Safety of Sildenafil in Premature Infants with Severe Bronchopulmonary Dysplasia (SILDI-SAFE)

Principal Investigator: Christoph Hornik, MD, PhD, MPH

Grantee Institution: Duke University

Version Number: 2.2

08 March 2022

NCT04447989

STATEMENT OF COMPLIANCE

The study will be conducted in accordance with the International Council for Harmonisation guidelines for Good Clinical Practice (GCP) (ICH E6) and the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46).The US Code of Federal Regulations (CFR) include but are not limited to:

- 45 CFR 46 (Human Subjects Protection)
- 45 CFR 160 and 164 Subparts A and E (Health Insurance Portability and Accountability Act [HIPAA] Privacy Rule)
- 21 CFR 312 (Investigational New Drug [IND] Application)
- 21 CFR 50 (Protection of Human Subjects, including Subpart D - Additional Safeguards for Children in Clinical Investigations)
- 21 CFR 56 (Institutional Review Boards [IRB])

National Institutes of Health (NIH)-funded investigators and all clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection, ICH GCP Training and are qualified to conduct this research.

PROTOCOL SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Clinical Site Investigator:

Signed: _____ Date: _____

Name:

Title:

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LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BPD	Bronchopulmonary Dysplasia
CFR	Code of Federal Regulations
CMP	Clinical Monitoring Plan
CPAP	Continuous Positive Airway Pressure
eCRF	Electronic Case Report Form
DCC	Data Coordinating Center
DCRI	Duke Clinical Research Institute
DSMB	Data Safety Monitoring Board
DMS	N-desmethylsildenafil
EDC	Electronic Data Capture System
ESI	Events of Special Interest
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Council for Harmonisation
ICMJE	International Committee of Medical Journal Editors
IND	Investigational New Drug Application
IRB	Institutional Review Board
ITT	Intention to Treat
IV	Intravenous
LAR	Legally Authorized Representatives
LPM	Liters per Minute
MAP	Mean Arterial Pressure
MOP	Manual of Procedures
N	Number (typically refers to participants)
NAVA	Neurally Adjusted Ventilatory Assist
NCPAP	Nasal Continuous Positive Airway Pressure
NHLBI	National Heart Lung and Blood Institute
NICHD	National Institute of Child Health and Human Development

NICU	Neonatal Intensive Care Unit
NIH	National Institutes of Health
NIPPV	Nasal Intermittent Positive Pressure Ventilation
PAH	Pulmonary Arterial Hypertension
PH	Pulmonary Hypertension
PI	Principal Investigator
PK	Pharmacokinetics
PMA	Postmenstrual Age (gestational age plus postnatal age)
PP	Per Protocol
PTN	Pediatric Trials Network
q	Every
ROP	Retinopathy of Prematurity
SAE	Serious Adverse Event/Serious Adverse Experience
SAR	Suspected Adverse Reaction
U/L	Units per Liter
US	United States

PROTOCOL SUMMARY

<table>
<tr><td>Title:</td><td>Safety of Sildenafil in Premature Infants with Severe Bronchopulmonary Dysplasia</td></tr>
<tr><td>Précis:</td><td>This is a multicenter, randomized, placebo-controlled, sequential dose-escalating, double-masked, safety study of sildenafil in premature infants with severe bronchopulmonary dysplasia (BPD).

Number of Participants (N) and Dosing Scheme
<table>
<tr><th rowspan="2">Cohort[1]</th><th colspan="2">Treatment Group</th><th colspan="2">Sildenafil Dosing[2] (mg/kg q 8 hours)</th><th rowspan="2">Total (N)</th></tr>
<tr><th>Placebo (N)</th><th>Sildenafil (N)</th><th>IV</th><th>Enteral</th></tr>
<tr><td>1</td><td>10</td><td>30</td><td>0.5</td><td>1</td><td>40</td></tr>
<tr><td>2</td><td>10</td><td>30</td><td>1</td><td>2</td><td>40</td></tr>
<tr><td>3</td><td>10</td><td>30</td><td>2</td><td>4</td><td>40</td></tr>
</table>
Abbreviations: IV, intravenous; N, number of participants; q, every
1 Participants will be enrolled into cohorts sequentially (i.e., Cohort 1 then Cohort 2 then Cohort 3) based on safety.
2 Route of administration should be via IV route if patient has an IV and IV administration is feasible. However, route of administration, IV or enteral, is left to investigator discretion.</td></tr>
<tr><td>Objectives:</td><td>Primary: Describe the safety of sildenafil as determined by incidence of hypotension in premature infants with severe BPD.

Secondary: Pharmacokinetics (PK) and preliminary effectiveness of sildenafil for the prevention of pulmonary hypertension in premature infants with severe BPD.</td></tr>
</table>

Population:	Premature infants with severe BPD (inpatient in neonatal intensive care units) will be randomized in a dose escalating approach 3:1 (sildenafil: placebo) sequentially, into each of 3 cohorts. There will be approximately 40 randomized and dosed participants in each cohort for a total of up to 120 participants. For the purposes of this study, severe BPD is defined as: • 32-44 weeks postmenstrual age at enrollment; and • Receiving respiratory support at enrollment o If 32 0/7–35 6/7 weeks postmenstrual age: mechanical ventilation (high frequency or conventional) o If 36 0/7–44 6/7 weeks postmenstrual age: mechanical ventilation (high frequency or conventional) OR continuous positive airway pressure (CPAP). Note: Exclusion from participation due to enrollment in other clinical trials, including IND trials, will be left to the judgement of the site principal investigator (PI). Investigator judgement should consider the safety of the participant.
Phase or Stage:	2
Number of Sites:	Approximately 30 sites
Description of Intervention:	Sildenafil citrate injection and powder for suspension
Study Duration:	Approximately 36 months enrollment period with an additional 24 months of data analyses (60 months total)
Subject Participation Duration:	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 postmenstrual age and/or at discharge.

1 KEY ROLES AND CONTACT INFORMATION

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2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Sildenafil is approved by the FDA for the treatment of pulmonary arterial hypertension in adults to improve exercise ability and delay clinical worsening.[1] The FDA does not recommend using sildenafil for pulmonary hypertension in children 1 to 17 years of age. Recommendations about sildenafil use in neonates and infants do not exist. Differences

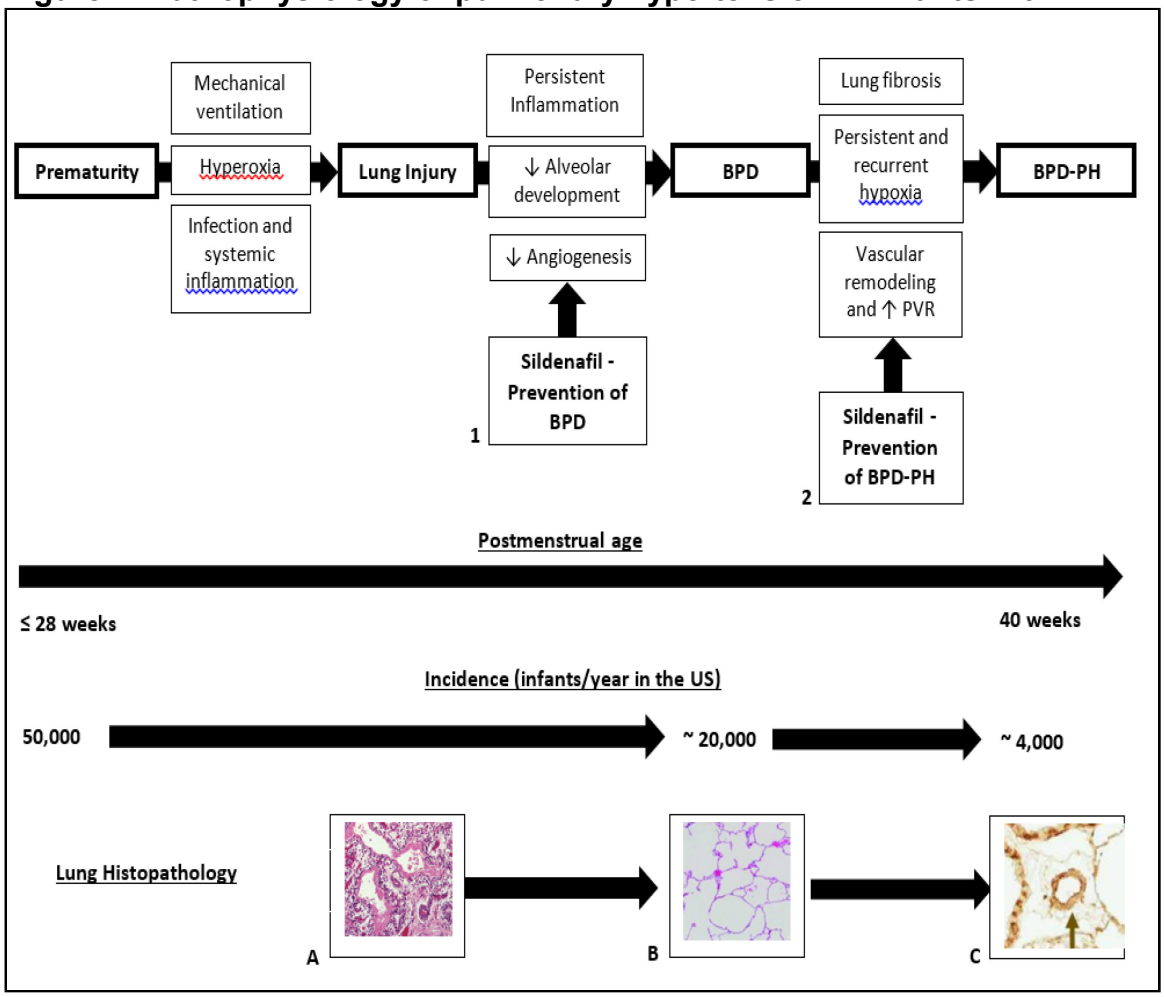
in the cardiopulmonary pathophysiology targeted to neonates and infants suggest that sildenafil may be beneficial in this population. As a result, pharmacokinetic (PK) and safety studies of sildenafil in neonates and infants have been or are currently being conducted under the Best Pharmaceuticals in Children's Act by the National Institute of Child Health and Human Development (NICHD)-funded Pediatric Trials Network (PTN).[2] The present study, supported by the National Heart Lung and Blood Institute (NHLBI), will generate additional safety, PK, and preliminary effectiveness data on sildenafil in a subpopulation of infants with severe bronchopulmonary dysplasia (BPD).

2.2 Rationale

Over 50,000 infants are born < 29 weeks gestational age each year in the US, of whom nearly 40% develop BPD.[3] BPD is defined by the NIH as either mild, moderate, or severe based on the respiratory support required at 36 weeks postmenstrual age.[4] Premature infants with severe BPD are particularly challenging and suffer from multiple morbidities including pulmonary hypertension (PH), prolonged hospitalization, death, and life-long morbidities.[5–7] Because of the prevalence and seriousness of the disease, neonatologists are dedicated to preventing BPD.[8] Vitamin A, caffeine, and postnatal steroids have all been shown to prevent BPD.[9–11] Members of this protocol team (Drs. Hornik, Laughon, and Jackson) are currently performing trials to prevent BPD using sildenafil (NCT03142568). However, once BPD has developed, there are no drugs to prevent its associated morbidities including PH. This study seeks to address this knowledge gap by evaluating the safety, PK, and preliminary effectiveness of sildenafil in this population and was designed based on the rationale outlined below.

Pulmonary hypertension is a deadly complication of severe BPD. Approximately 20% of premature infants with severe BPD develop PH, and up to 40% of premature infants with severe BPD and PH die, usually due to right heart failure.[5,12,13] In addition to higher mortality, infants with combined BPD and PH have longer hospitalizations and are more likely to require in-home respiratory support.[14,15] The temporal relationship between BPD and PH in premature infants is complex. Early PH without concurrent BPD can be present in the first week of life in infants at the highest risk of BPD, such as those with fetal growth restriction.[12] Only one-third of these infants will develop BPD later in life. In contrast, most infants with BPD who develop PH do so later in life: one prospective study of extremely low-birth-weight infants found that 65% of infants with BPD-related PH had normal echocardiograms at 4-6 weeks.[16] BPD-related PH occurs due to impaired lung development resulting in decreased pulmonary vessel branching and vascular remodeling (Figure 1).[17] Knowledge of this pathology and the delay in development of this severe complication offer an opportunity for prevention to decrease infant mortality.

