TRIAL STATISTICAL ANALYSIS PLAN

c37731570-01

BI Trial No.:	1346-0017
Title:	Effect of fluconazole on the pharmacokinetics of a single oral dose of BI 425809 in healthy male subjects (an open-label, two-period fixed-sequence design study) Final protocol (Version 2.0, c36040039-02)
Investigational Product:	BI 425809
Responsible trial statistician:	[redacted] Phone: +[redacted] Fax: +[redacted]
Date of statistical analysis plan:	10-Feb-2022 SIGNED
Version:	1

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2. LIST OF ABBREVIATIONS

Term	Definition / description
AE	Adverse Event
AESI	Adverse event of special interest
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
$AUC_{0\text{-}215h}$	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 to 215 hours post administration of BI 425809
$AUC_{0\text{-}\infty}$	Area under the concentration-time curve of the analyte in plasma over the time interval from 0 extrapolated to infinity
BI	Boehringer Ingelheim
C_{max}	Maximum measured concentration of the analyte in plasma
COVID	Coronavirus disease
CTP	Clinical Trial Protocol
CTR	Clinical Trial Report
CV	Arithmetic coefficient of variation
ECG	Electrocardiogram
gCV	geometric coefficient of variation
gMean	Geometric mean
ICH	International Conference On Harmonisation
iPD	Important protocol deviations
LLOQ	Lower limit of quantification
MedDRA	Medical Dictionary For Regulatory Activities
PK	Pharmacokinetics
PKS	Pharmacokinetic parameter analysis set
PR	Pulse rate
RAGe	Report appendix generator
REP	Residual effect period
RPM	Report Planning Meeting
SAE	Serious adverse event
SD	Standard Deviation
SOC	System Organ Class

Term	Definition / description
TS	Treated set
TSAP	Trial Statistical Analysis Plan
ULN	Upper limit of normal range

3. INTRODUCTION

As per ICH E9 (1) the purpose of this document is to provide a more technical and detailed elaboration of the principal features of the analysis described in the revised CTP, and to include detailed procedures for executing the statistical analysis of the primary variables and other data.

This TSAP assumes familiarity with the CTP. In particular, the TSAP is based on the planned analysis specification as written in CTP Section 7 "Statistical Methods and Determination of Sample Size". Therefore, TSAP readers may consult the CTP for more background information on the study, e.g., on study objectives, study design and population, treatments, definition of measurements and variables, planning of sample size, randomisation.

Study data as collected in the eCRF will be stored in a trial database within the RAVE EDC system. All study data also including external data will then be uploaded to the CDR data warehouse.

The statistical analyses will be performed within the validated working environment CARE, including SAS™ (current Version 9.4, by [redacted]), and a number of SAS™-based tools (e.g., macros for the analyses of AE data or laboratory data; Report Appendix Generator system (RAGe) for compilation/formatting of the CTR appendices).

PK parameters will be calculated using Phoenix WinNonlin™ software (version Phoenix 6.3, [redacted]).

4. CHANGES IN THE PLANNED ANALYSIS OF THE STUDY

All analyses described in this TSAP are in accordance with the statistical methods described in the CTP.

5. ENDPOINTS

5.1 PRIMARY ENDPOINT

Primary endpoints are the following PK endpoints of BI 425809 as defined in Section 2.1.2 of the **CTP**:

- $AUC_{0\text{-}215h}$ (area under the concentration-time curve of the analyte in plasma over the time interval from 0 to 215 hours post administration of BI 425809)

- C_{max} (maximum measured concentration of the analyte in plasma)

