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STATISTICAL ANALYSIS PLAN

Protocol BPR-CP-101

A phase 1, single-center, open-label, non-randomized, fixed-sequence, drug-drug interaction study to assess the effect of repeated doses of intravenous ceftobiprole on the pharmacokinetics of oral pitavastatin (OATP1B substrate) and on plasma levels of coproporphyrin I (OATP1B biomarker) in healthy subjects

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LIST OF ABBREVIATIONS

AE	Adverse event
BLQ	Below limit of quantification
BMI	Body mass index
CI	Confidence Interval
CP-I	Coproporphyrin I
CRF	Case report form
CTCAE	Common Terminology Criteria for Adverse Events
CV	Coefficient of variation
ECG	Electrocardiogram
eCRF	Electronic CRF
IV	Intravenous
MedDRA	Medical Dictionary for Regulatory Activities
OATP1B	organic anion-transporting polypeptide 1B
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
PT	Preferred Term
q8h	every 8 hours
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SD	Standard deviation
SOC	System Organ Class
TEAE	Treatment emergent adverse event
WHO DDE	World Health Organization Drug Dictionary Enhanced

1 INTRODUCTION

This document outlines the statistical methods to be implemented in the analysis of data collected within the scope of Basilea Pharmaceutica International Ltd, Allschwil, Protocol BPR-CP-101 (A phase 1, single-center, open-label, non-randomized, fixed-sequence, drug-drug interaction study to assess the effect of repeated doses of intravenous ceftobiprole on the pharmacokinetics of oral pitavastatin (OATP1B substrate) and on plasma levels of coproporphyrin I (OATP1B biomarker) in healthy subjects).

The purpose of this Statistical Analysis Plan (SAP) is to define the planned statistical methods in line with the study objectives. This plan should be read in conjunction with the study protocol (Version 1.0, dated 30 September 2024 at the time of finalization). All analyses will be conducted using SAS® Version 9.4 or higher.

2 OBJECTIVES

2.1 Primary objective

The primary objective of study BPR-CP-101 is to assess the pharmacokinetics (PK) of a single oral dose of pitavastatin, administered without and with intravenous (iv) ceftobiprole in healthy subjects.

2.2 Secondary objectives

The secondary objectives are:

- To assess the pharmacodynamic (PD) effect of iv ceftobiprole on the diurnal plasma level profile of coproporphyrin I (CP-I) in healthy subjects
- To describe the systemic exposure of iv ceftobiprole without and with oral administration of pitavastatin in healthy subjects
- To assess the single-dose plasma PK of the pitavastatin metabolite, pitavastatin lactone, after a single oral dose of pitavastatin, administered without and with iv ceftobiprole in healthy subjects
- To assess the safety and tolerability of ceftobiprole without and with administration of a single oral dose of pitavastatin in healthy subjects.

3 INVESTIGATIONAL PLAN

3.1 Overall study design

3.1.1 Brief summary

This is a Phase 1, single-center, open-label, non-randomized, fixed-sequence, drug-drug interaction study in healthy male or female subjects to assess if there is an inhibitory effect of ceftobiprole on the hepatic OATP1B activity. Pitavastatin is used as an OATP1B substrate in this study. The PK of oral pitavastatin is assessed when administered alone and when administered together with iv ceftobiprole in a study design including two treatment periods. In addition, the PD effect of repeated doses of iv ceftobiprole on the diurnal plasma

levels of CP-I is assessed in this study as CP-I is an endogenous biomarker for hepatic OATPB1 activity.

An overview of the study design is provided in protocol Section 3.1. The schedule of assessments and procedures for the different periods are provided in protocol Table 1.

3.1.2 Number of subjects

Twelve subjects will be enrolled in the study. If a subject does not complete the study for a reason not related to the study drug, the early-termination subject may be replaced.

