Statistical Analysis Plan

AM1476 - A Phase I, Double-blind, Placebo-controlled, Single- and Multiple-oral Dose, Safety, Tolerability, and Pharmacokinetic Study in Healthy Subjects

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LIST OF ABBREVIATIONS

Abbreviations pertain to the statistical analysis plan (SAP) only (not the tables, figures, and listings [TFLs]).

ADaM	Analysis Data Model
AE	adverse event
Ae_{t1-t2}	amount of the dose administered recovered over the time interval t1 to t2
ANOVA	analysis of variance
AUC	area under the concentration-time curve
$AUC_{0-\infty}$	area under the concentration-time curve from time 0 to infinity
$AUC_{0-tlast}$	area under the concentration-time curve from time 0 to the time of the last quantifiable concentration
$AUC_{0-\tau}$	area under the concentration-time curve over a dosing interval (τ)
BID	twice daily
BLQ	below the limit of quantification
CDISC	Clinical Data Interchange Standards Consortium
CI	confidence interval
CL/F	apparent total clearance
CL_R	renal clearance
C_{max}	maximum observed concentration
C_{min}	minimum observed concentration
COVID-19	coronavirus disease 2019
CRU	Clinical Research Unit
CSR	clinical study report
C-SSRS	Columbia-Suicide Severity Rating Scale
CV	coefficient of variation
$DAUC_{0-\infty}$	$AUC_{0-\infty}$ normalised by dose administered
$DAUC_{0-tlast}$	$AUC_{0-tlast}$ normalised by dose administered
$DAUC_{0-\tau}$	$AUC_{0-\tau}$ normalised by dose administered
DC_{max}	C_{max} normalised by dose administered
DMP	data management plan
ECG	electrocardiogram
eCRF	electronic case report form
fe_{t1-t2}	percentage of the dose administered recovered over the time interval t1 to t2
GLSM	geometric least squares mean
ICH	International Council for/Conference on Harmonisation
LLOQ	lower limit of quantification
ln	natural log

LSM	least squares mean
MedDRA	Medical Dictionary for Regulatory Activities
PK	pharmacokinetic(s)
QD	once daily
QTcB	QT interval corrected for heart rate using Bazett's formula
QTcF	QT interval corrected for heart rate using Fridericia's formula
R^2-adj	adjusted coefficient for determination of exponential fit
$RA_{AUC0-\tau}$	observed accumulation ratio based on $AUC_{0-\tau}$
RA_{Cmax}	observed accumulation ratio based on C_{max}
SAP	statistical analysis plan
SD	standard deviation
SDV	source document verification
$t_{1/2}$	apparent terminal elimination half-life
TEAE	treatment-emergent adverse event
TFL	table, figure, and listing
t_{last}	time of the last quantifiable concentration
t_{max}	time of the maximum observed concentration
V_z/F	apparent volume of distribution
WHODrug	World Health Organization Drug Dictionary
λ_z	apparent terminal elimination rate constant
λ_z Lower	start of exponential fit
λ_z N	number of data points included in the log-linear regression
λ_z Span Ratio	time period over which λ_z was determined as a ratio of $t_{1/2}$
λ_z Upper	end of exponential fit

1. INTRODUCTION

This SAP has been developed after review of the clinical study protocol (Final Version 5 dated 06 January 2021) and electronic case report form (eCRF).

This SAP describes the planned analysis of the pharmacokinetic (PK), safety, and tolerability data from this study. A detailed description of the planned TFLs to be presented in the clinical study report (CSR) is provided in the accompanying TFL shells document.

In general, the analyses are based on information from the protocol, unless they have been modified by agreement with AnaMar AB. A limited amount of information about this study (eg, objectives, study design) is given to help the reader's interpretation.

This SAP must be finalised prior to any unblinding of study data for analysis purposes (interim or final). Additionally, the SAP and TFL shells should be finalised prior to any programming activities commencing.

This SAP supersedes any statistical considerations identified in the protocol; where considerations are substantially different, they will be so identified. If additional analyses are required to supplement the planned analyses described in this SAP, they may be performed and will be identified accordingly in the CSR. Any substantial deviations from this SAP will be agreed with AnaMar AB and identified in the CSR.

This SAP is written with consideration of the recommendations outlined in the International Conference on Harmonisation (ICH) E3 guideline *Structure and Content of Clinical Study Reports*, ICH E8 guideline *General Considerations for Clinical Trials*, and ICH E9 guideline *Statistical Principles for Clinical Trials*.[1,2,3]

The document history is presented in Appendix 1.

2. STUDY OBJECTIVES

The primary objective of the study is:

- to determine the safety and tolerability of single and multiple oral doses of AM1476 in healthy subjects.

The secondary objectives of the study are:

- to determine the single- and multiple-oral dose PK of AM1476 in healthy subjects.
- to determine the effect of food on the single-oral dose PK of AM1476 in healthy subjects.

3. STUDY ENDPOINTS

3.1. Primary Endpoints

The primary safety endpoints for this study are as follows:

- incidence and severity of adverse events (AEs)
- incidence of laboratory abnormalities, based on haematology, clinical chemistry, coagulation, and urinalysis test results
- vital signs measurements
- 12-lead electrocardiogram (ECG) parameters
- telemetry
- neurological examinations
- Columbia-Suicide Severity Rating Scale (C-SSRS)
- physical examinations.

3.2. Secondary Endpoints

For Part A, the single-ascending dose and food (fed versus fasted dietary status at dosing), PK outcome endpoints of AM1476 are as follows:

- area under the concentration-time curve from time 0 to infinity ($AUC_{0-\infty}$)
- area under the concentration-time curve from time 0 to the time of the last quantifiable concentration ($AUC_{0-tlast}$)
- maximum observed concentration (C_{max})
- time of the maximum observed concentration (t_{max})
- apparent terminal elimination half-life ($t_{½}$)
- apparent total clearance (CL/F)
- apparent volume of distribution (V_z/F)
- renal clearance (CL_R)
- amount of the dose administered recovered over the time interval t1 to t2 (Ae_{t1-t2})
- percentage of the dose administered recovered over the time interval t1 to t2 (Fe_{t1-t2}).

For Part B, the multiple-ascending dose PK outcome endpoints of AM1476 are as follows:

- area under the concentration-time curve over a dosing interval ($AUC_{0-\tau}$)
- $AUC_{0-\infty}$ (Day 1 only)
- C_{max}
- minimum observed concentration (C_{min})
- t_{max}
- $t_{½}$
- CL/F
- V_z/F
- observed accumulation ratio based on $AUC_{0-\tau}$ ($RA_{AUC0-\tau}$)
- observed accumulation ratio based on C_{max} (RA_{Cmax})
- CL_R
- A_e
- F_e.