5.2 SECONDARY ENDPOINTS

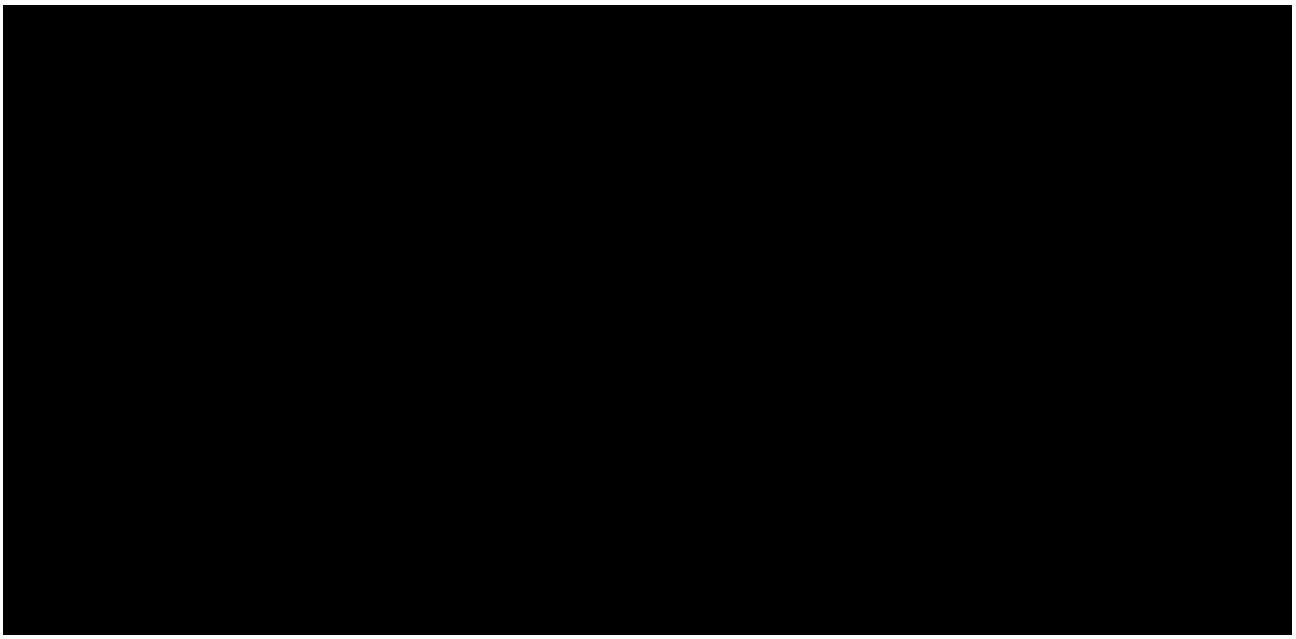
5.2.1 Key secondary endpoints

Not applicable.

5.2.2 Secondary endpoint

Secondary endpoint is the following PK endpoint of BI 425809 as defined in Section 2.1.3 of the **CTP**.

- $AUC_{0\text{-}\infty}$ (area under the concentration-time curve of the analyte in plasma over the time interval from 0 extrapolated to infinity)



6. GENERAL ANALYSIS DEFINITIONS

6.1 TREATMENTS

For basic study information on the treatment to be administered, and selection of dose, cf. Section 4 of the CTP.

Subjects will receive the following two treatments in a fixed sequence (reference treatment always followed by test treatment).

In Period Reference R, all subjects will receive 1 tablet containing

- 10 mg BI 425809 (Reference Treatment R) as a single dose

Afterwards, in Period Test T, all subjects will receive

- 2 capsules, each containing 200 mg Fluconazole, once daily for 13 days (Day -4 to Day 9) and 1 tablet containing 10 mg BI 425809 as a single dose on Day 1 (Test Treatment T).

For statistical analysis of AEs, the following analysis phases are defined for each subject.

Table 6.1: 1 Analysis phases for statistical analysis of AEs, and actual treatment for analysis of laboratory data and vital signs

Study analysis phase	Label	Start	End
Screening[1]	**Screening**	Date of informed consent	Date/time of first administration of BI 425809
On treatment	**BI 425809**	Date/time of first administration of BI 425809	Date/time of first administration of BI 425809 + REP (11 days) or 12:00 a.m. on day after last contact date (whichever occurs first)
Follow-up	**F/U BI 425809**	Date/time of first administration of BI 425809 + REP (11 days)	Date/time of first administration of Fluconazole or 12:00 a.m. on day after last contact date (whichever occurs first)
On treatment	**Fluconazole**	Date/time of first administration of Fluconazole	Date/time of second administration of BI 425809 or 12:00 a.m. on day after last contact date (whichever occurs first)
On treatment	**BI + Fluconazole**	Date/time of second administration of BI 425809	Date/time of second administration of BI 425809 + REP (14 days) or 12:00 a.m. on day after last contact date (whichever occurs first)
Follow-up	**F/U BI +Fluconazole**	Date/time of second administration of BI 425809 + REP (14 days)	12:00 a.m. on day after last contact date

