Statistical Analysis Plan	RICHMOND Pharmacology

SPONSOR'S REFERENCE NUMBER: APM/18/1702001

EUDRACT NUMBER: 2018-004968-60

RPL STUDY CODE: C18034

STATISTICAL ANALYSIS PLAN

A randomized, double-blind, placebo controlled parallel group study in heathy adult subjects to determine the tolerability and safety of atovaquone-proguanil (ATV-PG) co-administered with amodiaquine (AQ)

FINAL VERSION 1.0, 01 NOV 2019

Issued by: Dorothée Camilleri
Richmond Pharmacology
Biostatistician

Signature.....
Date...06.NOV.2019

Approved by: Stephan CHALON
Medicines for Malaria Venture
Head Experimental Medicine

Signature.....
Date...06. November 2019

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or Specialist Term	Explanation
%CV	Coefficient of Variation
AE	Adverse Event
AESI	Adverse events of special interest
AQ	Amodiaquine
ATV	Atovaquone
ATV-PG	Atovaquone-proguanil
AUC	Area under the Concentration Time Curve
$AUC_{0\text{-}inf}$	Area under the Plasma Concentration versus Time Curve from Time Zero Extrapolated to Infinity
$AUC_{0\text{-}t}$	Area under the Plasma Concentration versus Time Curve from Time Zero to the Last Quantifiable Concentration
BDRM	Blind Data Review Meeting
BLQ	Below the Level of Quantification
CG	Cycloguanil
CI	Confidence Interval
CL	Total Body Clearance
C_{max}	Maximum Observed Plasma Concentration
CSR	Clinical Study Report
DEAQ	Desethyl-amodiaquine
ECG	Electrocardiogram
HR	Heart rate
λ_z	Terminal Elimination Rate Constant
MedDRA	Medical Dictionary for Regulatory Activities
MMV	Medicines for Malaria Venture
PDF	Portable Document Format
PG	Proguanil
PK	Pharmacokinetic(s)
POM	Profile of Mood
PR	Pulse Rate
PROC	Procedure in SAS
QRS	Ventricular Conductance Time
QT	Cardiac Output
QTcF	QT interval corrected using Fridericia's formula
QTcF	QT interval corrected using Bazett's formula
RPL	Richmond Pharmacology Ltd
SAP	Statistical Analysis Plan
SAS	Statistical Analysis Software
SD	Standard Deviation
SOC	System Organ Class
SRC	Safety Review Committee
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_d (V_d/F)	Apparent Volume of Distribution

TABLE OF CONTENTS

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 29th of July 2019 was used to prepare this SAP.

Pharmacokinetic (PK) parameters calculations and statistical analyses will be the responsibility of Richmond Pharmacology Ltd (RPL). Tables, figures, and listings (TFLs) will be produced using Statistical Analysis Software (SAS), Version 9.3 or higher.

2. Study Objectives and Endpoints

2.1 Study objectives

The objectives of this study are:

Primary

- To assess the safety and tolerability of the approved curative dose of ATV-PG (once daily for 3 days) and the adult equivalent of the approved SMC dose of AQ (once daily for 3 days) when administered alone and in combination, in comparison with placebo.

Secondary

- To determine the pharmacokinetics (PK) of atovaquone (ATV), proguanil (PG) and cycloguanil (CG), and amodiaquine (AQ) and desethyl-amodiaquine (DEAQ) following administration of ATV-PG and AQ alone and in combination.
- To determine the relationship between AQ and DEAQ and ECG parameters and to evaluate any impact of the combination with ATV-PG on this relationship.

2.2 Endpoints

Primary

- Safety and tolerability as measured by the incidence of treatment-emergent adverse events (TEAEs) including the clinical signs, nausea, vomiting and diarrhoea, proportion of subjects with clinically relevant changes in laboratory safety tests (haematology, chemistry (in particular ALT, AST and bilirubin increases) and urinalysis), proportion of subjects with morphological and/or rhythm abnormalities on electrocardiogram (ECG), proportion of subjects with clinically significant changes in ECG time intervals (PR, QRS, QT and QTc intervals) and proportion of subjects 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 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 infinity (AUC_{0-inf}), terminal elimination rate constant (λ_z), terminal elimination half-life ($t_{1/2}$).
- Baseline corrected QTc (ΔQTc) as a function of the concentrations of AQ and DEAQ when administered alone, and when administered in combination with ATV-PG.

