Sponsor: Basilea Pharmaceutica International Ltd.

Study title: **An open-label study to evaluate the single-dose pharmacokinetics and safety of ceftobiprole in neonate and infant subjects aged up to 3 months undergoing treatment with systemic antibiotics**

Study number: BPR-PIP-001

Development Phase: Phase 1

SAP version: Final v2.0 – 17 Aug 2020

Trial Biostatistician:

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LIST OF ABBREVIATIONS

AUC	Area under the curve
AE	Adverse event
BLQ	Below the limit of quantitation
BMI	Body mass index
BSA	Body surface area
CI	Confidence interval
CL_S	Systemic clearance
C_{max}	Maximum observed plasma concentration
ITT	Intent-to-treat
IV	Intravenous
MIC	Minimum inhibitory concentration
PK	Pharmacokinetic(s)
SAE	Serious adverse event
SAP	Statistical Analysis Plan
$t_{1/2}$	Half-life
T > MIC	Time that concentration is above the MIC
V_{SS}	Volume of distribution at steady-state
WBC	White blood cell
RBC	Red blood cell

1 INTRODUCTION

This statistical analysis plan covers the detailed procedures for performing the statistical analyses and producing tables, listings and figures in the study described in Basilea Pharmaceutica International Ltd. (Basilea) Protocol BPR-PIP-001, version 5 dated 21st of March 2019..

2 STUDY OBJECTIVES, MAIN PARAMETERS AND DESIGN

2.1 Study Objectives

2.1.1 Primary objective

The primary objective of this study is to characterize the pharmacokinetics (PK) of a single dose of ceftobiprole in neonates and infants aged ≤ 3 months.

2.1.2 Secondary objective

The secondary objective of this study is to evaluate the safety and tolerability of ceftobiprole in neonates and infants aged ≤ 3 months.

2.2 Main Study Parameters

2.2.1 Efficacy

Not applicable for this study.

2.2.2 Safety and Tolerability

Adverse events, laboratory tests (hematology, biochemistry, urinalysis), vital signs, physical examination.

2.2.3 Pharmacokinetics

Non-compartmental analysis: C_{max}, $t_{1/2}$, AUC_{0-t}, $AUC_{0-\infty}$, CL_S, V_{SS}, T > MIC.
The relationship between exposure (C_{max} and AUC), derived PK parameters (CL_S, V_{SS}, $t_{1/2}$), gestational age, post-natal age, body weight, body mass index, body surface area, and calculated creatinine clearance will also be assessed.

2.3 Study Design

2.3.1 Study design and sample size

This is an open-label, single-fixed-dose, multicenter study carried out in three sequential cohorts. Each cohort consists of 15 neonates or infants aged ≤ 3 months (45 subjects total).
The study will be conducted in European and United States centers.

No sample size calculation has been performed for this study. Study drug treatment of 15 subjects per dosing cohort (total N = 45) is based on experience and is considered sufficient to fulfil the objectives of this study.

The study comprises:

I. A pre-dosing screening phase of up to 24 hours,
II. A 1-day dosing phase with a single-dose intravenous administration of ceftobiprole at 7.5 mg/kg, and
III. A post-dose follow-up safety assessment on Day 7 ± 3 after ceftobiprole administration.

2.3.1.1 Cohorts

The study population was initially planned to comprise of 3 cohorts, with 15 subjects in each cohort, based on gestational age (28 to 32 weeks [Cohort 3]; 33 to 36 weeks [Cohort 2]; ≥ 37 weeks [Cohort 1]).

Due to the very low rate of patient recruitment, only cohort 1 was successfully completed. There is no cohort 2 and cohort 3 population at the end of the study.

2.4 Assessments

The study assessments from screening to follow-up are summarized in Table 1 below:

Table 1: Schedule of assessments

	Screening Day −1 to 1	Dosing Day 1	Follow-up Day 7±3
SCREENING AND ADMINISTRATIVE PROCEDURES			
Written Informed consent[1]	X		
Inclusion/exclusion criteria	X		
Medical history	X		
Prior medications[2]	X		
CEFTOBIPROLE ADMINISTRATION			
Infusion of ceftobiprole[3]		X	
Collection of aliquot of infusion solution[4]		X	
PHARMACOKINETIC PROCEDURES			
Blood sample collection[5]		X	
Urine collection[6]		X	
SAFETY PROCEDURES			
Vital signs[7]	X	X	X
Physical examination	X	X*	X
Body weight and height[8]	X	X*	X
Laboratory tests[9]	X		X
Concomitant medication		X	X
Adverse events		< ----------X ---------->	

[1] Informed consent must be obtained within 3 days prior to dosing on Day 1.

