

# **Safety of Sildenafil in Premature Infants at Risk of Bronchopulmonary Dysplasia**

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Best Pharmaceuticals for Children Act

**STATISTICAL ANALYSIS PLAN**  
**FOR**  
**Safety of Sildenafil in Premature Infants at**  
**Risk of Bronchopulmonary Dysplasia**  
**Phase II Trial**

**Version 2.0**

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BPCA Data Coordinating Center  
The Emmes Company, LLC.  
Rockville, Maryland

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## SIGNATURE PAGE AND APPROVALS

Matthew M. Laughon, MD, MPH Professor of Pediatrics The University of North Carolina at Chapel Hill	Date
Perdita Taylor-Zapata, MD Medical Officer Obstetric and Pediatric Pharmacology and Therapeutics Branch Center for Research for Mothers and Children Eunice Kennedy Shriver National Institute of Child Health and Human Development	Date
Ravinder Anand, PhD Principal Investigator, BPCA DCC The Emmes Company, LLC.	Date
Karen Martz, MS Biostatistician, BPCA DCC The Emmes Company, LLC.	Date

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## ACRONYMS AND ABBREVIATIONS

AE	Adverse Event
ANCOVA	Analysis of Covariance
ALT	Alanine Transaminase
AST	Aspartate Transaminase
ATC	Anatomical Therapeutic Chemical Classification
AUC	Area Under the Curve
BPCA	Best Pharmaceuticals for Children Act
BPD	Bronchopulmonary Dysplasia
CFR	Code of Federal Regulations
CL	Clearance
CPAP	Continuous Positive Airway Pressure
CRF	Case Report Form
CSR	Clinical Study Report
DCC	Data Coordinating Center
DCRI	Duke Clinical Research Institute
DMC	Data Monitoring Committee
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture system
FDA	Food and Drug Administration
FiO <sub>2</sub>	Fraction of inspired oxygen
g	Grams
GA	Gestational Age
HFNC	High Flow Nasal Cannula
HFvent	High Frequency Ventilation
ICH	International Conference on Harmonization
IMV	Intermittent Mandatory Ventilation
Kg	Kilogram
L	Liter
LOCF	Last Observation Carried Forward
LPM	Liters per Minute
MAP	Mean Arterial Pressure
MedDRA®	Medical Dictionary for Regulatory Activities
Mg	Milligram
mm Hg	Millimeter of mercury
N	Number (typically refers to patients)
NCPAP	Nasal Continuous Positive Airway Pressure
NICHD	National Institute of Child Health and Human Development
NEC	Necrotizing Enterocolitis

NIH	National Institutes of Health
NRN	Neonatal Research Network
PI	Principal Investigator
PK	Pharmacokinetics
PMA	Post Menstrual Age
PNA	Postnatal Age
PT	Preferred term
PTN	Pediatric Trials Network
ROP	Retinopathy of Prematurity
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Suspected Adverse Reaction
SIMV	Synchronized Intermittent Mandatory Ventilation
SUSAR	Serious Unexpected Suspected Adverse Reaction
SOC	Standard of Care
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-2015-SIL02, “Safety of Sildenafil 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 SIL02 (Version 7.0, issued 03 April 2023).
- Case report forms (CRFs) for Protocol NICHD-2015-SIL02.
- 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

<b>Protocol Title</b>	<b>Safety of Sildenafil in Premature Infants at Risk of Bronchopulmonary Dysplasia (BPD)</b>
<b>Phase:</b>	II
<b>Protocol Version</b>	Version 7.0, 03 April 2023
<b>Product</b>	Sildenafil citrate injection and powder for suspension
<b>Objective</b>	Primary: Describe the safety of sildenafil in premature infants at risk of BPD Secondary: Preliminary effectiveness and pharmacokinetics (PK) of sildenafil
<b>Study Design</b>	Multi-center, randomized, placebo-controlled, sequential dose escalating, double masked, safety study