3. Trial Design

The study is a randomized, double-blind, placebo controlled, parallel group study to determine the tolerability and safety of ATV-PG + AQ, ATV-PG + AQ placebo, ATV-PG placebo + AQ, and ATV-PG placebo + AQ placebo administered once daily for 3 days to healthy adult male and female subjects.

3.1 Overall Design

Fifty-two subjects will be enrolled and randomized to one of the four treatment (Cohorts 1 to 4) in a ratio of 4:3:3:3 as described below:

- Treatment 1 (n=16) - ATV-PG 1000-400 mg + AQ 612 mg;
- Treatment 2 (n=12) - ATV-PG 1000-400 mg + AQ unmatched placebo;
- Treatment 3 (n=12) - ATV-PG unmatched placebo + AQ 612 mg;
- Treatment 4 (n=12) - ATV-PG unmatched placebo + AQ unmatched placebo.

Following the completion of dosing of the first 20 randomised subjects, Treatment 1 has been discontinued from further dosing in accordance with the Adverse Reaction rules of the protocol. Up to 24 further subjects will be included in the study, split in three cohorts of up to eight subjects.

The remaining three treatments will be re-randomised in such a way that across the whole study there will be still 12 subjects dosed with Treatment 2, 12 subjects with Treatment 3 and 12 Subjects with treatment 4. This is taking into account both the already dosed subjects with their originally randomised treatments and the newly randomised subjects yet to be included. Furthermore, within each cohort of up to eight subjects, each of the three treatments (2, 3, and 4) needs to be represented with a maximum of four and a minimum of one subject in the same cohort.

Due to randomization scheme with the initial 20 subjects, a total of 8 subjects have received treatment 1 before it was decided to terminate this arm.

Subjects will be screened within 20 days prior to entering the study on Day -1. Each subject will receive verbal and written information followed by signing of the Informed Consent Form (ICF) prior to any screening procedures taking place. Subjects will be admitted to the study unit on Day -1 and will be discharged on Day 4.

All subjects will attend the unit for an outpatient visit on Days 8, 15, 22, 29 and a follow-up visit on Day 36 +/- 1 day (Figure 1). All the assessments performed during the study are detailed in the study schedule of assessments. Study design features as well as number of subjects may be adapted according to the Adaptive Features. This study will use a sentinel dosing strategy.

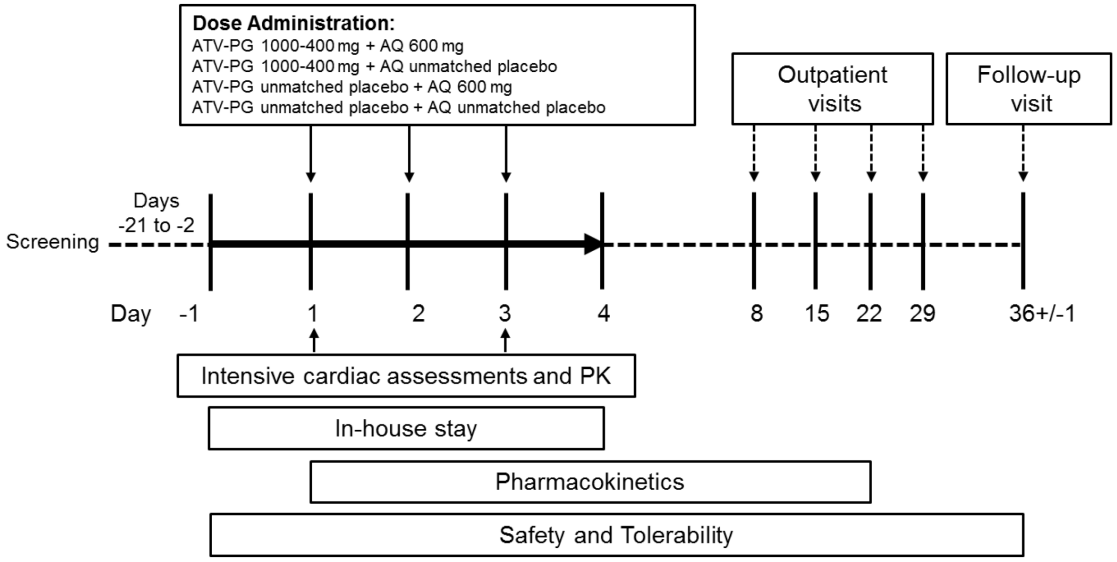

Figure 1. Study flow chart.