Figure 1 Pathophysiology of pulmonary hypertension in infants with BPD



Abbreviations: BPD, bronchopulmonary dysplasia; PH, pulmonary hypertension

Sildenafil reduces lung injury and preserves normal vasculature in preclinical BPD models. BPD and PH are characterized by lung injury, inflammation, abnormal alveolarization, and dysregulated vascular development. Preclinical models demonstrate that sildenafil might prevent ischemic-perfusion injury and improve lung function (rat[18]), suppress inflammatory mediators (mouse[19]; rat[20,21]; also matrix metalloproteinase-2 in cell cultures[22]) that contribute to lung injury and BPD, and improve alveolarization and lung vascular development. Sildenafil is a potent inhibitor of phosphodiesterase type 5, the predominant isoform in the lung, which metabolizes cyclic guanosine monophosphate and produces pulmonary vasodilation. Sildenafil may thus prevent the development of BPD-related PH in premature infants by reducing pulmonary vascular remodeling and lowering pulmonary vascular resistance, but clinical trial evidence is needed.

Sildenafil improves short-term outcomes in children and adults with pulmonary hypertension. Sildenafil is a potent inhibitor of type 5 phosphodiesterase, the

predominant isoform in the lung. Type 5 phosphodiesterase metabolizes cyclic guanosine monophosphate and produces pulmonary vasodilation. In adults, sildenafil is approved by the FDA for the treatment of pulmonary arterial hypertension.[1] Children with pulmonary arterial hypertension (either idiopathic or due to preexisting cardiac disease) treated with sildenafil had improved aerobic capacity after 16 weeks of treatment, and the effect appeared to be dose dependent.[23,24] In this study, children with BPD were excluded, and all participants were > 8 kg. Long-term follow-up demonstrated an increased risk of mortality in the highest sildenafil dosing group, (dosing was weight-based with the highest dose group receiving 20 mg thrice daily if weight was 8-20 kg, 40 mg thrice daily if weight was 21-45 kg and 80 mg thrice daily if weight was > 45 kg).[25] FDA does not recommend sildenafil use, particularly chronic use, in children and Health Canada states sildenafil is not indicated in pediatric patients.[1,26]

Neonatologists frequently use sildenafil to treat infants with pulmonary hypertension. Two case reports documented the successful resolution of PH, measured by echocardiogram, in premature infants treated with sildenafil.[27,28] In a small case series, enteral sildenafil at a dose range of 1.5-8 mg/kg/day in 25 premature infants with a median gestational age of 28 weeks (range 23-41 weeks) with lung disease (72% with BPD) resulted in improved hemodynamics by echocardiogram.[29] A retrospective case series of 23 preterm infants with median gestational age of 26 weeks treated with 1-7.3 mg/kg/day of oral sildenafil found a substantial improvement in BPD related PH based on echocardiography in 71% of patients for whom echocardiographic data were complete (15/21).[30] These data confirm that neonatologists usually treat PH in infants with BPD and are likely unwilling to randomize to placebo; thus, a prevention approach is needed.

Neonatologists are using sildenafil without safety data. Neonatologists are increasingly using sildenafil in premature infants despite a lack of sufficient safety data, including data related to hypotension and retinopathy of prematurity (ROP). Despite neonatal medicine's long history of catastrophic adverse events (AEs) resulting from inadequate study of drugs prior to their widespread use,[31–34] the majority of drugs used in premature infants, including sildenafil, have undergone insufficient study to receive FDA labeling for this population.[35–39] Extrapolating drug safety and dosing from adults underestimates the complicated physiology of premature infants, who have (1) larger extracellular fluid volume per unit body weight, (2) immature renal and hepatic function, and (3) disease-related changes in metabolizing organ function, each of which can alter drug disposition.[40,41] Improper use of drugs in these vulnerable patients leads to increased treatment failure, AEs, mortality, and long-term morbidities.[42] Sildenafil studies specifically designed for premature infants with BPD are urgently needed. These studies should initially focus on characterizing PK and safety in infants to inform the design of a pivotal efficacy trial.

Sildenafil is increasingly used off-label in premature infants with BPD: A review of data from the Mednax Clinical Data Warehouse, a real-world data source representing approximately 20% of US Neonatal Intensive Care Unit (NICU) admissions,[43] found an

exponential increase in sildenafil use by > 1000%, second highest of all drugs from 2005 to 2010.[44] Of the infants exposed to sildenafil, 79% had BPD and 84% had PH.

A phase 1 opportunistic pilot study of sildenafil in premature infants under IND (NCT01670136) has been completed. Conducted under IND by the PTN, the trial enrolled 34 infants ≤ 28 weeks gestational age with a median birth weight of 666 g receiving intravenous (IV) or enteral sildenafil per standard of care at doses between 0.13 mg/kg - 2.1 mg/kg. Pharmacokinetic and safety analyses were performed and are summarized below.

The infant population PK model identified safe sildenafil starting doses for early phase trials. Using 109 plasma samples collected in 34 infants in a multicenter phase 1 open-label PK study of sildenafil in premature infants, we developed a 2-compartment population PK model of sildenafil and its primary active metabolite N-desmethylsildenafil (DMS). Population estimates of parent sildenafil elimination clearance and central volume of distribution were 27.8 L/h/70kg and 116 L/70kg; relative DMS elimination clearance and volume of distribution, were 135 L/h/70kg and 1670 L/70kg.[2] The model simulated sildenafil a DMS exposures at different enteral and IV dosing regimens to identify safe doses for evaluation in a dose escalation trial (Table 1). Starting doses of 0.5 mg/kg IV (or 1 mg/kg enteral) resulted in combined $AUC_{0\text{-}24,SIL+DMS}$ comparable to a previously reported value of 2650 ng*hr/mL which has been associated with infant survival.[45] However, exposure targets for PH prevention in infants with severe BPD are unknown and will be further elucidated in this trial.

Table 1 Simulated Median Steady-State Area under the Concentration vs. Time Profile 0- 24 Hours

Intravenous Dosing (every 8 hours)	**0.5 mg/kg**	**1 mg/kg**
Sildenafil (ng*hr/ml)	3157	6314
DMS (ng*hr/ml)	669	1337
Sildenafil + 62.5%DMS (ng*hr/ml)	3581	7163
Enteral Dosing (every 8 hours)	**1 mg/kg**	**2 mg/kg**
Sildenafil (ng*hr/ml)	1825	3650
DMS (ng*hr/ml)	1608	3217
Sildenafil + 62.5%DMS (ng*hr/ml)	2941	5883

Abbreviation: DMS, N-desmethylsildenafil

The safety analysis identified overall incidence and events of special interest: The safety analysis included all 34 infants. There were 23 adverse events in 15 participants and 5 serious adverse events (SAEs) in 5 participants, including 1 episode of hypotension, the only event related to sildenafil, in an infant receiving IV sildenafil (Table 2). This event was determined to be related to a faster than expected infusion rate of IV sildenafil. The event led to a data monitoring committee (DSMB) review of available data, and a review of all clinical sites' infusion methods and equipment. The protocol was changed prior to enrolling the remaining Cohort 2 subjects to include a

longer infusion time (30 minutes) and the dose was lowered by 50% to 0.125 mg/kg. When the study reopened, 2 additional subjects were enrolled without any related AEs.

Table 2 Summary of All Adverse Events in Phase 1 Study of Sildenafil

	Cohort 1 (N = 25)	Cohort 2 (N = 9)
Number of Events/Participants		
Number of AEs	13	10
Participants with at least one AE	8 (32.0%)	7 (77.8%)
Number of SAEs	1	4
Participants with at least one SAE	1 (4.0%)	4 (44.4%)
Severity (All AEs)		
Mild	7	3
Moderate	4	3
Severe	2	4
Highest Severity per Participant		
Mild	4 (16.0%)	2 (22.2%)
Moderate	3 (12.0%)	1 (11.1%)
Severe	1 (4.0%)	4 (44.4%)
Relationship (All AEs)		
Not related	13	9
Related	0	1
Strongest Relationship per Participant		
Not related	8 (32.0%)	6 (66.7%)
Related	0	1 (11.1%)

Abbreviations: AE, adverse event; N, number of participants; SAE, serious adverse event

Echocardiography is a reliable surrogate endpoint for the diagnosis of pulmonary hypertension in premature infants: With support from NHLBI (R34HL14038), members of the current study team (Drs. Hornik and Laughon) assigned 3 blinded pediatric cardiologists to review 483 echocardiograms from 49 infants < 29 weeks gestational age at birth (range 22–28) admitted to the neonatal intensive care units of UNC or Duke. PH diagnosis was a priori defined using tricuspid regurgitation jet and, if unavailable, a composite score of echocardiographic measures. The proposed score had high inter (modified Fleiss Kappa 0.759 (0.771, 0.801) and intra-rater reliability (Fleiss Kappa 0.847 (0.75, 0.931), and acceptable internal consistency (Cronbach's alpha 0.64)[46]. Importantly, the score identified infants with PH with a prevalence (33%) and mortality (34%) comparable to prior reports. Mortality was significantly lower (10%) for infants not diagnosed with PH.

Based on the above data, sildenafil is a promising potential intervention to prevent the development of PH in premature infants with BPD. Prior to the completion of definitive efficacy trials, an evaluation of its safety, PK, and preliminary effectiveness are essential.

2.3 Potential Risks and Benefits

2.3.1 *Potential Risks*

2.3.1.1 Risks of Blood Sampling

There are small risks to blood sampling including minor pain or discomfort with the needle stick and blood loss. Every effort will be made to avoid needle sticks in excess of standard of care for this study and every effort will be made to time clinical blood draws to coincide with timed samples, using existing IV lines when possible.

2.3.1.2 Sildenafil

There are several risks associated with administering sildenafil in premature infants, including:

1. A theoretical increased risk of ROP associated with sildenafil has been suggested in a case report in the literature;[47] however, case-control and cohort studies have not found a significant association between sildenafil exposure and ROP.[48–50]
2. Sildenafil has vasodilatory properties that can result in mild and transient decreases in blood pressure. See sildenafil package insert for additional information.[1]

Adverse events were previously observed in the phase 1 opportunistic pilot study of sildenafil in premature infants (NICHD-2012-SIL0; see Table 2, Section 2.2). Safety data will also be reviewed prior to dose escalation between cohorts. Risks of ROP have been minimized by administering sildenafil during the time when screening for ROP begins (31-32 weeks adjusted age). Treatment of ROP is an exploratory outcome (Section 3.3.1).

2.3.2 *Potential Benefits*

Sildenafil may improve pulmonary mechanics, reduce exposure to mechanical ventilation, and prevent PH. Although these improvements are not proven, improved pulmonary function and decreased risk of PH are potential benefits for participants enrolled in the study who receive sildenafil. Conclusions drawn from this study will benefit infants receiving sildenafil in the future through better understanding of dose response and characterization of the safety profile of sildenafil.

3 OBJECTIVES AND OUTCOME MEASURES

3.1 Objectives

3.1.1 *Primary Objective*

Describe the safety of sildenafil in premature infants with severe BPD.

3.1.2 *Secondary Objectives*
Analyze the pharmacokinetics (PK) and preliminary effectiveness of sildenafil in premature infants with severe BPD.

3.1.3 *Tertiary/Exploratory Objectives*
Explore other events of interest in premature infants with severe BPD.

3.2 Outcome Measures

3.2.1 *Primary Outcome Measures*
Safety as determined by incidence of hypotension experienced by the participants through 28 days post last dose of study drug.

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.