Other PK parameters may also be reported.

4. STUDY DESIGN

This will be a double-blind, randomised, placebo-controlled, single- and multiple-oral dose study conducted in 2 parts.

4.1. Part A

Part A will comprise a single-ascending dose, sequential-group design incorporating a single-group, 2-period crossover arm incorporating a food-effect evaluation. Overall, 48 subjects will be studied in 6 groups (Groups A1 to A6), with each group consisting of 8 subjects.

Potential subjects will be screened to assess their eligibility to enter the study within 28 days prior to the first dose administration (within 35 days for Group A1). Each subject will participate in 1 treatment period only, except for those participating in a food-effect evaluation, where each subject will participate in 2 treatment periods separated by a minimum of 6 days.

Subjects will reside at the Clinical Research Unit (CRU) from Day -1 (the day before dosing) to Day 3 of each treatment period, as applicable. All subjects will return for a Follow-up visit 7 to 10 days after their final dose.

Based on the ongoing review of the safety, tolerability, and PK results, additional non-residential visits may be required. The number of additional visits per subject will not exceed 3 per period and will not extend beyond 28 days after each final dosing occasion.

In each of Groups A1 to A6, 6 subjects will receive AM1476 and 2 subjects will receive placebo.

Groups A1, A2, and A4 to A6
It is planned for each subject in Groups A1, A2, and A4 to A6 to receive only a single dose of AM1476 or placebo during the study. Doses will be administered in the fasted state in accordance with a randomisation schedule on the morning of Day 1.

Group A3
It is planned for each subject in Group A3 to have the same treatment in both treatment period such that each subject will receive 2 single doses of AM1476 or placebo during the study. On Day 1 in Treatment Period 1, doses will be administered in the fasted state in accordance with a randomisation schedule. On Day 1 in Treatment Period 2, doses will be given 30 minutes after starting a standard high-fat breakfast. Although a food-effect evaluation is planned to occur in Group A3, this may be subject to change depending upon ongoing review of the PK data.

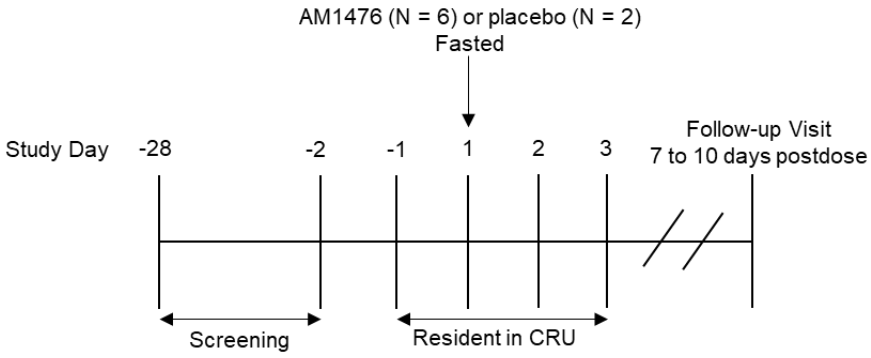
Additional Groups (Groups A7 to A9)
If it is decided to enrol additional groups, the effect of food on the PK of AM1476 may further be evaluated as described for Group A3. However, fasting requirements, meal compositions, and timing of doses will be determined following review of the available PK data.

Sentinel Dosing
All groups in Part A will be divided into 2 cohorts, with each cohort being dosed at least 24 hours apart. The first cohort will comprise 2 subjects, with 1 subject receiving AM1476 and 1 subject receiving placebo. The second cohort will comprise 6 subjects, with 5 subjects receiving AM1476 and 1 subject receiving placebo. For groups participating in a food-effect evaluation, sentinel dosing will only be utilised in Treatment Period 1 when AM1476 or placebo are administered in the fasted state. Dosing of subjects in the second cohort will not continue if any of the dose escalation stopping criteria are met by the sentinel subjects (first cohort).

The total duration of study participation for each subject (from Screening through Follow-up visit) is anticipated to be approximately 6 weeks for subjects not participating in the food-effect evaluation (approximately 7 weeks for Group A1) and approximately 7 weeks for those participating in the food-effect evaluation.

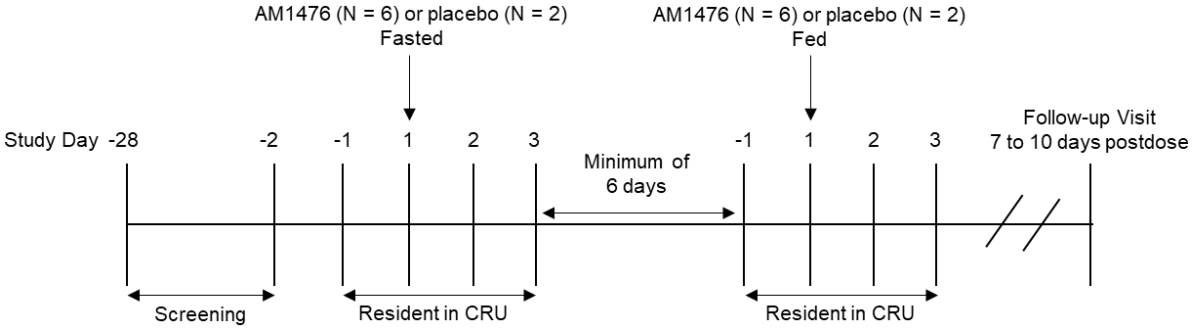
An overview of the study design is shown in Figure 1 and Figure 2, and the planned dose levels in Figure 3.

Figure 1: Study Schematic (Part A) for Groups A1, A2, and A4 to A6



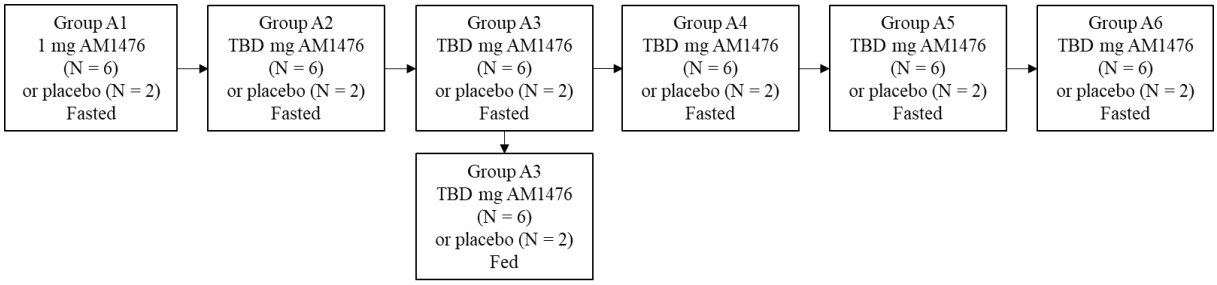
Abbreviations: CRU = Clinical Research Unit; N = number of subjects.
Note: Group A1: Screening period from Day -35 to Day -2 (approximately 5 weeks)

Figure 2: Study Schematic (Part A) for Food-effect Evaluation(s)



Abbreviations: CRU = Clinical Research Unit; N = number of subjects.