3.2 Duration of Study

The planned study duration is approximately 57 days; up to 21 days for screening and approximately 36 days for dosing and follow-up.

3.3 Sample Size

This is an exploratory study to evaluate tolerability and pharmacokinetics of each treatment group and is not based on formal statistical considerations. The numbers assigned to each treatment are deemed adequate to describe the tolerability, safety and pharmacokinetics.

3.4 Randomization and Blinding

Subjects in this study 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 on Day 1.

According to the original protocol subjects were randomly assigned to one of four treatments in a 4:3:3:3 ratio as described below:

- Treatment 1 (n=16) - ATV-PG 1000-400 mg + AQ 612 mg;
- Treatment 2 (n=12) - ATV-PG 1000-400 mg + AQ unmatched placebo;
- Treatment 3 (n=12) - ATV-PG unmatched placebo + AQ 612 mg;
- Treatment 4 (n=12) - ATV-PG unmatched placebo + AQ unmatched placebo.

As per the stipulations of the non-substantial amendment No 05 of the protocol (V1.0, dated 29JUL2019), following the completion of dosing of the first 20 randomised subjects, Treatment 1 has been discontinued from further dosing in accordance with the Adverse Reaction rules of the protocol. Up to 24 further subjects will be included in the study, split in three cohorts of up to eight subjects. The remaining three treatments will be re-randomised in such a way that across the whole study there will be still 12 subjects dosed with Treatment 2, 12 subjects with Treatment 3 and 12 Subjects with treatment 4. This is taking into account both the already dosed subjects with their originally randomised treatments and the newly randomised subjects yet to be included.

Furthermore, within each cohort of up to eight subjects, each of the three treatments (2, 3, and 4) needs to be represented with a maximum of four and a minimum of one subject in the same cohort.

This study will be conducted in a double-blind fashion whereby subjects and clinical study site staff are blinded.

Because the placebo tablets are not an exact match for the active treatment, tablets will be administered by an un-blinded member of clinical study staff, who will protect the blinding from both subjects and all other site staff. Dosing will take place behind a curtain and during IMP administration the subjects will be blindfolded.

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

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

Study numbers will not be reallocated once assigned.

4. 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 number of observations, arithmetic mean (n), standard deviation (SD), arithmetic coefficient of variation (%CV), geometric mean, geometric %CV, median, minimum and 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 will be reported with 2 more decimal places than the original data.

PK parameters will be presented as follows in the listing: C_{max} and T_{max} will be presented as given in the raw data; AUC_{0-t}, $AUC_{0-\infty}$, λz, $t_{1/2}$, CL or CL/F, and Vd or V_d/F 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. SAS v9.3 or higher or Phoenix WinNonLin v7 or higher may be used for the determination of Pharmacokinetic parameters.

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

Baseline will be defined as the last non-missing value among assessments recorded 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 in bookmarked PDF. Further details of page layout will be provided in the TFL shell document. Individual RTF files for tables may be provided to assist medical writing.

4.1 Interim Analysis

No interim analysis is planned for this study.

4.2 Analysis Sets

The data analysis will be based on different analysis sets according to the purpose of analysis. Inclusion and exclusion from each analysis set will be decided at the Blind Data Review Meeting (BDRM) prior to database lock. Further exclusions may be made from PK set based on the concentrations.

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

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

The PK set will consist of those subjects in the safety set with at least one evaluable concentration after IMP administration. Subjects who vomited within 30mn of dosing, and who were not subsequently re-dosed, will be excluded from the PK set. The PK set will be used for the presentation of the PK analyses.

ECG set for concentration-QTc analysis

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 QTc.

The analysis set for intensive cardiac assessment will be based on the intersection of the PK set and the ECG set. In addition, Below Limit of Quantification (BLQ) values and subjects on placebo will be included with plasma concentrations set to 0. Individual time points will be included only if there is a PK sample that allows the determination of the plasma concentration and a valid ECG assessment as defined above. Individual QTc/concentration pairs will be excluded from this set if the time between ECG (time of first replicate) and blood sampling is at most twice the allowable difference to the scheduled time as defined in the Data Handling Plan.

Time-point	ECG time window in the SOM	Allowed window for Concentration-QTc analysis
H-3 to H-2	±10mins	± 30 mins
H1 to H2	±10mins	± 30 mins
H3 to H4	±10mins	± 30 mins
H5	±10mins	± 30 mins

H6	±10mins	± 30 mins
H8	±10mins	± 30 mins
H12	±10mins	± 30 mins
H24 (DAY 4 Only)	±10mins	± 30 mins

4.3 Subject Disposition

All subjects will be included in the summary of subject disposition. This will present the overall number of subjects screened by treatment group and overall, 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.