3.1.3 Treatments

The following treatments are planned to be administered in two periods:

- Period 1 (from Day -1 to Day 3): pitavastatin single dose
    - Day 1: a single oral dose of 2 mg pitavastatin is administered in the morning under fasted conditions.
- Period 2 (from Day 4 to Day 8): pitavastatin single dose + ceftobiprole every 8 hours (q8h)
    - Day 4 to Day 7: 500 mg ceftobiprole (as the prodrug ceftobiprole medocaril sodium) is administered as a 2-hour iv dose q8h under fasted conditions in the morning, and irrespective of timing of food intake further on the day.
    - Day 6: a single oral dose of 2 mg pitavastatin is coadministered with ceftobiprole 30 minutes prior to the end of the first iv dose of 500 mg ceftobiprole under fasted conditions.

3.2 Study endpoints

3.2.1 Primary endpoints

The primary endpoints are the plasma PK parameters of pitavastatin including C_{max}, t_{max}, k_{el}, $t_{1/2}$, $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, CL/F, and V_z/F, as appropriate.

3.2.2 Secondary endpoints

The secondary endpoints are:

- Plasma exposure parameters of CP-I, including $AUEC_{0\text{-}25.5h}$, C_{max}, t_{max}, C_{trough}, and $C_{25.5h}$.
- Plasma exposure parameters of ceftobiprole, including C_{trough}, C_{max}, t_{max}, k_{el}, $t_{1/2}$, $AUC_{0\text{-}8h}$, CL, and V_z, as appropriate.
- Plasma PK parameters of pitavastatin lactone including C_{max}, t_{max}, k_{el}, $t_{1/2}$, $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, as appropriate.
- Ratios of C_{max}, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}inf}$ of pitavastatin lactone over parent drug.
- Type, frequency, severity, timing, and relationship to study drug of any adverse events (AEs).

- Abnormalities and changes from baseline in clinical laboratory, vital signs, and 12-lead electrocardiogram (ECG) parameters, as appropriate.

4 GENERAL STATISTICAL CONSIDERATIONS

Summary outputs will be presented overall, except adverse events that will be summarized by period (see Section 9.1 for details) and overall. No formal hypothesis testing will be performed. Continuous data, unless otherwise specified, will be described using descriptive statistics (n, mean, standard deviation [SD], median, minimum, and maximum). Categorical data will be described using the subject count and percentage in each category. Non-zero percentages will be rounded to one decimal place, except 100%, which will be displayed without any decimal places. For the summary statistics of all numerical variables unless otherwise specified, minimum and maximum will be displayed to the same level of precision as reported, up to a maximum of three decimal places. Mean and median will be displayed to one level of precision greater than the data collected, up to a maximum of three decimal places. Standard deviation will be displayed to two levels of precision greater than the data collected, up to a maximum of three decimal places.

When count data are presented, the percentage will be suppressed when the count is zero, to draw attention to the non-zero counts. A row denoted ‘Missing’ will be included in count tabulations where specified in the table shells, to account for dropouts and missing values. The denominator for all percentages will be the number of subjects in the population of interest, unless otherwise specified.

All data will be displayed in listings sorted by subject ID and treatment periods, when applicable.

4.1 Sample size justification

The sample size has been based on the desire to obtain adequate PK, PD, safety, and tolerability data to achieve the objectives of the study.

The intended sample size of 12 subjects is considered adequate to fully address the primary objective of the study, given the precedence of earlier studies with pitavastatin as probe substrate.

4.2 Analysis sets

4.2.1 Safety set

The safety set consists of all enrolled subjects who have received at least one dose of study drug.

4.2.2 Pharmacokinetic set

The PK set consists of all subjects who received at least one dose of study drug and provided sufficient bioanalytical assessment results to calculate reliable estimates of the PK parameters.

4.2.3 Pharmacodynamic set

The PD set consists of all subjects for whom the PD data are considered to be sufficient and interpretable.

4.3 Other important considerations

4.3.1 Definition of baseline

Unless otherwise specified, baseline is defined as the last non-missing assessment prior to the first study drug administration. Both scheduled and unscheduled visits and assessments will be used in determining baseline.