[1] See Section 6.7 for definition of baseline, which will be used in the statistical analyses of safety laboratory data and vital signs.
The on-treatment phase for 'BI + Fluconazole' includes both the REP for BI 425809 (14 days) and Fluconazole (6 days). Since the second administration of BI 425809 is scheduled at 9:00 am on Visit 3, Day 1, while the last administration of Fluconazole is scheduled at 8:00 am on the Visit 3, Day 9 (see CTP, Flow chart). Therefore, Date/time of the second administration of BI 425809 + REP (14 days) is one hour after Date/time of the last administration of Fluconazole + REP (6 days).

AE summary tables will present results for the on-treatment phases only. All AEs will be listed.

In AE tables in CTR Section 15.3 (but not in Appendix 16.1.13.1.8.1 and Appendix 16.1.13.1.8.2 AE tables), the following totals will be provided in addition:

- "**Total BI**", defined as the total over all on-treatment phases involving BI 425809
- "**Total Fluconazole**", defined as the total over all on-treatment phases involving Fluconazole
- **"Total on-trt"**, defined as the total over all on-treatment phases

Safety laboratory data, vital signs and PK parameters will be analyzed based on treatment periods (BI 425809 and BI+Fluconazole) with clear differentiation between baseline (cf. Section 6.7) and post-baseline measurements. Measurements will be considered on-treatment, if they were taken within the on-treatment phases of BI 425809 or BI+Fluconazole as defined in Table 6.1: 1. More details on the technical implementation of these analyses are provided in the ADS Plan of this TSAP.

6.2 IMPORTANT PROTOCOL DEVIATIONS

Consistency check listings (for identification of deviations from time windows) and a list of protocol deviations (e.g. deviations in drug administration, in blood sampling times, etc.) will be provided to be discussed at the Report Planning Meeting. At this meeting, it will be decided whether a discrepant data value can be used in analyses or whether it must be corrected in the clinical database. Each protocol deviation must be assessed to determine whether it is an important PD (iPD). For definition of iPDs, and for the process of identification of these, refer to the BI reference document "Identify and Manage Important Protocol Deviations (iPD)" (2) and the DV domain template.

If any iPDs are identified, they are to be summarised into categories and will be captured in the decision log. Categories which are considered to be iPDs in this trial are defined in the DV domain template. If the data show other iPDs, the definition in the DV domain template will be supplemented accordingly by the time of the Report Planning Meeting.

IPDs will be summarized and listed. Which kind of iPDs could potentially lead to exclusion from which analysis set is specified in the DV domain template. The decision on exclusion of subjects from analysis sets will be made at the latest at the Report Planning Meeting, after discussion of exceptional cases and implications for analyses.

Non-important COVID-19 related PDs will only be listed.

6.3 SUBJECT SETS ANALYSED

The treated set (TS) and pharmacokinetic parameter analysis set (PKS) will be used as defined in the CTP, Section 7.3.

Table 6.3: 1 Subject sets analyzed

	Subject set	
Class of endpoint	TS	PKS
Disposition	X	
iPDs	X	
Primary endpoints		X
Secondary PK endpoints		X
Further PK endpoints		X
Safety parameters	X	
Demographic/baseline characteristics	X	
Treatment exposure	X	

6.5 POOLING OF CENTRES

This section is not applicable, because the study was performed in only one center.

6.6 HANDLING OF MISSING DATA AND OUTLIERS

CTP: "*If a subject is removed from or withdraws from the trial prior to the administration of trial medication, the data of this subject will not be entered in the case report form (CRF) and will not be reported in the clinical trial report (CTR). If a subject is removed from or withdraws from the trial after the administration of trial medication, this will be documented and the reason for discontinuation must be recorded in the CRF. In addition, the data will be included in the CRF and will be reported in the CTR."*

CTP: "*It is not planned to impute missing values for safety parameters."*

One exception where imputation might be necessary for safety evaluation is AE dates. Missing or incomplete AE dates are imputed according to BI standards (3).

