Safety of Furosemide in Premature Infants at Risk of Bronchopulmonary Dysplasia

Statistical Analysis Plan

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Best Pharmaceuticals for Children Act

STATISTICAL ANALYSIS PLAN

FOR

NICHD-2014-FUR01 – FUROSEMIDE

Safety of Furosemide in Premature Infants at Risk of Bronchopulmonary Dysplasia

Phase II Trial

Version 1.0

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TABLE OF CONTENTS

ACRONYMS AND ABBREVIATIONS

AE	Adverse Event
ANCOVA	Analysis of Covariance
ALT	Alanine Transaminase
AST	Aspartate Transaminase
ATC	Anatomical Therapeutic Chemical Classification
BPCA	Best Pharmaceuticals for Children Act
BPD	Bronchopulmonary Dysplasia
BUN	Blood Urea Nitrogen
CFR	Code of Federal Regulations
CL	Clearance
CL	Confidence Level
CSR	Clinical Study Report
DCC	Data Coordinating Center
DCRI	Duke Clinical Research Institute
DMC	Data Monitoring Committee
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
GA	Gestational Age
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Conference on Harmonization
IRB	Institutional Review Board
kg	Kilogram
LOCF	Last Observation Carried Forward
mg	Milligram
MedDRA®	Medical Dictionary for Regulatory Activities
N	Number (typically refers to patients)
NICHD	National Institute of Child Health and Human Development
NEC	Necrotizing Enterocolitis
NIH	National Institutes of Health
NRN	Neonatal Research Network
PD	Pharmacodynamics
PI	Principal Investigator
PK	Pharmacokinetics
PMA	Post Menstrual Age
PNA	Postnatal Age
PTN	Pediatric Trials Network
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
V	Volume of Distribution
WHO	World Health Organization

1 SYNOPSIS

This Statistical Analysis Plan (SAP) describes the planned analysis and reporting for the Pediatric Trials Network protocol NICHD-2014-FUR01, "Safety of Furosemide in Premature Infants at Risk of Bronchopulmonary Dysplasia" sponsored by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD).

The structure and content of this SAP provides sufficient detail to meet the requirements identified by the Food and Drug Administration, European Medical Agency, and International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use: Guidance on Statistical Principles in Clinical Trials [1]. All work planned and reported for this SAP will follow internationally accepted guidelines, published by the American Statistical Association [2] and the Royal Statistical Society [3], for statistical practice.

The planned analyses identified in this SAP may be included in a clinical study report (CSR), other regulatory submissions, or future manuscripts. Also, post-hoc exploratory analyses not identified in this SAP may be performed to further examine study data. Any post-hoc, or unplanned, exploratory analyses performed will be clearly identified as such in the final CSR, if applicable.

The following documents were reviewed in preparation of this SAP:

- Clinical Research Protocol for FUR01 (Version 5.0, issued 7 April 2015).
- Case report forms (CRFs) for Protocol NICHD-2014-FUR01.
- ICH Guidance on Statistical Principles for Clinical Trials (E9).

The clinical protocol, and other identified documents, should be referenced for details on the planned conduct of this study. Operational aspects related to collection and timing of planned assessments are not repeated in this SAP unless relevant to the planned analyses.

1.1 Study Synopsis

Protocol Title	**Safety of Furosemide in Premature Infants at Risk of Bronchopulmonary Dysplasia (BPD)**
Phase:	II
Protocol Version	Version 5.0, 7 April 2015
Product	Furosemide
Objective	Primary: Describe the safety of furosemide in premature infants at risk of BPD Secondary: Preliminary effectiveness and pharmacokinetics (PK) of furosemide
Study Design	Multi-center, randomized, placebo-controlled, dose escalating, double masked, safety study

