the random effects are populated however the following should be carried through to this menu

- Subject (Sequence) should be in the random effects model field

Bioequivalence is demonstrated when the 90% confidence interval for parameter is contained between 80 and 125.

5.3 Interim Analysis

There will be one interim analysis after dosing of Groups 1 and 2, to review safety data obtained up to Day 15 and PK data obtained up to Day 8, to aid the transition from Part 1 of the study into Part 2.

- For interim analysis purposes the bioanalytical data and generated pharmacokinetic parameters will be tabulated and summarised according to the nominal timepoint in an interim report.
- The following PK parameters for the hard tablet (fasted) and the dispersible granule formulations (fasted) will be generated; C_{max}, $AUC_{0-24h,}$ AUC_{0-72h}, AUC_{0-t}, time to maximum plasma concentration (T_{max}).
- Interim analysis will be conducted using QC'd data available at the time of analysis and will use nominal sampling timepoints.

Summary statistics (i.e., mean, median, SD, CV%, minimum, maximum, n, geometric mean, geometric SD and geometric CV%) will be calculated for blood concentrations for each time point and treatment. The same will be done for the PK parameters (except t_{max}) which will be listed for each individual and summarised by treatment. The t_{max} summary statistics will be provided as n, minimum, median and maximum only.

Arithmetical mean blood concentration vs. time curve will be produced by treatment on both linear/linear and log10/linear scales. An arithmetic mean comparison plot of tablet fed and granule fed data will also be presented on both linear/linear and log10/linear scales.

Spaghetti plots of individual blood concentrations against nominal sampling times after dosing for each 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.

Bioequivalence on Part 1 data will be performed as described in section 4.2 for C_{max}, AUC_{0-72} and AUC_{0-t} and tabulated to form part of the interim PK report.

The interim report will contain a dose recommendation of PQP dispersible granule for use in Part 2 of the study, based upon ensuring that the C_{max} of PQP granule formulation administered with a high fat meal, will not be predicted to exceed the geometric mean of C_{max} 98 ng/ml with CV 39% obtained from PQP tablet (960 mg) administered in the fasted state (see study MMV_P21_01). An assumption will be made that the exposure of PQP in the dispersible granule formulation administered in the fasted state will increase 3-fold when administered with a high fat meal, in line with the exposure increase seen when film-coated tablets (Eurartesim®) are administered with a high fat/high calorie meal.

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