4.3.2 Study day calculation and visit windows

Visit windowing approaches will not be used for this study, and visit based summaries will include scheduled assessments only.

The following conventions will be used to calculate analysis study day:

- Day 1 is the day of first study drug administration. Day −1 is the day before Day 1. No Day 0 is defined for this study.
- Prior to Day 1, the algorithm is:

  Study Day = visit/examination date − date of first study drug administration
- For Day 1 and subsequent days, the algorithm is:

  Study Day = visit/examination date − date of first study drug administration + 1.

Summary data such as AEs will not be reported by visit. Tables that report abnormalities (e.g., laboratory shift tables) will include all assessments.

4.3.3 Missing and partial data

The following rules for missing data will be followed:

- AE date imputations will follow the rules described in Appendix 1.
- The causality assessment for AEs should not be missing and will be queried for a value. AEs with missing causality will be considered related to study drug.

4.3.4 Duration (e.g., for adverse events)

If date and time are collected, then duration is calculated as event end date and time minus event onset date and time. Duration will be displayed as days and fractions of days, or as hours and fractions of hours, as appropriate.

If only the date is collected, then the duration in days is calculated as event end date minus event onset date + 1.

4.3.5 Elapsed actual time

For PK blood sampling, elapsed actual time in hours is the number of hours elapsed from date and time of study drug administration on related day (Day 1 or Day 6 for pitavastatin PK and CP-I, Day 5 and Day 6 for ceftobiprole PK). This time is derived from actual dates and times recorded in the eCRF.

4.3.6 Coding dictionaries

AEs, medical history, and procedures are to be coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 27.0 or later. Previous and concomitant treatments are to be coded using the World Health Organization Drug Dictionary Enhanced (WHO DDE) dated 01MAR2024 or later (format B3).

5 SUBJECT DISPOSITION

5.1 Disposition

The number and percentage of subjects screened and included in each of the analysis sets will be summarized overall. The number and percentage of subjects who completed or discontinued from the study treatment and from study will also be presented, with the reason for discontinuation. All percentages will be based on the number of subjects in the safety set.

Reasons for screen failure will be listed only.

5.2 Protocol deviations

A protocol deviation is any change, divergence, or departure from the study design or procedures defined in the protocol. Protocol deviations will be documented by the clinical monitor throughout the course of monitoring visits. The protocol deviations will be categorized as important and non-important deviations.

All protocol deviations will be listed.

5.3 Inclusion and exclusion criteria

Full inclusion and exclusion criteria are listed in protocol Section 3.3.1 and 3.3.2, respectively. Unmet inclusion and met exclusion criteria for each subject will be listed.

6 DEMOGRAPHICS AND BASELINE CHARACTERISTICS

6.1 Demographics and general baseline characteristics

The following demographics and baseline characteristics data will be presented in tables using descriptive statistics for the Safety, PK, and PD sets:

- Age, sex, childbearing potential, race, ethnicity
- Height, weight, and BMI.

6.2 Medical history

Medical history will be listed.

7 TREATMENTS AND MEDICATIONS

7.1 Prior and concomitant medications

Prior medications are defined as medications with a stop date prior to the first dose of study drug. Concomitant medications are defined as medications that are either ongoing at the start of the first dose of study drug or start after the start of the first dose of study drug.

Prior and concomitant medications will be listed separately.

7.2 Study treatments

Exposure to study treatment will be listed.

8 PHARMACOKINETIC ANALYSIS

8.1 Plasma concentration

Plasma concentrations of pitavastatin, pitavastatin lactone, ceftobiprole, and CP-I will be analyzed at each time point of collection and will be summarized with descriptive statistics (n, mean, SD, coefficient of variation [CV%], minimum, median, maximum, and geometric mean) for the PK set (pitavastatin, pitavastatin lactone, and ceftobiprole) or PD set (CP-I).

Treatment of outliers

Individual concentration-time points, if considered anomalous, may be excluded from the analysis at the discretion of the pharmacokineticist and with approval from the Sponsor following a review of the available documentation. Any such exclusion will be outlined in the CSR.