<table>
<tr><td>Study Population</td><td>Inclusion Criteria
1. Documented informed consent from legal guardian, prior to study procedures
2. Receiving positive airway pressure (nasal continuous airway pressure, nasal intermittent positive pressure ventilation, or nasal cannula flow > 1LPM) or mechanical ventilation (high frequency or conventional)
3. < 29 weeks gestational age at birth
4. 7-28 days postnatal age at time of first study dose
Exclusion Criteria
1. Exposure to any diuretic ≤ 72 hours prior to first study dose
2. Previous enrollment and dosing in current study, “Safety of Furosemide in Premature Infants at Risk of Bronchopulmonary Dysplasia”
3. Hemodynamically significant patent ductus arteriosus, as determined by the investigator
4. Major congenital anomaly (e.g. congenital diaphragmatic hernia, congenital pulmonary adenomatoid malformation)
5. Meconium aspiration syndrome
6. Known allergy to any diuretic
7. Serum creatinine >1.7 mg/dl < 24 hours prior to randomization
8. BUN >50 mg/dl < 24 hours prior to randomization
9. Na <125 mmol/L < 24 hours prior to randomization
10. K ≤2.5 mmol/L < 24 hours prior to randomization
11. Ca ≤ 6 mg/dL < 24 hours prior to randomization
12. Indirect bilirubin >10 mg/dl < 24 hours prior to randomization
13. Any condition which would make the participant, in the opinion of the investigator, unsuitable for the study</td></tr>
<tr><td>Number of Participants</td><td>120</td></tr>
<tr><td>Number of Sites</td><td>Approximately 30 sites</td></tr>
<tr><td>Duration of Participation:</td><td>Up to 35 days (28 days of study drug plus 7 days of safety monitoring).
Information about hospitalization will be collected at 36 weeks post menstrual age and/or at discharge.</td></tr>
<tr><td>Dose Schedule:</td><td>Table. N and dosing scheme
<table>
<tr><th></th><th></th><th>N</th><th>Furosemide (IV)</th><th>Furosemide (enteral)</th><th>N</th><th>Cohort N</th></tr>
<tr><td>Cohort 1</td><td>Placebo</td><td>10</td><td>1 mg/kg q 24 hours</td><td>2 mg/kg q 24 hours</td><td>30</td><td>40</td></tr>
<tr><td>Cohort 2</td><td>Placebo</td><td>10</td><td>1 mg/kg q 6 hours</td><td>2 mg/kg q 6 hours</td><td>30</td><td>40</td></tr>
<tr><td>Cohort 3</td><td>Placebo</td><td>10</td><td>2 mg/kg q 6 hours</td><td>4 mg/kg q 6 hours</td><td>30</td><td>40</td></tr>
</table></td></tr>
</table>

2 STUDY OBJECTIVES AND OUTCOMES

2.1 Objectives

Primary: Describe the safety of furosemide in premature infants at risk of BPD.
Secondary: Preliminary effectiveness and pharmacokinetics (PK) of furosemide.

2.2 Outcomes

2.2.1 Primary Outcome Measures

Safety as determined by adverse events experienced by the participants.

2.2.2 Secondary Outcome Measures

2.2.2.1 Preliminary effectiveness: Risk of BPD

The outcome measure is change in moderate-severe BPD or death risk from baseline. Moderate-severe BPD or death risk will be defined by the NICHD Neonatal Research Network BPD outcome estimator[4]. (https://neonatal.rti.org/index.cfm?fuseaction=BPDCalculator.start).

2.2.2.2 Pharmacokinetics

A population PK analysis will be performed. Using the final population PK model, empirical Bayesian estimates of clearance (CL), volume of distribution (V), half-life, and exposure metrics (e.g. AUC, maximum concentration) will be generated for each patient.

2.2.3 Other safety and efficacy outcomes

Death, BPD, nephrocalcinosis/nephrolithiasis, and hearing loss will be assessed.

3 STUDY METHODS

3.1 Overall Study Design and Plan

This study is a multi-center, randomized, placebo-controlled, dose escalating, double masked, safety study.

This study will consist of a screening for eligibility, a 28-day treatment period, a 7-day safety follow-up period, a 36-week PMA assessment and a final study assessment.

3.2 Selection of Study Population

Premature infants (inpatient in neonatal intensive care units) will be randomized 3:1 to furosemide: placebo with increasing target doses of furosemide for each Cohort. There will be 40 randomized and dosed participants in each Cohort for a total of up to 120 participants.

3.3 Level of Masking

The study will remain double-masked throughout the complete study period. Individual participant treatment assignments will be maintained by DCC unblinded staff only. Any unplanned unmasking occurring during the study period will be documented and reported in the final CSR.