<b>Study Population</b>	<p><b>Inclusion Criteria</b></p> <ol style="list-style-type: none"> <li>1. Documented informed consent from parent or guardian, prior to study procedures</li> <li>2. Receiving positive airway pressure (nasal continuous airway pressure, nasal intermittent positive pressure ventilation, or nasal cannula flow &gt; 1LPM) or mechanical ventilation (high frequency or conventional) at the time of randomization*</li> <li>3. &lt; 29 weeks gestational age at birth</li> <li>4. 7-28 (inclusive) days postnatal age at time of randomization*</li> </ol> <p><b>Exclusion Criteria</b></p> <ol style="list-style-type: none"> <li>1. Previous enrollment and dosing in NICHD-2015-SIL02 <i>Safety of Sildenafil in Premature Infants at Risk of Bronchopulmonary Dysplasia</i></li> <li>2. Previous exposure to sildenafil within 7 days prior to randomization*</li> <li>3. Currently receiving vasopressors*</li> <li>4. Currently receiving inhaled nitric oxide*</li> <li>5. Baseline mean arterial pressure (MAP) &lt; gestational age (in weeks) plus postnatal age (in weeks) within 24 hours of randomization*; e.g. an infant at 25 weeks gestational age and 3 weeks postnatal age with MAP &lt; 28 mm Hg would be ineligible</li> <li>6. Known allergy to sildenafil</li> <li>7. Known sickle cell disease</li> <li>8. AST &gt; 225 U/L &lt; 72 hours prior to randomization*</li> <li>9. ALT &gt; 150 U/L &lt; 72 hours prior to randomization*</li> <li>10. Any condition which would make the participant, in the opinion of the investigator, unsuitable for the study*</li> </ol> <p><i>*Participant will be re-assessed prior to dosing to reconfirm eligibility criteria</i></p>
<b>Number of Participants</b>	Up to 120
<b>Number of Sites</b>	Approximately 30 sites
<b>Duration of Participation:</b>	Up to 62 days (up to 34 days of study drug plus 28 days of safety monitoring). Information about hospitalization will be collected at 36 weeks post menstrual age and/or at discharge.

<b>Dose Schedule:</b>	<b>Table. N and dosing scheme</b>					
			<b>N</b>	<b>Sildenafil (IV)</b>	<b>Sildenafil (enteral)</b>	<b>Cohort Total N</b>
	Cohort 1	Placebo	10	0.125 mg/kg q 8 hours	0.25 mg/kg q 8 hours	40
	Cohort 2	Placebo	10	0.5 mg/kg q 8 hours	1 mg/kg q 8 hours	40
	Cohort 3	Placebo	10	1 mg/kg q 8 hours	2 mg/kg q 8 hours	40
Note: Participants will be enrolled into cohorts sequentially (i.e. cohort 1 then cohort 2 then cohort 3) based on safety. Route of administration should be via IV route if patient has IV and is feasible, but choice is left to the discretion of investigator.						

## 2 STUDY OBJECTIVES AND OUTCOMES

### 2.1 Objectives

Primary: Describe the safety of sildenafil in premature infants at risk of BPD.

Secondary: Preliminary effectiveness and pharmacokinetics (PK) of sildenafil.

### 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 a reduction in moderate-severe BPD or death risk from first day of study drug to end of study drug administration. Moderate-severe BPD or death risk will be defined by the NICHD NRN BPD outcome estimator (<https://neonatal.rti.org/>).

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 (White/Hispanic/Black)
5. Postnatal day (1, 3, 7, 14, 21, 28)
6. Ventilation type (on the postnatal day of interest: HF vent, IMV/SIMV, CPAP, Cannula/hood or none)
7.  $\text{FiO}_2$  (%) (on the postnatal day of interest)

Note: The NICHD NRN BPD estimator provides an estimate of the risk of BPD (as defined by NIH: none, mild, moderate, severe) or death by postnatal day. The risk of BPD is presented as a percentage. For this protocol, we will dichotomize the outcome as above (none-mild vs. moderate-severe-death).

Risk of BPD or death as defined by the NICHD NRN BPD estimator will be collected on days 7, 14, 21 and 28 of study drug period. The BPD estimator rate will be calculated using the day closest to the participant's postnatal age. The BPD estimator includes infants up to 28 postnatal days; for infants >28 postnatal days, the 28 day estimates will be used.