3.2.2 *Secondary Outcome Measures*

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

3.2.2.2 Preliminary Effectiveness
The presence or absence of PH will be determined using central review by blinded pediatric cardiologists of echocardiograms performed at the end of the study period.

The developed population PK model will be linked to the diagnosis of PH made by echocardiography at the end of the study period to develop a population PK/PD model. We will use the model to simulate the effect of variable sildenafil exposures on the probability of developing PH.

3.3 Tertiary/Exploratory

3.3.1 *Other Safety and Efficacy Outcomes*

1. **Global Rank:** Clinically significant events ranked in order of decreasing perceived severity. Each participant will receive a rank based upon the lowest ranking (worst) endpoint as defined in the statistical analysis plan that they experienced during the study.

2. **Events of Special Interest**: See Section 9.2.4 for a complete list of events of special interest (ESI).

4 STUDY DESIGN

Premature infants (inpatient in NICUs) will be randomized in a dose escalating approach 3:1 (sildenafil: placebo) sequentially, into each of 3 cohorts. There will be approximately 40 randomized and dosed participants in each cohort for a total of up to 120 participants.

4.1 Screening/Baseline

Research staff will document informed consent from the parent/guardian for all participants who satisfy eligibility criteria. The following information will be recorded in the case report form (eCRF) from the clinical medical record:

1. Participant demographics, including birth weight and gestational age at birth
2. Maternal race/ethnicity
3. Medical history
4. Physical examination, including actual weight
5. All mean arterial pressure (MAP) obtained in the 24 hours before the first dose
6. Concomitant medications (within 24 hours prior to start of study drug; including medications of special interest [Section 6.5.1])
7. Respiratory assessment (Section 8.2.2.3)
8. Laboratory evaluations (Section 8.2)
9. Echocardiogram: If performed per local standard of care < 14 days prior to start of study drug, a study-specific echocardiogram need not be repeated. If not performed per local standard of care < 14 days prior to start of study drug, an echocardiogram will be required to confirm eligibility.
10. Cardiac catheterization reports, if performed per local standard of care < 14 days prior to start of study drug.
11. Adverse events following informed consent (Section 9.3)

4.2 Treatment Period

The treatment period will include Days 1-28 or last day of study drug if early withdrawal of study drug. The following information will be collected and recorded while the participant is on study drug:

1. Actual weight on study Days 7 (± 1 day), 14 (± 1 day), 21 (± 1 day), and 28 (± 1 day) of study drug administration
2. Date, time, amount, and route of study drug dose
3. All concomitant medications (including medications of special interest [Section 6.5.1])
4. MAP
    A. All MAP values obtained 24 hours after the first dose of study drug regardless of administration route.
    B. MAP values will be obtained at a minimum at the following time points
        i. Prior to the first dose of study drug or dose escalation: 2 hours (± 5 minutes), 1 hour (± 5 minutes), and 15 minutes (± 5 minutes)
        ii. If administration route is IV:
            a. During and following the first dose of study drug or dose escalation: MAP at start of infusion, every 15 minutes (± 5 minutes) during infusion, at end of infusion (inclusive of flush) (± 5 minutes), at 15 and 30 minutes (± 5 minutes) after end of infusion, hourly (± 15 minutes) for 4 hours, and once in the remaining 2 hours prior to the next dose.
            b. For subsequent IV doses, the lowest valid MAP value should be recorded daily while on study drug.
        iii. If the administration route is enteral:
            a. During and following the first dose of study drug or dose escalation: MAP at start of enteral administration, then every 15 minutes (± 5 minutes) for 90 minutes (1.5 hours), then every 30 minutes (± 5 minutes) for 60 minutes (1 hour), then hourly (± 15 minutes) for 4 hours, then once in the remaining 2 hours prior to the next dose.
            b. For subsequent enteral doses, the lowest valid MAP value should be recorded daily while on study drug.
5. Respiratory assessment, daily (Section 8.2.2.3)
6. Laboratory evaluations, at least once every 2 weeks (Section 8.2).
7. Echocardiograms and cardiac catheterization reports, if performed per local standard of care
8. PK sampling (after Day 7 [Section 8.2.2.1])
9. Adverse events (Section 9.3)

4.3 Weaning Period (Cohorts 2 and 3)

The weaning period will begin following Day 28 of study drug or, following the last day of study drug if participant was withdrawn from study drug prior to Day 28 and the dose escalated to ≥ 0.5 mg/kg IV or ≥ 1 mg/kg enteral.

The following information will be collected and recorded while the participant is weaning from study drug:

1. Date, time, amount and route of study drug dose
2. MAP (the lowest MAP value on last day of wean should be recorded).
3. Respiratory assessment, daily (Section 8.2.2.3)

4. Concomitant medications of special interest (Section 6.5.1)
5. Echocardiogram and cardiac catheterization reports, if performed per local standard of care
6. Adverse events (Section 9.3)

4.4 Follow-up Period

The follow-up period will include Days 1-28 after the last study drug dose; last study drug dose may occur prior to Day 28 for those participants who withdraw from study drug early; on Day 28 for those participants who complete the full treatment period; or after last weaning dose for those participants who require weaning. The following information will be reported in electronic data capture system (EDC) at Day 1 (+ 2 days) and 14 (± 2days) of the follow-up period (or days closest to and after Day 1 and 14, if >1 assessment is available), except for MAP, AEs, and SAEs (which will be reported from Days 1-28 post last study drug dose) and standard of care echocardiograms or cardiac catheterization reports:

1. Physical examination, including actual weight
2. MAP (the lowest valid MAP value on follow-up Day 1, 7, 14, 21, and 28 should be recorded).
3. Respiratory assessment, daily (Section 8.2.2.3)
4. Laboratory evaluations obtained per local standard of care (Section 8.2)
5. Concomitant medications of special interest (Section 6.5.1)
6. Echocardiogram on follow-up Day 1 (+2 days). If performed per local standard of care, a study-specific echocardiogram need not be repeated. If not performed per local standard of care on Day 1 (+2 days) of the follow-up period, an echocardiogram will need to be performed.
7. Echocardiograms and cardiac catheterization reports, if performed per local standard of care (during follow-up Days 1-28)
8. Adverse events and SAEs (during follow-up Days 1-28 [Sections 9.3])

4.5 Final Study Assessment

Final study assessment will occur at the time of discharge or transfer. The following information will be collected:

1. Physical examination, including actual weight
2. Respiratory assessment (Section 8.2.2.3))
3. Concomitant medications of special interest (Section 6.5.1)
4. Echocardiogram and cardiac catheterization reports, if performed per local standard of care on the day of discharge or transfer or up to 2 days prior.
5. Global rank (Section 8.2.2.4)
6. Discharge information
    A. Discharge or transfer
    B. Death
    C. Duration of hospitalization
7. Record if treatment for ROP was required

5 STUDY POPULATION

5.1 Participant Inclusion Criteria

To be eligible to participate in this study, an individual must meet all of the following criteria:

1. Documented informed consent from parent or guardian, prior to study procedures
2. < 29 weeks gestational age at birth
3. 32-44 weeks postmenstrual age
4. Receiving respiratory support at enrollment:
    - If 32 0/7–35 6/7 weeks postmenstrual age: mechanical ventilation (high frequency or conventional)
    - If 36 0/7–44 6/7 weeks postmenstrual age: mechanical ventilation (high frequency or conventional) OR continuous positive airway pressure (CPAP)

Note:
- Criteria 3 and 4 define severe BPD for the purposes of this study
- CPAP is defined as any of the following:
    - Nasal cannula > 2 liters per minute (LPM)
    - Nasal continuous positive airway pressure (NCPAP)
    - Nasal intermittent positive pressure ventilation (NIPPV)
    - Noninvasive neurally adjusted ventilatory assist (NAVA)
    - Any other device designed to provide positive pressure through a nasal device (e.g., RAM cannula, etc.)

5.2 Participant Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Previous enrollment and dosing in this study, protocol number (NHLBI-2019-SIL), “Safety of Sildenafil in Premature Infants with Severe Bronchopulmonary Dysplasia (BPD)”
2. Previous exposure to sildenafil within 7 days prior to randomization*
3. Previous exposure to vasopressors within 24 hours prior to randomization*
4. Previous exposure to inhaled nitric oxide within 24 hours prior to randomization*
5. Previous exposure to milrinone within 24 hours prior to randomization*
6. Evidence of pulmonary hypertension or moderate/large patent ductus arteriosus (PDA) on the most recent echocardiogram performed within 14 days prior to randomization
7. Known major congenital heart defect requiring medical or surgical intervention in the neonatal period
8. Known allergy to sildenafil
9. Known sickle cell disease

10. Aspartate aminotransferase (AST) > 225 U/L < 72 hours prior to randomization
11. Alanine aminotransferase (ALT) > 150 U/L < 72 hours prior to randomization
12. Any condition that would make the participant, in the opinion of the investigator, unsuitable for the study.

**Participant will be reassessed prior to dosing to reconfirm eligibility criteria.*

Note: Exclusion from participation due to enrollment in other clinical trials, including IND trials, will be left to the judgement of the site principal investigator (PI). Investigator judgement should consider the safety of the participant.

5.3 Strategies for Recruitment and Retention

The study will include up to 120 premature infants (< 29 weeks gestation) with severe BPD (will screen approximately 600 infants). Potential subjects will be identified in the NICU at participating sites. Due to the nature of the condition, a minority population is intrinsically overrepresented (minority population represents 30-40% of infants with BPD; https://www.marchofdimes.org/peristats). Therefore, the study team does not anticipate difficulty with inclusion of individuals commonly underrepresented in research studies.

Sites were selected based on prior participation in infant trials. Selected sites have research teams with extensive experience interacting with and enrolling the target population. Prior to and during the enrollment period for the study, sites will participate in conference calls to discuss recruitment and retention plans, including strategies for building rapport, increasing awareness, and enrolling the study population.

Study staff will remain in frequent contact with patients/parents to ensure that the follow-up data are collected pre the protocol schedule.

5.4 Treatment Assignment Procedures

5.4.1 *Randomization Procedures*

Randomization will occur on Day -1 among participants who satisfy all eligibility criteria. Participants will be centrally randomized via the EDC to maintain a 3:1 ratio within each cohort of sildenafil (n=30) to placebo (n=10). Participants from a multiple gestation will be randomized independently as they become eligible. If more than one infant from a multiple gestation is randomized, the sibling relationship between participants will be collected in the eCRF.

Reasons for premature discontinuation will be noted on the electronic case report form (eCRF). A randomized participant who prematurely discontinues from the study prior to receiving 7 days of study drug may be replaced with a new participant who will receive the same randomization assignment. Replacement will not be allowed if the reason for premature discontinuation is related to study drug (Safety Medical Monitor deems the event relationship as Possible, Probable, or Definite). The randomization process will not determine if an IV or enteral dose is used. Route of administration, IV or enteral, will

be left to investigator discretion. Participants should not have an IV placed specifically for study drug administration alone. However, if an investigator deems that a second IV would optimize the safe administration of other medications or fluids while the participant is receiving the study drug, then an additional IV is permissible. If the participant does not have an IV or if the clinical team elects to use enteral dosing, then the dose of study drug will be twice that of the IV dosing (from the product label). Infants may transition from enteral to IV sildenafil (and vice versa) throughout the duration of the study per the investigator's determination. Placebo will be administered either enterally or IV in the same manner.

Study randomization codes and replacement codes for all cohorts will be generated and uploaded prior to the enrollment of the first participant. One sequential dosing cohort will be available at a time for assignment. Subsequent cohort slots will only become available after completion of safety reviews. Unblinded results will only be shared with blinded members of the study team following database lock. Any unplanned unblinding of individual or treatment group-level data will be reported to the study PI. Unplanned unblinding will be reported to NHLBI Program Officer and the DSMB.