4 ANALYSES AND REPORTING

4.1 Dose Escalation and Halting Criteria

The trial will be halted (paused) for a safety review by the Data Monitoring Committee (DMC) if there are 4 or more Serious Adverse Reactions within a given Cohort (i.e., Cohort 1, 2, or 3). If there are <4 Serious Adverse Reactions total, the DMC will receive a summary of masked safety data and enrollment will continue in the next highest dose Cohort. Enrollment in the next highest dose Cohort will begin immediately after the 40th (or 80th) subject is enrolled and the following criteria have been met:

Table 1 Rules for advancing to next highest dose Cohort

Number of Serious Adverse Reactions	% total evaluable safety days* completed from lower dose Cohort
3	100%
2	95%
1	80%
0	70%

***% total evaluable safety days =** $\frac{sum\ of\ days\ of\ dosing + 7\ days\ of\ safety\ follow\ up\ for\ all\ participants}{\#\ of\ participants * 35}$

If a 4th Serious Adverse Reaction occurs in the lower dose Cohort after enrollment begins in higher dose Cohort, enrollment will be halted for safety review but current subject dosing will continue pending DMC review. If there are 4 or more Serious Adverse Reactions, then the DMC may choose to be unmasked to treatment assignment.

4.2 Interim Analyses

A masked interim safety analysis will be performed after completion of the safety follow up period of Cohort 1 participants, and again after completion of the safety follow up period of Cohort 2 participants to include data entered up to the cutoff time point (data from next Cohort will be included if entered before the cutoff time point). Enrollment will not be halted during the analyses. Halting may occur if analysis shows a positive finding. The number and percent of AEs and SAEs within each dose group will be summarized overall as well as by each Medical Dictionary for Regulatory Activities (MedDRA) system organ class and preferred term. BPD, nephrocalcinosis/nephrolithiasis, and hearing loss will be included in the summary. Also, laboratory adverse events will be tabulated by cohort.

4.3 Final Analysis

All final, planned analyses identified in this SAP will be performed only after the last subject has completed the last study visit and end of study assessments, and all relevant study data have been processed and integrated into the analysis database. In addition, no database may be locked or analyses completed until this SAP has been approved. Unmasking of treatment assignments will not occur until the data are locked.

5 SAMPLE SIZE DETERMINATION

The sample size of 30 in each dose group is sufficient to estimate AE or SAE incidence with sufficient precision. Table 2 provides widths for 95% Wilson confidence intervals in the dose groups of size 30 and the total furosemide treatment cohort of 90 with different incidence rates.

An event with an incidence rate of 0.05 has a 79% chance of being observed at least once in a dose group and a 99% chance of being observed at least once in the total furosemide Cohort.

Table 2. Widths for 95% Wilson confidence intervals.

N=30		N=90	
Rate	Width	Rate	Width
0.1	0.22	0.1	0.13
0.2	0.28	0.2	0.16
0.3	0.31	0.3	0.19

6 ANALYSIS POPULATION

The analysis populations are defined as follows:

Safety population will include all randomized and dosed participants.

PK population will include all participants with at least one interpretable PK sample.

7 GENERAL ISSUES FOR STATISTICAL ANALYSIS

Descriptive statistics such as number of observations, mean, median, standard deviation, standard error, minimum and maximum will be presented by dose groups for continuous variables (such as age, weight, etc.). The dose groups will include furosemide groups (by dose level and across all dose levels), placebo group and overall. Other descriptive statistics such as counts, proportions, and/or percentages will be presented by dose groups to summarize discrete variables (such as race, sex, etc.).

Missing data will not be imputed in the non-PK analysis of this study.

Patient profile graphs showing chronologically the dosing, PK sampling, laboratory results of interest, risk of BPD, and adverse events for each participant will be developed.

All confidence intervals and tests will use α=0.05. Analyses in this study are exploratory or descriptive in nature. No adjustments for multiplicity between endpoints are planned. Analyses will be performed using SAS Software version 9.2 or later.