Missing data and outliers of PK data are handled according to BI standards.

CTP: "*Pharmacokinetic parameters that cannot be reasonably calculated based on the available drug concentration-time data will not be imputed."*

6.7 BASELINE, TIME WINDOWS AND CALCULATED VISITS

For both periods, the last available value determined prior to administration of BI 425809 will be defined as baseline.

Time windows are defined in Section 6.1 of the CTP. Adherence to time windows will be checked at the Report Planning Meeting.

7. PLANNED ANALYSIS

The format of the listings and tables will follow the BI guideline "Standards for Reporting of clinical trials and project summaries" (6).

The individual values of all subjects will be listed. Listings will be sorted by subject number, visit and planned time point (if visit/ planned time point is applicable in the respective listing). The listings will be contained in Appendix 16.2 (SDL) of the CTR.

The following standard descriptive statistical parameters will be displayed in summary tables of continuous variables:

N	number of non-missing observations
Mean	arithmetic mean
SD	standard deviation
Min	minimum
Median	median
Max	maximum

For plasma concentrations as well as for all PK parameters the following descriptive statistics will additionally be calculated:

CV	arithmetic coefficient of variation
gMean	geometric mean
gCV	geometric coefficient of variation

For PK parameters the following descriptive statistics will additionally be calculated:

P10	10^{th} percentile
Q1	1^{st} quartile
Q3	3^{rd} quartile
P90	90^{th} percentile

The data format for descriptive statistics of plasma concentrations will be identical with the data format of the respective concentrations. The descriptive statistics of PK parameters will be calculated using the individual values with the number of decimal places as provided by the evaluation program. Then the individual values as well as the descriptive statistics will be reported with three significant digits in the CTR.

Tabulations of frequencies for categorical data will include all possible categories and will display the number of observations in a category as well as the percentage (%) relative to the respective treatment group. Percentages will be rounded to one decimal place. The category missing will be displayed if and only if there actually are missing values. Percentages will be based on all subjects in the respective subject set whether they have non-missing values or not.

7.1 DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

Only descriptive statistics are planned for this section of the CTR. These will be based on the TS.

7.2 CONCOMITANT DISEASES AND MEDICATION

Concomitant diseases will be coded according to the most recent version of MedDRA. Concomitant medication will be coded according to the most recent version of the World Health Organisation – Drug Dictionary. Concomitant non-drug therapies will be coded according to the most recent version of MedDRA.

Only descriptive statistics are planned for this section of the CTR.

CTP: *Previous and concomitant therapies will be presented without consideration of time intervals and treatment periods.*

A medication will be considered concomitant, if it

- is ongoing at the time of study drug administration, or
- starts within the on-treatment phase of the respective treatment (see Section 6.1 for a definition of treatments and analysis phases).

The relevance of the concomitant therapies to the evaluation of PK will be decided no later than at the RPM.

7.3 TREATMENT COMPLIANCE

Treatment compliance will not be analyzed as a specific endpoint. Any deviations from complete intake will be addressed in the Report Planning Meeting (cf. Section 6.2) and described in the CTR.

7.4 PRIMARY ENDPOINTS

7.4.1 Primary analysis of the primary endpoints

Descriptive statistics are planned for this section of the CTR. Furthermore, relative bioavailability will be evaluated using the following model based approach:

CTP:
Primary analyses
The statistical model used for the analysis of the primary endpoints (refer to Section 2.1.2) will be an analysis of variance (ANOVA) model on the logarithmic scale. That is, the PK endpoints will be log-transformed (natural logarithm) prior to fitting the ANOVA model. This model will include effects accounting for the following sources of variation: subjects and treatment. The effect 'subjects' will be considered as random, whereas the effect 'treatment' will be considered as fixed. The model is described by the following equation:
$y_{km} = \mu + s_m + \tau_k + e_{km}$, *where*

y_{km} = logarithm of response measured on subject m receiving treatment k,
μ = the overall mean,
s_m = the effect associated with the m_{th} subject, m = 1, 2, ..., 15
τ_k = the k^{th} treatment effect, k = 1, 2,
e_{km} = the random error associated with the m^{th} subject who received treatment k. where $s_m \sim N(0, \sigma_R^2)$) i.i.d., $e_{km} \sim N(0, \sigma_W^2)$i.i.d. and s_m, e_{km} are independent random variables.