5.4.2 *Masking Procedures – Enteral Dosing*

Infants randomized to the placebo treatment group and receiving enteral study drug will receive the equivalent volume of dextrose 5% appropriate for enteral use. Staff accessing participant outcomes will be masked to treatment. Because prepared enteral sildenafil and placebo are visually slightly different, the pharmacy at each site will prepare and dispense the study drug into appropriate sized syringes in a masked manner (e.g., amber syringe, syringe overlay, etc.) in order to maintain masking of staff. Unmasking procedures are described in detail in the Manual of Procedures (MOP).

5.5 Participant Withdrawal or Discontinuation from Study Procedures/Intervention

5.5.1 *Reasons for Participant Withdrawal or Discontinuation from Study Procedures/Intervention*

The participant's parent or guardian may withdraw an infant from participation in the study at any time. Parents or guardians can choose to have infant discontinue study drug and continue to be followed or they can withdraw consent for further participation at any time.

The site investigator may choose to suspend study drug dosing once for up to 48 hours for any reason. If study drug is resumed by the site investigator, the dose and interval of the study drug will be the same as the prior dose. If applicable, subsequent doses will follow the dose escalation or weaning schedule as specified in section 6.1. If study drug is suspended for > 48 hours, the study drug administration will be discontinued and the participant will proceed directly into the safety follow-up period, during which all remaining assessments will be collected. If study drug is suspended for > 48 hours for a documented safety concern, the suspension will not be considered a protocol deviation.

The site investigator will withdraw a participant from receiving further study interventions, including study drug administration, and the participant will enter the 28-day safety-monitoring period if any of the following occur:

- During the course of the study, the participant meets safety-related criteria that would have led to initial exclusion from study participation (i.e., ALT > 150 U/L, AST > 225 U/L, or infant started on vasopressor or inhaled nitric oxide [Section 5.2]).
- Any clinically significant AE (e.g., hypotension) that is deemed by the site investigator to require discontinuation of study drug (see Section 9.5.3 for the definition of hypotension).
- Unmasking of the participant treatment occurs. All unmasking events should be recorded on a protocol deviation form in the EDC system within 24 hours of the unmasking. The decision to unmask will be made in consultation with the medical monitor.

Before discontinuing a participant from the study drug, the site investigator must contact the medical monitor or study PI, except in emergencies. All participants who have study drug suspended will continue to be in the study and complete all remaining study procedures in the safety follow-up period.

5.5.2 *Handling of Participant Withdrawals from Study or Participant Discontinuation of Study Intervention*

A randomized participant (Section 5.4.1) who prematurely discontinues from the study prior to receiving 7 days of study drug may be replaced with a new participant who will receive the same randomization assignment. Replacement will not be allowed if the reason for premature discontinuation is related to study drug (i.e., Safety Medical Monitor deems the event relationship as Possible, Probable, or Definite).

Participants who are prematurely discontinued from receiving study drug for any reason will be followed for 28 days after last study drug dose for safety monitoring, and will have final study assessments collected.

Reasons for participant withdrawal of consent or discontinuation from study drug will be appropriately documented by the site and captured in the appropriate eCRF, as instructed in the study eCRF Instructions provided to sites.

The participant’s parent or guardian is not obligated to state the reason for withdrawal. The reasons for withdrawal, or decision not to provide a reason, must be documented by the investigator on the completion/withdrawal section of the corresponding eCRF. Participants/guardians who withdraw consent will not have any additional data entered in the EDC system.

5.6 Premature Termination or Suspension of Study

This study may be terminated at any time by the IND sponsor after consultation with FDA, NIH, and/or the Data Safety Monitoring Board DSMB. If the study is terminated, notification to FDA and US investigators will be made in accordance with 21 CFR 312.56(d).

If the study is discontinued, enrolled participants will continue to be followed for safety assessments through 28 days after last dose of study drug. All AEs must be followed through resolution. If the study is discontinued, regulatory authorities, IRBs and participant parents/guardians will be notified as required by regulation (e.g., U.S. 21 CFR 312.56).

6 STUDY INTERVENTION

6.1 Study Product Description

In the US, sildenafil, in both IV and for oral suspension, has been approved for use in other populations and for other indications by the FDA[1].

Study dosing and escalation are described in Table 3. Refer to Section 6.1.3 for study drug packaging and labeling.

Table 3 Number of Participants (N) and Dosing Scheme

	Treatment Group		Sildenafil Dosing[2] (mg/kg q 8 hours)		
Cohort[1]	Placebo (N)	Sildenafil (N)	IV	Enteral	Total (N)
1	10	30	0.5	1	40
2	10	30	1	2	40
3	10	30	2	4	40

Abbreviations: IV, intravenous; N, number of participants; q, every

1 Participants will be enrolled into cohorts sequentially (i.e., Cohort 1 then Cohort 2 then Cohort 3) based on safety.

2 Route of administration, IV or enteral, is left to investigator discretion.

Dose escalations for each sequential cohort will proceed as summarized in Table 4. DSMB safety review (Section 10) will be required prior to enrolling the next cohort.

Table 4 Dose Escalations for Each Sequential Cohort

	Study Days (Dose Numbers)							
	1-2 (1-6)	3-4 (7-12)	5-6 (13-18)	7-8 (19-24)	9-10 (25-30)	11-12 (31-36)	13-14 (37-42)	15 + (43 +)
Sildenafil Dose (mg/kg)								
Cohort 1								
IV	0.25	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Enteral	0.5	1	1	1	1	1	1	1
Cohort 2								
IV	0.25	0.5	0.75	1	1	1	1	1
Enteral	0.5	1	1.5	2	2	2	2	2
Cohort 3								
IV	0.25	0.5	0.75	1	1.5	1.75	2	2
Enteral	0.5	1	1.5	2	3	3.5	4	4

Abbreviation: IV, intravenous

Dose escalation will occur if the infant is:

1. Receiving exogenous oxygen or respiratory support (nasal cannula or positive pressure from any device); and
2. AST < 225 U/L and ALT < 150 U/L at last check (within 14 days) prior and closest to dose increase.

An infant will meet respiratory criteria for dose escalation only if the infant is receiving exogenous oxygen or respiratory support (nasal cannula or positive pressure from any device) for at least 12 hours before the dose escalation. Infants who do not initially qualify for escalation should be reevaluated daily to ascertain whether they meet these criteria, and escalation must occur within 24 hours of an infant meeting all dose escalation criteria (respiratory and laboratory). If escalation does not occur within this timeframe, then a protocol deviation must be recorded.

Adjusting dose for weight

The actual weight should be reviewed weekly (plus or minus one day, Section 4.2) and the dose of the study drug may be adjusted.

Weaning Period Schedule

Sildenafil is commonly used at higher doses to treat PH in infants. In clinical practice, it is recommended to wean from these higher doses to prevent rebound effects that may be seen with abrupt discontinuation of sildenafil. Although sildenafil used in this study is for prevention of PH, the target doses in Cohorts 2 and 3 are similar to those used for the treatment of PH.

For Cohorts 2 and 3, weaning of study sildenafil or placebo will begin following the last study dose on Day 28 or if the dose escalates to a dose of ≥ 0.5 mg/kg IV or ≥1 mg/kg enteral and the participant is withdrawn from the study. Wean by 25% of last study dose every 2 days until off. Participants should complete weaning after 6 days (Table 5).

Table 5 Weaning Schedule for Study Sildenafil or Placebo in Cohorts 2 and 3

Weaning Days	IV	Enteral
1-2	75% of last study dose	75% of last study dose
3-4	50% of last study dose	50% of last study dose
5-6	25% of last study dose	25% of last study dose
7	Discontinue	Discontinue

Abbreviation: IV, intravenous

If a participant in Cohort 2 or 3, escalates to a dose of ≥ 0.5 mg/kg IV (or ≥ 1 mg/kg enteral) and then is withdrawn from study drug, they will be withdrawn using the weaning schedule outlined above. Deviation from the weaning schedule for documented safety concerns will not result in a protocol deviation.

Weaning is not required for participants in Cohort 1 as the risk of rebound effects is likely minimal for infants receiving low-dose sildenafil.

6.1.1 *Study Placebos*

Intravenous placebo is sterile saline or dextrose 5% and enteral placebo is dextrose 5%.

6.1.2 *Acquisition*

Study drug and placebo will be acquired from the hospital pharmacy. Enteral sildenafil will be supplied to the site pharmacy by Pfizer, Inc. and will be labelled for investigational use. IV sildenafil will be supplied by the site using commercially available products (“off the shelf”).

6.1.3 *Formulation, Packaging, and Labeling*

Intravenous formulation: Only marketed IV formulation for IV administration will be used. This protocol will not specify the brand of product. Each product will be “off the shelf” as provided by the site’s pharmacy. Placebo will be an equal volume of sterile saline or dextrose 5%.

Enteral formulation: The enteral formulation of sildenafil (Revatio®) will be provided for the study by Pfizer, Inc. Study drug will be shipped to each site pharmacy by the drug distribution vendor, Almac. Placebo will be an equal volume of dextrose 5%.

Any requisite clinical trial materials will be provided with labeling in accordance with applicable regulatory requirements. Information regarding dosage, preparation and administration of study drug is detailed in Section 6.2 below.

6.1.4 *Product Storage and Stability*

All study drugs must be stored in a secure area with access limited to the investigator and authorized site staff and under physical conditions that are consistent with product-specific requirements provided in the product labeling.

Detailed information on handling storing and reconstitution is found in the package insert.

6.2 Dosage, Preparation and Administration of Study Product

The investigational pharmacist will be unmasked, and will prepare masked study drug. Detailed information will be included in the MOP. The pharmacy at each site will prepare and distribute the study drug in a masked manner and drug will be administered by the bedside nurse.

Intravenous doses of study drug will be administered as infusion over 60 minutes followed by 30 minutes of flush.

Enteral doses will be administered with feedings. The enteral formulation, if used, will be administered enterally either by mouth, orogastric, nasogastric, gastrostomy tube, or other enteral tubes. For enteral administration in infants receiving bolus feedings, mixing and timing of administration may follow the institutional policy. If there is no specific institutional policy, it is recommended that the study drug be mixed in 10 mL of feedings to be given at the end of the feed. If feeds are administered on pump, timing of administration may follow the institutional policy. If there is no specific institutional policy, it is recommended that study drug be mixed in the last 30 minutes of feeding volume.

If the participant experiences signs or symptoms deemed by the investigator to be clinically significant hypotension or study drug-related SAE, the infusion or feeding will be stopped. Continued study drug administration will be decided by the clinical team.

Refer to the MOP for specific details and recommendations for the use of IV and enteral dilutions for small drug volumes.

6.3 Modification of Study Product Administration for a Participant

As noted in Section 6.1, dosing may be adjusted according to any weight changes determined at baseline, and study Days 7, 14 and 21. DSMB safety review is required prior to enrolling participants to the next cohort (Section 10).

6.4 Accountability Procedures for the Study Product

Site pharmacies will have accountability for the study drug and placebo. Monitoring will be conducted to review compliance.

6.5 Concomitant Medications/Treatments

All drug and/or treatments are permitted while on study. Concomitant medications received within 24 hours prior to start of study drug and those given during the 28-day treatment period will be recorded.

6.5.1 Concomitant Medications of Special Interest

The following medications of special interest will be recorded for the entire study period:

- Furosemide
- Bumetanide
- Chlorothiazide
- Hydrochlorothiazide
- Spironolactone
- Inhaled nitric oxide
- Bosentan
- Epoprostenol sodium
- Milrinone
- Sildenafil

7 STUDY SCHEDULE

7.1 Table 6 Schedule of Study Procedures

	Screen/ Baseline	Treatment	Weaning[1] Cohort 2 and 3	Follow-up	Final study assessment
Time (Day)	Predose[2]	1-28[3] (± 1 Day)	Weaning Day 1-6	Day 1-28 post last study drug dose	Discharge or Transfer
Informed consent	X				
Randomization[4]	X				
Demographics[5]	X				
Physical examination	X			X (weekly)	X
Medical history	X				
Actual Weight	X	X (weekly)		X (weekly)	X
Mean arterial pressure	X	X	Last day of wean	X (weekly)	
Respiratory assessment	X	X[6] (daily)	X[6] (daily)	X[6] (daily)	X
Laboratory evaluations[7]	X	X (every 2 weeks)	X	X	
Study drug administration		X[8]	X		
Concomitant medications	X	X	X		
Concomitant medications of special interest[9]	X	X	X	X	X
Adverse events (including SAEs and ESIs)[10]	X	X	X	X	
Global Rank					X
Echocardiogram	X	X[11]	X[11]	X	X[11]
Cardiac catheterization reports[11]	X	X	X	X	X
PK sampling		X (after Day 7)			
Discharge information, including ROP[12]					X

Abbreviations: AE, adverse event; PK, pharmacokinetics; PMA, postmenstrual age; ROP, retinopathy of prematurity; SAE, serious adverse event

[1] Weaning for Cohort 2 and 3 will begin following Day 28 or following the last day of study drug if participant was withdrawn from study drug prior to Day 28 and the dose was escalated to ≥ 0.5 mg/kg IV or ≥ 1 mg/kg enteral.