8 STUDY PARTICIPANTS AND DEMOGRAPHICS

8.1 Disposition of Participants and Withdrawals

All safety participants will be accounted for in this study. Number and percentage by Cohorts and dose groups for the safety population and PK population will also be presented. Also, summaries will include the number and percentage of participants who completed or early terminated the study, classified by reasons for early termination; the number of participants from each study site; and the number and percentage of participants who completed or early terminated the study at each study site. Early termination is defined as:

1. Participant is randomized but does not receive at least 7 days of study product.

2. Participant receives 7-28 days of study product, but does not complete required follow up assessments.

8.2 Demographics and Baseline Characteristics

Demographic and baseline characteristics will be collected during the screening visit. Variables include gestational age (GA) at birth, postnatal age (PNA), post menstrual age (PMA), maternal race, maternal ethnicity, sex, and birth weight. The PMA is calculated by adding up the completed weeks of GA and PNA. Also, respiratory assessment will be conducted at baseline visit. Surfactant therapy administration and antenatal betamethasone use by the participant's mother will also be summarized.

Medical history will be MedDRA coded and summarized by system organ class and preferred term. Medical history will also be summarized in a participant listing which will also include the actual reported medical conditions. The listing will also include system organ class, preferred term and onset date.

8.3 Protocol Violations and Deviations

All protocol deviations will be reported by site and category of deviation and reason for the deviation. A detailed listing of all protocol deviations by participant will be included. Non-participant specific protocol deviations are also collected and these will also be reported by site and category of deviation.

9 SAFETY ANALYSES

Safety will be assessed following initial study-specific procedure e.g., screening blood draws, dosing through 7 days post last study dose and will be assessed by frequency and incidence of all AEs and SAEs. Other safety parameters include clinical laboratory measurements of interest, concomitant medications of interest, physical examination abnormalities and special events of interest: hearing loss, nephrocalcinosis/nephrolithiasis, and respiratory assessment.

Safety summaries will be provided by dose groups based on data from pre-dose and post-dose visits for the safety population. Frequency counts and descriptive statistics will be provided.

9.1 Adverse Events

An **adverse event** (AE) is any untoward medical occurrence in humans, whether or not considered drug-related, which occurs during the conduct of a clinical trial. (Any change in clinical status, routine labs, x-rays, physical examinations, etc.), that is considered clinically significant by the study investigator is considered an AE.

Suspected adverse reaction is any adverse event for which there is a reasonable possibility that the drug caused the adverse event. A reasonable possibility implies that there is evidence that the drug caused the event.

Adverse reaction is any adverse event caused by the drug.

Serious adverse event or **serious suspected adverse reaction** or **serious adverse reaction** as determined by the investigator or the sponsor is any event that results in any of the following outcomes:

1. Death at or before 36 weeks PMA

2. Life-threatening AE ("life-threatening" means that the study participant was, in the opinion of the investigator or sponsor, at immediate risk of death from the reaction as it occurred and required immediate intervention)
3. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
4. Inpatient hospitalization or prolongation of existing hospitalization
5. Important medical event that may not result in one of the above outcomes, but may jeopardize the health of the study participant or require medical or surgical intervention to prevent one of the outcomes listed in the above definition of serious event

All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) central coding dictionary. All AEs and SAEs will be summarized by dose groups.

9.2 Clinical Laboratory Evaluations

Hematology and chemistry parameters will be summarized at baseline (prior to first dose) and last dose, as well as change from baseline to last dose. Tables (by dose groups) will present n, mean, median, minimum, and maximum at baseline, last dose, last day of 7-day safety follow-up period, 36 weeks PMA and change from baseline to last dose. A summary of result assessment shifts from baseline to last dose will be given for each dose group for each parameter. Each parameter will be coded as abnormal and clinically significant ("ABN, CS") or abnormal and not clinically significant ("ABN, NCS"). The number and percentage of subjects in each dose group in each shift category from baseline to last dose will be shown for each parameter.

Laboratory values at baseline, last dose and changes from baseline to last dose will also be summarized descriptively by dose groups using box plots. The box will represent the 75th and 25th percentiles and the upper and lower whiskers will represent the 95th and 5th percentiles. Mean, median and outliers will be identified. On each box plot, the number available, mean and median will also be presented numerically.

This will be completed for hematology laboratory test (including platelets) and chemistry laboratory test (including alkaline phosphatase, bicarbonate, bilirubin (direct), bilirubin (total), bilirubin (indirect), BUN, caffeine, calcium (ionized), calcium (total), chloride, creatinine, magnesium, phosphorus, potassium, sodium).