$\text{FiO}_2$  % will be the blended  $\text{O}_2$  for HV ventilation, IMV/SIMV, CPAP and cannula/hood with flow rate >1LMP. For cannula/hood with flow rate <1 LPM the  $\text{FiO}_2$  will be calculated from the STOP-ROP Effective  $\text{FiO}_2$  Conversion Tables for Infants on Nasal Cannula.

##### 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 participant. PK analysis plans will be detailed in a separate document.

### 2.2.3 Other Safety and Effectiveness Outcomes

1. *Death*: All infants who died at or before 36 weeks PMA will be included.
2. *BPD*: BPD is defined as using a method modified from the NICHD NRN BPD estimator. We will define BPD as a dichotomous (none/mild vs. moderate, severe or death) variable and as a categorical variable (none/mild, moderate, severe or death) at 36 weeks PMA. For the purpose of this study we will define BPD as follows:
  - No/Mild BPD: participant is not receiving supplemental O<sub>2</sub> at 36 weeks PMA
  - Moderate BPD: participant is receiving O<sub>2</sub> supplementation >21% but <30% at 36 weeks PMA, but not receiving positive pressure at 36 weeks PMA.
  - Severe BPD: participant is receiving O<sub>2</sub> supplementation ≥30% and/or positive pressure at 36 weeks PMA.
3. *Death or BPD (moderate or severe)*
4. *ROP*: Retinopathy (retinopathy of prematurity) will be defined as treatment for ROP (laser photocoagulation, cryotherapy, or intraocular injections such as bevacizumab).
5. *Pulmonary hypertension*: Pulmonary hypertension will be defined as present or not present per echocardiogram and/or cardiac catheterization reports, as provided per standard of care. Echocardiogram and cardiac catheterization reports will be reviewed centrally by a single Duke pediatric cardiologist.

## 3 STUDY METHODS

### 3.1 Overall Study Design and Plan

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

This study will consist of a screening for eligibility, a 28-day treatment period, an up to 7 day weaning period and a 14-day (Cohort 1) or-28-day (Cohort 2 & 3) safety follow-up period, a 36-week PMA assessment and a final study assessment.

### 3.2 Selection of Study Population

The study population will include 120 premature infants (inpatient in neonatal intensive care units) from about 30 sites who are less than 29 weeks gestational age at birth, 7-28 days postnatal age and receiving positive airway pressure. These participants will be randomized in a dose escalating approach 3:1 (sildenafil: placebo) sequentially, into each of three cohorts. There will be approximately 40 randomized and dosed participants in each cohort for a total of up to 120 participants.

### 3.3 Method of Treatment Assignment, Randomization and Masking

Participants who satisfy all eligibility criteria will be randomized in a dose escalating approach 3:1 (sildenafil: placebo) into three cohorts with escalating doses of sildenafil. All three cohorts will use the same randomization scheme. The participant's randomized treatment assignment will be obtained through the Advantage eClinical® enrollment module. If a participant is randomized but does not receive study drug, that participant will not count towards total sample size and will be replaced by a new participant who, in turn, will be assigned a new identification number and receive treatment corresponding to the new identification number. The randomization process will not determine if an IV or enteral dose is used. Randomization is stratified by site.

The study will remain double-masked throughout the study period. Individual participant treatment assignments will be maintained by DCC unmasked 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 DMC must complete a review of the masked safety data. Cohort 1 will be followed out to 14 days after the last participant in the cohort completes treatment and Cohort 2 will be followed out to 28 days after the last participant in the cohort completes treatment. Enrollment to the next highest dose will not begin until after the DMC has completed their review and provided a recommendation that the study proceed with enrollment to the next cohort.

An unscheduled DMC review of safety data will be triggered if: (a)  $\geq 3$  patients in a cohort have treatment discontinued or an infusion stopped due to the same type AE, or (b) if  $\geq 3$  patients in a cohort have a similar SAE, or (c)  $\geq 3$  patients in a cohort have a related SAE. Enrollment will be suspended during DMC review, though study activities will be allowed to proceed on previously enrolled subjects if applicable.

The DMC may receive unmasked safety data if safety concerns are identified at any point during the study.