Entire individual treatment profiles for a subject may be excluded following review of the available documentation. However, PK analyses may be performed with and without the excluded profiles. Any such exclusion will be clearly listed along with justification for exclusion.

Non-quantifiable concentrations

All concentration values reported as no results (not collected or not determined) values will be treated as missing. For the calculation of concentration summaries, all concentrations below the quantifiable limit (BLQ) will be treated as 0. Values that are BLQ will be displayed as ‘BLQ’ in listings.

Plasma concentrations will also be summarized with following figures:

- Plot of individual concentrations versus actual sampling time, using linear and semi-log scale by treatment period.
- Combined individual plasma concentrations versus actual sampling time, using linear and semi-log scale by treatment period.
- Plot of geometric mean versus scheduled sampling time, using linear and semi-log scale by treatment period.
- Boxplot of geometric mean versus scheduled sampling time by treatment period.

8.2 PK parameters

Individual PK parameters will be estimated using non-compartmental methods with WinNonlin® .

The plasma PK parameters will be estimated from the concentration-time profiles. In estimating the PK parameters, BLQ values at the beginning of the profile will be set to zero. BLQ values that occur after the first quantifiable point will be considered missing. Values that are embedded between BLQs, or quantifiable values occurring after two or more BLQs, will be set to missing at the discretion of the pharmacokineticist and in agreement with the Sponsor. Actual sampling times, rather than scheduled sampling times, will be used in all computations involving sampling times. If the actual time or dose time is missing, the scheduled time may be substituted in order to calculate the PK parameter.

The PK parameters to be determined or calculated using noncompartmental analysis from the plasma concentration-time data are:

- For pitavastatin and pitavastatin lactone on Day 1 and Day 6: C_{max}, t_{max}, k_{el}, $t_{1/2}$, $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, AUC_{extrap}, adjusted R-squared, CL/F (pitavastatin only), V_z/F (pitavastatin only), as appropriate; ratios of C_{max}, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}inf}$ of pitavastatin lactone over parent drug; AUC and C_{max} ratio Day 6/Day 1 for pitavastatin and pitavastatin lactone.
- For ceftobiprole on Day 5 and Day 6: C_{trough}, C_{max}, t_{max}, k_{el}, $t_{1/2}$, $AUC_{0\text{-}8h}$, AUC_{extrap}, adjusted R-squared, CL, and V_z, as appropriate.

Following administration, C_{max} and t_{max} will be obtained directly from the experimental observations. If multiple maxima occur at equal concentrations, the first temporal value will be taken. AUC will be calculated using the linear up/log down trapezoidal rule, using actual elapsed time since the start of administration. The number of data points included in the regression of k_{el} and $t_{1/2}$ after single dose will be determined by visual inspection, but a minimum of 3 data points in the terminal phase, excluding C_{max}, will be required to estimate k_{el}. The k_{el} values (and consequently $t_{1/2}$, CL/F, V_z/F, $AUC_{0\text{-}inf}$) will be considered unreliable estimates if the period of time is over which an individual k_{el} was estimated is less than twice the resultant $t_{1/2}$ or if the adjusted coefficient of determination R-squared is less than or equal to 0.75.

The proportion of $AUC_{0\text{-}inf}$ due to extrapolation (AUC_{extrap}) will also be calculated and expressed as a percentage. The value of AUC_{extrap} should be less than or equal to 20% for

$AUC_{0\text{-}inf}$ to be considered to be well estimated. If AUC_{extrap} is higher than 20%, then the values of $AUC_{0\text{-}inf}$, CL/F, and V_z/F will be considered unreliable and therefore excluded from the summaries.

All PK parameters will be summarized with descriptive statistics (n, mean, SD, CV, minimum, median, maximum, geometric mean, and geometric CV) for the PK set. For t_{max}, only n, median, minimum and maximum will be presented. All parameters will be listed by subject and period, parameters that meet the inclusion criteria for PK analysis will be accompanied by an indication that each criteria is met.