The laboratory events of interest will be summarized by dose groups. The laboratory adverse events of interest are listed in Table 3. Any abnormal laboratory values indicating serum electrolytes, renal dysfunction and other dysfunction are captured as laboratory adverse events of interest.

Table 7: Laboratory event values [4]

Laboratory values	Adverse event	Serious adverse event
Serum electrolytes		
Hypernatremia(Sodium)	150–159 mmol/L	> 159 mmol/L
Hyponatremia(Sodium)	120–124 mmol/L	<120 mmol/L
Hyperchloremia(Chloride)	110-120 mmol/L	> 120 mmol/L
Hypochloremia(Chloride)	80-90 mmol/L	< 80 mmol/L
Hyperkalemia(Potassium)	7.0–7.9 mmol/L	>7.9 mmol/L
Hypokalemia(Potassium)	2.0–2.5 mmol/L	<2.0 mmol/L
High Bicarbonate levels (Bicarbonate)	30–45 mmol/L	> 45 mmol/L

Low Bicarbonate levels(Bicarbonate)	12–14 mmol/L	< 14 mmol/L
Hypercalcemia (iCa)	1.3–1.6 mmol/L	>1.6 mmol/L
Hypocalcemia (iCa)	0.7–1.05 mmol/L	< 0.7 mmol/L
Hypermagnesemia (Magnesium)	3.0-6.0 mg/dL	> 6.0 mg/dL
Hypomagnesemia (Magnesium)	1.0-1.5 mg/dL	< 1.0 mg/dL
Hypophosphatemia(Phosphorus) Hyperphosphatemia(Phosphorus)	1.0-3.0 mg/dL 10.5-12.5 mg/dL	<1.0 mg/dL >12.5 mg/dL
Hyperbilirubinemia (Bilirubin)	20-30 mg/dL	>30 mg/dL
Renal dysfunction (i.e. azotemia)		
Elevated BUN	60–100mg/dL	> 100 mg/dL
Elevated creatinine	1.5–2.5 mg/dL	> 2.5 mg/dL
Gastrointestinal		
Elevated alkaline phosphatase	1000–1400 U/L	>1400 U/L
Hematology		
Thrombocytosis(Platelets)	450–1000 × 10^9/L	>100010 × 10^9/L
Thrombocytopenia(Platelets)	50–100 × 10^9/L	<50 × 10^9/L

9.3 Concomitant Medications of Interest

All concomitant medications, including intravenous potassium boluses or enteral electrolyte supplements, and treatments (including other diuretics) will be reported during the study drug administration period. All concomitant medications will be coded using the World Health Organization (WHO) drug classification system and presented by Anatomical Therapeutic Chemical Classification system first level (ATC1) drug classification and drug name. Frequency distributions of concomitant medications will be listed by ATC1 drug classification, drug name, and dose groups. Any diuretics used other than study furosemide during study period will be summarized separately. The cumulative dose, maximum daily dose and mean dose for caffeine at participant level will be summarized in mg/kg by dose groups. A listing of concomitant medications by participant will include start and end dates (or ongoing), and indication. Also, a listing of caffeine doses will be included.

9.4 Physical Examination

Results of the physical examination will be presented by system. Abnormal clinically significant and non-clinically significant findings will be summarized by system, visit, and dose groups. Also, the physical examination findings change from baseline to last dose will be summarized.

9.5 Special Safety Events of Interest

Hearing Loss

Hearing loss will be identified by hearing test (e.g. BAER) prior to discharge. Any failure of the hearing test (unilateral or bilateral) will be considered as hearing loss. If multiple tests are done, the last test post last study dose will be considered as the final result and any positive result during the study period will be reported. If only one hearing test is obtained, it can be considered as a final result if it was performed after the last study dose.

Nephrocalcinosis/nephrolithiasis

Nephrocalcinosis/nephrolithiasis will be determined by renal ultrasound. The summary will include nephrocalcinosis, nephrolithiasis and any of nephrocalcinosis or nephrolithiasis. A renal ultrasound is required per protocol at final study assessment. Results of standard of care renal ultrasounds will also be collected during the treatment period, follow-up period, 36-week PMA assessment. The study central radiologist (CR) will review any positive findings for nephrocalcinosis / nephrolithiasis reported by the site. If the primary study CR CONCURS with the site radiologist's findings, study image results will be documented and no further action will be taken; otherwise, a secondary study CR will review the images for final determination of results. Majority rules from up to 3 reviewers will be applied for the final renal ultrasound results. If multiple ultrasounds are received, the last ultrasound that is available post last study dose will be considered as the final result. If only one ultrasound is obtained, it can be considered as a final result if it was performed after the last study dose. In addition, any positive findings during the study period will be summarized as a separate endpoint.