4.4 Demographic Characteristics

Individual subject demographics (including age, sex, race and ethnicity) and body measurement data (height, body weight and body mass index) at screening will be listed and summarized by each treatment group and overall for the safety set. If the number of subjects in other analysis sets is different from the safety set by more than 5%, separate demographic tables will be produced.

Height will be measured in centimeters and weight in kilograms. Body mass index will be given in kg/m^2.

4.6 Inclusion and Exclusion Criteria

The inclusion and exclusion criteria will be listed together with the overall eligibility for each subject.

4.7 Protocol Deviations

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

4.8 Medical History

Medical history data will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) dictionary Version 21.0 (or higher) and listed individually. Surgical history data will be listed separately.

4.9 Study Drug Administration

Study drug administration data will be listed individually.

4.10 Prior and Concomitant Medications

All prior and concomitant medications will be coded using the World Health Organization Drug Dictionary version June 2018 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.

Prior and concomitant medications with missing start (end) date or time will be classified as concomitant medication.

5. Safety Analysis

Safety analyses will be performed on the safety set. Safety parameters will be summarized by treatment groups and, when relevant, overall.

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.

5.1 Adverse Events

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

An adverse events of special interest (AESI) is an adverse event of scientific or medical concern specific to the sponsor or the particular product or program, for which ongoing monitoring and rapid communication by the investigator to the sponsor may be appropriate.

The incidence of TEAEs (after dosing) will be summarized using the safety set. The MedDRA dictionary Version 21.0 (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, AESIs (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. For severity tables, a subject's most severe event within a category will be counted. For relationship tables, a subject's event with greatest relationship to study drug within a category will be counted. 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.

5.2 Laboratory Data

Clinical laboratory parameters (including blood chemistry, hematology, coagulation, urinalysis and other laboratory results) will be listed and abnormal parameters will be flagged as high (H) or low (L) according to reference ranges. Absolute (observed) values and changes from baseline (continuous variables) will be summarized for each parameter and scheduled time point by treatment group. The last 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.

Laboratory parameter values will be graded according to the Common Terminology Criteria for Adverse Events. Shift tables by treatment group will be produced for these laboratory parameters. These tables will summarize the number of subjects with each baseline grade relative to the normal ranges and changes to the worst highest grade assessed post-dose during the study.

The qualitative urinalysis data will be listed only.

5.3 Electrocardiograms (unadjudicated)

ECG analyses will be performed on two sets of ECGs: all ECGs prior adjudication and selected triplicates from each timepoint after adjudication.

All un-adjudicated ECG data (PR, QRS, QT, QTcB, QTcF and HR) including H/L flags and overall ECG evaluation will be listed. The QT interval will be corrected using Fridericia's formula (QTcF)(QTcF=QT/RR^1/3) and and Bazett's formula (QTcB)(QTcB=QT/RR^1/2).

For ECG variables, 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 three pre-dose QTcF values of Day 1.

Absolute (observed) values and changes from baseline in the ECG variables will be summarized by treatment group and timepoint.

Reference ranges for ECGs are as follows:

ECG Parameter	Normal Range Pulse
Rate (supine)	40–100 bpm Respiratory
Rate (supine)	8-20 breaths per min Heart Rate
(supine)	40-100 bpm
PR interval	120–200 ms
QRS duration	≤120 ms
QTcF	≤450 ms

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

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

Mean value of QTcF parameters will be plotted by dose group and time point.

Likewise, categorical analyses of other quantitative ECG parameters will be given. More specifically, an increase in PR from predose baseline >25% to a PR>200 ms; an increase in QRS from predose baseline >25% to a QRS >120 ms; a decrease in HR from predose baseline >25% to a HR <50 bpm or an increase in HR from predose baseline >25% to a HR >100 bpm will be summarised.

5.4 Analysis of Adjudicated ECG

Intensive cardiac assessments will be performed using food effects on the ECG to establish assay sensitivity. Analysis of drug related QT/QTc interval changes relative to plasma PK concentrations will be conducted. The principles of this analysis follow the statistical methods described by Garnett et al., 2018 [1] . The ECG utilised for this analysis require adjudication by qualified cardiologists in accordance with principles set out in the ICH E14 guideline and subsequent Q&A documents. All ECG recordings are in triplicate and will be compliant with the correct recording and manual adjudication of ECG in thorough QT/QTc studies. Below ECG analyses will be based on adjudicated selected triplicates from each time point.