Figure 3: Planned Dose Levels (Part A)



Abbreviations: N = number of subjects; PK = pharmacokinetic(s); TBD = to be determined.
NOTE: dose levels may be adjusted based on the ongoing review of the safety, tolerability, and PK data. Doses will be administered in an escalating manner following satisfactory review by the Sponsor and Investigator of the safety and tolerability data (up to 48 hours post-final dose) and plasma PK data (up to 24 hours post-final dose [ie, Day 1 in Part A]) from the lower dose levels.
Although the food-effect evaluation is planned to occur in Group A3, this may be subject to change depending upon ongoing review of the PK data.

4.2. Part B

Part B will comprise a multiple-ascending dose, sequential-group design. Overall, it is planned for 24 subjects to be studied in 3 groups (Groups B1 to B3), with each group consisting of 8 subjects. Part B may start following review of the safety and tolerability and PK data for Group A4, at a dose equal or less than given in Groups A1 to A4, provided the predicted exposure is not expected to exceed an exposure already shown to be safe and well tolerated. The dose level cannot be equal to a dose level from Part A if that dose met any of the dose escalation stopping rules.

Potential subjects will be screened to assess their eligibility to enter the study within 28 days prior to the first dose administration. Each subject will participate in 1 treatment period only and reside at the CRU from Day -1 until the morning of Day 12. All subjects will return for a Follow-up visit 7 to 10 days after their final dose.

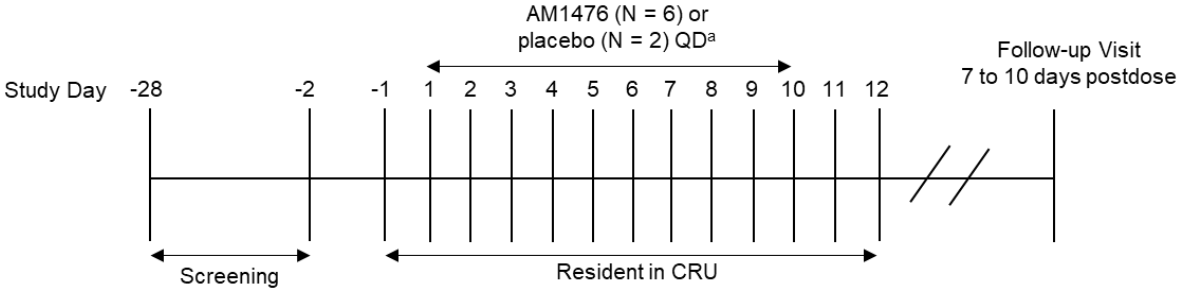
Based on the ongoing review of available safety, tolerability, and PK data, up to 3 additional non-residential visits may be required. The number of additional visits per subject will not exceed 3 and will not extend beyond 28 days after each final dosing occasion.

In each of Groups B1 to B3, 6 subjects will receive AM1476 and 2 subjects will receive placebo. The dietary state for dosing in Part B will be subject to review of the PK data from the fed/fasted comparison in Part A. For all subjects, dosing is planned to be once daily (QD) on Days 1 to 10, inclusive. However, the dietary state (including fasting requirement and meal compositions), dosing duration, and dosing frequency may be changed following review of safety, tolerability, and PK data from Part A or earlier groups in Part B. The dose regimen will comprise no less than once every 2 days and will not exceed twice daily (BID) dosing. The predicted total daily exposure at the selected dose administered will not exceed an exposure shown to be safe and well-tolerated in Part A. The dosing duration will comprise no fewer than 7 consecutive days and will not exceed 28 consecutive days. There will be a minimum of 6 days between dose escalations for each group.

The total duration of study participation for each subject (from Screening through Follow-up visit) is anticipated to be approximately 7 weeks.

An overview of the study design is shown in Figure 4 and the planned dose levels in Figure 5.

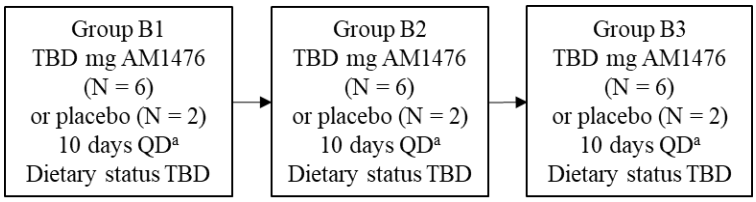
Figure 4: Study Schematic (Part B)



Abbreviations: BID = twice daily; CRU = Clinical Research Unit; N = number of subjects; QD = once daily.
a. Dosing is planned to be QD on Days 1 to 10, inclusive. The dosing regimen in Part B may be changed following review of data from groups in Part A or earlier groups in Part B. The dose regimen will comprise no less than once every

2 days and will not exceed BID dosing. The dosing duration will comprise no fewer than 7 consecutive days and will not exceed 28 consecutive days.

Figure 5: Planned Dose Levels (Part B)



Abbreviations: BID = twice daily; N = number of subjects; QD = once daily; TBD = to be determined.
NOTE: doses will be administered in an escalating manner following satisfactory review by the Sponsor and Investigator of the safety and tolerability data (up to 48 hours post-final dose) and plasma PK data (up to 24 hours post-final dose [ie, Day 1 in Part A and Day 10 in Part B]) from Part A or earlier groups in Part B.
a. Dosing is planned to be QD on Days 1 to 10, inclusive. The dosing regimen in Part B may be changed following review of data from groups in Part A or earlier groups in Part B and will not exceed BID dosing.

5. SAMPLE SIZE JUSTIFICATION

No formal statistical assessment, in terms of sample size, has been conducted as this is the first time AM1476 is being administered to humans. However, the number of subjects in each part of the present study is common in early clinical pharmacology studies and is considered sufficient to achieve the objectives of the study.

6. STUDY TREATMENTS

The study treatment names and ordering to be used in the TFLs for Part A are presented in Table 1 and for Part B in Table 3. The study treatment sequence names and ordering to be used in listings for food-effect group(s) in Part A only are presented in Table 2.

