Safety of Sildenafil in Premature Infants at Risk of Bronchopulmonary Dysplasia

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Pediatric Trials Network: Best Pharmaceuticals for Children Act

Safety of Sildenafil in Premature Infants at Risk of Bronchopulmonary Dysplasia

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Phase 2 Trial

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STATEMENT OF COMPLIANCE

This trial will be conducted in compliance with the protocol, International Council for Harmonisation (ICH) E6 (R2) Guideline for Good Clinical Practice, and the applicable regulatory requirements of the United States and Canada. The US regulations include but are not limited to 45 CFR 46 (Human Subjects Protection); 45 CFR 160 and 164 Subparts A and E (HIPAA Privacy Rule); 21 CFR 312 (Investigational New Drug Application); 21 CFR 50 (Protection of Human Subjects, including Subpart D - Additional Safeguards for Children in Clinical Investigations); and 21 CFR 56 (Institutional Review Boards [IRB]). Applicable Canadian legislation includes: the Food and Drug Act, the Food and Drug Regulations Part C Division 5, and Federal and Provincial Acts and Regulations respecting privacy and/or protection of personal information including health and genetic information.

All individuals responsible for the design and/or conduct of this study have completed Human Subjects Protection Training and are qualified to be conducting this research.

SITE PRINCIPAL INVESTIGATOR STATEMENT

I have read the protocol, including all appendices, and the investigator brochure or product label, and I agree that it contains all necessary details for my staff and me to conduct this study as described. I will personally oversee the conduct of this study as outlined herein and will make a reasonable effort to complete the study within the time designated. I agree to make all reasonable efforts to adhere to the attached protocol.

I will provide all study personnel under my supervision with copies of the protocol and access to all information provided by the sponsor or the sponsor's representative. I will discuss this material with study personnel to ensure that they are fully informed about the efficacy and safety parameters and the conduct of the study in general. I am aware that, before beginning this study, the Institutional Review Board (IRB) or Research Ethics Board (REB) responsible for such matters must approve this protocol in the clinical facility where it will be conducted.

I agree to obtain informed consent forms from participants' parent/legal guardian, as required by government regulations and International Conference on Harmonisation guidelines. I further agree to report to the sponsor or its representative any adverse events in accordance with the terms of this protocol; the U.S. Code of Federal Regulations, Title 21, part 312.64 (US sites); Part C Division 05.014 and C.05.012 (3) (f)(i) of the Canadian Food and Drug Regulations; and ICH GCP 4.11.

_____
Principal Investigator Name (Print)

_____ _____
Signature Date

STUDY PRINCIPAL INVESTIGATOR AND IND SPONSOR SIGNATURE

The signature below documents the review and approval of this protocol and the attachments (e.g., package inserts), and provides the necessary assurances that this clinical 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 to the principles outlined in applicable U.S. federal regulations or Canadian federal and provincial laws and regulations (as applicable) and ICH guidelines.

Matthew M. Laughon, MD, MPH

Pediatric Trials Network Study Principal Investigator Name

Signature

Date

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LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	Adverse Event
ALT	Alanine Aminotransferese
AST	Aspartate Aminotransferese
AUC_{0-24}	Area Under the Concentration Time Curve 0-24 hours
AUC_{ss}	Area Under the Concentration Time Curve at Steady State
BPD	Bronchopulmonary Dysplasia
CFR	Code of Federal Regulations
CL	Clearance
C_{max}	Maximum Concentration
CRF	Case Report Form
DCC	Data Coordinating Center
DCRI	Duke Clinical Research Institute
DSMB	Data Safety Monitoring Board
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture system
FiO_2	Fraction of Inspired Oxygen
FDA	Food and Drug Administration
GA	Gestational Age
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
HFNC	High Flow Nasal Cannula
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDES	Internet Data Entry System
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug Application
IRB	Institutional Review Board
IV	Intravenous
Kg	Kilogram
L	Liter
LPM	Liters per Minute
MedDRA®	Medical Dictionary for Regulatory Activities
mg	Milligram
µg	Microgram
MAP	Mean Arterial Pressure
mm Hg	Millimeter of mercury
MOP	Manual of Procedures
N	Number (typically refers to participants)
NCPAP	Nasal Continuous Positive Airway Pressure
N-DMS	N-Des-methyl Sildenafil
NICU	Neonatal Intensive Care Unit

Abbreviation	Definition
NIH	National Institutes of Health
NRN	Neonatal Research Network
PAH	Pulmonary Arterial Hypertension
PDA	Patent Ductus Arteriosus
PI	Principal Investigator
PK	Pharmacokinetics
PMA	Postmenstrual age (gestational age plus postnatal age)
PNA	Postnatal age
PO	Per os (by mouth)
PTN	Pediatric Trials Network
q	Every
ROP	Retinopathy of Prematurity
SAE	Serious Adverse Event
SAR	Suspected Adverse Reaction
SUSAR	Serious Unexpected Suspected Adverse Reaction
SOC	Standard of Care
TORO	Transfer of Regulatory Obligations
t1/2	Half-life
U/L	Units per liter
WHO	World Health Organization

PROTOCOL SYNOPSIS

Protocol Title:	**Safety of Sildenafil in Premature Infants at Risk of Bronchopulmonary Dysplasia (BPD)**
Phase:	2
Product:	Sildenafil citrate injection and powder for suspension
Objectives:	Primary: Describe the safety of sildenafil in premature infants at risk of BPD Secondary: Preliminary effectiveness and pharmacokinetics (PK) of sildenafil
Study Design:	Multi-center, randomized, placebo-controlled, sequential dose escalating, double masked, safety study
Study Population:	**Inclusion Criteria** 1. Documented informed consent from parent or guardian, prior to study procedures 2. Receiving positive airway pressure (nasal continuous airway pressure, nasal intermittent positive pressure ventilation, or nasal cannula flow > 1LPM) or mechanical ventilation (high frequency or conventional) at the time of randomization* 3. < 29 weeks gestational age at birth 4. 7-28 (inclusive) days postnatal age at time of randomization* **Exclusion Criteria** 1. Previous enrollment and dosing in NICHD-2015-SIL02 Safety of Sildenafil in Premature Infants at Risk of Bronchopulmonary Dysplasia" 2. Previous exposure to sildenafil within 7 days prior to randomization* 3. Currently receiving vasopressors* 4. Currently receiving inhaled nitric oxide* 5. Baseline mean arterial pressure (MAP) < gestational age (in weeks) plus postnatal age (in weeks) within 24 hours of randomization*; e.g. an infant at 25 weeks gestational age and 3 weeks postnatal age with MAP < 28 mm Hg would be ineligible 6. Known allergy to sildenafil 7. Known sickle cell disease 8. AST > 225 U/L < 72 hours prior to randomization* 9. ALT > 150 U/L < 72 hours prior to randomization* 10. Any condition which would make the participant, in the opinion of the investigator, unsuitable for the study* **Participant will be re-assessed prior to dosing to reconfirm eligibility criteria*
Number of Participants:	Up to 120

<table>
<tr><td>Number of Sites:</td><td>Approximately 30 sites</td></tr>
<tr><td>Duration of Participation:</td><td>Up to 62 days (up to 34 days of study drug plus 28 days of safety monitoring). Information about hospitalization will be collected at 36 weeks post menstrual age and/or at discharge.</td></tr>
<tr><td>Dose Schedule:</td><td>
Table. N and dosing scheme
<table>
<tr><th></th><th></th><th>N</th><th>Sildenafil (IV)</th><th>Sildenafil (enteral)</th><th>N</th><th>Cohort Total N</th></tr>
<tr><td>Cohort 1</td><td>Placebo</td><td>10</td><td>0.125 mg/kg q 8 hours</td><td>0.25 mg/kg q 8 hours</td><td>30</td><td>40</td></tr>
<tr><td>Cohort 2</td><td>Placebo</td><td>10</td><td>0.5 mg/kg q 8 hours</td><td>1 mg/kg q 8 hours</td><td>30</td><td>40</td></tr>
<tr><td>Cohort 3</td><td>Placebo</td><td>10</td><td>1 mg/kg q 8 hours</td><td>2 mg/kg q 8 hours</td><td>30</td><td>40</td></tr>
</table>
Note: Participants will be enrolled into cohorts sequentially (i.e. cohort 1 then cohort 2 then cohort 3) based on safety. Route of administration should be via IV route if patient has IV and is feasible, but choice is left to the discretion of investigator.
</td></tr>
</table>