Hearing loss, nephrocalcinosis and nephrolithiasis event rates will be summarized by dose groups and the percentage of participants with these events may be compared using a Fisher's exact test.

Respiratory assessment

The following information will be collected at baseline, on day 7, 14, 21, and 28 (+/- 1 day) of study drug administration, once during the follow-up period, once at 36 weeks PMA, and once at discharge:

1. Maximum F_iO_2 (sustained for > 30 minutes to account for suctioning, unless it is known to be a temporary (<2 hour) increase in F_iO_2)
2. Ventilation type:
    a. High frequency ventilator
    b. Conventional mechanical ventilator
    c. NCPAP (or equivalent, see inclusion criteria in protocol synopsis table)
    d. Cannula/Hood
    e. None (room air with no support)

All respiratory assessments (including assessments outside the window) will be summarized by visit and dose groups.

10 EFFICACY ANALYSES

10.1 Incidence of BPD

BPD will be defined by the NICHD BPD estimator as a dichotomous (none-mild vs. moderate-severe) variable and as a categorical variable (none, mild, moderate, or severe) among survivors by modifying the NIH consensus definition of BPD to include infants transferred prior to 36 weeks and the need for oxygen at 36 weeks PMA (or discharge, if sooner than 36 weeks PMA). The severity of BPD is defined as follows:

1. **No BPD**: receiving > 21% supplemental oxygen (O_2) for ≤28 days and not at 36 weeks PMA
2. **Mild BPD**: receiving > 21% O_2 for ≥28 days but not at 36 weeks PMA
3. **Moderate BPD**: receiving > 21% O_2 for ≥28 days plus treatment with <30% O_2 at 36 weeks PMA

4. **Severe BPD**: receiving > 21% O_2 for ≥28 days plus ≥30% O_2 and/or positive pressure at 36 weeks PMA

Note that the day in the definition above is postnatal day but not treatment day. Final assessment of BPD at 36 weeks PMA will be summarized by dose groups.

BPD will be evaluated through logistic regression models by whether the event occurred or did not occur at 36 weeks PMA assessment. Independent factors in the model will include gestational age, birth weight, dose groups. Confidence intervals will be calculated using the profile-likelihood.

10.2 Risk of BPD

The preliminary effectiveness analysis will be on the risk of BPD which is provided by BPD estimator. The risk of BPD is presented as a % and the dichotomized the outcome will be used in the analysis (none-mild vs. moderate-severe-death). The BPD outcome estimator uses the following information to provide individual risk of BPD:

1. Gestational age (weeks)
2. Birth weight (g)
3. Sex
4. Maternal Race/Ethnicity
5. Postnatal day
6. Ventilation type (on the postnatal day of interest)
7. F_iO_2 (%) (on the postnatal day of interest)

For example, a 25 week Hispanic female with birth weight of 689 g on postnatal day 14 on mechanical ventilation with a FiO_2 of 0.45 has risk of no BPD of 1.6%, mild 27.9%, moderate 28.9%, severe 30.5%, and death 11%. Thus, none-mild risk is 29.5% and moderate-severe-death risk is 70.5%.

The risk of BPD or death as defined by the NICHD Neonatal Research Network (NRN) BPD estimator will be evaluated at baseline, on days 7, 14, 21 and 28 of study drug, the postnatal days closest to these days will be used in the BPD estimator. The postnatal day will be re-grouped to fit the BPD estimator: day 0 and day 1 will be re-grouped as day 1, day 2 and day 3 as day 3, 4-7 days as day 7, 8-14 as day 14 and 15-21 as day 21. The BPD estimator includes infants up to 28 postnatal days; for infants older than that, the 28 day estimates will be used. The birth weight used in the BPD estimator is up to 1250g, for infants with birth weight greater than 1250g, 1250g will be used. The BPD estimator includes white, black, or Hispanic infants; infants with other race/ethnicity will be excluded from the estimation. Sensitivity analyses may be done by removing infants with birth weight greater than 1250g.