Table 1: Presentation of Study Treatments in TFLs (Part A)

Group	Study Treatment	Order in TFLs
A1 to A9	Placebo[a]	1
A1	1 mg AM1476 (Fasted)	2
A2	XX mg AM1476 (Fasted)	3
A3	XX mg AM1476 (Fasted)	4
	XX mg AM1476 (Fed)	5
A4	XX mg AM1476 (Fasted)	6
A5	XX mg AM1476 (Fasted)	7
A6	XX mg AM1476 (Fasted)	8
A7[b]	XX mg AM1476 (Fasted)	9
	XX mg AM1476 (Fed)[c]	10
A8[b]	XX mg AM1476 (Fasted)	11
	XX mg AM1476 (Fed)[c]	12
A9[b]	XX mg AM1476 (Fasted)	13
	XX mg AM1476 (Fed)[c]	14

a. Placebo will be pooled across all groups and fasting conditions
b. Optional group, to be included if required
c. Optional group may or may not include food-effect evaluation

Table 2: Presentation of Study Treatment Sequences in Listings (Food-effect Group(s) in Part A Only)

Group	Study Treatment Sequence	Order in TFLs
A3	Placebo (Fasted) / Placebo (Fed)	1
	XX mg AM1476 (Fasted) / XX mg AM1476 (Fed)	2
A7[a,b]	Placebo (Fasted) / Placebo (Fed)	3
	XX mg AM1476 (Fasted) / XX mg AM1476 (Fed)	4
A8[a,b]	Placebo (Fasted) / Placebo (Fed)	5
	XX mg AM1476 (Fasted) / XX mg AM1476 (Fed)	6
A9[a,b]	Placebo (Fasted) / Placebo (Fed)	7
	XX mg AM1476 (Fasted) / XX mg AM1476 (Fed)	8

a. Optional group, to be included if required
b. Optional group may or may not include food-effect evaluation

Table 3: Presentation of Study Treatments in TFLs (Part B)

Group	Study Treatment	Order in TFLs
B1 to B6	Placebo[a]	1
B1	XX mg AM1476 (QD) (Fasted)	2
B2	XX mg AM1476 (QD) (Fasted)	3
B3	XX mg AM1476 (QD) (Fasted)	4
B4[b]	XX mg AM1476 (QD) (Fasted)	5
B5[b]	XX mg AM1476 (QD) (Fasted)	6
B6[b]	XX mg AM1476 (QD) (Fasted)	7

Abbreviations: QD = once daily
a. Placebo will be pooled across all groups
b. Optional group, to be included if required

All TFLs will be based on actual treatments (eg, if subject was assigned to receive placebo but was wrongfully dosed with active treatment they would be summarised and listed under active treatment).

All dose levels described above are the potential dose levels, and therefore are subject to change. The TFLs will reflect the dose levels utilised in the study, and these will be displayed in increasing order.

All fasting conditions described above are the potential fasting conditions, and therefore are subject to change. The TFLs will reflect the fasting conditions utilised in the study.

All food-effect groups described above are the potential food-effect groups, and therefore are subject to change. The TFLs will reflect the food-effect groups utilised in the study.

7. DEFINITIONS OF POPULATIONS

Any protocol deviations, including those due to coronavirus disease 2019 (COVID-19) and related restrictions (see Section 8.1.1), will be considered prior to database lock for their importance and taken into consideration when assigning subjects to populations.

7.1. All Subjects Population

The all subjects population will include all subjects who signed the informed consent form and had any study assessment recorded in the database per the protocol.

7.2. Safety Population

The safety population will include all subjects who received at least 1 dose of study treatment (AM1476 or placebo).

7.3. Pharmacokinetic Population

The PK population will include all subjects who received at least 1 dose of active study treatment (AM1476) and have at least 1 valid PK concentration.

8. STATISTICAL METHODOLOGY

8.1. General

Listings will be provided for all data captured in the database, with the exception of medical history. Listings will include all subjects assigned to the all subjects population and include data up to the point of study completion or discontinuation. Subjects are generally considered to have completed the study if they complete the scheduled follow-up visit (rather than early termination visit). Any subject who discontinues the study will be identified accordingly in the listings. Summaries and statistical analyses will include the subjects assigned to the relevant population based on data type.

Analysis Data Model (ADaM) datasets will be prepared using Clinical Data Interchange Standards Consortium (CDISC) ADaM Version 2.1 (or higher if a new version is issued during the study) and CDISC ADaM Implementation Guide Version 1.1 (or higher if a new version is issued during the study). Pinnacle 21 Community Validator Version 3.0.2 (or higher if a new version is issued during the study) will be utilised to ensure compliance with CDISC standards.

For all statistical analyses, the hypothesis testing will be 2-sided and carried out on 0.05 significance level, unless specifically stated otherwise.

Caution should be used when interpreting results from the statistical analyses conducted in this study because the sample size is not based on power calculations.

Where reference is made to ‘valid’ data, this refers to non-missing data which meet the predetermined criteria (eg, are not flagged for exclusion).

Where reference is made to ‘all calculations’, this includes, but is not limited to, summary statistics, statistical analyses, baseline derivation, and changes from baseline.

All figures will be produced on linear-linear or discrete-linear scales, as applicable, unless specifically stated otherwise.

8.1.1. Handling of Data Quality Issues Due to Coronavirus Disease 2019 and Related Restrictions

Due to COVID-19 and related restrictions, there is a high risk for impact to data integrity, with the recognised potential for:

- Missed visits, caused by, for example:
  - Subject unable to travel to site due to restrictions, the need to quarantine, or COVID-19 infection
  - Subject unwilling to go to site due to fear of COVID-19 infection
  - Site postponing subject’s visit due to investigator not being available (eg, if they have been dispatched to hospital handling COVID-19 infections)

- Site unable to replenish supply of investigational product
- Incomplete data entry by sites due to limited resources to support study or no access to source documents or to eCRF
- Outstanding source document verification (SDV) due to sponsor or country restrictions on remote SDV, or no or limited access to site(s) for on-site visits
- Unanswered queries

At the time of the reporting of the study results, all protocol deviations due to COVID-19 or related restriction will be assessed for their severity and impact on the analyses. If needed, appropriate statistical methods will be applied as a mitigating action (eg, data might be categorised into 2 analysis groups, with and without COVID-19 and related restrictions impact); however, this will exclude any imputations of the missing values. Any mitigating actions will be agreed with AnaMar AB in advance and identified in the CSR.

8.1.2. Calculation of the Summary Statistics

For continuous data the following rules will be applied:

- Missing values will not be imputed, unless specifically stated otherwise.
- Unrounded data will be used in the calculation of summary statistics.
- In general, as early termination data are not associated with any scheduled timepoint, they will be excluded from all calculations of summary statistics. Exceptions may be made where justified.