1. KEY ROLES

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2. BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Section 409I of the Public Health Service Act, also known as the Best Pharmaceuticals for Children Act (BPCA), mandates the National Institutes of Health (NIH) to prioritize therapeutic areas in critical need for pediatric labeling; to sponsor pediatric clinical trials; and to submit data to FDA for consideration for labeling changes. Under the BPCA, the National Institute for Child Health and Human Development (NICHD) awarded a contract to Duke University, which established the Pediatric Trials Network (PTN) through its Duke Clinical Research Institute (DCRI). The NICHD awarded a separate contract to The Emmes Company, LLC to serve as the BPCA Data Coordinating Center (DCC).

2.2 Scientific Rationale

Bronchopulmonary dysplasia (BPD) is defined by the NIH as mild, moderate, or severe based on required respiratory support at 36 weeks postmenstrual age.[1] An NIH workshop proposed a severity -based definition that classifies BPD into mild, moderate or severe based on either postnatal age (PNA) or postmenstrual age (PMA) (**Table 2-1**). Ehrenkranz et al[2] validated the NICHD severity -based definition of BPD by comparing it to the more traditional definitions of BPD such as supplemental oxygen at 28 days and at 36 weeks PMA. The NICHD consensus severity based scale better identified infants who are at most risk for poor pulmonary outcomes as well as neurodevelopment impairment than the traditional definitions.[2]

Table 2-1. NICHD severity-based definition of BPD for premature infants at 36 weeks post menstrual age (or discharge)

No BPD	Receiving > 21% supplemental oxygen (O2) for ≤28 days and not at 36 weeks PMA
Mild BPD	Receiving > 21% O2 for ≥28 days but not at 36 weeks PMA
Moderate BPD	Receiving > 21% O2 for ≥28 days plus treatment with <30% O2 at 36 weeks PMA
Severe BPD	Receiving > 21% O2 for ≥28 days plus ≥30% O2 and/or positive pressure at 36 weeks PMA

BPD is characterized by clinical signs and symptoms as well as pathologic findings in the lung. The most common form of BPD is mild or moderate BPD. Animal studies suggest that the histology of infants with mild or moderate BPD have diffuse disease, minimal areas of lung hyperinflation, and most strikingly a reduction in alveoli and capillaries with little fibrosis.[3,4] Premature infants with severe BPD are generally exposed to prolonged mechanical ventilation and oxygen-often for weeks to months. These infants have characteristic areas of hyperinflation alternated with areas of focal collapse, as well as hyperplasia of the bronchial epithelium.[5,6] Radiography of these infants showed areas of heterogeneity throughout the lung fields and coarse scattered opacities in the most severe of infants.[7]

Each year, approximately 17,500 premature infants in the U.S. and approximately 500 premature infants in Canada develop BPD each year. Although rare in the general population, BPD is the most common pulmonary morbidity associated with prematurity and is increasing.

Approximately 50,000 infants in the U.S. and 1,500 infants in Canada are born each year at ≤ 28 weeks gestational age, and ~35% of these infants develop BPD. The incidence of BPD varies widely between centers even after adjusting for potential risk factors. Data from 2010 from the Vermont Oxford Network shows the rates of BPD vary from 12% to 32%, depending on center, among infants born less than 32 weeks gestation. The rising number of infants with BPD might be due to the improvement in the survival of extremely low gestational age infants that leads to an increase in the numbers of preterm infants who survive with BPD.[8,9] With increased survival of more very low birth

weight infants, due to several pre-and post-natal interventions, the number of infants at risk for developing BPD is increasing.[10]

BPD is associated with life-long problems. Premature infants with BPD have a longer initial hospitalization than their peers without BPD[11] and BPD remains a substantial lifelong burden. Premature infants with severe BPD are particularly challenging for clinicians and frequently suffer from multiple morbidities such as pulmonary hypertension, prolonged hospitalization and death.[12-14] The costs of the disorder are both social and economic and are measured in impaired childhood health and quality of life, family stress and economic hardship, and increased healthcare costs.[15-17]

The strongest risk factor for BPD is prematurity. Epidemiologic studies of cohorts of premature infants consistently find that lower gestational age and lower birth weight are associated with an increased risk of BPD. [18,19] Rojas et al[20] identified low birth weight and presence of a patent ductus arteriosus (PDA) as risk factors for BPD in premature infants between 500 and 1000 g. Marshall et al[21] identified gestational age, birth weight, nosocomial infection, fluid intake on day 2, PDA, and ventilation at 48 hours of life as risk factors for BPD. A secondary analysis of the Neonatal Research Network (NRN) Glutamine trial identified lower birth weight, lower gestational age, male sex, lower 1 and 5-minute Apgar scores, higher oxygen requirement at 24 hours of age, longer duration of assisted ventilation, use of postnatal steroids for BPD, presence of severe intraventricular hemorrhage, proven necrotizing enterocolitis, PDA, and late onset sepsis as risk factors for BPD.[22] In preterm infants with respiratory failure enrolled in the NRN Inhaled Nitric Oxide trial, the authors found that the risk of BPD was associated with lower birth weight, higher oxygen requirement, male sex, additional surfactant doses, higher oxygenation index and outborn status.[23] Until recently, using these risk factors for prediction of BPD has been challenging.

Accurate prediction of BPD risk is now possible: The NICHD NRN developed an online, publicly available BPD prediction tool (https://neonatal.rti.org or enter NICHD BPD estimator in a search engine) that accurately predicts the risk of developing BPD by postnatal day using only 7 readily available variables: postnatal day, gestational age, birth weight, sex, race/ethnicity, type of ventilator support, and F_iO_2 (%) (on the postnatal day of interest). Previous BPD prediction scoring systems have not been widely adopted, and each has had significant limitations. Some have satisfactory sensitivity and specificity but use a now outdated definition of BPD as oxygen therapy at 28 postnatal days.[24-26] Some include radiographs as part of the scoring system, which introduces subjectivity and reduces generalizability. Other limits to the utility of these models are the inclusion of ventilated infants only, the lack of categorization of BPD by severity, the exclusion of infants who die, and under-utilization of antenatal corticosteroids and surfactant therapy.[27-34] Most importantly, none examined models by postnatal day through the first 28 postnatal days. The prediction tool is internally and externally validated and the models classify infants in the internal validation sample into the correct level of BPD or death in more than 8 out of 10 cases.

Recently, investigators identified risk factors for BPD, and the competing outcome of death, by postnatal day in 3,636 infants 23-30 weeks gestation at six postnatal ages (postnatal day 1, 3, 7, 14, 21, and 28) using gestational age, birth weight, race and ethnicity, sex, respiratory support, and F_iO_2.[35] The investigators developed a web-based estimator using this clinical information to predict risk of BPD or death (https://neonatal.rti.org). BPD was defined as a categorical variable (none, mild, moderate, or severe). Using this estimator, typical risks of moderate to severe BPD or death are presented in **Table 2-2**. Based on data used to develop the BPD estimator, the cohort of infants meeting the inclusion criteria for this study are at high risk (generally >50 %) of developing moderate or severe BPD, or death.