The change of risk of BPD (as a percentage) from baseline to the end of study (day 28 or LOCF) will be assessed using an analysis of covariance (ANCOVA) model, with percentage change from baseline as the dependent variable. Independent factors include baseline risk percentage and dose groups.

As an exploratory analysis, a longitudinal mixed effect model may be used to test the risk of BPD at baseline and days 7, 14, 21 and 28 (with no LOCF). This longitudinal analysis will use all available risk of BPD percentages at each time point. Fixed effects will include treatment groups, dose level and dosing days; random effects will include participant. Linear and nonlinear

trends of the drug effect for both overall and individual level data will be evaluated. An unstructured covariance matrix will be used initially to model the co-variance of repeated measures within participants, but other co-variance structures will be explored and the best structure will be chosen based on Akaike's Information Criteria.

Potential biomarkers (i.e. IL-1, IL-6, IL-8, TNF-α, and RANTES) may be included in the models mentioned above.

11 PHARMACOKINETICS ANALYSIS

A population PK analysis will be performed to determine population PK parameters (e.g. CL and V) using nonlinear mixed effects modeling approach and the software NONMEM (version 7.2 or later, Icon Solutions., MD). Different structural PK models (e.g. 1 and 2 compartment) will be fitted to the data.

A covariate analysis will be performed to determine the influence of baseline factors (e.g., sex, race) and clinical factors (e.g., GA, PNA, PMA, serum creatinine, weight, and concomitant medications) on PK parameters. In addition, inter-subject variability and residual variability will be quantified. Empirical Bayesian estimates of PK parameters (e.g. CL, V, and half-life) will then be estimated for each patient.

Models will be evaluated based on successful minimization, goodness-of-fit plots, precision of parameter estimates. In addition, the precision of the final population PK model estimates will be evaluated by non-parametric bootstrapping using Perl-speaks-NONMEM. The final PK model will also be evaluated by visual predictive check.

Simulations of the study dosing regimens will be performed to generate exposure metrics (e.g. AUC, maximum concentration) for each patient using the final population PK model. Simulations of alternative dosing regimens may be performed as needed.

A detailed pharmacokinetic analysis plan will be addressed in a separate PK Analysis Plan. The PK analysis results and conclusions will be reported separately.

12 REFERENCES

1. US Federal Register. (1998) International Conference on Harmonization; Guidance on Statistical Principles for Clinical Trials. Department of Health and Human Services: Food and Drug Administration [Docket No. 97D-0174]. *Federal Register Volume 63, Number 179, pages 49583-49598. September 16, 1998.*
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13 TABLES, LISTINGS, AND FIGURES

Table Number	Population	Table Title / Summary	Supporting Listing Number
14.1 DEMOGRAPHIC DATA			
14.1.1.1	All Participants	Participant Accounting and Study Disposition by Dose Groups	16.2.1
14.1.1.2	Safety	Participant Enrollment by Site and Dose Groups	16.2.1
14.1.2.1	Safety	Demographic and Baseline Summary by Dose Groups	16.2.3
14.1.2.2	Safety	Summary of Medical History by System Organ Class, Preferred Term and by Dose Groups	16.2.4
14.1.3.1	All Participants	Participant-Specific Protocol Deviation Classification by Site	16.2.5.1
14.1.3.2	All Participants	Participant-Specific Protocol Deviation Classification by Reason for Deviation	16.2.5.1
14.1.3.3	All Sites	Site-Specific Protocol Deviations by Reason for Deviation	16.2.5.2
14.2 EFFICACY			
14.2.1	Safety	Analysis of BPD at 36 Weeks PMA Assessment	16.2.8.6
14.2.2	Safety	Analysis of Change of Risk of BPD from Baseline to End of Study	16.2.8.5
14.2.3	Safety	Longitudinal Analysis for Risk of BPD	16.2.8.5
14.3 SAFETY			
14.3.1.1	Safety	Summary of Adverse Events by Dose Groups (including Summary of AEs, and SAEs)	16.2.8.1, 16.2.8.2, 16.2.8.3
14.3.1.2	Safety	Adverse Events by System Organ Class, Preferred Term and by Dose Groups	16.2.8.1
14.3.1.3	Safety	Serious Adverse Events by System Organ Class, Preferred Term and by Dose Groups	16.2.8.2
14.3.1.4	Safety	Serious Adverse Reactions by System Organ Class, Preferred Term and by Dose Groups	16.2.8.3
14.3.1.5	Safety	Adverse Events by System Organ Class, Preferred Term, Severity and by Dose Groups	16.2.8.1
14.3.1.6	Safety	Adverse Events by System Organ Class, Preferred Term, Relationship to Study Medication and by Dose Groups	16.2.8.1
14.3.1.7	Safety	Adverse Events Listed by Most Frequent Preferred Term and by Dose Groups	16.2.8.1
14.3.1.8	Safety	Summary of Special Safety Events of Interest by Dose Groups	16.2.8.4, 16.2.8.5, 16.2.8.6
14.3.2	Safety	Summary of Laboratory Events by Dose Groups	16.2.9.1. 16.2.9.2
14.3.3.1	Safety	Summary of Clinical Laboratory Hematology Measurements by Visit and Dose Groups (platelets)	16.2.9.1