Point estimates for the ratios of the geometric means (test/reference) for the primary endpoints (see Section 2.1) and their two-sided 90% confidence intervals (CIs) will be provided.
For each endpoint, the difference between the expected means for log(T)-log(R) will be estimated by the difference in the corresponding adjusted means (Least Squares Means). Additionally their two-sided 90% confidence intervals will be calculated based on the residual error from the ANOVA and quantiles from the t-distribution. These quantities will then be back-transformed to the original scale to provide the point estimate and 90% CIs for each endpoint.

Further exploratory analyses
The same statistical model as stated above will be repeated for the primary endpoints but with all sources of variation (‘subjects‘, ‘treatment’) considered as fixed effects.
In addition to the model based approach all parameters will be calculated and analyzed descriptively.

Analyses of primary endpoints will be based on the PKS.

Exclusion of PK parameters

The ADS ADPP contains column variables APEXC and APEXCO indicating inclusion/exclusion (APEXC) of a PK parameter and an analysis flag comment (APEXCO). All analyses based on the PKS are based on PK parameter values which are not flagged for exclusion, i.e. with APEXC equal to "Included".

CTP: *Plasma concentration data and parameters of a subject which is flagged for exclusion will be reported with its individual values but will not be included in the statistical analyses.*

Exclusion of plasma concentrations
The ADS ADPC (PK concentrations per time-point or per time-interval) contains column variables ACEXC or ACEXCO indicating inclusion/exclusion (ACEXC) of a concentration and an analysis flag comment (ACEXCO). Exclusion of a concentration depends on the analysis flag comment ACEXCO. For example, if ACEXCO is set to "ALL CALC", the value will be excluded for all types of analyses based on concentrations. If ACEXCO is set to "DESC STATS" the value will be excluded from descriptive evaluations per planned time point/time interval. If ACEXCO contains the addition "TIME VIOLATION" or "TIME DEVIATION", the value can be used for further analyses based on actual times. If ACEXCO is set to "HALF LIFE", the value will be excluded from half-life calculation only; the value is

included for all other analyses. Excluded concentration itself will be listed in the CTR associated with an appropriate flag.

Further details are given in "Noncompartmental Pharmacokinetic/ Pharmacodynamic Analyses of Clinical Studies" (4) and "Description of Analytical Transfer Files and PK/PD Data Files" (5).

7.5 SECONDARY ENDPOINTS

7.5.1 Key secondary endpoints

This section is not applicable as no key secondary endpoint has been specified in the protocol.