A scatter plot of individual (plus mean and median) PK parameters C_{max}, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}inf}$ by treatment (i.e., without or with ceftobiprole) for pitavastatin and pitavastatin lactone will be provided.

A mixed-effects model with subject as a random effect and treatment (i.e., without or with ceftobiprole) as a fixed effect will be performed on the following natural logarithm (ln)-transformed PK parameters of pitavastatin and pitavastatin lactone: C_{max}, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}inf}$. From this model, the back-transformed least-squares means (LSMeans) for each treatment will be presented. The ratio of least-squares geometric means between the test treatment (coadministration of pitavastatin + ceftobiprole) to the reference (pitavastatin alone) and the corresponding 90% confidence interval (CI) will be presented.

8.3 Exposure parameters of CP-I

Plasma exposure parameters of CP-I will be estimated using similar methods than PK parameters. Estimated plasma exposure parameters of CP-I are: C_{max}, t_{max}, $C_{through}$, $C_{25.5h}$, $AUEC_{0\text{-}25.5h}$. In addition, C_{max}, $AUEC_{0\text{-}25.5h}$, C_{trough} and $C_{25.5h}$ ratios between Days 5 to 6/Days -1 to 1, and shift (if any) of t_{max} on Days 5 to 6 versus Days -1 to 1 will be calculated.

All PD parameters will be summarized with descriptive statistics (n, mean, SD, CV, minimum, median, maximum, geometric mean, and geometric CV) for the PD set. For t_{max}, only median, minimum and maximum will be presented. All parameters will be listed by subject and period. Parameters that meet the inclusion criteria for PD analysis will be accompanied by an indication that each criteria is met.

A scatter plot of individual (plus mean and median) PK parameters C_{max}, $AUC_{0\text{-}25.5h}$, $AUC_{0\text{-}t}$, and $C_{through}$ by treatment for CP-I will be provided, as appropriate.

A mixed-effects model with subject as a random effect and treatment (i.e. without or with ceftobirpole) as a fixed effect will be performed on the following natural logarithm (ln)-transformed PD parameters of CP-I: C_{max} and $AUEC_{0\text{-}25.5h}$. From this model, the back-transformed least-squares means (LSMeans) for each treatment will be presented. The ratio of least-squares geometric means between the test treatment (coadministration of pitavastatin + ceftobiprole) to the reference (pitavastatin alone) and the corresponding 90% CI will be presented.

9 SAFETY ANALYSIS

All Safety analyses will be conducted using the Safety population.

9.1 Adverse events

Adverse events are defined in protocol Appendix 8.2.

A treatment-emergent AE (TEAE) is defined as any event not present prior to the first administration of the study drug, or any event already present that worsens in either severity or frequency following exposure to the study drug.

An AE that occurs prior to the first administration of the study drug will be considered a non-treatment emergent AE.

TEAEs will be classified and summarized as follow:

- TEAEs in pitavastatin period: TEAEs with a start date/time between first dose of pitavastatin on Day 1 and before first dose of ceftobiprole on Day 4.
- TEAEs in ceftobiprole period: TEAEs with a start date/time between first dose of ceftobiprole on Day 4 and before first dose of pitavastatin on Day 6.
- TEAEs in ceftobiprole + pitavastatin period: TEAEs with at start date/time on or after first dose of pitavastatin on Day 6.
- Overall: all TEAEs starting on or after first dose of pitavastatin on Day 1.

An overview summary of number and percentage of subjects will be provided for any TEAE, study-drug-related TEAEs, serious TEAEs, study drug-related serious TEAEs, TEAEs leading to treatment discontinuation, study drug-related TEAEs leading to treatment discontinuation, TEAEs leading to treatment interruption, study drug-related TEAEs leading to treatment interruption, TEAEs leading to death, study drug-related TEAEs leading to death. In summary tables, relationship and action taken with study drug refer to ceftobiprole or pitavastatin.

All TEAEs, non-TEAEs, serious AEs (SAEs), and AEs leading to death will be presented in separate listings.