### **4.2 Interim Analyses**

A masked interim safety analysis will be performed after completion of enrollment and the 14 day safety follow up period of Cohort 1 participants, and again after completion of the enrollment and 28 day safety follow up period of Cohort 2 participants. Enrollment will be halted/paused during the analyses and DMC review. Halting may occur if analysis shows a positive finding.

The tables will be stratified by cohort and total, see Appendix 2 for a listing of tables.

The number and percent of AEs and SAEs within each Cohort will be summarized overall as well as by each Medical Dictionary for Regulatory Activities (MedDRA) system organ class and preferred term. Low blood pressure, hypotension and BPD status at 36 weeks PMA will be included in the summary. Also, laboratory adverse events (AST, ALT and other select lab types based on data availability) will be summarized.

### **4.3 Final Analysis**

All final, planned analyses identified in this SAP will be performed only after the last participant has completed the last study visit, safety period and had 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 5-1 provides widths for 95% Wilson confidence intervals in the dose groups of size 30 and the total sildenafil treatment cohort of 90 with different incidence rates. An event with an incidence rate of 0.05 has a 78% chance of being observed at least once in a dose group and a 99% chance of being observed at least once in the total sildenafil cohort.

**Table 5-1. Widths for 95% Wilson confidence intervals.**

N=30			N=90		
Rate	Width	95% CI	Rate	Width	95% CI
0.1	0.22	0.04-0.26	0.1	0.13	0.05-0.18
0.2	0.28	0.10-0.37	0.2	0.16	0.13-0.29
0.3	0.31	0.17-0.48	0.3	0.19	0.22-0.40

## 6 ANALYSIS POPULATION

The analysis populations are defined as follows:

- All randomized participants will be described in the participant disposition section.
- The safety population will include all randomized and dosed participants.
- The effectiveness population includes all randomized and dosed participants with at least one post-dose follow-up respiratory assessment.
- The PK population will include all randomized and dosed participants with at least one interpretable PK sample.

## 7 GENERAL ISSUES FOR STATISTICAL ANALYSIS

Analyses will be stratified by cohort/dose groups which will include: Sildenafil/Cohort1, Sildenafil/Cohort 2, Sildenafil/Cohort 3, Total Sildenafil and Total Placebo.

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

Missing data will be imputed for BPD status at 36 weeks PMA as a sensitivity analysis as described in Section 10 below.

Data for calculating the BPD risk that is outside of the calculator range will be imputed as described in Section 10 below.

Imputation of data in the PK analysis of this study will be described in the separate PKAP.

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

All confidence intervals and tests will use  $\alpha=0.05$ . 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 9.4 or later.

## 8 STUDY PARTICIPANTS AND DEMOGRAPHICS

### 8.1 Disposition of Participants and Withdrawals

All randomized participants will be accounted for in this study. Number and percentage by cohort/dose groups for the randomized population, safety population, effectiveness population and PK population will also be presented. Also, summaries will include the number and percentage of all randomized participants who completed or did not complete the study, classified by reasons for non-completion; the number of participants from each study site; and the number and percentage of participants who completed or did not complete the study at each study site. A study completer was considered to have received at least 7 days of study drug plus up to 6 weaning days (Cohorts 2 and 3 only), completion of 14 days (Cohort 1) or 28 days (Cohorts 2 & 3) of safety monitoring after last dose of study drug, and completion of all final study 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 the GA and PNA. Rounding down of GA and PNA will not occur. For example: GA = (25 weeks + 6 days) + PNA = (1 week + 3 day) therefore PMA = 27 weeks and 2 days.

Also, respiratory assessment will be conducted at baseline visit.

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 14 /28 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, MAP and respiratory assessment.

Safety summaries will be provided by cohort/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, version 22 or higher.

All AEs and SAEs will be summarized for the following endpoints:

1. The total number of AEs and SAEs experienced
2. The number and percentage of participants who experienced at least one AE, number who experienced at least one SAE and then number with at least 1 related SAE.
3. The number and percentage of AEs and SAEs at each level of severity
4. The number and percentage of participants by the greatest severity of AE and SAE experienced
5. The number and percentage of AEs and SAEs by relationship to study drug

6. The number and percentage of participants by the strongest relationship to study medication of AE and SAE experienced
7. The number and percentage of participants who experienced AEs or SAEs in each MedDRA system organ class and preferred term.