[2] Prior medications administered within the 7 days prior to dosing will be collected.

[3] Ceftobiprole must be administered via a separate infusion line. No other intravenous infusion may be given through the same line during the 4-hour ceftobiprole infusion, and – if feasible – attempts should be made to not simultaneously administer any other antibiotic treatment (i.e., through any infusion line) during the ceftobiprole infusion. If feasible, attempts should be made to allow for a minimum period of 30 minutes between the end of administration of any other antibiotic treatment and the start of ceftobiprole medocaril infusion.

[4] An aliquot of approximately 3 mL of infusion solution will be collected after completion of the ceftobiprole infusion and frozen at below −65 °C immediately after sampling.

[5] Blood samples of 200–300 µL will be obtained for PK analysis at the times described in Table 2.

6 Attempts should be made to obtain urine at the times described in Table 2; NB: Full portions of urine will be collected from subjects in whom a urinary catheter has been placed for their standard clinical care. Urinary catheters will not be placed for the purpose of the study. For subjects without a urinary catheter, one or more portions of urine may be obtained before, during, and after dosing.

[7] Vital signs (body temperature, respiratory rate, pulse rate, and systolic and diastolic blood pressures) will be measured at the times described in Table 2.

[8] Height must be recorded at screening only. The body weight determination on Day 1 will be used for dose calculation and should be obtained within 6-hours prior to dosing.

[9] Safety laboratory tests include hematology, biochemistry, and urinalysis (see protocol Section 5.2.3.2).

*Physical examination and body weight obtained during the screening evaluation do not need to be repeated as Day 1 assessments if they were performed within 6 hours prior to dosing on Day 1.

The schedule of PK sampling and vital signs on Day 1 are summarized in Table 2 below:

Table 2: Schedule of PK sampling and vital signs on Day 1

Time	Vital signs	Blood samples	Urine collection*	Infusion solution aliquot collection
−2 to 0 h			X	
−15 min	X			
Pre-dose		X		
0 to 2 h			X	
1 h	X			
2 h		X		
2 h 15 min	X			
2 to 4 h			X	
4 h (end of infusion)		X		X
4 to 8 h			X	
6 h	X	X		
8 h		X		
8 to 12 h			X	
12 h		X		

* NB: Full portions of urine will be collected from subjects in whom a urinary catheter has been placed for their standard clinical care. Urinary catheters will not be placed for the purpose of the study. For subjects without a urinary catheter, one or more portions of urine may be obtained before, during, and after dosing.

2.4.1 Interim analyses

No hypothesis-testing interim analysis is planned. PK data from early dosing cohorts may be analyzed before dosing in later cohorts is started or completed. Safety data will be reviewed on an ongoing basis.

3 ANALYSIS POPULATIONS

3.1 Pharmacokinetics Population

Consists of all subjects who receive study drug and have adequate samples for determination of time-plasma concentration profiles of ceftobiprole. Subjects may be excluded from the PK analysis if they have significant protocol deviations, including eligibility criteria deviations, or if data which may influence the PK analysis are unavailable.

3.2 Safety Population

All subjects who receive any quantity of the study drug.

4 STATISTICAL CONSIDERATIONS AND ANALYSIS

4.1 General Considerations

No formal hypothesis testing will be performed. Summary outputs, where possible, will be presented for cohort 1.

4.2 Derived Variables

The following derived variables will be applied throughout the study:

- Baseline is defined as the last available assessment prior to start of study drug administration (including unscheduled assessments).
- Last/Final for safety is the first available value after study drug administration.
- Adverse event duration (in days, hours or fractions of hours) will be calculated as:
  - (<event end date.time> minus <event onset date.time>) in days or hours.
    If only date is collected:
  - ((<event end date> minus <event onset date>) + 1) in days
- The following algorithm will be used for the study day determination:
  - Day 1 = Day of study drug administration. The day before day 1 is Day -1.
  - Prior to Day 1 the algorithm is (<visit/examination date> minus <date of first study drug administration >)
  - Day 1 and subsequent days = (<visit/examination date> minus <date of first study drug administration >) + 1.
- Elapsed Times Between Examination And Treatment Administration will be calculated as

  (<examination date.time> minus <treatment administration date.time>)
- Post-natal age expressed in days will be computed as

  ((<consent date> minus <date of birth>) + 1) .Gestational age collected by the investigator will be used for analysis.