Table 2-2. Typical risk of moderate or severe BPD, or death, by postnatal day and respiratory support in premature infants 23-27 weeks gestation[35]

	Mechanical ventilation				NCPAP or HFNC			
	Postnatal day				Postnatal day			
Gestational age (wks)	7	14	21	28	7	14	21	28
23-27 weeks, % risk	65-80	71-77	75-78	81-83	47-69	55-65	52-57	60-64

There are currently no therapies indicated by the U.S. FDA or Health Canada that prevent BPD or are available to treat BPD symptoms. To date, only vitamin A and caffeine prevent BPD without known significant long term adverse events.[35-37] Postnatal steroids reduce BPD, but increase the risk of cerebral palsy.[38,39] Although inhaled nitric oxide is beneficial in term infants with hypoxic respiratory failure, the majority of studies demonstrate that it does not prevent BPD in premature infants, although there was a great deal of heterogeneity in the patient populations, dose, and duration of inhaled nitric oxide.[40] One problem with the vast majority of trials of drugs tested to prevent BPD is that they did not establish the safety, preliminary effectiveness, PK, pharmacodynamics, or dose prior to implementation of phase 3 randomized, controlled trials.[41]

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 (PAH). In pediatric patients 1 to 17 years old, sildenafil is approved by the FDA for the treatment of PAH (WHO Group I) to improve exercise ability and, in pediatric patients too young to perform standard exercise testing, pulmonary hemodynamics thought to underly improvements in exercise.[42] The safety and effectiveness of sildenafil has not been established in pediatric patients younger than 1 year of age. Health Canada states sildenafil is not indicated in pediatric patients. The effect of sildenafil on premature infants at risk for BPD is unknown.

Sildenafil reduces lung injury and preserves normal vasculature in preclinical models. BPD is characterized by lung injury, inflammation, abnormal alverolarization, and dysregulated vascular development. Preclinical models demonstrate that sildenafil might prevent ischemic-perfusion injury and improve lung function (rat[43]), suppress inflammatory mediators (mouse[17,18]; also, matrix metalloproteinase-2 in cell cultures[44]) that contribute to lung injury and BPD, and improve alveolarization and lung vascular development.[17,18] Thus, sildenafil may prevent the development of BPD.

Case reports suggest sildenafil improves outcomes in premature infants with BPD and pulmonary hypertension. Two case reports documented the successful resolution of pulmonary hypertension, measured by echocardiogram, in premature infants with BPD and pulmonary hypertension who were treated with sildenafil.[45,46] In a small case series, enteral sildenafil at 1.5-8 mg/kg/day in 22 premature infants with a median gestational age of 28 weeks (range 23-41 weeks) with lung disease (72% with BPD) resulted in improved hemodynamics determined by echocardiogram.[47] A retrospective case series including 23 preterm infants with median gestational age of 26 weeks (interquartile range 23-29 weeks) treated with 1-7.3 mg/kg/day of oral sildenafil for BPD-associated pulmonary hypertension in a level 3 NICU found a significant improvement in pulmonary hypertension based on echocardiography in the majority (71%) of patients, with a minority (35%) of patients demonstrating a significant clinical response as defined by oxygen

requirement and mean airway pressure.[48] This report reflects the common clinical practice of using echocardiograms to follow premature infants with severe BPD and pulmonary hypertension. These reports do not establish the efficacy or safety of sildenafil in premature infants; more data is required from well-designed clinical trials.

Sildenafil is increasingly being used off-label in premature infants. Data was examined from the Pediatrix Medical Group, a U.S. consortium of NICUs which encompasses 20% of all NICUs and ~90,000 admissions/year. Sildenafil use increased exponentially from 2001-2010[49] (Figure 1). Infants exposed to sildenafil increased by >1000% (#2 among all drugs used in the NICU) from 2005-2010, from 0.2 to 2.3 per 1000 admissions. Sildenafil is increasingly being used in premature infants with little efficacy or safety data.

Table 2-3. Top 5 % change drugs among 450,386 infants in 305 NICUs

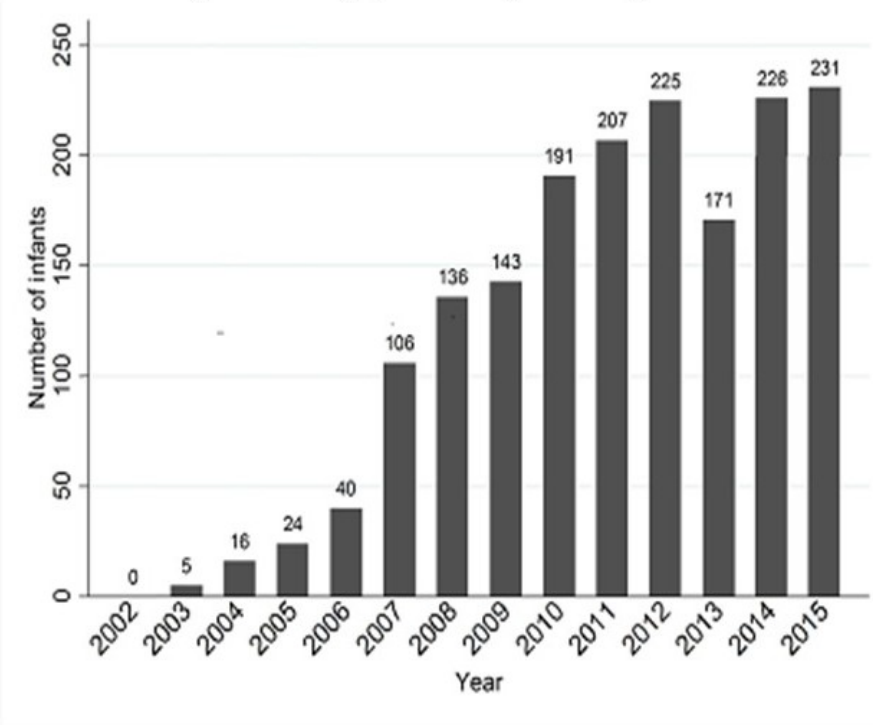
Rank	Medication	% Change	Use (2005) *	Use (2010) *
1	Azithromycin	2900	0.1	3.0
2	**Sildenafil**	**1050**	**0.2**	**2.3**
3	Milrinone	900	0.4	4.0
4	Ibuprofen	650	1.4	11
5	Carnitine	517	0.6	3.7

*Per 1000 infants
Source: Pediatrix Medical Group

Figure 2-1. Infants exposed to sildenafil in the Pediatrix Medical Group[52]

	Median	IQR
GA at birth (wks)	28	25-37
Birthweight (g)	940	610-2872
Post-natal age (days)	65	12-115

GA-gestational age, IQR-interquartile range



Source: Pediatrix Medical Group

Sildenafil pharmacokinetic and safety have not been adequately studied in premature infants. In adults, sildenafil is rapidly absorbed after oral administration, with a mean absolute bioavailability of 41%.[50-52] Maximum observed plasma concentrations are reached within 30–120 minutes (median 60 minutes) of oral dosing. In term infants with persistent pulmonary hypertension, sildenafil lowers mortality.[53] Doses of up to 3 mg/kg/dose up to four times per day (12 mg/kg/day) have been reported in term infants.[54]

Table 2-4. Adult, child, and infant clinical pharmacology parameters of sildenafil[50-52,54,55]

	Adult, *FDA label*	Children, *N=235*	Term, *N=36*	Premature
Postnatal Age	N/A	N/A	<3 days	Infants
Volume of distribution	105-140 L/70 kg	150 L/70 kg	456 L/70 kg	*Unknown*
Clearance	31 L/hr/70 kg	13.7L/hr/70 kg	62 L/hr/70 kg	*Unknown*
Half-life	3-4 hours	4.7 hours	48-56 hours	*Unknown*

The efficacy target is unknown, however, an AUC_{24} of 2650 ng*hr/mL (sildenafil plus the active metabolite) is equivalent to an oral adult dose of 20 mg three times daily, which decreases symptoms of pulmonary hypertension in adults.[56] Efficacy and safety data from premature infants are lacking.