For categorical data the following rules will be applied:

- For ordered categorical data (eg, AE severity), all categories between the possible minimum and maximum categories will be included, even if n = 0 for a given category.
- For non-ordered categorical data (eg, race), only those categories for which there is at least 1 subject represented will be included; unless specifically stated otherwise.
- Missing values will not be imputed, unless specifically stated otherwise. A ‘missing’ category will be included for any parameter for which information is missing. This will ensure that the population size totals are consistent across different parameters.

8.1.3. Triplicate Readings

For vital signs data only, where triplicate readings are taken, the median of triplicate readings will replace the separate individual triplicate readings in all calculations.

For 12-lead ECG data only, where triplicate readings are taken, the mean of triplicate readings will replace the separate individual triplicate readings in all calculations.

In case of incomplete triplicate readings (eg, only 2 out of 3 readings were recorded), the mean and/or medians will be calculated, as appropriate, based on the number of readings available.

8.1.4. Repeat and Unscheduled Readings

For vital signs and 12-lead ECG data only, any predose value recorded in addition to the original value or a postdose value recorded within 15 minutes of the original value will be defined as a repeat value; any postdose value recorded more than 15 minutes after the original value will be defined as an unscheduled value. For all other data types (eg, laboratory parameters), any value recorded in addition to the original value will be defined as an unscheduled value.

The original value will be replaced by the last associated repeat value in all calculations, with the exception of the 12-lead ECG outlier analysis (see Section 8.6.4).

As unscheduled values are not associated with any scheduled timepoint, they will be excluded from all calculations, with the exception of the baseline derivation (see Section 8.1.5) and 12-lead ECG outlier analysis (see Section 8.6.4).

8.1.5. Definitions of Baseline and Change from Baseline

The baseline will be defined as the last value recorded prior to the first dose. If the date/time of the value is incomplete or missing, it will be excluded from the baseline calculation, unless the incomplete date/time indicates the value was recorded prior to the first dose.

Individual changes from baseline will be calculated by subtracting the individual subject's baseline value from the value at the postdose timepoint.

The summary statistics for change from baseline will be derived from individual subjects' values (eg, mean change from baseline will be the mean of the individual changes from baseline for all subjects, rather than difference between the mean value at the postdose timepoint and mean value at baseline).

See Section 8.1.4 for more detail on handling repeat and unscheduled readings in the calculations. See Section 8.1.3 for more detail on handling of triplicate readings in the calculations.

8.2. Subject Disposition and Population Assignment

Subject disposition and population assignment will be listed. A summary table by treatment will be provided, based on the safety population.

8.3. Screening Demographics

The screening demographics including age, sex, height, body weight, and body mass index will be listed. A separate summary table by treatment will be provided, based on the safety and PK populations.

8.4. Prior and Concomitant Medication

Prior medication will be defined as medication that ends prior to the first dose. Concomitant medication will be defined as medication that starts during or after the first dose or starts but does not end prior to the first dose.

Prior and concomitant medications will be coded using the World Health Organization Drug Dictionary (WHODrug) Global, Format B3, Version September 2020 (or later if a new version is issued during the study; see the data management plan [DMP] for more details). Prior and concomitant medications will be listed.

8.5. Pharmacokinetic Assessments

8.5.1. Pharmacokinetic Analysis

The following PK parameters will be determined where possible from the plasma and urine concentrations of AM1476 using noncompartmental methods in validated software program Phoenix WinNonlin (Certara, Version 8.1 or higher):

Part A (Single Dose)

Parameter	Units[a]	Definition
$AUC_{0\text{-}tlast}$	ng*h/mL	area under the concentration-time curve from time 0 to the time of the last quantifiable concentration (t_{last})[b]
$AUC_{0\text{-}\infty}$	ng*h/mL	area under the concentration-time curve from time 0 extrapolated to infinity [b,c]
C_{max}	ng/mL	maximum observed concentration
t_{max}	h	time of the maximum observed concentration
t_{last}	h	time of the last quantifiable concentration
$t_{1/2}$	h	apparent terminal elimination half-life
CL/F	L/h	apparent total clearance
V_Z/F	L	apparent volume of distribution during the terminal phase
CL_R	L/h	renal clearance
$Ae_{t1\text{-}t2}$	mg	amount of the dose administered recovered over the time interval t1 to t2
$fe_{t1\text{-}t2}$	%	percentage of the dose administered recovered over the time interval t1 to t2
$DAUC_{0\text{-}tlast}$	h*ng/mL/mg	$AUC_{0\text{-}tlast}$ normalised by dose administered [d]
$DAUC_{0\text{-}\infty}$	h*ng/mL/mg	$AUC_{0\text{-}\infty}$ normalised by dose administered [d]
DC_{max}	ng/mL/mg	C_{max} normalised by dose administered [d]

Abbreviations: AUC = area under the concentration-time curve; PK = pharmacokinetic(s).
a. Units are based on concentration units (provided by the bioanalytical lab or preferred units for presentation of PK parameters) and dose units used in the study.
b. The AUC will be calculated using the linear trapezoidal rule for increasing concentrations and the logarithmic rule for decreasing concentrations (linear up/log down rule).
c. Based on the last observed quantifiable concentration
d. Calculated by dividing the parameter by the dose (mg)

Part B (Multiple-dose)

Parameter	Units[a]	Definition
$AUC_{0-\tau}$	ng*h/mL	area under the concentration-time curve over a dosing interval (τ) [b]
$AUC_{0-\infty}$	ng*h/mL	area under the concentration-time curve from time 0 to infinity (Day 1 only) [b,c]
C_{max}	ng/mL	maximum observed concentration
C_{min}	ng/mL	minimum observed concentration
t_{max}	h	time of the maximum observed concentration
$t_{1/2}$	h	apparent terminal elimination half-life
CL/F	L/h	apparent total clearance
V_Z/F	L	apparent volume of distribution during the terminal phase
$RA_{AUC0-\tau}$		observed accumulation ratio based on $AUC_{0-\tau}$
RA_{Cmax}		observed accumulation ratio based on C_{max}
CL_R	L/h	renal clearance
Ae_{t1-t2}	mg	amount of the dose administered recovered over the time interval t1 to t2
fe_{t1-t2}	%	percentage of the dose administered recovered over the time interval t1 to t2
$DAUC_{0-\tau}$	h*ng/mL/mg	$AUC_{0-\tau}$ normalised by dose administered [d]
DC_{max}	ng/mL/mg	C_{max} normalised by dose administered [d]

Abbreviations: AUC = area under the concentration-time curve; PK = pharmacokinetic(s).
a. Units are based on concentration units (provided by the bioanalytical lab or preferred units for presentation of PK parameters) and dose units used in the study.
b. The AUC will be calculated using the linear trapezoidal rule for increasing concentrations and the logarithmic rule for decreasing concentrations (linear up/log down rule).
c. Based on the last observed quantifiable concentration
d. Calculated by dividing the parameter by the dose (mg)

Additional PK parameters may be calculated, as appropriate.