[2] Refers to < 24 hours (except laboratory evaluations) and < 14 days (for echocardiogram) prior to start of study drug that these procedures may be conducted and may be the same calendar date as Day 1; informed consent may be obtained any time prior to the initiation of any study procedures.

[3] Collect all safety follow-up and remaining assessments if early withdrawal of study drug occurs during the 28-day treatment period. AE and SAE follow-up is through 28 days post last dose of study drug.

[4] Randomization will occur on Day -1 (1 day prior to first day of treatment).

[5] Participant demographics (including birthweight, gestational age at birth) and maternal race/ethnicity will be collected.

[6] Recorded daily during the treatment, weaning, and follow-up periods.

[7] If not performed per standard of care, AST and ALT must be performed within 72 hours prior to randomization and at least every 2 weeks during the treatment period. When available, the following labs obtained during the treatment, weaning, and follow up periods will be recorded in the EDC if obtained per standard of care: ALT, AST, platelets, phosphorus, chloride, alkaline phosphatase, and caffeine levels.

[8] Dosing may be adjusted according to any weight changes determined at baseline, study Days 7, 14, and 21.

[9] Concomitant medications of special interest will be recorded during the entire study period.

[10] AEs will be collected following informed consent through 28 days post last dose of study drug.

[11] If performed per local standard of care

[12] Transfer, discharge, duration of hospitalization or death; record if treatment for ROP was required.

8 STUDY PROCEDURES/EVALUATIONS

8.1 Study Procedures/Evaluations

- A focused medical history of the participant will be extracted from the medical record.
- Concomitant medication information including name, route of administration, and dose will be extracted from the medical record.
- Physical examination including the vital signs (MAP) listed under the study schedule will be extracted from the medical record.
- Echocardiogram images will be uploaded for central review and interpretation at the coordinating center.
- Echocardiogram dates and reports will be collected in the EDC.
- Cardiac catheterization dates and reports will be collected in the EDC.
- Biological specimen collection will be performed per local procedures. See the MOP for further detail about biological specimen handling, storage, and shipping.
- Respiratory assessments will be extracted from the medical record (see Section 8.2.2.3 for more details).
- Discharge information will be extracted from the medical record.
- Protocol-specified MAP values will be obtained per local procedures.
- Blood pressure will be determined using MAP. For each participant, MAPs will be taken as noted in Section 4.2. Mean arterial pressure will be measured using an appropriate size cuff or with an intra-arterial placed catheter that measures continuous blood pressure.

8.2 Laboratory Procedures/Evaluations

8.2.1 *Clinical Laboratory Evaluations*

Laboratory evaluations for ALT and AST, if not already obtained per standard of care during the times indicated, must be conducted within 72 hours prior to randomization, and at least once every 2 weeks during the treatment period (at any time during the 2 week period).

If laboratory evaluations for ALT and AST are obtained more frequently than required by protocol, all results must be entered into the EDC system.

Standard of Care laboratory evaluations: When available, all values for ALT, AST, platelets, phosphorus, chloride, alkaline phosphatase, and caffeine levels within 72 hours prior to randomization through 28 days following the last dose of study drug (include weaning doses) will be recorded.

8.2.2 *Special Assays or Procedures*

8.2.2.1PK Sampling

Table 7 below provides the optimal PK sampling collection windows. Blood samples will be collected after any dose following the completion of 7 days (168 hours) of study drug administration at the target dose for that cohort. Every effort should be made to collect plasma samples within the windows; however, samples obtained outside of the sampling windows will not be considered protocol deviations. Sample collection windows are relative to the end of the infusion and flush (for IV administration); all samples should be collected after the flush. Blood samples should not be drawn during infusions or during the flush. Elimination samples will only be obtained around the last dose of study drug.

Table 7 Target PK Sampling Times

	Intravenous or enteral per clinical care	
	Time*	
PK #	*Enteral***	*Intravenous***
1	0–15 minutes	0–15 minutes
2	30–60 minutes	30–60 minutes
3	1-2 hours	1-2 hours
4	2–3 hours	2–3 hours
5	3–4 hours	3–4 hours
6	4-5 hours	4-5 hours
7	15 minutes prior to next dose	15 minutes prior to next dose
8^ (elimination)	16–24 hours	16–24 hours

* Time is relative to the end of flush, which must be less than or equal to 30 minutes
** May be drawn around more than 1 dose.
**Sample is taken after dose of study drug (and flush if given by IV).
^ Sample taken 16-24 hours after last dose.

8.2.2.2 Pharmacogenomics

Because the major sildenafil metabolizing enzyme, CYP3A4/CYP3A5, is known to exhibit significant genetic polymorphisms altering its activity, we will evaluate polymorphisms as covariates in our PK model. Samples will be obtained from leftover cellular components of plasma samples. DNA samples will be identified by a code number, and other identifying information will be removed. Targeted genetic testing for CYP3A gene locus single nucleotide polymorphisms will be employed.

8.2.2.3Minimizing Blood Loss

Blood samples will be collected in approximately 500 µL blood aliquots. To minimize the amount of blood sampling, a limited sampling scheme will be employed such that no more than 8 timed PK samples (4.0 mL of blood) are obtained from each participant for analysis. This amount of blood loss is safely within accepted 24-hour volumes for infants. Participants in all treatment groups (including placebo) will have samples

collected. Infants assigned to the placebo treatment group will have biomarkers of bronchopulmonary dysplasia measured instead of PK levels at a central laboratory (details in MOP; biomarkers may include NT-proBNP, IL-1, IL-6, IL-8, TNF-α).

8.2.2.4Respiratory Assessment

The following information will be recorded daily during the treatment, weaning, and follow-up periods per the schedule of procedures tables:

1) Fraction of inspired oxygen (FiO2): Blended O_2 is defined as the maximum blended O_2 on day of assessment, unless it is known to be a temporary (< 2 hour) increase in blended O_2.
2) Highest level of ventilation type on day of assessment:
    a. High-frequency ventilator
    b. Conventional mechanical ventilator
    c. NCPAP (or equivalent, see Section 5.1)
    d. NIPPV
    e. Noninvasive NAVA
    f. Nasal cannula LPM
    g. None (room air with no support)
3) Highest Mean Airway Pressure on day of assessment (if receiving invasive mechanical ventilation)

8.2.2.5Global Rank

Each participant will receive a rank based upon the lowest ranking endpoint (1 = worst) that they experienced during their participation in the study.

Details on clinical events and rank included in the global rank endpoint will be provided in the Statistical Analysis Plan.

8.2.3 *Specimen Preparation, Handling, and Storage*

See the MOP for further detail about biological specimen handling, storage, and shipping.

8.2.4 *Specimen Shipment*

See the MOP for further detail about specimen shipment.

9 ASSESSMENT OF SAFETY

9.1 Methods and Timing for Assessing, Recording and Analyzing Safety Parameters

The sponsor has engaged Duke Clinical Research Institute (DCRI) Safety Surveillance to oversee real-time SAE collection, evaluation, and expedited regulatory reporting for this study. A DCRI Safety Medical Monitor will be responsible for evaluating site reported SAEs to confirm protocol-specific serious reporting criteria, causality

assessment, and expectedness compared to the product label. Details of this process can be found in the study-specific Safety Management Plan.

Safety will be assessed following informed consent through 28 days post last study drug dose (last study drug dose includes weaning doses). Safety will be assessed by frequency and incidence of the primary safety outcome (hypotension), events of special interest (ESI), and SAEs. A DSMB will be convened to review data and safety information from study participants throughout the study and prior to opening of Cohorts 2 and 3.

Monitoring for hypotension, ESIs, and SAEs will occur from informed consent through 28 days post last study drug dose (including the weaning doses).

9.2 Specification of Safety Parameters

Safety parameters to be assessed include physical examination, weight, MAP, respiratory assessment, laboratory evaluations, echocardiograms, and cardiac catheterization reports.

Safety events will be assessed and reported as described in the following sections.

9.2.1 *Unanticipated Problems*

The Office for Human Research Protections considers unanticipated problems involving risks to subjects or others to include, in general, any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- Related or possibly related to participation in the research (“possibly related” means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

9.2.2 *Serious Adverse Events*

An AE or Suspected Adverse Reaction (SAR) or Adverse Reaction is considered “Serious” if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Results in death
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred)

- Results in inpatient hospitalization or prolongation of existing hospitalization
- Results in a persistent or significant disability or incapacity
- An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

9.2.3 *Serious Adverse Event Reporting Timelines*

Hypotension and SAEs will be entered into the EDC system within 24 hours of a site's knowledge of the event.

If the EDC system is temporarily unavailable, the SAE, including site investigator-determined relationship assessment (Section 9.4.1), should be reported to the sponsor's Safety Surveillance via a paper back-up SAE form. Upon return of the availability of the EDC system, the most current SAE information must be entered on the AE/SAE eCRF.

Events of special interest are to be entered into the EDC system within 7 days of a site's knowledge of the event.

Investigators must also submit safety reports locally as required by their IRB.

9.2.4 *Events of Special Interest*

The patients enrolled in this trial are initially critically ill and are deemed to require prolonged NICU care. Such individuals are known to have a high morbidity and mortality rate. The following events will not be included in the primary safety outcome, but they are clinical ESI that may relate to safety and that will be tracked and reported in the trial results. These AEs are to be reported to study EDC, regardless of whether these events meet serious criteria or unexpectedness:

- Sepsis, defined as positive blood culture with an organism not typically considered a contaminant.
- Urinary tract infections, defined as a positive urine culture with an organism not typically considered a contaminant
- Bacterial meningitis, defined as positive cerebrospinal fluid culture with an organism not typically considered a contaminant
- Retinopathy of prematurity (ROP), defined as treatment with laser photocoagulation or an anti-VEGF drug
- Seizures, determined by the treating provider
- Abnormal hearing test results
- Systemic arterial or deep venous thrombosis requiring treatment with anticoagulation (e.g., heparin or LMW heparin),
- Direct hyperbilirubinemia, defined as conjugated serum bilirubin > 2.0 mg/dL
- ALT > 150 U/L or AST > 225 U/L
- Endotracheal intubation and transition from non-invasive to invasive ventilation, not due to planned surgical procedure (e.g., inguinal hernia repair or ROP treatment)

9.2.5 *Respiratory Support Escalation*

Sites will record the escalation from non-invasive ventilation to invasive ventilation in the EDC within 24 hours of knowledge of the escalation to invasive ventilation including the event, unless the escalation is due to a planned surgical procedure. If the reason for escalation to invasive ventilation met serious adverse event criteria, the site will record the final diagnosis as an SAE on the SAE eCRF. The DSMB will be notified of the escalation within 1-2 business days by the sponsor or designee.

9.2.6 *Time Period and Frequency for Event Assessment and Follow-Up*

The site investigator will record all SAEs and ESIs with a start date occurring from informed consent through 28 days after the last dose of study drug (last study drug dose includes weaning doses).

All hypotension and SAEs (study-drug related or not) must be followed until resolution. Events that cannot be resolved by 30 days after the safety-monitoring period will have the status of the ongoing event entered in the EDC system at that time.