The risks of "excessive" rates of administration of sildenafil are unknown. Studies of IV administration with higher rates of infusion (median dose – 0.35 mg/kg (range 0.125, 0.45 over 20 minutes: 5.5 times higher dose and 10 minute faster infusion time than the proposed study) in older children (median age 3.3. years) were not associated with hypotension.[57,58] One infant in the SIL01 study (see below) who received an IV dose of sildenafil at higher rate of infusion than specified in the protocol suffered from lower blood pressure. The SIL02 protocol uses intensive blood pressure monitoring, a lower starting dose of sildenafil, and a gradual dose escalation to the target dose to minimize the risk of high infusion rate associated low blood pressure.

Investigators completed a pilot randomized study of enteral sildenafil in premature infants <28 weeks of gestation at risk of BPD and enrolled 20 participants: 10 received oral sildenafil 3 mg/kg/day (mean gestational age 24 + 5 weeks (SD 4.9 days), mean weight 692 g (SD 98 g)) and 10 received placebo (mean gestational age 24 + 5 weeks (SD 6.5 days), mean weight 668 g (SD 147 g)).[59] Short term respiratory parameters (duration of mechanical ventilation, duration of NCPAP) were not statistically significant between groups. (clinicaltrials.gov; NCT00431418). This study did not collect PK data.

The Pediatric Trials Network conducted a Phase 1 study of sildenafil in premature infants with BPD (NICHD-2012-SIL01,IND 112,374,NCT01670136, Protocol Chair Matthew Laughon): The trial enrolled 25 premature infants ≤28 weeks gestational age (range 22-27 weeks) who were receiving sildenafil per local standard of care at doses between 0.5-2.25 mg/kg in cohort 1. The trial enrolled 9 premature infants ≤ 28 weeks gestational age (range 23-27 weeks) and postnatal age range 7-40 days who received a single intravenous dose of sildenafil 0.25 mg/kg or 0.125 mg/kg in cohort 2. Separate interim PK analyses were completed on samples from the 12 participants in Cohort 1 and samples from the 7 participants in Cohort 2.

Cohort 1 (N=12): all subjects received drug PO, with 39 quantifiable PK samples collected for sildenafil and N-desmethyl sildenafil concentration. All subjects received at least 6 doses prior to PK sampling.

Median (range) sildenafil and N-des-methyl sildenafil (DMS) concentrations were 60.4 ng/mL (4.7-369.4) and 46.9 ng/mL (5.6-275.2), respectively. The median DMS: sildenafil concentration ratio was 0.7 (0.2-4.7).

Analysis of this preliminary data indicates that a one compartment body model best described the sildenafil PK data. Population estimates of CL/F and V/F, both scaled to a 70-kg adult weight, were 50.7 L/h and 357 L, respectively (**Table 2-5**).

Table 2-5. Parameter estimates for the base population PK model for sildenafil.

Parameter	Estimate	RSE (%)	Shrinkage (%)	2.5th %ile	Bootstrap* Median	97.5th %ile
Structural Model						
K_A (H^{-1})	2.4 FIXED	-	-	-	-	-
CL/F_{70KG} (L/H)	50.7	14.1	-	37.6	50.7	68.6
V/F_{70KG} (L)	357	20.1	-	264.4	357.9	575.2
Inter-Individual Variability (%CV)						
CL/F	38.6	52.1	1.7	15.2	37.5	55.9
Residual error						
Proportional Error (%)	41.7	34.7	9.5	24.9	40.2	53.9

*1,000 bootstrap runs were performed

Assuming a bioavailability of 41%, a DMS: sildenafil area under the concentration versus time curve (AUC) ratio of 0.7 following oral administration, and that DMS has 50% the activity of the parent compound, individual estimates of CL/F and dose were used to predict steady-state $AUC_{24,(SIL+DMS)}$ for the 12 subjects in cohort 1. The median (range) predicted steady-state $AUC_{24,\ (SIL+DMS)}$ was 1203 (502-3,480) ng*h/mL. If a 0.5 mg/kg intravenous dose is administered to 12 premature infants of the same weight, and assuming a 15% DMS: sildenafil AUC ratio following intravenous administration,[9] predicted steady-state $AUC_{24,(SIL+DMS)}$ would be 1335 (841-3,583) ng*h/mL.

Cohort 2 (N=7): All subjects received sildenafil via intravenous administration at a dose of 0.25 mg/kg. A total of 26 PK samples were collected, 4 (15%) of which were scavenged samples. There was one sample (not scavenged) that was below the limit of quantification (BQL) for sildenafil, whereas there were no BQL samples for DMS. The median (range) number of samples collected per subject was 3 (3-4). The median (range) dose was 0.251 mg/kg (0.247-0.254) or 0.180 mg (0.170-0.304).

The median (range) sildenafil and DMS concentrations (not including scavenged samples) were 25.40 ng/mL (0.28-193.07) and 2.84 ng/mL (0.21-17.95), respectively. The median DMS: sildenafil concentration ratio was 0.18 (0.0064-16.10). One infant experienced hypotension, which was deemed to be related to sildenafil. This subject had two PK samples collected and the sildenafil (0.29 ng/L and BQL) and DMS (0.87 and 4.67 ng/mL) concentrations were within the range of values observed for the other six subjects (median [range] sildenafil and DMS were 27.40 ng/mL [0.28-193.07] and 2.84 ng/mL [0.21-17.95], respectively).

Following a single intravenous dose of 0.25 mg/kg, sildenafil exposures were within the range of estimates previously reported for infants following multiple oral dosing. Additional data collected in this study will provide the opportunity to perform a more robust PK analysis and provide meaningful estimates of sildenafil disposition in premature infants following both oral and intravenous administration.

Adverse Events, Cohorts 1 and 2 (Table 2-6): All enrolled participants in cohorts 1 and 2 completed the study. In cohort 1, there were 13 adverse events with 1 serious adverse event occurring in 1 participant. Eight of the 25 participants experienced at least one adverse event. The adverse events were not found to be related to the study drug. In cohort 2, there were 10 adverse events with 4 serious adverse events. Seven of the 9 participants experienced at least one adverse event and 4 of these experienced at least one serious adverse event. One of the 4 serious adverse events was found to be related to the study. The 1 related event was an episode of hypotension which was determined to be related to a faster than expected infusion rate. The event led to a Data Safety Monitoring Board (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 0.125mg/kg. When the study re-opened 2 additional subjects were enrolled without any related adverse events.

Table 2-6. 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 Adverse Events	13	10
Participants with at least one AE	8 (32.0%)	7 (77.8%)
Number of Serious Adverse Events	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%)

Determining the safety of sildenafil in premature infants is an urgent, unmet public health need. Investigating sildenafil addresses a research gap in neonatology because: 1) premature infants with BPD often die or have life-long problems; 2) there are no drugs that are FDA or Health Canada indicated for preventing BPD; and 3) sildenafil is commonly used off-label to treat or prevent BPD without adequate safety or efficacy data.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

2.3.1.1 Risks of Blood Drawing

There are small risks to blood sampling, usually some pain/discomfort with the blood stick and blood loss. Every effort will be made to avoid additional (to standard of care) sticks for this study and will time clinical blood draws to coincide with timed samples, using existing intravenous lines when possible.

2.3.1.2 Sildenafil

1. Per the FDA, the safety and effectiveness of sildenafil have not been established in pediatric patients younger than 1 year of age. Health Canada states sildenafil is not indicated in pediatric patients (< 18 years old).
2. There is a theoretical risk of an increase in retinopathy of prematurity (ROP) that has been reported in a case report in the literature; this has been refuted by cohort studies.[60]
3. Sildenafil has vasodilatory properties that can result in mild and transient decreases in blood pressure. See sildenafil package insert for additional information.
4. The most common side effect observed in children is an erection that lasts for more than 4 hours (priapism). See sildenafil package insert for additional information
5. See also Section **2.2**. (**Table 2-6**) Scientific Rationale Adverse Events, Cohorts 1 and 2 for AEs in study NICHD-2012-SIL01.