## **9.2 Low Blood Pressure and Hypotension**

Blood pressure will be determined using mean arterial pressure (MAP). For each participant, MAP will be measured using an appropriate size cuff or with an intra-arterial placed catheter that measures continuous blood pressure.

### **9.2.1 Primary Definition of Hypotension**

Hypotension will be defined as any clinically significant low blood pressure event deemed by the treating physician to require intervention with a fluid bolus or the initiation or escalation of inotropic, vasopressor, or systemic steroid therapy with the specific intent to raise blood pressure. To identify primary hypotension events, all adverse events with a MedDRA Preferred Term of "Hypotension" will be reviewed, and if the review determines the reported AE as requiring medical intervention, it will be considered primary hypotension. Medical intervention status will be determined utilizing AE comments, as well as reported concomitant medications starting on the same day as the event and indicated for hypotension.

### **9.2.2 Presumed low blood pressure and presumed hypotension**

Presumed low blood pressure and presumed hypotension will be evaluated during periods of intensive MAP collection using 4 different methods as described below. Intensive MAPs are collected at first dose and during dose escalation.

Presumed low blood pressure will be considered as one valid MAP (in mmHg) and presumed hypotension will be defined as 2 valid MAPs (in mmHg) taken 15-60 minutes apart, that meet the following criteria:

1. MAP(s) < gestational age at birth (in weeks) plus postnatal age (in weeks) in mmHg
2. MAP(s) < gestational age at birth (in weeks) in mmHg
3. MAP(s) < 30 mmHg
4. MAP(s) >30% lower than baseline. Baseline MAP is the mean of the 3 valid MAPs prior to the first dose or dose escalation (i.e. the mean of the following 3 MAPs: 2 hours [+/- 5 minutes], 1 hour [+/- 5 minutes], and 15 minutes [+/- 5 minutes]).

## **9.3 Clinical Laboratory Evaluations**

Chemistry parameters (AST and ALT) will be summarized at baseline (prior to first dose) and weekly, as well as change from baseline to last dose. Tables (by cohort/dose groups) will present n, mean, median, minimum, and maximum.

Other laboratory evaluations of interest: platelets, phosphorus, chloride, alkaline phosphatase, and caffeine levels will also be summarized if sufficient numbers are recorded.

A Listing of all clinical laboratory evaluations will also be included. Box plot figures will be provided for select lab types based on data availability.

#### **9.4 Concomitant Medications of Interest**

All concomitant medications will be reported within 24 hours prior to start of study drug and during the 28-day treatment 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 cohort/dose groups. A listing of concomitant medications by participant will include start and end dates (or ongoing), and indication.

#### **9.5 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 cohort/dose groups. A listing of physical examination results by participant and visit will also be included.

## 10 EFFECTIVENESS ANALYSES

### 10.1 Incidence of BPD

BPD will be defined by the NICHD BPD estimator as a dichotomous (none/mild vs. moderate, severe or death) variable and as a categorical variable (none/mild, moderate, severe or death). The severity of BPD is defined as follows:

1. *No/mild BPD*: participant is not receiving supplemental O<sub>2</sub> at 36 weeks PMA
2. *Moderate BPD*: participant is receiving O<sub>2</sub> supplementation >21% but <30% at 36 weeks PMA, but not receiving positive pressure at 36 weeks PMA
3. *Severe BPD*: participant is receiving O<sub>2</sub> supplementation ≥30% and/or positive pressure at 36 weeks PMA
4. *Death*: participant dies at or before 36 weeks PMA

Final assessment of BPD at 36 weeks PMA will be summarized by cohort/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 for consideration in modeling will include gestational age, birth weight, sex, maternal race/ethnicity, and cohort/dose groups. Additional models will be considered that 1) combine the sildenafil dose groups and compare with placebo; 2) replace treatment with cumulative sildenafil dose. Ordinal logistic regression with three levels of BPD as the dependent variable may be explored (no/mild BPD, moderate BPD and severe BPD/death).