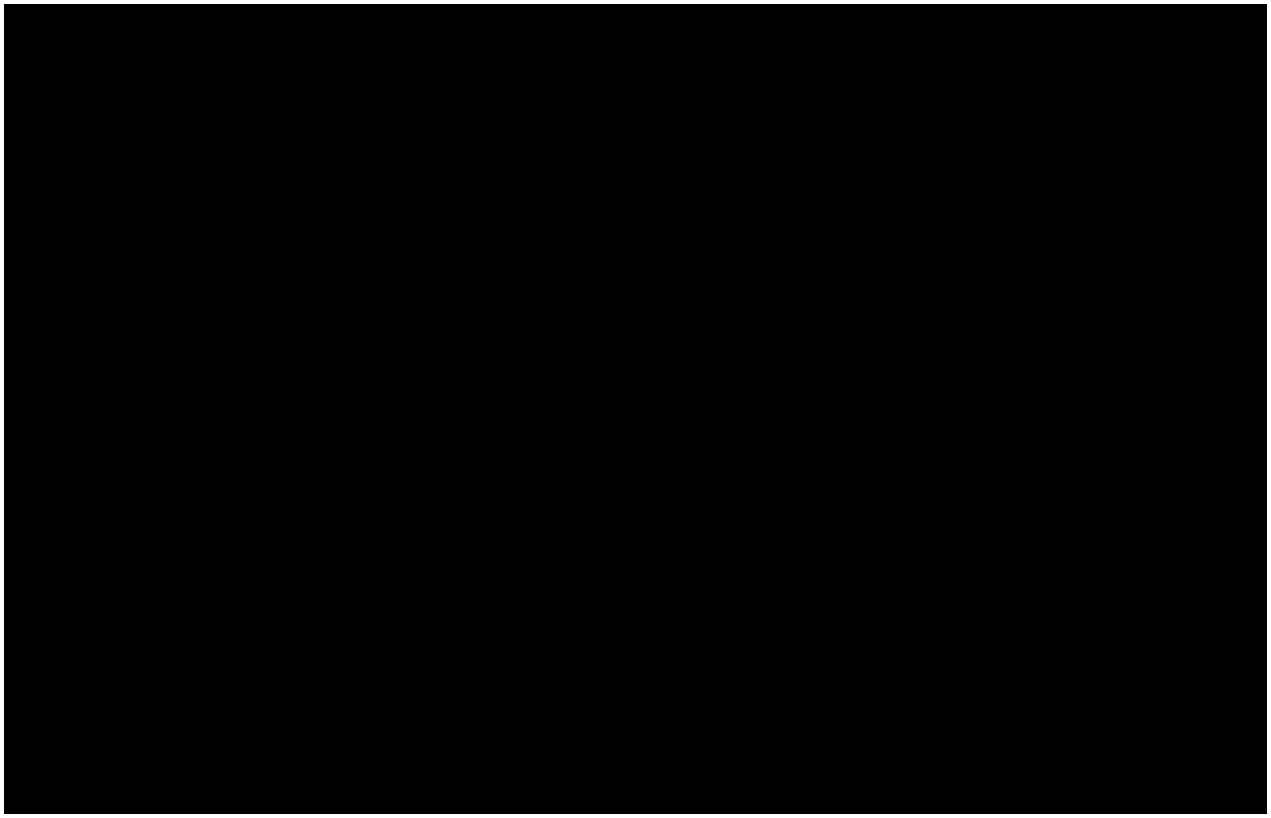
7.5.2 Secondary endpoints

The analysis of secondary PK endpoints will be based on the PKS.

7.5.2.1 Secondary endpoint analysis

CTP: *The secondary endpoints (refer to Section 2.1.3) will be calculated according to the relevant BI internal procedures and will be assessed statistically using the same methods as described for the primary endpoints.*

Exclusion of PK parameters and exclusion of plasma concentrations are handled as described in Section 7.4.1.



7.7 EXTENT OF EXPOSURE

Descriptive statistics are planned for this section of the report.

7.8 SAFETY ANALYSIS

All safety analyses will be performed on the TS.

7.8.1 Adverse Events

AEs will be coded with the most recent version of MedDRA.

The analyses of AEs will be descriptive in nature. All analyses of AEs will be based on the number of subjects with AEs and not on the number of AEs.

For further details on summarization of AE data, please refer to "Analysis and Presentation of Adverse Event Data from Clinical Trials" (7) and "Handling of missing and incomplete AE dates" (3).

The analysis of AEs will be based on the concept of treatment emergent AEs. That means that all AEs will be assigned to screening, on-treatment or follow-up phases as defined in Section 6.1. AEs will be analysed based on actual treatments, as defined in Table 6.1: 1.

An overall summary of AEs will be presented. This overall summary will comprise summary statistics for the class of AESIs.

CTP: *Hepatic injury is considered an AESI in this trial. A hepatic injury is defined by the following alterations of hepatic laboratory parameters:*

- *An elevation of AST (aspartate transaminase) and/or ALT (alanine transaminase) ≥3 fold ULN combined with an elevation of total bilirubin ≥2 fold ULN measured in the same blood sample, or*
- *Aminotransferase (ALT, and/or AST) elevations ≥10 fold ULN*

The investigator had to classify on the eCRF whether an observed AE was an AESI or not.