Minimizing risk: We have minimized these risks by studying sildenafil in a different age group and a different disease state (premature infants at risk for BPD). We will review safety data prior to dose escalation between cohorts (see Section **10.3**). Risks of retinopathy of prematurity have been minimized by administering sildenafil before the proliferative period of retinopathy of prematurity (32-34 weeks adjusted age). We will collect treatment of ROP as an outcome.

2.3.2 Potential Benefits

Sildenafil may improve pulmonary mechanics, reduce exposure to mechanical ventilation, and prevent BPD. Although these improvements are not proven, increase in pulmonary function and decrease risk of BPD 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 at risk of BPD.

3.1.2 Secondary Objective

Preliminary effectiveness and pharmacokinetics (PK) of sildenafil.

3.2 Outcome Measures

3.2.1 Primary Outcome Measures

Safety as determined by adverse events experienced by the participants.

3.2.2 Secondary Outcome Measures

3.2.2.1 Preliminary effectiveness: Risk of BPD

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

The BPD outcome estimator uses the following information to provide individual risk of BPD:

1. Gestational age (weeks)
2. Birth weight (g)
3. Sex
4. Maternal Race/Ethnicity (White/Hispanic/Black)
5. Postnatal day (1, 3, 7, 14, 21, 28)
6. Ventilation type (on the postnatal day of interest: HFvent, IMV/SIMV, CPAP, Cannula/hood or none)
7. F_iO_2 (%) (on the postnatal day of interest)

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

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

3.2.2.2 Pharmacokinetics

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

3.2.3 Other Safety and Efficacy Outcomes

1. **Death:** All infants who died at or before 36 weeks PMA will be included.
2. **BPD:** BPD is defined as using a method modified from the NICHD NRN BPD estimator. We will define BPD as a dichotomous (none-mild vs. moderate-severe- death) variable and as a

categorical variable (none/mild, moderate, severe or death)among survivors by modifying the NIH consensus definition of BPD[20, 21] to include infants transferred prior to 36 weeks and the need for oxygen at 36 weeks PMA (or discharge, if sooner than 36 weeks PMA).[29] For the purpose of this study we will define BPD as follows:

No/Mild BPD: participant is not receiving supplemental O_2 at 36 weeks PMA

Moderate BPD: participant is receiving O_2 supplementation >21% but <30% at 36 weeks PMA, but not receiving positive pressure at 36 weeks PMA.

Severe BPD: participant is receiving O_2 supplementation ≥30% and/or positive pressure at 36 weeks PMA.

3. Death or BPD (moderate or severe)
4. ROP: Retinopathy (retinopathy of prematurity) will be defined as treatment for ROP (laser photocoagulation, cryotherapy, or intraocular injections, such as bevacizumab).
5. Pulmonary hypertension: Pulmonary hypertension will be defined as present or not present per echocardiogram and/or cardiac catheterization reports, as provided per standard of care. Echocardiogram and cardiac catheterization reports will be reviewed centrally by a single Duke pediatric cardiologist.

4. STUDY DESIGN

Multi-center, randomized, placebo-controlled, sequential cohort dose escalating, double masked, safety study.

5. STUDY POPULATION

5.1 Selection of the Study Population

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

5.2 Inclusion/Exclusion Criteria

5.2.1 Inclusion Criteria

1. Documented informed consent from parent or guardian, prior to study procedures
2. Receiving positive airway pressure (nasal continuous airway pressure, nasal intermittent positive pressure ventilation, or nasal cannula flow > 1LPM) or mechanical ventilation (high frequency or conventional) at time of randomization*
3. < 29 weeks gestational age at birth
4. 7-28 (inclusive) days postnatal age at time of randomization*

5.2.2 Exclusion Criteria

1. Previous enrollment and dosing in NICHD-2015-SIL02 “Safety of Sildenafil in Premature Infants at Risk of Bronchopulmonary Dysplasia”
2. Previous exposure to sildenafil within 7 days prior to randomization*
3. Currently receiving vasopressors*
4. Currently receiving inhaled nitric oxide*
5. Baseline mean arterial pressure < gestational age (in weeks) plus postnatal age (in weeks) within 24 hours of randomization*; e.g. an infant at 25 weeks gestational age and 3 weeks postnatal age with MAP <28 mm Hg would be ineligible
6. Known allergy to sildenafil
7. Known sickle cell disease
8. AST > 225 U/L < 72 hours prior to randomization*
9. ALT > 150 U/L < 72 hours prior to randomization*
10. Any condition which would make the participant, in the opinion of the investigator, unsuitable for the study*

** Participant will be re-assessed prior to dosing to reconfirm eligibility criteria if >24 hours elapse between randomization and first study dose.*

5.3 Treatment Assignment Procedures

5.3.1 Additional Participants

If a participant is randomized (Section **5.3.2**) and receives < 7 days of study drug, then additional participants may be enrolled.

5.3.2 Randomization Procedures

Participants who satisfy all eligibility criteria will be randomized 3:1 (sildenafil: placebo). All three cohorts will use the same randomization scheme. The participant’s randomized treatment assignment will be obtained through the Advantage eClinical® enrollment module. In the event that Advantage eClinical® is not available at the time of randomization, a back-up system specified in the Manual of Procedures (MOP) will be used. If a participant is randomized but does not receive study drug, that

participant will not count towards total sample size and will be replaced by a new participant who, in turn, will be assigned a new identification number and receive treatment corresponding to the new identification number. The reason for not dosing the participant will be noted on the electronic case report form (CRF). The randomization process will not determine if an IV or enteral dose is used. Investigators are encouraged to use intravenous dosing if the patient has IV access. Participants should not have an IV placed specifically for study drug administration alone. However, if a site deems that a second IV would optimize the safe administration of other medications or fluids while 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.

5.3.3 Masking Procedures

Infants randomized to the placebo treatment group will receive the equivalent volume of dextrose 5% appropriate for IV use or enteral use (if receiving enteral study drug). The pharmacy at each site will prepare and dispense the study drug into appropriate sized syringes in a masked manner (e.g. amber syringe), staff accessing participant outcomes will be masked to treatment.

5.3.4 Reasons for Participant Withdrawal

The site investigator may choose to suspend study drug dosing for up to 48 hours for any reason. If study drug is suspended for >48 hours, the study drug administration will be stopped and the participant will proceed directly into the safety follow-up period (and will have all remaining assessments 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:

1. During the course of the study, the participant fits safety-related criteria for which they would have been excluded from the study in the first place (ALT > 150 U/L, AST > 225 U/L, or infant started on vasopressor or inhaled nitric oxide (Section **5.2**).
2. Any clinically significant adverse event (e.g. hypotension) that is deemed by the site investigator to require discontinuation of study drug (Section **8.1.4** for definition of hypotension.)
3. A participant develops hypotension during the study drug infusion.
4. Unmasking of the participant treatment is performed. All unmasking events should be recorded on a protocol deviation form in electronic data capture (EDC) system within 24 hours of the unmasking. The decision to unmask will be made in consultation with the Medical Monitor if possible.
5. Requested by the NIH, FDA, PTN, Health Canada or DSMB.

Before discontinuing a participant from the study drug, the local investigator must contact the medical monitor or Study PI except in emergency situations. Participants who are prematurely discontinued from the study drug for any reason will be followed for 28 days for safety monitoring, and will have 36 weeks PMA and final study assessments collected.

The participant's parent or guardian may withdraw voluntarily from participation in the study at any time. The participant's parent or guardian is not obligated to state the reason for withdrawal. The reasons for

withdrawal, or failure to provide a reason, must be documented by the investigator on the completion/withdrawal section of the corresponding CRF. Participants/guardians who withdraw consent will not have any additional data entered in the EDC system.