In cases when the participant has been transferred to another facility or discharged home, researchers may access test, treatment, and outcome information related to the event from the new treating facility and/or contact the participant/guardian.

9.3 Characteristics of an Adverse Event

Each hypotension event, SAE and other clinical ESI will be assessed by the site investigator for the characteristics in the following sections.

9.3.1 *Relationship to Study Intervention*

The investigator will use the following question when assessing relatedness of an event of hypotension, ESI, or an SAE to study drug and study procedures, where an affirmative answer designates the event is a SAR:

- Is there a reasonable possibility that the drug caused the event?

‘‘Reasonable possibility’’ means that there is evidence to suggest a causal relationship between the drug and the adverse event.

To assess relationship of an event to study intervention the following guidelines are used:

1. Related (Possible, Probable, Definite)
    a. The event is known to occur with the study intervention, and/or
    b. There is a temporal relationship between the intervention and event onset and/or
    c. The event abates when the intervention is discontinued, and/or
    d. The event reappears upon a re-challenge with the intervention.
2. Not Related (Unlikely, Not Related)
    a. There is no temporal relationship between the intervention and event onset, and/or
    b. An alternate etiology has been established.

9.3.2 *Severity of Event*

The investigator should use the following definitions when assessing intensity of an event:

1. Mild: Participant is aware of symptoms or has minor findings but tolerates them well, and no or minimal intervention required
2. Moderate: Participant experiences enough symptoms or findings that may warrant intervention
3. Severe: Participant experiences symptoms or findings that significantly affect clinical status and warrant intervention

9.4 Adverse Event Reporting Procedures

All hypotensive events and SAEs, as defined above, based on physical examination, laboratory findings, MAP measurements, or other means, will be reported in the EDC system within 24 hours of first knowledge of the event. The investigator or designated site personnel will record the required information regarding the hypotension, ESI, or SAE on the appropriate eCRF data fields including, relationship assessment to study drug, outcome, and whether the event met serious criteria.

The DSMB will review hypotension and SAEs on a regular basis. In addition, a qualified and experienced clinician not otherwise associated with this protocol will serve as the Safety Medical Monitor. The Safety Medical Monitor will review valid SAEs at the time they are reported. If safety concerns are identified, the medical monitor may request a meeting of the DSMB to review safety data. At a minimum, the medical monitor will comment on the outcomes of the SAE and causal relationship of the SAE to the study drug. If no SAEs prompt review at an earlier time point, the DSMB will review safety events at the regularly scheduled meeting. Additionally, the DSMB will periodically review interim safety analyses (Section 10). The DSMB will convene and make recommendations on continuation of the study based on review of safety reports and halting rules.

9.4.1 *Expectedness*

The expectedness of a drug-related event (hypotension or SAE) will be determined according to the reference documents containing safety information (the package insert and the protocol). Any SAE that is not identified in nature, severity, or specificity in the current study package insert or protocol is considered unexpected. Events that are mentioned in the package insert or investigational plan as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but not specifically mentioned as occurring with the particular drug under investigation are considered unexpected. The study PI and/or study-appointed, clinically/medically -responsible individual will determine whether an AE is expected or unexpected.

9.4.2 *Reporting of Safety Events to FDA*

The Sponsor or designee will notify the FDA and all participating investigators in a written IND safety report of an SAR that, based on the opinion of the investigator or sponsor, is serious, is related to study drug, and is unexpected (per the sponsor) as soon as possible, but not later than 15 calendar days after the sponsor has determined the serious, unexpected SAR (SUSAR) qualifies for expedited reporting. ESI will not be reported as SUSARs. The sponsor will identify all safety reports previously filed with the IND concerning a similar SAR, and will analyze the significance of the SAR in light of the previous, similar reports. Follow-up reports will be sent to investigators to inform and update them about an important SAR if it significantly affects the care of the patients or conduct of the study.

The site investigator will be responsible for reporting adverse events and unanticipated problems involving risks to patients to their local IRBs/IECs in accordance with local regulations.

9.4.3 *Reporting of Hypotension*

If a hypotension event meets serious criteria, it will be reported as an SAE.

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.

9.4.4 *Reporting of Pregnancy*

As the participants of this clinical trial are neonates, this section is not applicable.

9.5 Halting Rules

9.5.1 *Discontinuation of a Participant Due to Adverse Events*

Participants may be withdrawn from the study at any time. Participants withdrawn from the study due to a hypotensive event, ESI, or SAE must be followed by the investigator until the clinical outcome from the event is determined, or 28 days after the safety-monitoring period, whichever comes first. Any participant who experiences an event may be withdrawn at any time from the study at the discretion of the investigator. The AE(s) resulting in discontinuation should be noted on the appropriate eCRFs, and the participant's progress should be followed until the AE is resolved or considered stable. The medical monitor or project manager must be notified. If the AE may relate to overdose of study treatment, the package insert should be consulted for details of any specific actions to be taken.

9.5.2 *Unscheduled DSMB Review and Study Discontinuation*

An unscheduled DSMB review of safety data will be triggered if:

- ≥ 3 patients in a cohort have treatment discontinued or an infusion stopped due to the same type of event; or
- ≥ 3 patients in a cohort have a SAR.

Enrollment will be suspended during DSMB review, although study activities will proceed on previously enrolled subjects as applicable.

If the study is discontinued, enrolled participants will continue to be followed for safety assessments for 28 days.

10 STUDY OVERSIGHT

An independent DSMB will monitor the progress of the study to ensure study participant safety. Responsibilities of the DSMB will include review of accumulated safety data and assessments to ensure the safety of participating study participants and overall integrity of the study, provide recommendations regarding the further conduct of the study and identify any safety issues that may suggest risk to the participants enrolled in the study

or prospective participants. The DSMB will complete an unblinded review of the safety data after 80% of infants within the cohort complete 14 days of treatment. Enrollment to the next cohort will not begin until after the DSMB has completed their review and provided a recommendation that the study proceed.

A DSMB charter will outline the membership, responsibilities, scope of activities, meeting frequency and communication plan between committee, sponsor, DCRI, and study sites.

11 CLINICAL SITE MONITORING

Clinical site monitoring will be conducted to ensure that human participant protection, study procedures, laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, GCP/ICH, and regulatory guidelines, and that the study is conducted in accordance with the protocol and sponsor standard operating procedures. The IND sponsor, or its designee will conduct site-monitoring visits as detailed in the Clinical Monitoring Plan (CMP).

Most monitoring activities will be performed remotely, while other monitoring will take place at the study sites. Study team staff from the DCRI will conduct monitoring activities and provide reports of the findings and associated action items in accordance with the details described in the CMP. Documentation of monitoring activities and findings will be provided to the site study team.

12 STATISTICAL CONSIDERATIONS

12.1 Study Endpoints

Primary safety endpoints are the incidence of hypotension. Secondary endpoints include PK and preliminary effectiveness (PK/PD).

12.2 Sample Size Considerations

The sample size of 30 (sildenafil) in each dose group is sufficient to estimate hypotension incidence with sufficient precision. Table 8 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. If hypotension occurs with an incidence rate of 0.05, it 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 8 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

Abbreviations: CI, confidence interval; N, number of participants

Populations for Analysis

Intention to Treat (ITT) Population: Participants whose parent or guardian signs informed consent and are randomized.

Per Protocol (PP) Population: Participants who receive at least 7 days of study medication within 9 days (to allow for one pause in medication administration for up to 48 hours).

Safety Population: Participants who receive at least 1 dose of study medication.

PK Population: All participants who have at least one interpretable PK sample.

12.3 Planned Interim Analyses

12.3.1 *Safety Review*

The DSMB will complete an unblinded review of the safety data after 80% of infants within the cohort complete 14 days of treatment. Enrollment to the next cohort will not begin until after the DSMB has completed their review and provided a recommendation that the study proceed.

See Section 9.6 for additional details for halting rules due to safety concerns.

12.4 Final Analysis Plan

General Analysis Conventions

A comprehensive overview of planned statistical analyses will be provided in a separated statistical analysis plan.

Analysis results will be presented by placebo versus sildenafil, overall and by dose cohort. Data from participants assigned to placebo across cohorts will be pooled in result presentations.

Descriptive statistics such as number of observations, mean, median, standard deviation, minimum and maximum will be presented by treatment groups for continuous variables (such as age, weight, etc.). Other descriptive statistics such as counts, proportions, and/or percentages will be presented by group to summarize discrete variables (such as race, sex, etc.).

Participant Disposition and Study Drug Exposure

Study disposition, demographic and baseline characteristics, and study medication exposure will be summarized for the ITT Population. Study drug administration will be summarized by route (IV vs enteral), number of days of dosing, and reasons for final discontinuation of study drug.

Demographic and baseline characteristics

Demographic and baseline characteristics will include race, age, sex, and selected clinical variables recorded prior to initiation of study drug.

Safety

The primary safety endpoint is number and percent of hypotension events and will be summarized for the PP and Safety Population.

Serious adverse events will be presented overall and by each Medical Dictionary for Regulatory Activities (MedDRA) system organ class and preferred term. Severity and relationship to sildenafil or placebo will be provided.

Events of special interest will be presented overall and summarized by participant characteristics.

Prior and concomitant medications will be summarized by World Health Organization (WHO) drug class.

Laboratory data, including change from baseline, will be tabulated by treatment groups.

PK Analysis Plan

A comprehensive overview of planned PK analyses will be provided in a separated PK analysis plan.

Pharmacokinetic parameters will be estimated by population PK approach using nonlinear mixed effects modeling in NONMEM. The influence of covariates on PK parameters will be explored.

Biomarkers

We will relate the concentration of biomarkers (Section 8.2) to the development of and severity of PH in participants exposed to placebo.

Preliminary Effectiveness

A multivariable mixed-effects model will be used to explore the relationship between the maximum and total dose of sildenafil and change in PH, death, and death or PH at 28 days of study drug and at the end of hospitalization or 52 weeks PMA, whichever comes first. We will use 52 weeks PMA because we found that 95% of infants with severe BPD died or were discharged home by this time.[7]

Planned sensitivity analyses of the primary endpoint

To address the potential effect of multiples enrolled in the study on the primary endpoint, we will conduct the following 2 sensitivity analyses:

- Sensitivity analysis #1 using only the first randomized sibling from any multiple gestations.
- Sensitivity analysis #2 using generalized estimating equations (GEE) with log link random effect for siblings to compute ratios of hypotension between the sildenafil and placebo groups.

13 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

An eCRF will be used to record participation data. The eCRF will be used for the recording of all historical participant information and study data as specified by this protocol. The eCRF must be completed by designated and trained study personnel.

According to ICH E6 (R2), source data are defined as all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies). Source documents are defined as original documents, data, and records (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, participants' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, participant files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial).

It will be the responsibility of the investigators to ensure that the regulatory binder at the site is maintained. The study file will contain, but will not be limited to:

- Current package inserts, and all previous versions
- Current study protocol
- Protocol amendments (if applicable)
- Manual of Procedures
- Informed permission form (blank)
- IRB approved informed permission form
- Revised informed permission forms and/or all addenda (blank)
- IRB registration or other documentation of IRB compliance with applicable regulations
- Documentation of IRB/REB approval of protocol, permission form, any protocol amendments, and any permission form revisions
- Annual IRB/REB updates and approvals
- All correspondence between the investigator and IRB/REB

Each participating site will maintain appropriate medical and research records for this trial, in compliance with Section 4.9 of ICH E6 (R2), and regulatory and institutional requirements for the protection of confidentiality of participants. Each site will permit authorized representatives of the sponsor, its designees, and appropriate regulatory agencies to examine (and, when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress. These representatives will be permitted access to all source data.

14 QUALITY CONTROL AND QUALITY ASSURANCE

The site investigator will provide direct access to all trial-related locations, source data/documents, eCRFs, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The site investigator will

ensure that all study personnel are appropriately trained and applicable documentations are maintained on site.