A first sensitivity analysis will be done using imputed BPD at 36 weeks PMA for participants without a 36-week PMA respiratory assessment. BPD will be imputed as follows: for participants ≥28 days at assessment, the closest respiratory assessment to 36 weeks PMA will be used as the imputed BPD status. If the participant is <28 days PMA at the respiratory assessment used to impute BPD status, the imputed BPD status is none or mild BPD for participants receiving room air or receiving FiO<sub>2</sub> 0.21–0.30 and moderate or severe BPD for participants receiving FiO<sub>2</sub> >0.31. Additional sensitivity analysis will consider any reported deaths as within the BPD death categorization.

### 10.2 Risk of BPD

The preliminary effectiveness analysis will be on the risk of BPD which is provided by NICHD Neonatal Research Network (NRN) 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. FiO<sub>2</sub> (%) (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  $\text{FiO}_2$  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 70.4%.

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 respiratory assessments on postnatal days 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. Other data necessary for the BPD calculator will be imputed as follows: 1) GA <23 weeks will be analyzed as 23 weeks; 2) birth weight <501g will be analyzed as 501g; 3) birth weight >1249g will be analyzed as 1249g; 4) participants of white race and Hispanic ethnicity will be analyzed as white race; participants of black race and Hispanic ethnicity will be analyzed as black race; 5) PNA >28 days will be analyzed as PNA equal 28 days. Sensitivity analyses may be done by removing all imputed data. Ventilation types include high-frequency ventilator, conventional mechanical ventilator, NCPAP or equivalent, nasal cannula (<1 LPM/hood) and room air with no support (21%),

As an exploratory analysis, a longitudinal mixed effect model may be used to test the risk of BPD 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 cohort/dose groups and baseline BPD risk; random effects will include participant. If the longitudinal model does not fit, the change from baseline in risk of BPD will be assessed using an analysis of covariance (ANCOVA) model, with percent change from baseline as the dependent variable and independent factors of baseline risk and cohort/dose groups.

An unstructured covariance matrix (if possible) 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.

### **10.3 Incidence of Retinopathy of Prematurity (ROP)**

Retinopathy of prematurity will be defined as treatment for ROP (laser photocoagulation, cryotherapy, or intraocular injections such as bevacizumab) at or before 36 weeks PMA.

Final assessment of ROP at 36 weeks PMA will be summarized by cohort/dose groups.

If numbers suffice, ROP will be evaluated through logistic regression models by whether the event occurred or did not occur during the study period. Independent factors for consideration in modeling will include gestational age, birth weight, sex, maternal race/ethnicity, and cohort/dose groups.

### **10.4 Pulmonary Hypertension**

Pulmonary hypertension will be defined as present or not present per echocardiogram and/or cardiac catheterization reports, as provided per standard of care. Echocardiogram and cardiac catheterization reports will be reviewed centrally by a single Duke pediatric cardiologist.

Distribution of factors in reports providing information permitting any determination of Pulmonary Hypertension (pHTN) will be summarized, including tricuspid regurgitation, flattening septum and other evidence of pulmonary hypertension.

If numbers suffice, pHTN will be evaluated through logistic regression models by whether the event occurred or did not. Independent factors for consideration in modeling will include gestational age, birth weight, sex, maternal race/ethnicity and cohort/dose groups.

## 11 PHARMACOKINETICS ANALYSIS

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.*
2. American Statistical Association. Ethical Guidelines for Statistical Practice. Prepared by the Committee on Professional Ethics. April 2016.
3. Royal Statistical Society. The Royal Statistical Society: Code of Conduct. 2014.
4. England A, Wade K, Smith PB, Berezny K, Laughon M. Optimizing operational efficiencies in early phase trials: The Pediatric Trials Network experience. *Contemp Clin Trials*. 2016 Mar;47:376-8.
5. Laughon MM, Langer JC, Bose CL, et al. Prediction of Bronchopulmonary Dysplasia by Postnatal Age in Extremely Premature Infants. *Am J Respir Crit Care Med*. Jun 2011; 183(12): 1715-1722.
6. Supplemental Therapeutic Oxygen for Prethreshold Retinopathy of Prematurity (STOP-ROP), a randomized, controlled trial. I: Primary outcomes. *Pediatrics*. 2000 Feb;105(2):295–310.