5.3.5 Termination of Study

This study may be terminated at any time by FDA, NIH, Health Canada, the Investigational New Drug Application (IND) or Clinical Trial Application (CTA) sponsor, or the Data Safety Monitoring Board (DSMB). If the study is terminated, notification to FDA and U.S. investigators will be made in accordance with 21 CFR 312.56(d) and Health Canada and Canadian investigators will be made in accordance with Canadian Food and Drug Regulation C.05.015.

6. STUDY PROCEDURES

6.1 Summary of Procedures

Table 6-1. Schedule of study procedures

	Screen/ Baseline	Treatment	Weaning[9] Cohort 2 and 3	Follow-up	SAE Follow-up	36 weeks PMA assessment[6]	Final study assessment
Time (Day)	Pre-dose[1]	1-28[7] (± 1 Day)	Weaning Day 1-6	Day 1-14 post last study dose[11]	Day 15-28 post last study dose[11]	36 weeks PMA (+ 6 days)	Discharge or Transfer
Informed consent	X						
Demographics[10]	X						
Physical examination	X			X (WEEKLY)		X	X
Medical history	X						
Actual Weight	X	X (WEEKLY)		X (WEEKLY)		X	X
Mean Arterial Pressure	X	X	LAST DAY OF WEAN	X (WEEKLY)			
Respiratory assessment	X	X (WEEKLY)	LAST DAY OF WEAN	X (WEEKLY)		X	X
Laboratory evaluations[2]	X	X (WEEKLY)		X (WEEKLY)		X	
Study drug administration		X[4]	X				
Concomitant medications	X	X					
Adverse Events (including SAEs)	X[5]	X	X	X			
SAEs Only					X		
Echocardiogram and Cardiac catheterization reports[8]	X	X	X	X		X	X
PK sampling		X (AFTER DAY 7)					
Discharge information, including ROP[3]							X

[1] Refers to <24 hours prior to start of study drug that these procedures (except labs) 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.
[2]If not performed per standard of care (SOC), labs must be taken within 72 hrs. prior to randomization and weekly (at any time) during treatment; weekly during the first 14 days of the follow-up period.
[3]Transfer, discharge, duration of hospitalization or death; record results of ROP, if treatment required.
[4] Dosing may be adjusted according to any weight changes determined at baseline, study days 7, 14, and 21.
[5] AEs will be collected following initial study-specific procedure (e.g., screening blood draws, dosing)
[6]Assessment must occur between 36 weeks 0 days PMA to 36 weeks 6 days PMA.
[7]Collect all safety follow-up and remaining assessments if early withdrawal of study drug occurs during the 28 day treatment period.
[8] If performed per local standard of care.
[9]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.
[10]Maternal race/ethnicity will be collected.
[11] Includes weaning during cohort 2 and 3.

6.2 Screen/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 CRF from the clinical medical record:

1. Participant demographics, including birth weight and gestational age at birth
2. Maternal Race/Ethnicity (White/Hispanic/Black)
3. Medical history
4. Physical examination, including actual weight
5. MAP A. Obtained at screening/baseline. All mean arterial pressure (MAP) values obtained 24 hours before the first dose
6. Concomitant medications (within 24 hours prior to start of study drug)
7. Respiratory assessment (Section **6.8**)
8. Laboratory evaluations (Section **6.9**)
9. Echocardiogram and Cardiac catheterization reports, if performed per local standard of care
10. Adverse events following initial study-specific procedure (Section **6.11**)

6.3 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
4. MAP
   A. All mean arterial pressure (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 (one hour), then hourly (+/- 15 minutes) for 4 hours, then and 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, weekly (Section **6.8**)
6. Laboratory evaluations, at least once a week (Section **6.9**).
7. Echocardiogram and Cardiac catheterization reports, if performed per local standard of care
8. PK sampling (after Day 7 – Section **6.10**)
9. Adverse events (Section **6.11**)

6.4 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. Adverse events (Section **6.11**)
3. MAP (the lowest valid MAP value on last day of wean should be recorded).
4. Respiratory assessment on last day of wean (Section **6.8**)
5. Echocardiogram and Cardiac catheterization reports, if performed per local standard of care

6.5 Follow-up Period

Follow-up period will include days 1-28 after last study dose, last study 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 EDC at Day 7 and 14 of the follow-up period (or day closest to and after Day 7 and 14, if >1 assessment is available), except for SAEs (which will be reported from days 1-28):

1. Physical examination, including actual weight
2. MAP (the lowest valid MAP value on Day 7 and Day 14 should be recorded)
3. Respiratory assessment (Section **6.8**)
4. Laboratory evaluations (Section **6.9**)
5. Echocardiogram and Cardiac catheterization reports, if performed per local standard of care
6. Adverse events (only during follow-up period days 1-14 – Section **6.11**)
7. Serious adverse events (during follow-up period days 1-28 – Section **6.11**)

6.6 36-week PMA Assessment

The following information will be reported at 36 weeks (+ 6 days) PMA if available. If the participant is

discharged before 36 weeks, record assessment closest to discharge date. If more than one 36-week assessment is available record results obtained closest to Week 36, 0 days:

1. Physical examination, including actual weight
2. Respiratory assessment (Section **6.8**)
3. Laboratory evaluations (Section **6.9**)
4. Echocardiogram and Cardiac catheterization reports, if performed per local standard of care

6.7 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 **6.8**)
3. Echocardiogram and Cardiac catheterization reports, if performed per local standard of care
4. Discharge information
    A. Discharge or transfer
    B. Death
    C. Duration of hospitalization
5. Record results of ROP, if treatment required

6.8 Respiratory Assessment

The following information will be collected at baseline, on day 7 (+/- 1 day), 14 (+/- 1 day), 21 (+/- 1 day), and 28 (+/- 1 day) of initial study drug administration, once during the weaning period, twice during the follow-up period, once at 36 weeks PMA, and once at discharge (if applicable): Maximum blended O_2 (blended O_2 for each assessment 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.)

1. Ventilation type:
    A. High frequency ventilator
    B. Conventional mechanical ventilator
    C. NCPAP (or equivalent, see inclusion criteria in protocol synopsis table)
    D. Cannula/Hood
    E. None (room air with no support)
    F. Other: presence of Tracheostomy or Extracorporeal Membrane Oxygenation (ECMO)

6.9 Laboratory Evaluations

The following laboratory evaluations must be conducted (if not obtained per standard of care [SOC]) within 72 hours prior to randomization, and at least once per week during the treatment and weekly during the first 14 days of the follow-up period (at any time during the week). The lab values will also be recorded at 36 weeks PMA if available.

1. AST
2. ALT

If the above laboratory evaluations are obtained more frequently than required by protocol, all test results must be entered into the EDC system. SOC laboratory evaluations: We will also record all values for platelets, phosphorus, chloride, alkaline phosphatase, and caffeine levels within 72 hours prior to randomization through 14 days following the last dose of study drug (include weaning doses).

6.10 PK Sampling

Table 6-2 below provides the optimal PK sampling collection windows. Blood samples will be collected after any dose following completion of 7 days (168 hours) of study drug administration. 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; 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 6-2. Target PK Sampling Times (time in relation to end of infusion and flush)

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

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

6.10.1 Minimizing 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.

Cohort 1 and 2 Infants assigned to the sildenafil treatment group in cohorts 1 and 2, will only have PK analysis conducted.

Infants assigned to the placebo treatment group in cohorts 1 and 2, will only have biomarkers of bronchopulmonary dysplasia measured in a central laboratory (details in MOP; biomarkers may include: BNP, IL-1, IL-6, IL-8, TNF-α) instead of PK levels.

Cohort 3 All infants in cohort 3 (placebo and active arm) will have biomarkers of bronchopulmonary dysplasia measured in a central laboratory (details in MOP; biomarkers may include: NT-proBNP, MR-proANP, in addition to BNP, IL-1, IL-6, IL-8, TNF-α). Only infants assigned to the active treatment group will have PK levels assessed.