Pharmacokinetic analysis will be carried out where possible using actual blood sampling times postdose. If an actual time is missing, the sample concentration result will be treated as missing unless there is scientific justification to include the result using the nominal time.

C_{max}, C_{min}, t_{last} and t_{max} will be obtained directly from the concentration-time profiles. If C_{max} occurs at more than 1 timepoint, t_{max} will be assigned to the first occurrence of C_{max}.

The accumulation ratio(s) (AR_{AUC} and AR_{Cmax}) will be calculated as follows:

$RA_{AUC0-\tau}$ = $AUC_{0-\tau}$ profile day 10 / $AUC_{0-\tau}$ profile day 1

RA_{Cmax} = C_{max} profile day 10 / C_{max} profile day 1

The parameter $AUC_{0-tlast}$ or other common partial area may be used to determine $RA_{AUC0-\tau}$ if $AUC_{0-\tau}$ cannot be reliably calculated for the majority of subjects.

8.5.1.1. Criteria for the Calculation of Apparent Terminal Elimination Rate Constant and Half-Life

The start of the terminal elimination phase for each subject will be defined by visual inspection and generally will be the first point at which there is no systematic deviation from the log-linear decline in concentrations.

The apparent terminal elimination rate constant (λ_z) will only be calculated when a reliable estimate can be obtained using at least 3 data points, preferably not including C_{max}, and the adjusted coefficient for determination of exponential fit (R^2-adj) of the regression line is ≥0.7. Parameters requiring λ_z in their calculation (eg, $AUC_{0-\infty}$, $t_{1/2}$, CL/F [profile day 1 only] and V_z/F) will only be calculated if the R^2-adj value of the regression line is ≥0.7.

The following regression-related diagnostic PK parameters will be determined where possible:

Parameter	Units[a]	Definition
λ_z	1/h	apparent terminal elimination rate constant
λ_z Upper	h	end of exponential fit
λ_z Lower	h	start of exponential fit
λ_z N		number of data points included in the log-linear regression
λ_z Span Ratio		time period over which λ_z was determined as a ratio of $t_{1/2}$
R^2-adj		adjusted coefficient for determination of exponential fit

Where possible, the span of time used in the determination of λ_z (ie the difference between λ_z Upper and λ_z Lower) should be ≥2 half-lives. If the λ_z Span Ratio is <2, the robustness of the $t_{1/2}$ values will be discussed in the CSR.

8.5.1.2. Criteria for Calculation and Reporting of Area Under the Concentration-time Curve

The minimum requirement for the calculation of area under the concentration-time curve (AUC) will be the inclusion of at least 3 consecutive concentrations above the lower limit of quantification (LLOQ). If there are only 3 consecutive concentrations, at least 1 should follow C_{max}.

If the extrapolated area is >20%, $AUC_{0-\infty}$ (and derived parameters) may be excluded from summary statistics and statistical analysis at the discretion of the sponsor or pharmacokineticist.

If $AUC_{0-\infty}$ cannot be determined reliably for all subjects and/or dose levels, an alternative AUC measure, such as AUC to a fixed timepoint or $AUC_{0-tlast}$, may be used in the statistical analysis of dose proportionality/food-effect.

If the end of the dosing interval PK blood sample is collected slightly early (ie, the 24 hour sample), the actual sampling time of the scheduled τ sample (24 hours) may be used for the

calculation of $AUC_{0-\tau}$. However, the $AUC_{0-\tau}$ parameter will be calculated if the τ sample is within 60 minutes of the nominal sampling time.

8.5.1.3. Calculation of Urinary Parameters

The amount of drug excreted in urine (Ae) in urine for AM1476 and urine collection interval (t_1-t_2) will be calculated as the product of urine AM1476 concentration and urine volume. Where only urine sample weight is supplied, a specific gravity of 1 g/mL will be assumed, and it will be considered equivalent to urine volume. A total cumulative $Ae_{0-x\ h}$ will be calculated by summing the Ae_{t1-t2} values over the 0-x h interval, where x = the end of the last collection time interval.

The percentage of the dose administered over the time interval t1 to t2 (fe_{t1-t2}) as AM1476 will be calculated for each urine collection interval as follows:

$fe_{t1-t2} = (Ae_{t1-t2} / dose) \times 100$.

Cumulative $fe_{0-x\ h}$ will be calculated by summing the fe_{t1-t2} values over the 0-x h period in the same manner as $Ae_{0-x\ h}$.

Renal clearance (CL_R) will be calculated over 0-t2 according to the following formula, where cumulative Ae and AUC are calculable to the same end time (t2):

$CL_R = Ae_{0-t2} / AUC_{0-t2}$.

Alternatively, $AUC_{0-\infty}$ may be used (applicable to profile day 1 only) if urinary excretion of the dose is considered to be complete and $AUC_{0-\infty}$ is well characterised.

8.5.1.4. Criteria for Handling Concentration Below the Limit of Quantification or Missing Concentrations for Pharmacokinetic Analysis

Plasma concentrations below the limit of quantification (BLQ) will be assigned a value of 0 before the first measurable concentration and thereafter BLQs will be treated as missing. The following rules apply with special situations defined below:

- If an entire concentration-time profile is BLQ, it will be excluded from PK analysis.
- Where 2 or more consecutive concentrations are BLQ at the end of a profile, the profile will be deemed to have terminated and any further quantifiable concentrations will be set to missing for the calculation of the PK parameters, unless they are considered to be a true characteristic of the profile of the drug.
- If a predose plasma concentration is missing, it may be set to 0 by default.
- For multiple dose occasion parts of the study only (profile day 10), if the concentration at τ (C_τ) is missing, this may be substituted with the predose concentration. Similarly, if the predose concentration is missing, this may be substituted with C_τ.

Urine concentrations that are BLQ will be set to 0 for the calculation of $Ae_{t1-t2.}$

8.5.1.5. Treatment of Outliers in Pharmacokinetic Analysis

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 CSR.

Any quantifiable predose concentration value on the first dosing day of all parts of the study (profile day 1) will be considered anomalous and set to missing for the PK analysis. This will be set to 0 by default in Phoenix WinNonlin.

8.5.2. Presentation of Pharmacokinetic Data

All PK concentrations and parameters will be listed.