- Gestational age collected as weeks and days by the investigator will be computed as weeks rounded to 1 decimal for analysis.

4.3 Handling of Missing Data and/or Invalid Data and Outliers

Incomplete/partial dates will be replaced by derived variables and imputed using the following rules:

- if the day of the month is missing it is imputed to be the 15th if not in the month of study treatment. In case this leads to inconsistencies with other available patient's data, the imputation values will be handled case-by-case.
- if both the day and month are missing, they are imputed to be June 30 if not in year of study treatment. In case this leads to inconsistencies with other available patient's data, the imputation values will be handled case-by-case.
- missing years will be left as missing.
- missing times will be replaced by '00:00' for start times and '23:59' for end times if time is required.
- missing minutes will be replace by '00' for start times and '59' for end times times if time is required.

5 STATISTICAL PLAN AND METHODS

The statistical analysis will be performed using the software package SAS version 9.3 or higher (SAS Institute Inc., Cary, NC 27513, USA). Validation will be done by double programming.

All individual data as well as results of statistical analyses will be presented in individual patient data listings and statistical summary tables.

In general, continuous variables will be summarized using the following standard descriptive summary statistics: mean, standard deviation, median, minimum, maximum and number of observations. Categorical data will be described using frequency and percentage. Shift tables will be provided, where appropriate. One additional decimal point for mean, median, Q1, Q3 and two additional decimal points for SD will be used.

PK parameters will be presented by individual listings and descriptive summary statistics including arithmetic and geometric means, arithmetic and geometric coefficients of variation, standard deviation, minimum, median, and maximum.

Percentages will be rounded to one decimal place or more if most results are close to 0 or 100. Unscheduled assessments will only be listed and will not be included in the tables, unless otherwise specified. Any changes in the planned statistical methods will be documented in the clinical study report.

To assess the relationship between pairs of continuous variables (including PK parameters), the Pearson product moment correlation will be computed. This will however, only be applied when each variable has at least 5 non-missing observations. This correlation computation will apply to C_{max}, AUC, CL_s, V_{ss}, $t_{1/2}$, gestational and post-natal age, weight, BMI, BSA, and calculated creatinine clearance.

The following international dictionaries will be used for medical coding:

- Medical History events: MedDRA (version 20.1)
- Medications: WHO Drug Dictionary Enhanced (March 2018 B2 version)
- Adverse events: MedDRA (version 20.1)

Data from all participating study centers will be combined for analysis.

5.1 Background Characteristics

5.1.1 Patient disposition

Enrollment and disposition data will be presented for each patient in data listings and summarized by frequency tables.

Patient enrollment eligibility and inclusion and exclusion criteria deviations will be presented in data listings.

5.1.2 Protocol deviations

All protocol deviations reported by the clinical team will be presented in a data listing sorted by center, category and patient.

Protocol deviations captured in the database such as:

- Non-adherence to study inclusion and exclusion criteria
- Failure to obtain informed consent prior to beginning the study
- Wrong study treatment received, or an incorrect dose
- Other data deviations as observed upon review of the data

Major protocol deviations are defined as those that could potentially bias either the PK or safety conclusions of the study. All other protocol deviations are defined as minor protocol deviations. Protocol deviations will be reviewed prior to database lock and classified as major or minor based on medical review.

5.2 Demographics and Other Baseline Characteristics

5.2.1 Demographics

Descriptive statistics of baseline data will be presented for cohort 1. The following baseline demographic data will be presented:

- Post-natal Age [days] at Day 1 / Gestational Age [weeks]
    - Continuous summary
- Sex
    - Categorical summary (Male, Female)
- Race
    - Categorical summary (White, Black or African American, Asian, American Indian or Alaska Native, Native Hawaiian or Other Pacific Islander, Other)
- Height [cm]
    - Continuous summary
- Weight [g]
    - Continuous summary

5.2.2 Medical history

Medical history of diseases will be summarized in tables by SOC, and PT, including number of subjects and percentage relative to the numbers of subjects in the corresponding study groups.