According to ICH E3 (8), in addition to Deaths and Serious Adverse Events, 'other significant' AEs need to be listed in the clinical trial report. These will be any non-serious adverse event that led to an action taken with study drug (e.g. discontinuation or dose reduced or interrupted).

The frequency of subjects with AEs will be summarised by treatment, primary SOC and preferred term. AEs which were considered by the investigator to be drug related will be summarised separately. Separate tables will also be provided for subjects with SAEs and subjects with AESIs. AEs will also be summarized by maximum intensity.

The SOCs and preferred terms within SOCs will be sorted by descending frequency.

For disclosure of AE data on ClinicalTrials.gov, the frequency of subjects with non-serious AEs occurring with an incidence of greater than 5 % (in preferred terms) will be summarised by primary SOC and preferred term. The frequency of subjects with SAEs will also be summarised.

For disclosure of AE data in the EudraCT register, the frequency of AEs, the frequency of non-serious AEs with an incidence of greater than 5 % (in preferred terms) and the frequency of SAEs will be summarized.

For support of lay summaries, the frequency of subjects with drug-related SAEs will be summarized by treatment, primary SOC and preferred term.

7.8.2 Laboratory data

The analyses of laboratory data will be descriptive in nature and will be based on BI standards "Display and Analysis of Laboratory Data" (9).

Analyses will be based on normalised values, which means transforming to a standard unit and a standard reference range. The original values will be analysed if the transformation into standard unit is not possible for a parameter.

Descriptive statistics of laboratory values over time and for the difference from baseline (see Section 6.7) will be provided. Frequency tables of changes between baseline and last value on treatment with respect to the reference range will be presented.

Unscheduled measurements of laboratory data will be assumed to be repeat measurements of the most recent scheduled measurement (e.g. for follow-up or confirmation of a particular

value). Therefore, unscheduled measurements will be assigned to the planned time point of the previous scheduled measurement. Descriptive statistics will be calculated by planned time point based on the worst value of the subject at that planned time point (or assigned to that planned time point).

Clinically significant abnormal laboratory values are only those identified either in the Investigator's comments or at the Report Planning Meeting at the latest. It is the Investigator's responsibility to decide whether a lab value is clinically significant abnormal or not.

Laboratory data will be compared to their reference ranges. Values outside the reference range as well as possibly clinically significant values will be highlighted in the listings. Possibly clinically significant laboratory values will be listed in Section 15.4.1.

Clinically relevant findings in laboratory data will be reported as baseline conditions (prior to first administration of study treatment) or as AEs (after first administration of study treatment) if judged clinically relevant by the investigator, and will be analyzed as such.

7.8.3 Vital signs

The analyses of vital signs (blood pressure and pulse rate) will be descriptive in nature. Descriptive statistics of vital signs over time and for the difference from baseline (see Section 6.7) will be provided.

Unscheduled measurements of vital signs will be assigned to planned time points in the same way as described above for laboratory data. However, for vital signs, descriptive statistics will be calculated by planned time point based on the last value of the subject at that planned time point (or assigned to that planned time point). If the time of measurement is missing for a scheduled post-baseline measurement, the scheduled measurement will be used in calculation of descriptive statistics (as time difference between scheduled and unscheduled cannot be assessed).

If the time of measurement is missing for an unscheduled measurement, this measurement will be listed but will be ignored for the calculation of descriptive statistics.

Clinically relevant findings in vital signs data will be reported as baseline conditions (prior to first administration of study treatment) or as AEs (after first administration of study treatment) if judged clinically relevant by the investigator, and will be analyzed as such.

7.8.4 ECG

Abnormal findings in ECG will be reported as baseline conditions (at screening) or as AEs (during the trial) if judged clinically relevant by the investigator, and will be analyzed as such. No separate listing or analysis of ECG data will be prepared.