6.10.2 Specimen Preparation, Handling, and Shipping

Detailed information will be in the MOP.

6.11 Adverse Event

AEs will only be collected following initial study-specific procedure (e.g., screening blood draws, drug administration), through 14 days post last study dose (which includes weaning doses). SAEs will be collected following initial study-specific procedure (e.g., screening blood draws, drug administration), through 28 days post last study dose (which includes weaning doses). Refer to Section **6** for safety information.

7. STUDY DRUG DESCRIPTION

7.1 Dosage and Study Drug Information

In the U.S., sildenafil, in both intravenous (IV) and for oral suspension, has been approved for use in other populations and for other indications by the FDA. For the indication of PAH in adults, the oral dose is 20 mg three times a day or 10 mg three times a day if administered as an intravenous bolus injection. In pediatric patients (ages 1-17), the approved recommended oral dosing is 10 mg three times a day (<20 kg), 20 mg three times a day (20-45 kg), 20 mg three times a day (>45 kg). In pediatric patients >45 kg, doses may be increased up to 40 mg three times a day based on symptoms and tolerability.[42]

In Canada, only the intravenous and tablet formulation have marketing approval, and sildenafil is not approved for use in pediatric patients < 18 years old. Pfizer Inc. and Mylan Inc., a Viatris Inc. company will provide the oral suspension (enteral) formulation to U.S. and Canadian sites. In Canada, the enteral formulation will be provided to Canadian sites as investigational product under a CTA.

Study dosing and escalation is described in **Table 7-2**. Refer to Section **7.3** for study drug packaging and labeling.

Table 7-1. Treatment Period Dosing

Table. N and dosing scheme						
		N	**Sildenafil (IV)**	**Sildenafil (enteral)**	**N**	**Cohort N**
Cohort 1	Placebo	10	0.125 mg/kg q 8 hours	0.25 mg/kg q 8 hours	30	40
Cohort 2	Placebo	10	0.5 mg/kg q 8 hours	1 mg/kg q 8 hours	30	40
Cohort 3	Placebo	10	1 mg/kg q 8 hours	2 mg/kg q 8 hours	30	40

Dosing escalation: Participants in Cohort 1 will start at 0.0625 mg/kg IV (or 0.125 mg/kg enteral) every 8 hours and the dose will increase to 0.125 mg/kg IV (or 0.25 mg/kg enteral) after 6 doses (48 hours). Participants enrolled in Cohort 2 and Cohort 3 will initially start at 0.125 mg/kg IV (or 0.25 mg/kg enteral) every 8 hours and the dose will increase by 0.125 mg/kg IV (or 0.25 mg/kg enteral) to the target dose every 6 doses. This means that the dose will increase every 48 hours. 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. Infants who do not initially qualify for escalation should be reevaluated daily for whether they meet these criteria, and escalation must occur within 24 hours of an infant meeting all criteria (respiratory and laboratory). If escalation does not occur within this timeframe, then a protocol deviation must be recorded.

Note: DSMB safety review (Section **10.3**) is required prior to enrolling to next cohort.

Table 7-2. Dosing escalations for each sequential cohort

		Cohort 1		Cohort 2		Cohort 3	
Day #	Dose #	IV (mg/kg)	Enteral (mg/kg)	IV (mg/kg)	Enteral (mg/kg)	IV (mg/kg)	Enteral (mg/kg)
1-2	1-6	0.0625	0.125	0.125	0.25	0.125	0.25
3-4	7-12	0.125	0.25	0.25	0.5	0.25	0.5
5-6	13-18	0.125	0.25	0.375	0.75	0.375	0.75
7-8	19-24	0.125	0.25	0.5	1	0.5	1
9-10	25-30	0.125	0.25	0.5	1	0.625	1.25
11-12	31-36	0.125	0.25	0.5	1	0.75	1.5
13-14	37-42	0.125	0.25	0.5	1	0.875	1.75
15 +	43 +	0.125	0.25	0.5	1	1	2

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 weekly check (within 7 days) prior and closest to dose increase.

7.1.1 Adjusting dose for weight:

The actual weight should be reviewed weekly (plus or minus one day, Section **6.4**) and the dose of the study drug may be adjusted. DSMB safety review (Section **10.3**) is required prior to enrolling to next cohort.

7.2 Weaning Period Schedule:

Sildenafil is commonly used at higher doses to treat pulmonary hypertension 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 BPD, target doses in cohorts 2 and 3 are similar to those used for the treatment of pulmonary hypertension.

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 participant is withdrawn from the study. Wean by 25% of last study dose every 2 days until off. Participants will complete the wean after 6 days.

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

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

Weaning for a withdrawn participant: If a participant in cohort 2 or 3, escalates to a dose of ≥0.5 mg/kg IV (or ≥1mg/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.

7.3 Formulation, Packaging, and Labeling

IV 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.

Enteral Formulation: U.S. commercially available REVATIO sildenafil citrate powder for suspension (Manufactured by: Fareva Amboise) will be distributed for use to all sites and labeled in accordance with 21 CFR 312.6.

Any requisite clinical trial materials will be provided with labeling in accordance with all applicable regulatory requirements. Detailed information regarding formulation, packaging and labeling will be included in the MOP.

For Canada, enteral drug product will be labeled for investigational use in accordance with Health Canada regulations (C.05.011), prior to shipment to Canadian sites under a CTA.

7.4 Preparation and Administration of Study Intervention/Study Drug

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. The investigational pharmacist will be unmasked and will prepare masked study drug. Detailed information will be included in the MOP. If the participant experiences signs or symptoms deemed by the investigator to be clinically significant hypotension or other type of SAR, the infusion will be stopped. Resumption of 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.

7.5 Product Storage and Stability

All investigational products 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 investigational product-specific requirements provided by the Sponsor.

Detailed information on handling storing and reconstitution is found in the Package Insert.

7.6 Concomitant Medications/Treatments

All drug and/or treatments are permitted while on study. All concomitant medications and treatments will be reported within 24 hours prior to the start of study drug and during the 28-day treatment administration period.

8. ASSESSMENT OF SAFETY

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

Safety will be assessed following initial study-specific procedure e.g., screening blood draws, dosing through 14 days post last study dose (last study dose includes weaning doses) and it will be assessed by frequency and incidence of AEs and SAEs. A safety monitoring committee (DSMB) will be convened by NIH to review data and safety information from study participants throughout the study and prior to opening of cohorts 2 and 3 (see Sections **8.4** and **10.3**).

Monitoring for SAEs will continue for a total of 28 days post last study drug dose (including the weaning doses).

8.1.1 Adverse Event

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

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

Adverse reaction is any adverse event caused by the drug.

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

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

8.1.2 Unexpected Adverse Event or Reaction

This is defined as any adverse event not listed in the package insert or investigational brochure or investigational plan or is not listed at the specificity or severity in the package insert or investigational brochure or investigational plan.

8.1.3 Identification of Events and Timeframe for Reporting

As all participants in this study will have pre-existing medical conditions and may be currently hospitalized, those pre-existing conditions will not be considered as adverse events. New events that occur or pre-existing conditions that worsen in terms of frequency or intensity will be reported as

adverse events.

All reportable events as defined above, determined to be an AE based on physical examination, laboratory findings, or other means, will be reported in the electronic case report form (e-CRF). The investigator will provide the date of onset and resolution, intensity, frequency, action(s) taken, changes in study drug dosing, relationship to study drug, and outcome.

8.1.4 Low Blood Pressure and Hypotension

Blood pressure will be determined using mean arterial pressure (MAP). For each participant, MAPs will be taken as noted in Section **6.4**. MAP will be measured using an appropriate size cuff or with an intra-arterial placed catheter that measures continuous blood pressure.

8.1.5 Primary Definition of Hypotension

Hypotension will be defined as any clinically significant low blood pressure event deemed by the treating physician to require intervention with a fluid bolus or the initiation or escalation of inotropic, vasopressor, or systemic steroid therapy with the specific intent to raise blood pressure.