5.3 Prior and Concomitant Medications

Data concerning concomitant medications and procedures will be collected throughout the study. These data will be obtained at scheduled or unscheduled study visits, based on information provided by the subject's parent or legal guardian.

The coding of medications will be performed using the WHODRUG enhanced dictionary and partial start and/or end dates will be imputed as described in section 4.3.

Medications will be classified based on the observed or imputed start dates into:

- A medication is classified as prior if it started and stopped before the reference start date of the subject.
- A medication is classified as concomitant medication if it started on or after the reference start date of the subject.
- A medication is classified as ongoing if it started before the reference start date of the subject and is still ongoing after the reference start date.

Prior medications, concomitant and ongoing medications will be presented in a listing.

Concomitant and ongoing medications will be summarized in tables by Anatomical Therapeutic Chemical (ATC) level 4 code, and Preferred Term (PT), including number of subjects and percentage relative to the numbers of subjects in the corresponding study groups.

5.4 Treatment Compliance and Exposure

Exposure to study drug will be summarized. The total dose taken will be presented.

5.4.1.1 Pharmacokinetic analysis

Plasma concentrations at each time point of measurement will be evaluated by descriptive statistics, including arithmetic mean, standard deviation (SD), minimum, maximum, and median. Actual sampling times will be used to calculate PK parameters. If ≤ 50% of the values at a given time point are BLQ, these values will be set to zero for mean value calculation. If more than 50% of the values at a given time point are BLQ, no mean value will be calculated. Mean plasma concentration profiles of cohort 1 will be generated according to these criteria.

For PK evaluation, BLQ-values at infusion start will be set to zero, BLQ-values at the end of the sampling period will be disregarded for PK assessment. Single BLQ values during blood sampling will be taken as 'missing values', if the following sample(s) show a concentration above the BLQ value.

5.5 Safety Analysis

Definitions of adverse events (AEs) and serious adverse events (SAEs) as well as information on

reporting procedures for AEs/SAEs are provided in the clinical trial protocol. AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA version 20.1) and partial start and/or end dates will be imputed as described in section 4.3.

Frequency tables summarizing the incidence and observed number of treatment-emergent adverse events by treatment and by System Organ Class and Preferred Term will be prepared with 3 levels of detail:

- All adverse events
- Adverse events by severity (mild/moderate/severe)
- Adverse events by relationship to study drug (not related/related)

An adverse event is classified as treatment emergent if the following conditions are met:

- Start date and end date of adverse event is missing.
- Start date is missing but end date is not before reference start date of treatment
- Start date is missing but end date is after reference start date
- Start date is on or after reference start date.

Summary tables for all adverse events and for adverse events by severity will be presented for cohort 1.

5.6 Clinical Laboratory Evaluations

The laboratory parameters collected per protocol will be described using summary statistics and shift tables. Summary statistics will be presented for absolute values and absolute change from baseline.

Reference ranges (low/high, negative/positive, normal/abnormal) will be presented in shift tables to show shifts from baseline values at the different time points.

5.7 Vital Signs

The actual and change from baseline values of the following vital signs will be summarized using descriptive statistics

- Systolic blood pressure
- Diastolic blood pressure
- Pulse rate
- Temperature
- Respiration rate

In addition marked reference changes from baseline will be presented for vital signs. Marked reference change will be defined as follow:

- Diastolic Blood Pressure: <60 or >100 mmHg.
- Systolic Blood Pressure: <80 or >180 mmHg.

- Pulse: <40 or >120 beats/min.
- Temperature: <36.0 or >38.5 degree Celsius.

6 REFERENCES

- Basilea SOP-GLO-000416 (3.0) Statistical Analysis Plan
- Basilea LIS-GLO-000412 (3.0) Preferred Units and Marked Factors
- EMEA. CPMP/ICH/363/96: Note for Guidance on Statistical Principles for Clinical Trials - ICH Topic E9. London: EMEA; 1998.
- Integrated Addendum to ICH E6 (R1), GUIDELINE FOR GOOD CLINICAL PRACTICE E6 (R2), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, 9 November 2016.