14.3.3.2	Safety	Summary of Clinical Laboratory Serum Chemistry Measurements by Visit and Dose Groups (alkaline phosphatase, bicarbonate, bilirubin (direct), bilirubin (total), bilirubin (indirect), BUN, caffeine, calcium (ionized), calcium (total), chloride, creatinine, magnesium, phosphorus, potassium, sodium)	16.2.9.2
14.3.4	Safety	Summary of Concomitant Medications of interest by Drug Classification, Drug Name and Dose Groups	16.2.10
14.3.5	Safety	Summary of Physical Examination Findings by Body System and Dose Groups	16.2.11

Figure Number	Population	Table Title / Summary	Supports Table Number(s)
14.4.1	Safety	Box Plot of Hematology Laboratory Measurement by Visit and Dose Groups (platelets)	16.2.9.1
14.4.2	Safety	Box Plot of Each Chemistry Laboratory Measurement by Visit and Dose Groups (alkaline phosphatase, bicarbonate, bilirubin (direct), bilirubin (total), bilirubin (indirect), BUN, caffeine, calcium (ionized), calcium (total), chloride, creatinine, magnesium, phosphorus, potassium, sodium)	16.2.9.2
14.4.3	Safety	Patient Profile	16.2.1, 16.2.6, 16.2.8.5, 16.2.9.1. 16.2.9.2

Listing Number	Population	Listing Title / Summary	Supports Table Number(s)
16.2.1	All Participants	Final Study Disposition	14.1.1.1, 14.1.1.2
16.2.2	All Participants	Inclusion/Exclusion Criteria	N/A
16.2.3	Safety	Demographic Characteristics	14.1.2.1
16.2.4	Safety	Medical History	14.1.2.2
16.2.5.1	All Participants	Participant-Specific Protocol Deviations	14.1.3.1, 14.1.3.2
16.2.5.2	All Sites	Site-Specific Protocol Deviations	14.1.3.3
16.2.6	Safety	Study Medication Dose Administration	N/A
16.2.7	Safety	PK Concentrations and Sampling	N/A
16.2.8.1	Safety	Adverse Events	14.3.1, 14.3.1.5, 14.3.1.6, 14.3.1.7
16.2.8.2	Safety	Serious Adverse Events	14.3.1.3
16.2.8.3	Safety	Serious Adverse Reactions	14.3.1.4
16.2.8.4	Safety	Hearing Screen	14.3.1.8
16.2.8.5	Safety	Respiratory Assessment	14.2.2, 14.2.3
16.2.8.6	Safety	BPD at 36 Weeks PMA Assessment	14.2.1
16.2.8.7	Safety	Ultrasound Review	14.3.1.8
16.2.9.1	Safety	Hematology Laboratory Measurements	14.3.2.1

Listing Number	Population	Listing Title / Summary	Supports Table Number(s)
16.2.9.2	Safety	Chemistry Laboratory Measurements	14.3.2.2
16.2.10	Safety	Concomitant Medications	14.3.4
16.2.11	Safety	Physical Examination Findings	14.3.5