Two complementary sets of analyses will be performed: a per timepoint analysis and a concentration-QTc analysis. While the first 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-QTc analysis has shown to have this power, but it is based on more restrictive assumptions.

5.4.1 Heart rate correction

QTcF will be used for ECG analyses, unless there is a substantial heart rate effect, i.e. changes in HR compared to placebo of more than 10 bpm, in which case the correction used for QTc (the most appropriate heart rate correction) will be calculated. More specifically, a study specific correction (QTcS) will be calculated by fitting a linear mixed effects model of the form

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

to the data from the individual time-points of the pre-dose data of all subjects. This model will have a random intercept and term for log(RR). The study specific effect will be determined from the fixed effect β of the log(RR) term and will result in a parabolic correction of the form

$$QTc = QT*RR^{-\beta}$$

To determine the primary correction method in this case, a linear mixed effects model of the form

$$QTc \sim RR + 1$$

with random intercept and RR per subject will be fitted to all non-baseline data using QTcF and QTcS. The mean of the squared individual slopes (MSS) (i.e. the individual regression coefficients) will be calculated and the correction method with the smaller MSS will be selected as primary.

Scatter plots of QT and QTc against RR will be produced to visualise the best correction formula.

Note: The study protocol mentioned that an individual correction of QTc would be applied but due to the small sample-size a study specific correction of QTc will be the method of choice if QTcF cannot be used.

5.4.2 Baseline

The average over the three pre-dose QTcF values of Day 1 will be used as baseline.

5.4.3 Descriptive Statistics

For the quantitative ECG parameters, descriptive statistics will be given by timepoint for absolute values and change from baseline by treatment group.

5.4.4 By timepoint analysis for QTc

A linear model of the form: $\Delta QTc \sim treat + BL$ will be fitted for each timepoint and the difference between each of the active treatments and placebo will be estimated based on this model with two-sided 90 % confidence intervals. In this model, treat is a factor with four levels: placebo, the two monotherapies and combination therapy, and BL is the centered baseline (i.e. the mean baseline across subjects is subtracted from each individual baseline).

5.4.5 Primary linear model

A series of linear mixed effects model will be fitted. The most comprehensive of these models will be of the form

$$\mathbf{\Delta QTcF \sim C_{ATV} + C_{AQ} + C_{PG} + C_{CG} + C_{DEAQ} + treat + T + BL}$$

where ΔQTcF is the change from baseline of QTcF, C_{ATV} the plasma concentration of ATV, C_{AQ} the plasma concentration of AQ, C_{PG} the plasma concentration of PG, C_{CG} the plasma concentration of CG, C_{DEAQ} the plasma concentration of DEAQ, treat is a four-level factor with levels placebo, ATV-PG, AQ and ATV-PG + AQ (for placebo, the two monotherapies and the combination therapy respectively) and T is a discrete time effect with one level for each timepoint across study days – i.e. the same time at different days will have different values. This model will allow for treatment and time as categorical fixed effect factors. Concentrations of AQ, DEAQ and relevant analytes/metabolites will be used as covariates.

Note that including a treatment effect will allow some judgement on the appropriateness of the model, as a well-fitting linear model should have a non-significant treatment effect. BL is baseline value for each subject, with the mean across subjects subtracted. No fixed intercept will be allowed.

The model will have random effects per subject for the intercept and the concentration levels. An unstructured covariance matrix will be assumed. If this model does not converge, some or all of the random slopes will be removed, and further simplifications may be performed to reach convergence. Parameter estimation will be using the REML (Restricted Maximum Likelihood) method.

The Kenward-Roger approximation will be used to calculate degrees of freedom and any t-test-based quantities, in particular two-sided 90% confidence intervals for the model parameters.

From the above most comprehensive model, simplified models will be derived by omitting one or more concentration parameters. This will be performed in a stepwise way, by removing the concentration with the smallest absolute t-value from the model. As a result, a series of 5 models with 5 down to 1 concentration will be obtained. For each model, the AIC and the F-value of the treatment effect from an ANOVA will be obtained. If this F-value is significant at the 5 % level, the model will be excluded from the candidate set. For the models remaining, the model with smallest AIC will be chosen as primary.