Clinical monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements. Clinical monitoring reports will be submitted to the PI.

The DCRI will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the site(s) for prompt clarification and resolution.

15 ETHICS/PROTECTION OF HUMAN SUBJECTS

15.1 Institutional Review Board

Prior to enrollment of participants into this trial, the protocol, the informed parental permission form, and any materials or advertisements presented to participants or potential participants, will be reviewed and approved by the appropriate IRB.

A copy of the letter of approval will be provided by the site study team to the sponsor. The institution's federal-wide assurance number will be provided to the sponsor.

If amendments to the protocol are required, the amendments will be written by the sponsor and provided to the investigator for submission and approval to the IRB.

15.2 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation in this study will be provided to the parents/legal guardians/legally authorized representatives (LARS) of participants in this study. Permission forms describing in detail the study purpose, duration, nature of participation and responsibilities of participants, procedures and risks are given to participants' parents/guardians/LARs, and written documentation of informed permission is required prior to enrolling in the study.

The participants' parents/legal guardians/LARs will be asked to read and review the document, and the investigator will explain the research study and answer any questions that may arise. The participants' parents/legal guardians/LARs will provide informed permission prior to the conduct of any study procedures. The participant's parents/legal guardians/LARs should have the opportunity to think about the study prior to providing permission for the child to participate. The participants' parents/legal guardians/LARs may withdraw permission at any time throughout the course of the study. A copy of the informed permission document will be given to the parent/legal guardian/LAR for their records. The rights and welfare of the participants will be protected by emphasizing to parents/legal guardians/LARs that the quality of medical care will not be adversely affected if they decline permission for the child to participate or withdraw from participating in this study.

Permission forms will be IRB-approved, and the IRBs will determine whether one parent or both parents must provide permission. It is the sponsor's opinion, that because sildenafil is not part of the usual care for the patients who are eligible for enrollment that only one parent's permission is needed. This is based on the following risk assessment:

- Participants randomized to the placebo arm: These participants are receiving usual/standard of care which is no more than minimal risk. Per §46.404 only one parent's permission is needed;
- Participants randomized to the sildenafil arm: These participants are at more than minimal risk but are presented with the prospect of direct benefit. Per §46.405 only one parent's permission is needed.

Site staff may employ IRB-approved recruitment efforts prior to parent/legal guardian/LAR permission; however, before any protocol-specific procedures are performed to determine protocol eligibility, informed permission must be obtained and properly executed.

By signing the permission form, the participant's parent/legal guardian/LAR agrees that the participant will complete all evaluations required by the trial, unless the participant's parent/legal guardian/LAR withdraws the participant voluntarily or the participant is withdrawn from the trial for any reason.

The consent process will be documented in the research record.

15.3 Exclusion of Women, Minorities, and Children (Special Populations)

This is a study of the safety of sildenafil in premature infants with severe BPD. Individuals of any gender or racial/ethnic group who meet the inclusion/exclusion criteria for the study may participate.

15.4 Subject Confidentiality

Participant confidentiality is held strictly in trust by the participating investigators, their staff, and the study sponsor and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests, in addition to the clinical information relating to participating participants. Participants will be assigned unique code numbers and will not be identifiable. Birth dates and date of death or discharge are collected in this study.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the study sponsor.

The clinical study site will permit access to all documents and records that may require inspection by the sponsor, Regulatory Authorities or its authorized representatives, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study.

In the US, the Health Insurance and Portability and Accountability (HIPAA) Privacy Rule applies. The rule provides US federal protection for the privacy of protected health information sent to or collected in the US for the purposes of this research by implementing standards to protect and guard against the misuse of individually identifiable health information of participants participating in clinical trials. “Authorization” is required from each research participant (i.e., specific permission granted by an individual to a covered entity for the use or disclosure of an individual’s protected health information). A valid authorization must meet the implementation specifications under the HIPAA Privacy rule.

Certificate of Confidentiality

To protect further the privacy of study participants, the Secretary, Health and Human Services (HHS), has issued a Certificate of Confidentiality to all researchers engaged in biomedical, behavioral, clinical, or other human subjects research funded wholly or in part by the federal government. Recipients of NIH funding for human subjects research are required to protect identifiable research information from forced disclosure per the terms of the NIH Policy (*https://humansubjects.nih.gov/coc/index*). As set forth in 45 CFR Part 75.303(a) and NIHGPS Chapter 8.3, recipients conducting NIH-supported research covered by this Policy are required to establish and maintain effective internal controls (e.g., policies and procedures) that provide reasonable assurance that the award is managed in compliance with Federal statutes, regulations, and the terms and conditions of award. It is the NIH policy that investigators and others who have access to research records will not disclose identifying information except when the participant consents or in certain instances when federal, state, or local law or regulation requires disclosure. NIH expects investigators to inform research participants of the protections and the limits to protections provided by a Certificate issued by this Policy.

NIH Data Sharing Policies

As described in Section 17, it is NIH policy that the results and accomplishments of the activities that it funds should be made available to the public (see *https://grants.nih.gov/policy/sharing.htm*). PIs and funding recipient institutions will ensure that all mechanisms used to share data include proper plans and safeguards to protect the rights and privacy of individuals who participate in NIH-sponsored research.

15.5 Future Use of Stored Specimens and Other Identifiable Data

Biological samples collected as part of this study may be shipped periodically to the central laboratory(s) for protocol defined testing (e.g., drug concentration measurements of PK samples).

After the study is completed, residual biological samples (e.g., plasma) from this study will be submitted to an NIH storage facility in accordance with applicable privacy laws and/or IRB determinations. These samples will not include any personal identifiers. They will be labeled with a unique code. The NIH repository will not have access to any personally identifying participant information. With NIH approval, the de-identified study samples may be made available to other researchers.

Parents/legal guardians/LARs are asked to provide informed consent/permission for the biological sample collection process, specimen repository, and potential for future research prior to the child's participation in the study.

16 DATA HANDLING AND RECORD KEEPING

US investigators are obligated to conduct this study in accordance with US Federal Regulation 21 CFR 312.60-69 as specified on the signed form FDA 1572, applicable local laws, and the ICH: GCP: Consolidated Guidance (ICH E6[R2]).

The investigator is responsible for informing the IRB of any safety issues related to the study and the study drug, including reports of SAEs, if required, and all expedited safety reports.

The investigator is responsible for ensuring that attributable, legible, contemporaneous, original, accurate, and complete data or records are recorded and reported.

Data reported in the eCRF should be consistent with the source documents, or the discrepancies should be documented. The sponsor and/or its designee will provide guidance to investigators on making corrections to the eCRFs and eCRF.

16.1 Data Management Responsibilities

All eCRFs and laboratory reports must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. Adverse events must be graded, assessed for severity and causality, and reviewed by the site PI or designee. Data collection is the responsibility of the study staff at the site under the supervision of the site PI. During the study, the investigator must maintain complete and accurate documentation for the study.

The DCRI will be responsible for data management, quality review, analysis, and reporting of study data.

16.2 Data Capture Methods

Clinical data (including SAEs and concomitant medications) will be entered into a 21 CFR Part 11-compliant internet data entry system provided by the DCC. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from source documents.

16.3 Types of Data

We will collect clinical and pharmacokinetic laboratory data. Safety data will include blood pressure measures, hypotension events, events of special interest and SAEs. Outcome data includes clinical diagnoses, echocardiograms and their reports, and cardiac catheterization reports.

16.4 Schedule and Content of Reports

Not applicable

16.5 Study Records Retention

The investigator must retain all study records and source documents for a minimum of 2 years following the completion of the study, unless the sponsor requests longer retention, or local regulations and institutional policies require longer retention.

If the investigator withdraws from the study (e.g., relocation, retirement), the records shall be transferred to a mutually agreed upon designee (e.g., another investigator, IRB). Notice of such transfer will be given in writing to the sponsor.

16.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol. The noncompliance may be on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with GCP:

4.5. Compliance with Protocol, Sections 4.5.1, 4.5.2, and 4.5.3
5.1. Quality Assurance and Quality Control, Section 5.1.1
5.2. Noncompliance, Sections 5.20.1, and 5.20.2

It is the responsibility of the site to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation or within 5 working days of the scheduled protocol-required activity. All deviations must be promptly reported to the sponsor, via the DCC's EDC.

All deviations from the protocol must be addressed in study eCRFs. A completed copy of the protocol deviation form must be maintained in the regulatory file. Protocol deviations must be submitted to the local IRB per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB requirements.

17 PUBLICATION/DATA SHARING

Following completion of the study, the investigator may publish the results of this research in a scientific journal. All public presentations (abstracts, manuscripts, slides and text of oral or other presentations, and text of any transmission through any electronic media) by participating investigators, participating institutions, and DCC, will be reviewed by the Publication Committee per the Publication Committee charter.

The Publication Committee guarantees that the study results are presented by experts in the field that have working knowledge of the study design, implementation, data synthesis/analysis, and interpretation. The committee goals are to ensure that any confidential or proprietary information is protected, and that all appropriate statistical analyses have been included.

The Publication Committee will adhere to the trials registration policy adopted by the International Committee of Medical Journal Editors (ICMJE) member journal. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Other

biomedical journals are considering adopting similar policies. It is the responsibility of the IND holder to register this trial in an acceptable registry.

All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. Refer to *http://publicaccess.nih.gov/ and http://grants.nih.gov/grants/guide/notice-files/NOT-OD-08-033.html*.

This study will comply with all applicable NIH Data Sharing Policies. See *https://grants.nih.gov/policy/sharing.htm* for policies and resources.

NIH Public Access Policy

The NIH *Public Access Policy* requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to *PubMed Central* immediately upon acceptance for publication. This ensures that the public has access to the published results of NIH funded research.

NIH Policy on the Dissemination of NIH-Funded Clinical Trial Information

The study is a clinical trial and will comply with the NIH policy that establishes the expectation that all investigators conducting clinical trials funded in whole or in part by the NIH will ensure that these trials are registered at ClinicalTrials.gov, and that results of these trials are submitted to ClinicalTrials.gov.

Food and Drug Administration Amendments Act of 2007 (FDAAA) and the Final Rule for Clinical Trials Registration and Results Information Submission

This study is an applicable clinical trial and will comply with *U.S. Public Law 110-85* (Food and Drug Administration Amendments Act of 2007 or FDAAA), Title VIII, Section 801 and *42 CFR Part 11* (HHS Final Rule for Clinical Trials Registration and Results Information Submission), which mandate that a "responsible party" (i.e., the sponsor or designated principal investigator) register and report results of "applicable clinical trials."

18 LITERATURE REFERENCES

1. Revatio (sildenafil) [package insert]. New York, NY: Pfizer Labs; 2014. http://labeling.pfizer.com/ShowLabeling.aspx?id=645.