7.8.5 Others

7.8.5.1 Physical examination

Physical examination findings will be reported as relevant medical history/baseline condition (if a condition already exists before first administration of study treatment) or as AE (if condition emerges after first administration of study treatment) and will be summarized as such. No separate listing or analysis of physical examination findings will be prepared.

7.8.5.2 Body weight

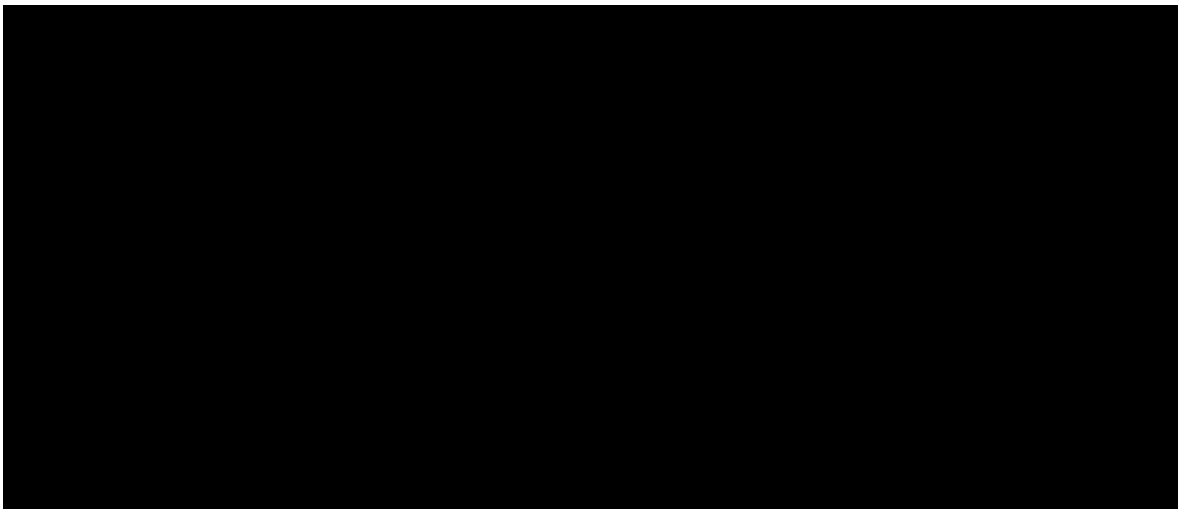
Body weight will only be measured at screening and therefore will only be listed.

8. TIMEPOINT OF RELEASE OF TREATMENT INFORMATION

The treatment information will be loaded into the trial database at trial initiation.

9. REFERENCES

1	*CPMP/ICH/363/96*: "Statistical Principles for Clinical Trials", ICH Guideline Topic E9; Note For Guidance on Design, Conduct, Analysis and Evaluation of Clinical Trials, current version
2	*001-MCS-40-413:* "Identify and Manage Important Protocol Deviations (iPD)", current version; KMED
3	*KM Asset BI-KMED-BDS-HTG-0035:* "Handling of missing and incomplete AE dates", current version; KMED
4	*KM Asset BI-KMED-TMCP-MAN-0014*: "Noncompartmental PK/PD Analyses of Clinical Studies", current version; KMED
5	*KM Asset BI-KMED-TMCP-MAN-0010:* "Description of Analytical Transfer Files, PK/PD Data Files and ADA files", current version; KMED
6	*KM Asset BI-KMED-BDS-HTG-0045:* "Standards for Reporting of Clinical Trials and Project Summaries", current version; KMED
7	*KM Asset BI-KMED-BDS-HTG-0066:* "Analysis and Presentation of Adverse Event Data from Clinical Trials", current version; KMED
8	*CPMP/ICH/137/95*: "Structure and Content of Clinical Study Reports", ICH Guideline Topic E3; Note For Guidance on Structure and Content of Clinical Study Reports, current version
9	*KM Asset BI-KMED-BDS-HTG-0042:* "Handling, Display and Analysis of Laboratory Data", current version; KMED



11. HISTORY TABLE

Table 11: 1 History table

Version	Date (DD-MMM-YY)	Author	Sections changed	Brief description of change
Final	**10-FEB-2022**	[redacted]	None	This is the final TSAP