## 13 TABLES, FIGURES AND LISTINGS

See [Appendix A](#) for a complete list of Tables, Figures and Listings.

## Appendix A: Tables/Listings/Figures for Final Analysis

Table Number	Population	Table Title / Summary	Supporting Listing Number
<b>14.1 DEMOGRAPHIC DATA</b>			
14.1.1.1	Randomized	Participant Accounting and Study Disposition by Dose Group	16.2.1.2
14.1.1.2	Randomized	Participant Enrollment by Site and Dose Group	16.2.1.2
14.1.2.1	Safety	Demographic and Baseline Summary by Dose Group	16.2.2
14.1.2.2	Safety	Summary of Medical History by System Organ Class, Preferred Term and by Dose Group	16.2.3
14.1.3.1	Safety	Protocol Deviation Classification by Site	16.2.4.1
14.1.3.2	Safety	Protocol Deviation Classification by Reason for Deviation	16.2.4.1
14.1.3.3	All Sites	Non-Participant-Specific Protocol Deviations Listing	16.2.4.2
14.1.4	Safety	Summary of Study Doses	16.2.5
14.1.5	Safety	Summary of PK samples and concentrations	16.2.6
<b>14.2 EFFECTIVENESS</b>			
14.2.1.1	Effectiveness	Summary of BPD at 36 Week PMA Assessment	16.2.7.1
14.2.1.2	Effectiveness	Logistic Regression results of BPD at 36 Week PMA Assessment	16.2.7.1
14.2.3.1	Effectiveness	Summary of BPD risk assessment during the treatment period	16.2.7.2
14.2.3.2	Effectiveness	Analysis of Change of Risk of BPD from Baseline to End of Study	16.2.7.2
14.2.3.3	Effectiveness	Longitudinal Analysis for Risk of BPD	16.2.7.2
14.2.4.1	Effectiveness	Summary of ROP during the study period	16.2.7.3
14.2.4.2	Effectiveness	Logistic Regression results of ROP during the study period, performed if numbers suffice	16.2.7.3
14.2.5.1	Effectiveness	Summary of Pulmonary Hypertension during the study period	16.2.7.4
14.2.5.2	Effectiveness	Logistic Regression results of pHTN, during the study period, performed if number suffice	16.2.7.4

Table Number	Population	Table Title / Summary	Supporting Listing Number
<b>14.3 SAFETY</b>			
14.3.1.1	Safety	Summary of Adverse Events by Dose Group (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 Group	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	Related Serious Adverse Events by System Organ Class, Preferred Term and by Dose Group	16.2.8.3
14.3.1.5	Safety	Adverse Events by System Organ Class, Preferred Term, Severity and by Dose Group	16.2.8.1
14.3.1.6	Safety	Adverse Events by System Organ Class, Preferred Term, Relationship to Study Medication and by Dose Group	16.2.8.1
14.3.1.7	Safety	Adverse Events Listed by Most Frequent Preferred Term and by Dose Group	16.2.8.1
14.3.1.8	Safety	Summary of Participant Deaths	16.2.8.4
14.3.2	Safety	Summary of Low Blood pressure, presumed low blood pressure, hypotension and presumed hypotension by Dose Group	16.2.9.1, 16.2.9.2, 16.2.9.3, 16.2.9.4
14.3.3.1 14.3.3.2	Safety	Summary of Laboratory Events by Dose Group AST (14.3.3.1) and ALT (14.3.3.2); additional laboratory events will be considered if data are sufficient	16.2.10.1, 16.2.10.2
14.3.4	Safety	Summary of Vital Signs by Dose Group	16.2.11
14.3.5	Safety	Summary of Concomitant Medications of interest by Drug Classification, Drug Name and Dose Group	16.2.12
14.3.6	Safety	Summary of Physical Examination Findings by Body System and Dose Group	16.2.13