Summary tables, arithmetic mean (+ standard deviation [SD]) figures, overlaying individual figures, and individual figures by treatment and time postdose will be provided for plasma PK concentrations. All figures will be produced on both linear-linear and linear-logarithmic scales, with the exception of figures across all days, which will be produced on the linear-linear scale only. The +SD bars will only be displayed on the linear-linear scale.

Summary tables by treatment will be provided for all PK parameters, with the exception of diagnostic regression-related PK parameters. Separate summary tables by treatment and time interval will be provided for excretion parameters and cumulative excretion parameters.

A subject may be excluded from the PK summary statistics and statistical analysis if the subject has an AE of vomiting that occurs at or before 2 times the median t_{max}.

Any exclusions from PK summary statistics and/or statistical analysis will be identified by pharmacokineticist and will be agreed with AnaMar AB.

If the actual time of sample collection deviates from the nominal time by more than ±10%, the concentration will be flagged and excluded from summary statistics. Individual concentrations that are deemed to be anomalous will be flagged in the listings and excluded from the summary statistics.

For PK concentration data the following rules will apply:

- Values that are BLQ will be set to 0 for the calculation of summary statistics.
- Arithmetic mean or median values that are BLQ will be presented as 0.

For PK parameters the following rule will apply:

- Geometric mean and coefficient of variation (CV) will not be calculated for t_{last} and t_{max}.

8.5.3. Pharmacokinetic Statistical Methodology

8.5.3.1. Dose Proportionality Assessment

A statistical analysis will be conducted to investigate the dose proportionality of $AUC_{0\text{-tlast}}$, $AUC_{0\text{-}\infty}$, and C_{max} on profile day 1 for Part A and $AUC_{0\text{-}\tau}$ and C_{max} on profile day 10 for Part B (only for plasma AM1476).

The PK parameters will be analysed using a power model[4] that will have the following form:

$$parameter = intercept \times dose^{slope} \times random\ error$$

Using the natural log (ln) transformation,[5] a power model can be expressed as a linear regression equation:

$$ln(parameter) = intercept + slope \times ln(dose) + random\ error$$

For dose proportionality, the slope of the regression line is equal to 1; for dose independence, it is equal to 0.

For each PK parameter separately, a pooled estimate (across all doses) of slope, corresponding 95% confidence interval (CI), and between-subject CV will be calculated. Figures (on the logarithmic-logarithmic scale) containing individual values, power model line (95% CI), and dose proportionality line (defined as the power model line with slope of 1) will be created for each PK parameter. Additionally, figures (on the logarithmic-linear scale) containing individual values and geometric means will be created for each corresponding PK parameter normalised by dose administered.

The lack of fit test will be conducted for the statistical assessment of linearity assumption, and thus appropriateness of a power model. The lack of fit model will be the same as the power model fitted, but with dose included as additional fixed effect. The statistical assessment will rule the linearity assumption acceptable if the diagnostic plots appear reasonable and the lack of fit 2-sided p-value >0.05 (dose effect is not significant at the 0.05 level of significance). The assessment of linearity assumption may also occur via visual examination of the figures by the pharmacokineticist. This assessment may override the statistical assessment; where this occurs, it will be detailed in the CSR.

It will be concluded that PK parameter is dose proportional for the dose range studied if the assumption of linearity is ruled acceptable and the 95% CI for the slope spans 1.

If the assumption of linearity is ruled unacceptable for any PK parameter, its corresponding PK parameter normalised by dose administered will be ln-transformed and analysed using an analysis of variance (ANOVA) model.[6] The model will include dose as a factor.

For each PK parameter separately, the geometric least squares mean (GLSM) for each dose, p-values for the overall, and pairwise dose comparisons will be calculated. Residual plots will be produced to assess the adequacy of the model(s) fitted.

Examples of the SAS code that will be used are as follows:

Power Model Analysis

```
proc mixed data = <data in> alpha = 0.05;
 by parcat1n parcat1 pkday paramn param;
 model lpk = ldose / cl residual ddfm = kr2;
 ods output solutionf = <data out>;
run;
```

Power Model Analysis (Between-subject Variability)

```
proc mixed data = <data in> covtest alpha = 0.05;
 by parcat1n parcat1 pkday paramn param;
 class ldose;
 model lpk = ldose / cl residual ddfm = kr2;
 ods output covparms = <data out>;
run;
(Note: Pooled Geometric CV (%) = 100*(sqrt(exp(estimate)-1)))
```

Power Model Analysis (Lack of Fit Test)

```
proc mixed data = <data in>;
 by parcat1n parcat1 pkday paramn param;
 class dose;
 model lpk = ldose dose / htype = 1 ddfm = kr2;
 ods output tests1 = <data out>;
run;
```

ANOVA Model Analysis

```
proc mixed data = <data in> alpha = 0.05;
 by parcat1n parcat1 pkday paramn param;
 class dose;
 model ldnpk = dose / cl residual ddfm = kr2;
 lsmeans dose / cl pdiff;
 ods output lsmeans = <data out>;
 ods output diffs = <data out>;
 ods output tests3 = <data out>;
run;
```

8.5.3.2. Food-effect Assessment

A statistical analysis will be conducted to investigate the food-effect on the treatment by comparing AM1476 (fed) treatment to AM1476 (fasted) at the same dose level.

The hypothesis testing will be 2-sided and carried out on both 0.05 and 0.1 significance levels.

The ln-transformed[5] $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$, and C_{max} on profile day 1 for food-effect group(s) in Part A (only for plasma AM1476) will be analysed using a mixed model.[7] The model will include actual treatment as fixed effect and subject as a random effect.

For each PK parameter separately, the least squares mean (LSM) for each treatment, difference in LSMs between the fed and fasted treatments, and corresponding 90% and 95% CIs will be calculated; these values will then be back-transformed to give the GLSM, ratio of GLSMs, and corresponding 90% and 95% CIs.

Additionally, the pooled estimate (across treatments) of the within-subject CV will be calculated, and residual plots will be produced to assess the adequacy of the model(s) fitted.

Examples of the SAS code that will be used are as follows:

Mixed Model Analysis

```
proc mixed data = <data in>;
 by parcat1n parcat1 pkday paramn param;
 class trtan usubjid;
 model lpk = trtan / cl residual ddfm = kr2;
 lsmeans trtan / cl pdiff = control('1') alpha = 0.1;
 lsmeans trtan / cl pdiff = control('1') alpha = 0.05;
 random intercept / subject = usubjid;
 ods output lsmeans = <data out>;
 ods output diffs = <data out>;
 ods output covparms = <data out>;
run;
```

8.6. Safety and Tolerability Assessments

8.6.1. Adverse Events

All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 23.1 (or higher if a new version is issued during the study; see the DMP for more details).