Predictions of the effects on QTc of each monotherapy and for the combination therapy will be made at the geometric means of the individual C_{max} values and given together with two-sided 90 % confidence intervals. Predictions will be given for the primary model, and may also be given for other models of interest.

5.4.6 Checking model assumptions

The key assumptions for the above model to be applicable are linearity of the concentration-QTc-relationship and the absence of hysteresis. These assumptions apply only if there is an effect of plasma concentrations on QTc. Therefore, if the predictions described above exclude an effect of more than 10 ms (i.e. the two-sided 90 % confidence interval for the prediction is completely below 10 ms) and

the per timepoint analysis described in 5.4.4 do not indicate a different result, the primary model will be accepted.

If, however, a prolongation beyond 10 ms cannot be excluded, the following will be performed:

Hysteresis: Simultaneous displays of the time courses of the placebo adjusted mean ΔQTcF (ΔΔQTcF) and the (arithmetic) mean plasma concentrations will be prepared. Likewise, hysteresis loops, i.e. a plot of mean ΔΔQTc against the mean concentration for each timepoint will be presented. If there is a delay of the maximum of the mean ΔΔQTcF with respect to the mean concentration of all moieties included in the primary model, this is an indication for hysteresis. If there is a delay, it will be assumed that the model used is not appropriate.

Linearity: Linearity will be assessed from the decile plots (see below). In addition, if the treatment effect of all candidate models is significant on the 5 % level, a nonlinear emax model will be fitted to the data. More specifically, this will be a model where each of the concentration terms in the linear model with lowest AIC is replaced by an emax-term aC(1+bC). In this model the only random effect per subject will be the intercept. Predictions will be based on this model or on the primary linear model, whichever fits better according to the Akaike Information Criterion and the t-value of the treatment effect. More specifically, if the emax model has a non-significant treatment effect, while the linear one has a significant one, the emax model will be used, otherwise, the one with the smaller AIC will be used.

5.4.7 Presentation of Results

Model parameters and predictions will be presented in tables. In addition, data will be plotted as scatter plot with concentration on the abscissa and ΔQTc on the ordinate, for the concentrations of AQ, DEAQ, ATV, CG and PG. A regression line corrected for the time effect will be added together with a 90 % confidence band. In addition, a decile plot of the residuals by concentration will be given.

Additionally, goodness of fit plots will be produced.

5.4.8 Assay sensitivity

The anticipated effect of the meal at -1 h before drug administration will be used to show assay sensitivity [2]. This meal is expected to produce a shortening of QTcF at the 1, 2 and 3 h timepoints after the meal by 5 – 10 ms. This shortening will be investigated by looking at the estimates of the time effect in the primary model at these timepoints. If the two-sided 90 % CI for all three of these estimates are completely below nought for both days, assay sensitivity will be deemed shown. If this is not the case, a Hochberg procedure will be applied testing the null hypothesis that at least one of the six contrasts is negative. More specifically, for Day 1, the estimates of the time effect will be used directly, while for Day 3, the difference of these timepoints to the estimate for the -35 min predose timepoint will be used.

The time course of the time effect will also be displayed graphically together with two-sided 90 % CI.

Note: The protocol mentioned that assay sensitivity would be evaluated using the estimates of the time effect representing the effects of breakfast and lunch of Days 1 and 3. The effect of breakfast on the two days only will be used, as the first timepoint after lunch is already at 8 h, i.e. 3 hours after the meal.

5.4.9 PK/J-Tpeak statistical modelling

Similarly to the analysis done for QTc and using the same primary linear model on adjudicated ECGs, an exploratory analysis of J-Tpeak using JTcJ (J-Tpeakc = J-Tpeak/RR power 0.58 with RR in seconds) will be performed to explore the drug related JTcJ interval changes relative to plasma PK concentrations.

5.5 Telemetry and Holter evaluations

Cardiac telemetry and Holter data will be listed.

5.6 Vital Signs

Vital signs data (systolic and diastolic blood pressure, pulse rate, respiratory 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.

5.7 Physical Examination

Physical examination data will be listed individually.

5.8 Profile of Mood Scale questionnaire

Data from the Profile of Mood Scale (POM) questionnaire will be used to calculate a general score called the Total Mood Disturbance score, as well as scores for the 7 items which are Tension, Anger, Fatigue, Depression, Esteem-related Affect, Vigour and Confusion. The scoring approach is presented in the Appendix 1.