Figure Number	Population	Figure Title / Summary	Supports Table/Listing Number(s)
14.4.1	Safety	Patient Profile	16.2.2, 16.2.5, 16.2.7.1, 16.2.8.1, 16.2.10.1, 16.2.10.2
14.4.2	Effectiveness	Risk of Moderate BPD, Severe BPD or Death during the Treatment Period	14.2.3.1 16.2.7.2
14.4.3	Effectiveness	Linear Regression Model Estimates of BPD during the Treatment Period	14.2.3.3
14.4.4.1 14.4.4.2	Safety	Box Plot of Laboratory Results AST and ALT; additional laboratory events will be considered if data are sufficient	14.3.3 16.2.10.1, 16.2.10.2

Listing Number	Population	Listing Title / Summary	Supports Table Number(s)
16.2.1.1	Randomized	Inclusion/Exclusion Criteria	N/A
16.2.1.2	Randomized	Final Study Disposition	14.1.1.1, 14.1.1.2
16.2.2	Safety	Demographic Characteristics	14.1.2.1
16.2.3	Safety	Medical History	14.1.2.2
16.2.4.1	Safety	Protocol Deviations	14.1.3.1, 14.1.3.2
16.2.4.2	All Sites	Non-participant-Specific Protocol Deviations	14.1.3.3
16.2.5	Safety	Study Medication Dose Administration	14.1.4
16.2.6	Safety	PK Samples and Concentrations	14.1.5
16.2.7.1	Effectiveness	Respiratory Assessments	14.2.1.1, 14.2.1.2
16.2.7.2	Effectiveness	BPD Risk	14.2.3.1, 14.2.3.2, 14.2.3.3
16.2.7.3	Effectiveness	ROP Assessment	14.2.4.1, 14.2.4.2
16.2.7.4	Effectiveness	Pulmonary Hypertension Assessment	14.2.5.1, 14.2.5.2
16.2.8.1	Safety	Adverse Events	14.3.1.1, 14.3.1.2, 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	Related Serious Adverse Events	14.3.1.4
16.2.8.4	Safety	Deaths	14.3.1.8
16.2.9.1	Safety	Pre-Initial Dose MAPs	14.3.2
16.2.9.2	Safety	Post Initial Dose MAPs	14.3.2
16.2.9.3	Safety	Intensive MAP Monitoring	14.3.2
16.2.9.4	Safety	Daily MAP Monitoring	14.3.2
16.2.10.1	Safety	Chemistry Laboratory Measurements	14.3.3
16.2.10.2	Safety	Hematology Laboratory Measurements	14.3.3
16.2.11	Safety	Vital Signs	14.3.4
16.2.12	Safety	Concomitant Medications	14.3.5
16.2.13	Safety	Physical Examination Findings	14.3.6

## Appendix B: Interim Analysis Tables

Tables for the SIL02 BLINDED interim analysis created at completion of Cohort 1 (before opening Cohort2) and at completion of Cohort 2 (before opening Cohort 3). All tables (except protocol deviation tables 3 & 4 and listings) will be stratified by Cohort and total, data will not be presented by randomized treatment arm.

Table 1	Participant Accountability and Study Disposition – All Randomized Participants
Table 2	Participant Enrollment by Site – Safety Population
Table 3A	Protocol Deviation Classification by Site – Safety Population
Table 3B	Protocol Deviation Classification by Reason for Deviation – Safety Population
Table 3C	Listing of Site Specific Protocol Deviations – All Participating Sites
Table 4	Baseline Demographics – Safety Population
Table 5	Summary of All Adverse Events – Safety Population
Table 6	Adverse Events by System Organ Class and Preferred Term – Safety Population
Table 7	Listing of all Serious Adverse Events – Safety Population
Table 8	Listing of all Related Serious Adverse Events – Safety Population
Table 9	Summary of BPD at 36 weeks PMA – Safety Population
Table 10	Summary of Low Blood Pressure and Hypotension – Safety Population
Table 11	Summary of Laboratory Events – Safety Population
Table 12	Summary of Dosing – Safety Population

Appendix 1 Listing of Protocol Deviations (safety population)

Appendix 2 BPD Status Definition

Appendix 3 Participant Profiles (safety population)

Appendix 4 SAE Narratives