2. Gonzalez D, Laughon MM, Smith PB, et al. Population pharmacokinetics of sildenafil in extremely premature infants. *Br J Clin Pharmacol*. December 2019:bcp.14111. doi:10.1111/bcp.14111

3. Stoll BJ, Hansen NI, Bell EF, et al. Trends in Care Practices, Morbidity, and Mortality of Extremely Preterm Neonates, 1993-2012. *JAMA*. 2015;314(10):1039. doi:10.1001/jama.2015.10244

4. Jobe AH, Bancalari E. Bronchopulmonary Dysplasia. *Am J Respir Crit Care Med*. 2001;163(7):1723-1729. doi:10.1164/ajrccm.163.7.2011060

5. Khemani E, McElhinney DB, Rhein L, et al. Pulmonary Artery Hypertension in Formerly Premature Infants With Bronchopulmonary Dysplasia: Clinical Features and Outcomes in the Surfactant Era. *PEDIATRICS*. 2007;120(6):1260-1269. doi:10.1542/peds.2007-0971

6. Morrow CB, McGrath-Morrow SA, Collaco JM. Predictors of length of stay for initial hospitalization in infants with bronchopulmonary dysplasia. *J Perinatol*. 2018;38(9):1258-1265. doi:10.1038/s41372-018-0142-7

7. Jackson W, Hornik CP, Messina JA, et al. In-hospital outcomes of premature infants with severe bronchopulmonary dysplasia. *J Perinatol*. 2017;37(7):853-856. doi:10.1038/jp.2017.49

8. Poets CF, Lorenz L. Prevention of bronchopulmonary dysplasia in extremely low gestational age neonates: current evidence. *Arch Dis Child - Fetal Neonatal Ed*. 2018;103(3):F285-F291. doi:10.1136/archdischild-2017-314264

9. Tyson JE, Wright LL, Oh W, et al. Vitamin A Supplementation for Extremely-Low-Birth-Weight Infants. *N Engl J Med*. 1999;340(25):1962-1968. doi:10.1056/NEJM199906243402505

10. Schmidt B, Roberts RS, Davis P, et al. Caffeine Therapy for Apnea of Prematurity. *N Engl J Med*. 2006;354(20):2112-2121. doi:10.1056/NEJMoa054065

11. Doyle LW, Ehrenkranz RA, Halliday HL. Early (< 8 days) postnatal corticosteroids for preventing chronic lung disease in preterm infants. In: The Cochrane Collaboration, ed. *Cochrane Database of Systematic Reviews*. Chichester, UK: John Wiley & Sons, Ltd; 2014:CD001146.pub4. doi:10.1002/14651858.CD001146.pub4

12. Mourani PM, Sontag MK, Younoszai A, et al. Early Pulmonary Vascular Disease in Preterm Infants at Risk for Bronchopulmonary Dysplasia. *Am J Respir Crit Care Med*. 2015;191(1):87-95. doi:10.1164/rccm.201409-1594OC

13. Kim D-H, Kim H-S, Choi CW, Kim E-K, Kim BI, Choi J-H. Risk Factors for Pulmonary Artery Hypertension in Preterm Infants with Moderate or Severe Bronchopulmonary Dysplasia. *Neonatology*. 2012;101(1):40-46. doi:10.1159/000327891

14. Stuart BD, Sekar P, Coulson JD, Choi SEJ, McGrath-Morrow SA, Collaco JM. Health-care utilization and respiratory morbidities in preterm infants with pulmonary hypertension. *J Perinatol*. 2013;33(7):543-547. doi:10.1038/jp.2012.170

15. Check J, Gotteiner N, Liu X, et al. Fetal growth restriction and pulmonary hypertension in premature infants with bronchopulmonary dysplasia. *J Perinatol*. 2013;33(7):553-557. doi:10.1038/jp.2012.164

16. Bhat R, Salas AA, Foster C, Carlo WA, Ambalavanan N. Prospective Analysis of Pulmonary Hypertension in Extremely Low Birth Weight Infants. *PEDIATRICS*. 2012;129(3):e682-e689. doi:10.1542/peds.2011-1827

17. Ambalavanan N, Mourani P. Pulmonary hypertension in bronchopulmonary dysplasia: Pulmonary Hypertension in BPD. *Birt Defects Res A Clin Mol Teratol*. 2014;100(3):240-246. doi:10.1002/bdra.23241

18. Liu Z, Fang L. Phosphodiesterase-5 inhibitor and rat lung ischemia-reperfusion injury. *Asian Cardiovasc Thorac Ann*. 2012;20(1):42-47. doi:10.1177/0218492311429867

19. Ladha F, Bonnet S, Eaton F, Hashimoto K, Korbutt G, Thébaud B. Sildenafil Improves Alveolar Growth and Pulmonary Hypertension in Hyperoxia-induced Lung Injury. *Am J Respir Crit Care Med*. 2005;172(6):750-756. doi:10.1164/rccm.200503-510OC

20. de Visser YP, Walther FJ, Laghmani EH, van Wijngaarden S, Nieuwland K, Wagenaar GTM. Phosphodiesterase-4 inhibition attenuates pulmonary inflammation in neonatal lung injury. *Eur Respir J*. 2008;31(3):633-644. doi:10.1183/09031936.00071307

21. de Visser YP, Walther FJ, Laghmani EH, Boersma H, van der Laarse A, Wagenaar GT. Sildenafil attenuates pulmonary inflammation and fibrin deposition, mortality and right ventricular hypertrophy in neonatal hyperoxic lung injury. *Respir Res*. 2009;10(1):30. doi:10.1186/1465-9921-10-30

22. Sun X-Z, Li Z-F, Liu Y, Fang P, Li M-X. Inhibition of cGMP phosphodiesterase 5 suppresses matrix metalloproteinase-2 production in pulmonary artery smooth

muscles cells. *Clin Exp Pharmacol Physiol*. 2010;37(3):362-367. doi:10.1111/j.1440-1681.2009.05304.x

23. Barst RJ, Ivy DD, Gaitan G, et al. A Randomized, Double-Blind, Placebo-Controlled, Dose-Ranging Study of Oral Sildenafil Citrate in Treatment-Naive Children With Pulmonary Arterial Hypertension. *Circulation*. 2012;125(2):324-334. doi:10.1161/CIRCULATIONAHA.110.016667

24. Russell S, Beghetti M, Oudiz R, Balagtas C, Zhang M, Ivy D. Effects of oral sildenafil on exercise capacity in children with pulmonary arterial hypertension: a randomised trial. *Open Heart*. 2019;6(2):e001149. doi:10.1136/openhrt-2019-001149

25. Barst RJ, Beghetti M, Pulido T, et al. STARTS-2: Long-Term Survival With Oral Sildenafil Monotherapy in Treatment-Naive Pediatric Pulmonary Arterial Hypertension. *Circulation*. 2014;129(19):1914-1923. doi:10.1161/CIRCULATIONAHA.113.005698

26. Revatio (sildenafil) [product monograph]. Kirkland, Quebec: Pfizer Canada; 2017. https://pdf.hres.ca/dpd_pm/00041956.PDF.

27. Caputo S, Furcolo G, Rabuano R, et al. Severe pulmonary arterial hypertension in a very premature baby with bronchopulmonary dysplasia: normalization with long-term sildenafil: *J Cardiovasc Med*. 2010;11(9):704-706. doi:10.2459/JCM.0b013e328332e745

28. Hon KE, Cheung K, Siu K, et al. Oral Sildenafil for Treatment of Severe Pulmonary Hypertension in an Infant. *Neonatology*. 2005;88(2):109-112. doi:10.1159/000085646

29. Mourani PM, Sontag MK, Ivy DD, Abman SH. Effects of Long-Term Sildenafil Treatment for Pulmonary Hypertension in Infants with Chronic Lung Disease. *J Pediatr*. 2009;154(3):379-384.e2. doi:10.1016/j.jpeds.2008.09.021

30. Trottier-Boucher MN, Lapointe A, Malo J, et al. Sildenafil for the Treatment of Pulmonary Arterial Hypertension in Infants with Bronchopulmonary Dysplasia. *Pediatr Cardiol*. 2015;36(6):1255-1260. doi:10.1007/s00246-015-1154-0

31. Andersen DH, Blanc WA, Crozier DN, Silverman WA. A difference in mortality rate and incidence of kernicterus among premature infants allotted to two prophylactic antibacterial regimens. *Pediatrics*. 1956;18(4):614-625.

32. Stewart DJ. THE EFFECTS OF TETRACYCLINES UPON THE DENTITION. *Br J Dermatol*. 1964;76(8-9):374-378. doi:10.1111/j.1365-2133.1964.tb14546.x

33. Burns LE, Hodgman JE, Cass AB. Fatal Circulatory Collapse in Premature Infants Receiving Chloramphenicol. *N Engl J Med*. 1959;261(26):1318-1321. doi:10.1056/NEJM195912242612604

34. Yeh TF, Lin YJ, Huang CC, et al. Early Dexamethasone Therapy in Preterm Infants: A Follow-up Study. *PEDIATRICS*. 1998;101(5):e7-e7. doi:10.1542/peds.101.5.e7

35. Aranda J, Clarkson S, Collinge J. Changing Pattern of Drug Utilization in a Neonatal Intensive Care Unit. *Am J Perinatol*. 2008;1(01):28-30. doi:10.1055/s-2007-1000047

36. Du W, Warrier I, Tutag Lehr V, Salari V, Ostrea E, Aranda J. Changing Patterns of Drug Utilization in a Neonatal Intensive Care Population. *Am J Perinatol*. 2006;23(5):279-286. doi:10.1055/s-2006-946719

37. Avenel S, Bomkratz A, Dassieu G, Janaud JC, Danan C. Incidence des prescriptions hors autorisation de mise sur le marché en réanimation néonatale. *Arch Pédiatrie*. 2000;7(2):143-147. doi:10.1016/S0929-693X(00)88083-5

38. O'Donnell CPF, Stone RJ, Morley CJ. Unlicensed and Off-Label Drug Use in an Australian Neonatal Intensive Care Unit. *PEDIATRICS*. 2002;110(5):e52-e52. doi:10.1542/peds.110.5.e52

39. 't Jong GW, Vulto AG, de Hoog M, Schimmel KJM, Tibboel D, van den Anker JN. A Survey of the Use of Off-Label and Unlicensed Drugs in a Dutch Children's Hospital. *PEDIATRICS*. 2001;108(5):1089-1093. doi:10.1542/peds.108.5.1089

40. Rodriguez W, Selen A, Avant D, et al. Improving Pediatric Dosing Through Pediatric Initiatives: What We Have Learned. *PEDIATRICS*. 2008;121(3):530-539. doi:10.1542/peds.2007-1529

41. Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, Kauffman RE. Developmental Pharmacology — Drug Disposition, Action, and Therapy in Infants and Children. Wood AJJ, ed. *N Engl J Med*. 2003;349(12):1157-1167. doi:10.1056/NEJMra035092

42. Benjamin DK, Smith PB, Sun MJM, et al. Safety and Transparency of Pediatric Drug Trials. *Arch Pediatr Adolesc Med*. 2009;163(12). doi:10.1001/archpediatrics.2009.229

43. Spitzer AR, Ellsbury DL, Handler D, Clark RH. The Pediatrix BabySteps® Data Warehouse and the Pediatrix QualitySteps Improvement Project System—Tools for "Meaningful Use" in Continuous Quality Improvement. *Clin Perinatol*. 2010;37(1):49-70. doi:10.1016/j.clp.2010.01.016

44. Thompson E, Perez K, Hornik C, et al. Sildenafil Exposure in the Neonatal Intensive Care Unit. *Am J Perinatol*. 2019;36(03):262-267. doi:10.1055/s-0038-1667378

45. Ahsman MJ, Witjes BC, Wildschut ED, et al. Sildenafil exposure in neonates with pulmonary hypertension after administration via a nasogastric tube. *Arch Dis Child - Fetal Neonatal Ed*. 2010;95(2):F109-F114. doi:10.1136/adc.2009.168336

46. McCrary AW, Barker PCA, Torok RD, et al. Agreement of an echocardiogram-based diagnosis of pulmonary hypertension in infants at risk for bronchopulmonary dysplasia among masked reviewers. *J Perinatol*. 2019;39(2):248-255. doi:10.1038/s41372-018-0277-6

47. Fuwa K, Hosono S, Nagano N, Takahashi S, Nakashima M. Retinopathy of prematurity after sildenafil treatment. *Pediatr Int*. 2017;59(3):360-361. doi:10.1111/ped.13187

48. Fang AYW, Guy KJ, König K. The effect of sildenafil on retinopathy of prematurity in very preterm infants. *J Perinatol*. 2013;33(3):218-221. doi:10.1038/jp.2012.84

49. Samiee-Zafarghandy S, van den Anker JN, Laughon MM, et al. Sildenafil and retinopathy of prematurity risk in very low birth weight infants. *J Perinatol*. 2016;36(2):137-140. doi:10.1038/jp.2015.126

50. Aboudi D, Swaminathan N, Brumberg H, et al. Sildenafil and Retinopathy of Prematurity in Preterm Infants with Bronchopulmonary Dysplasia. *J Pediatr*. 2018;199:16-21. doi:10.1016/j.jpeds.2018.04.005