Results from the POM questionnaire will be listed and along with changes from baseline will be summarised using descriptive statistics (n, mean, median, standard deviation, minimum, maximum) for the Total Mood Disturbance score followed by the score for each of the 7 items.

5.9 Basic Neurological Examination

Basic Neurological Examination data will be listed only.

6. Pharmacokinetic Analysis

All plasma concentration data will be listed for each individual subject and summarized at each time point by treatment group. Individual and mean concentrations versus nominal time on linear and semi-log scales will be presented graphically. The PK set will be used to present the summary of PK parameters.

6.1 Values Below the Limit of Quantification and Missing Values

If a Below the Level of Quantification (BLQ) value occurs in a profile before the first measurable concentration, it will be assigned a value of zero concentration. If a BLQ value occurs after a measurable concentration in a profile and is followed by a value above the lower level of quantification, then the BLQ value will be omitted following visual inspection of the plasma concentration versus time profile to assess the appropriateness of this assignment. If a BLQ value occurs at the end of a collection profile (after the last quantifiable concentration), the value will be treated as missing data. If 2 BLQ values occur in succession, the profile will be deemed to have terminated at the first BLQ value and any subsequent concentrations will be omitted from PK calculations following visual inspection of the plasma concentration versus time profile to assess the appropriateness of this assignment.

Samples with no reportable value due to a bioanalytical issue or missing samples will be set to missing, and will not be included in the PK calculations.

When calculating the mean or median value 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 omitted.

For tabulation, graphical representation, and calculation purposes, all samples with no reportable value (or missing samples) observed after dosing will be set to missing.

6.2 Evaluation of Pharmacokinetic Parameters

Non-compartmental analysis will be used for estimation of pharmacokinetic parameters.

The following pharmacokinetic parameters will be calculated for atovaquone (ATV), proguanil (PG), cycloguanil (CG), amodiaquine (AQ) and desethyl-amodiaquine (DEAQ):

C_{max}	Maximal observed plasma concentration • For multiple peaks, the highest post-dose concentration will be reported as C_{max}
t_{max}	Time at which the maximum plasma concentration occurs • In case that multiple peaks are of equal magnitude, the earliest T_{max} will be reported
AUC_{0-t}	Area under the plasma concentration curve from time zero up to the last quantifiable concentration

$AUC_{0\text{-}inf}$	Area under the plasma concentration-time curve from time zero extrapolated to infinity
λ_z	Terminal elimination rate constant
$t_{1/2}$	Terminal elimination half-life • Calculated as ln(2)/ λ_z

For individual plasma concentration data, the actual time of ATV-PG and AQ administration and actual blood sampling time will be used in the derivation of the PK parameters. If there is any doubt in the actual time a sample was taken, then the scheduled time will be used.

AUC values will be calculated using the linear/log trapezoidal method, applying the linear trapezoidal rule up to C_{max} and the log trapezoidal rule for the remainder of the curve. Samples below limit of quantification (LOQ) prior to the first quantifiable concentration will be set to zero. Samples with concentrations below LOQ after the first quantifiable concentration will be set to 'missing' and omitted from the analysis. Other pharmacokinetic parameters will be calculated according to standard equations.

PK parameters will be listed for each individual subject and summarized by treatment group. Descriptive statistics for PK parameters will include number of observations (n), arithmetic mean (Mean), standard deviation (SD), arithmetic coefficient of variation (%CV), geometric mean, geometric %CV, median, minimum and maximum.

If a value is considered to be anomalous due to being inconsistent with the expected PK profile, it may be appropriate to exclude this point from the PK analysis. However, the exclusion of data must have strong justification and will be documented in the raw data and in the clinical study report. The following conditions for PK parameter inclusion may also be applied:

- AUC, λ_z and $t_{1/2}$ to be excluded if the regression does not include at least 3 different time points in the terminal phase (after C_{max}, but excluding the concentration at T_{max})
- $T_{1/2}$, λ_z to be excluded if the coefficient of determination, Adj_Rsq in the regression is less than 0.80
- $AUC_{0\text{-}inf}$ is excluded if the %extrapolated is greater than 20%

Additional PK parameters may be calculated, as appropriate.

6.3 Statistical Analysis on PK Parameters

The PK parameters will be listed for each subject and summarized using descriptive statistics (N - the number of subjects, arithmetic mean, SD - standard deviation, CV - coefficient of variation, geometric mean, geometric CV, median, minimum, maximum).

It is noted that for reporting purposes, Tmax will be described using Median (min, max), and all other parameters will be described using mean, geometric mean and CVs.