A treatment-emergent adverse event (TEAE) will be defined as an AE that starts during or after the first dose, or starts prior to the first dose and increases in severity after the first dose.

A treatment-related TEAE will be defined as a TEAE with a relationship of possibly related or related to the study treatment, as determined by the investigator.

All AEs will be listed. In addition to the data recorded in the database, the listings will include derived onset time and duration. Onset time will be calculated from the time of the last associated dose for TEAEs only. Where the last associated dose is referring to the last dose received prior to the start of a TEAE.

The frequency of subjects with TEAEs and the number of TEAEs will be summarised for the following categories:

- TEAEs (overall, serious, leading to discontinuation, and leading to death) by treatment
- TEAEs by severity and treatment
- Treatment-related TEAEs (overall, serious, leading to discontinuation, and leading to death) by treatment
- Treatment-related TEAEs by severity and treatment

The frequency of subjects will be summarised separately for TEAEs and treatment-related TEAEs by the following:

- System organ class, preferred term, and treatment
- Preferred term and treatment

For the AE data the following rules will apply:

- For the derivation of relationship (applicable to AEs captured on the 'change in the severity' eCRF form only): The relationship to study treatment is not captured on the AE change in severity eCRF form because it is always assumed to be the same as the relationship captured on the first eCRF form completed for this AE (initial severity). Therefore, for these cases the missing relationship will be set to that captured on the first form completed for this AE.

- For the derivation of treatment-emergent status (applicable to all AEs): If the start date/time of an AE is incomplete or missing, an AE will be assumed to not be a TEAE, unless the incomplete start date/time or the end date/time indicates an AE started after the first dose.

- For the derivation of treatment-related status (applicable to TEAEs only): If the study treatment relationship for a TEAE is missing, a TEAE will be assumed to not be a treatment-related TEAE.

- For the derivation of onset time (applicable to TEAEs only): If the start date/time of a TEAE is missing, onset time will not be calculated. If the start date/time of a TEAE is incomplete, where possible, the minimum possible onset time will be calculated and presented in '≥DD:HH:MM' format (eg, if the date/time of the last associated dose is 01MAY2019/08:00 and recorded start date/time of a TEAE is 03MAY2019, then the minimum possible onset time will be calculated by assuming a TEAE started at the first hour and minute of 03MAY2019 [03MAY2019/00:00], thus will be presented as onset time ≥01:16:00 in the listing). If the start date of a TEAE is the same as the date of the last associated dose but the start time of a TEAE is missing, an onset time will be presented as '≥00:00:01'. Any clock changes will be accounted for in the derivation.

- For the derivation of duration (applicable to all AEs): If the end date/time of an AE is missing, duration will not be calculated. If the start or end date/time of an AE is incomplete, where possible, the maximum possible duration will be calculated and presented in '≤DD:HH:MM' format (eg, if the start of an AE date/time is 01MAY2019/08:00 and its recorded end date/time is 03MAY2019, then the maximum possible duration will be calculated by assuming an AE ended at the last hour and minute of 03MAY2019 [03MAY2019/23:59], thus will be presented as duration ≤02:15:59 in the listing). Any clock changes will be accounted for in the derivation.

- For the calculation of TEAE summary statistics: If the severity of a TEAE is missing, that TEAE will be counted under the 'missing' category.

- For the calculation of TEAE summary statistics: If a subject experienced multiple TEAEs with the same preferred term for the same treatment, this will be counted as 1 TEAE for that treatment under the maximum severity recorded.

8.6.2. Clinical Laboratory Parameters

All clinical laboratory parameters will be listed; any value outside the clinical reference range will be flagged. Separate listings will be provided for any parameter for which there is any individual subject value outside the respective clinical reference range.

Summary tables and boxplots by treatment and timepoint will be provided for clinical chemistry, haematology, and coagulation parameters.

Values recorded as $<x$, $\leq x$, $>x$, or $\geq x$ will be displayed in the listings as recorded. For the derivation of listing flags, all calculations, and presentation in the figures, $<x$ and $\leq x$ values will be set to half of x, whereas $>x$ and $\geq x$ values will be set to x.

8.6.3. Vital Signs Parameters

For systolic and diastolic blood pressure and pulse rate, orthostatic parameters will be calculated as the supine value (recorded prior to the standing value) subtracted from the standing value. If the time between the supine and standing values used for derivation of the orthostatic value is <2 min, this orthostatic value will be flagged and excluded from all calculations.

All vital signs parameters and their changes from baseline will be listed, as applicable; any value outside the clinical reference range will be flagged. This will include any instances of orthostatic systolic blood pressure <-20 mmHg, orthostatic diastolic blood pressure <-10 mmHg, and orthostatic pulse rate >30 beats/min.

Summary tables and boxplots by treatment and timepoint will be provided for all vital signs parameters and their changes from baseline, as applicable.

8.6.4. 12-lead Electrocardiogram Parameters

All 12-lead ECG parameters and their changes from baseline will be listed; any value outside the clinical reference range will be flagged.

Summary tables and boxplots by treatment and timepoint will be provided for all 12-lead ECG parameters and their changes from baseline.

An outlier analysis will be performed for QT interval corrected for heart rate using Bazett's formula (QTcB) and QT interval corrected for heart rate using Fridericia's formula (QTcF). The analysis will include all individual original, repeat, and unscheduled postdose values.

The maximum postdose values will be summarised by treatment according to the following categories:

- $\leq$450 ms

- >450 and ≤480 ms (all instances flagged in the listing)
- >480 and ≤500 ms (all instances flagged in the listing)
- >500 ms (all instances flagged in the listing)

The maximum increases from baseline will be summarised by treatment according to the following categories:

- ≤30 ms
- >30 and ≤60 ms (all instances flagged in the listing)
- >60 ms (all instances flagged in the listing)

8.6.5. Other Assessments

Medical history will not be listed.

Telemetry, neurological examinations, and C-SSRS data will be listed.

All other safety and tolerability assessments not detailed in the above sections will be listed only.

8.6.6. Safety and Tolerability Statistical Methodology

No inferential statistical analyses are planned.

9. INTERIM ANALYSES

No formal interim analyses are planned for this study.

10. SIGNIFICANT CHANGES FROM THE PROTOCOL-SPECIFIED ANALYSES

There were no significant changes from the protocol-specified analyses.

11. REFERENCES

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2. ICH. ICH Harmonised Tripartite Guideline: General considerations for clinical trials (E8). 17 July 1997.
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12. APPENDICES

Appendix 1: Document History

Status and Version	Date of Change	Summary/Reason for Changes
Final Version 1.0	NA	NA; the first version.

NA = not applicable