9.1.1 Incidence of adverse events

If appropriate, the number and percentage of subjects reporting AEs will be tabulated by SOC and PT for all TEAEs, and separately for study-drug-related TEAEs. Subjects with multiple events will be counted only once within each category. SOC and PT will be sorted in descending order of frequency overall.

9.1.2 Relationship of adverse events to study drug

A summary of TEAEs will also be presented by relationship to study drug, SOC and PT. If a subject reports multiple occurrences of the same AE, only the worst relationship category will be counted.

Adverse events that are missing an assessment of relationship to study drug will be counted in the study-drug-related AEs summary table, but will be presented in the summary table by relationship and in listing with a ‘Missing’ relationship.

9.1.3 Severity of adverse event

Severity of AEs is assessed by the investigator according to CTCAE (version 5.0) as 'Mild' (Grade 1), 'Moderate' (Grade 2), 'Severe' (Grade 3), 'Life-threatening' (Grade 4), or 'Death' (Grade 5). A summary of TEAEs by severity will be presented in a table. If a subject reports multiple occurrences of the same AE, only the most severe will be counted. AEs that are missing severity will be presented in tables as severity 'Unknown'.

9.2 Physical examination

Physical examination results for all subjects will be presented in a listing.

9.3 Clinical laboratory evaluations

Local laboratory tests include hematology, chemistry, urinalysis, serology, and drug and alcohol screen parameters in accordance with the protocol Schedule of Assessments and protocol Section 3.5.1.3.2.

Summary tables for hematology and chemistry parameters including actual values and change from baseline values will be presented for baseline and post-baseline scheduled assessments.

Hematology and chemistry parameters data will also be categorized as following:

- Each subject's laboratory safety parameter values, when appropriate, will be categorized based on CTCAE version 5.0.
- Laboratory parameter values will be flagged as 'low', 'normal', or 'high' relative to the normal ranges of the laboratory.

These categorical data will be summarized in shift tables comparing relevant post-baseline visits with those at the baseline visit.

If any laboratory value falls above or below the upper or lower level of quantification, the following rule will be applied for summary statistics: values reported as < XX or ≤ XX will be analyzed as XX/2; values reported as > XX or ≥XX will be analyzed as XX.

All clinical laboratory parameters will be presented in a listing including normal ranges and indicating if the value is out of range. Laboratory data collected at unscheduled visits will be included in listings only.

9.4 Vital signs

Summary tables of observed values and changes from baseline, will be presented for vital signs data, including body temperature (°C), respiration rate (breaths/minute), pulse rate (bpm), systolic blood pressure (mmHg), diastolic blood pressure (mmHg), at each visit on a given timepoint, in the Safety set.

Vital signs data collected at unscheduled visits will be included in listings only.

9.5 Electrocardiogram

Summary tables of observed values and changes from baseline, will be presented for 12-lead data, including heart rate (bpm), PR interval (msec), QRS duration (msec), QT interval (msec), QTcF interval (msec), at each visit on a given timepoint, in the Safety set.

Interpretation and ECG data collected at unscheduled visits will be included in listings only.

10 INTERIM ANALYSES

No interim analyses will be performed.

11 CHANGES IN THE PLANNED ANALYSIS

There are no changes between the protocol-defined statistical analyses and those presented in this SAP.

12 APPENDICES

Appendix 1 Adverse event and prior/concomitant medication date imputations

Imputation rules for partial dates

Adverse event start date imputation

Parameter	Missing	Additional conditions	Imputation
Start date for AEs	D	M and Y same as M and Y of first dose of study drug	Date of first dose of study drug
		M and/or Y not same as date of first dose of study drug	First day of month
	D and M	Y same as Y of first dose of study drug	Date of first dose of study drug
		Y is after Y of first dose	1 January
	D, M, Y	None – date completely missing	Date of first dose of study drug

D = day; M = month; Y = year; AE = adverse event.
Note: In all cases, if an estimated start date is after a complete stop date, use the first day of the stop date month.