7. Methods for Withdrawals, Missing Data and Outliers

The individual plasma concentration data and the actual timing of study drug administration and blood sampling will be used throughout the analyses. If there is any doubt about the actual time at which a sample was taken, then the scheduled time will be used. For PK data analysis, please see Section 4.13.1 regarding the handling of missing and BLQ values.

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

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.

There will be no further imputation of missing data (i.e., subjects who prematurely discontinue from the study will not be included in summary statistics or analyses beyond the time of discontinuation).

Depending on the extent of missing values, the appropriateness of the methods described for handling missing data may be reassessed prior to database lock (to examine the sensitivity of results to handling of missing data).

References

[1] Garnett, C., Bonate, P.L., Dang, Q. et al. Garnett C, Bonate PL, Dang Q, Ferber G, Huang D, Liu J, Mehrotra D, Riley S, Sager P, Tornoe C, Wang Y. Correction to: Scientific white paper on concentration-QTc modelling. J Pharmacokinet Pharmacodyn. 2018b; 45 (3): 399.

[2] Ferber G, Fernandes S, Täubel J: Estimation of the Power of the Food Effect on QTc to Show Assay Sensitivity. J Clin Pharmacol 58 (2018), 81 - 88. DOI: 10.1002/jcph.975.

Appendix 1: Analysis of the POM Questionnaire

Abbreviated POMS (Revised Version)

***** SCORING KEY *****

Scores for the seven subscales in the abbreviated POMS are calculated by summing the numerical ratings for items that contribute to each subscale. The correspondence between items and subscales is shown below.

Item	Scale	Not At All	A Little	Moderate	Quite a lot	Extremely
Tense	TEN	0	1	2	3	4
Angry	ANG	0	1	2	3	4
Worn Out	FAT	0	1	2	3	4
Unhappy	DEP	0	1	2	3	4
Proud	ERA	0	1	2	3	4
Lively	VIG	0	1	2	3	4
Confused	CON	0	1	2	3	4
Sad	DEP	0	1	2	3	4
Active	VIG	0	1	2	3	4
On-edge	TEN	0	1	2	3	4
Grouchy	ANG	0	1	2	3	4
Ashamed	ERA	Reverse-score this item [0 = 4, 1 = 3, 2 = 2, 3 = 1, 4 = 0]				
Energetic	VIG	0	1	2	3	4
Hopeless	DEP	0	1	2	3	4
Uneasy	TEN	0	1	2	3	4
Restless	TEN	0	1	2	3	4
Can't concentrate	CON	0	1	2	3	4
Fatigued	FAT	0	1	2	3	4
Competent	ERA	0	1	2	3	4
Annoyed	ANG	0	1	2	3	4
Discouraged	DEP	0	1	2	3	4
Resentful	ANG	0	1	2	3	4
Nervous	TEN	0	1	2	3	4
Miserable	DEP	0	1	2	3	4

Confident	**ERA**	0	1	2	3	4
Bitter	**ANG**	0	1	2	3	4
Exhausted	**FAT**	0	1	2	3	4
Anxious	**TEN**	0	1	2	3	4
Helpless	**DEP**	0	1	2	3	4
Weary	**FAT**	0	1	2	3	4
Satisfied	**ERA**	0	1	2	3	4
Bewildered	**CON**	0	1	2	3	4
Furious	**ANG**	0	1	2	3	4
Full of Pep	**VIG**	0	1	2	3	4
Worthless	**DEP**	0	1	2	3	4
Forgetful	**CON**	0	1	2	3	4
Vigorous	**VIG**	0	1	2	3	4
Uncertain…	**CON**	0	1	2	3	4
Bushed	**FAT**	0	1	2	3	4
Embarrassed	**ERA**	Reverse-score this item [0 = 4, 1 = 3, 2 = 2, 3 = 1, 4 = 0]				

TEN = Tension	Note that 2 of the items on the Esteem-related Affect (ERA) subscale are reverse-scored prior to being combined with the other items. **Total Mood Disturbance (TMD)** is calculated by summing the totals for the negative subscales and then subtracting the totals for the positive subscales: TMD = [TEN+DEP+ANG+FAT+CON] – [VIG+ERA]. A constant (e.g., 100) can be added to the TMD formula in order to eliminate negative scores.
ANG = Anger	
FAT = Fatigue	
DEP = Depression	
ERA = Esteem-related Affect	
VIG = Vigour	
CON = Confusion	