8.1.6 Presumed low blood pressure and presumed hypotension

Presumed low blood pressure will be considered as one valid MAP (in mmHg):

- < gestational age at birth (in weeks) plus postnatal age (in weeks) in mmHg
- < gestational age at birth (in weeks) in mmHg
- < 30 mmHg
- During times of intensive MAP recording (Section **6.3**), one valid MAP of >30% lower than baseline. Baseline MAP is the mean of the 3 valid MAPs prior to the first dose or dose escalation (i.e. the mean of the following 3 MAPs: 2 hours [+/- 5 minutes], 1 hour [+/- 5 minutes], and 15 minutes [+/- 5 minutes]).

Presumed hypotension will be defined as 2 valid MAPs (in mmHg), taken 15-60 minutes apart:

- < gestational age at birth (in weeks) plus postnatal age (in weeks) in mmHg
- < gestational age at birth (in weeks) in mmHg
- < 30 mmHg
- During times of intensive MAP recording (Section **6.3**), two valid MAPs of >30% lower than baseline. Baseline MAP is the mean of the 3 MAPs prior to the first dose or dose escalation (i.e. the mean of the following 3 valid MAPs: 2 hours [+/- 5 minutes], 1 hour [+/- 5 minutes], and 15 minutes [+/- 5 minutes]).

If an infant has presumed hypotension as defined above i.e. < gestational age at birth (in weeks) plus postnatal age (in weeks) in mmHg that is not considered a suspected adverse reaction, then that event will be flagged by the EDC system and will be reviewed by the Study Team for possible DSMB review.

8.1.7 Follow-up of Adverse Events

All events (study-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.

All serious suspected adverse reactions and severe adverse events will be followed until resolution or until the patient is medically stable. Any adverse event beginning more than 14 days after the last dose of study drug and any serious adverse event beginning more than 28 days after the last dose of study drug will not be captured.

In cases when the baby 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 to contact the participant/guardian.

8.1.8 Guidelines for Assessing Intensity of an Adverse Event

The investigator should use the following definitions when assessing intensity of an adverse 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 to require intervention
3. **SEVERE**: Participant experiences symptoms or findings that require significant intervention

8.1.9 Guidelines for Determining Causality

The investigator will use the following question when assessing causality of an adverse event to study drug, where an affirmative answer designates the event as a suspected adverse reaction: 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.

8.1.10 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 an AE, whether serious or non-serious, must be followed by the investigator until the clinical outcome from the AE is determined. Any participant who experiences an AE may be withdrawn at any time from the study at the discretion of the investigator. The AE(s) should be noted on the appropriate CRFs, 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.

8.1.11 Investigator Reporting Procedures

Serious events will be entered into the data system immediately and no later than within 24 hours of identification. Non-serious adverse events will be entered into the safety data system within 7 days of identification. If there are any technical difficulties, the SAE will be reported by direct communication with the medical monitor.

8.2 Serious Adverse Events

Any serious adverse event entered in the safety database will generate an automatic email notification to the IND sponsor or the in-country designee and funding sponsor. The DCC medical monitor will review all SAEs at the time that they are reported. Investigators must also submit safety reports locally as required by their IRB/REB.

8.3 Regulatory Reporting

Any event that requires reporting to Regulatory Authorities (i.e. Serious Unexpected Suspected Adverse Reactions or SUSARS) based on applicable national regulations will be forwarded to the sponsor in time to meet reporting requirements, (e.g. 7 days for fatal and life-threatening (expedited) initial reports, with follow up reports within another 8 days, 15 days for all other SUSARs). The sponsor or the in-country representative will submit safety reports (e.g. IND safety reports) to the regulatory agencies as necessary and will inform the investigators of such regulatory reports. Site investigators must submit safety reports as required by their IRB/REB. Documentation of the submission and receipt by the IRB/REB must be retained for each expedited safety report.

All serious events irrespective of their designation as “related” or “not related” to study drug(s) will be

reported to the FDA at least annually in a summary format.

8.4 Safety Oversight

The DSMB will review serious adverse events on a monthly basis. In addition, a qualified and experienced clinician not otherwise associated with this protocol will serve as the medical monitor. The medical monitor will review all 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. The medical monitor will also indicate whether he/she concurs with the details of the report provided by the study investigator. If no SAEs prompt review at an earlier time point, the DSMB will review AEs and SAEs at the regularly scheduled meeting. Additionally, DSMB will periodically review interim safety analyses (Section **10.3**). The DSMB will convene and make recommendations on termination of the study based on review of safety reports and halting rules. The safety data will be compiled by DCC. Based on the recommendations of the DSMB, and NIH, the IND sponsor will make a decision to terminate or continue the study.

8.5 Halting Criteria: Safety Concerns

An unscheduled DSMB review of safety data will be triggered if: (a) ≥3 patients in a cohort have treatment completely stopped due to the same AE with a causal relationship to study treatment, or (b) if ≥3 patients in a cohort have the same SAE, or (c) ≥3 patients in a cohort have a Serious Adverse Reaction. Enrollment will be suspended during DMC review, though study activities will be allowed to proceed on previously enrolled subjects if applicable. If there are 3 or more Serious Adverse Reactions in a cohort, then we will also inform the NICHD, FDA and Health Canada, and the DSMB may choose to be unmasked to treatment assignment. The DSMB may receive unmasked safety data if safety concerns are identified at any point during the study.

9. CLINICAL MONITORING

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 DCRI sponsor standard operating procedures. The IND/CTA sponsor, or as detailed in the Transfer of Regulatory Obligations (TORO), the BPCA DCC, NIH, or its designee will conduct site-monitoring visits as detailed in the monitoring plan or in the Manual of Procedures (MOP).

Site visits will be conducted at standard intervals as defined by the site monitoring plans and may be made more frequently as directed by the IND sponsor. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, CRFs, informed consent forms, medical and laboratory reports, and protocol compliance. Study monitors will meet with investigators to discuss any problems and actions to be taken and document visit findings and discussions.

10. STATISTICAL CONSIDERATIONS

10.1 Study Endpoints

Primary safety endpoints are the incidence of AEs and SAEs. Secondary endpoints include change in BPD risk, BPD incidence, death rate, incidence of death or BPD, incidence of ROP, and incidence of pulmonary hypertension.

10.2 Sample Size Considerations

The sample size of 30 in each dose group is sufficient to estimate AE or SAE incidence with sufficient precision. **Table 10-1** provides widths for 95% Wilson confidence intervals in the dose groups of size 30 and the total sildenafil treatment cohort of 90 with different incidence rates. An event with an incidence rate of 0.05 has a 78% chance of being observed at least once in a dose group and a 99% chance of being observed at least once in the total sildenafil cohort.

Table 10-1. Widths for 95% Wilson confidence intervals

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

Population for Analysis
All participants enrolled, randomized, and dosed will be included in the safety population and the safety analyses. All participants who had at least one interpretable PK sample will be included in the PK analysis.

10.3 Interim Safety Analysis

The DSMB must complete a review of the safety data after completion of each cohort prior to opening enrollment into the next cohort. For cohort 1, this review was initiated once the 14 day follow up period post treatment for the last participant was completed. For cohort 2, the DSMB will review safety data once the 28 day follow up period post treatment is completed for the 36th participant. Approval for opening cohort 3 to enrollment will begin only after the DMC has completed their review of these safety data up to, and including, the 36th participant, and provided a recommendation that the study proceed with enrollment to the next cohort. Enrollment of the remaining four participants in cohort 2 will continue while the DSMB review of the safety data is ongoing. Cohort 3 enrollment will not begin until cohort 2 is closed. The remaining safety data among the last four participants will be reported to the DSMB for review per the current study process (see Section **8.4**).

The DSMB will continue to review the safety report on participants at regular DSMB meetings that occur two to three times a year. The number and percent of AEs and SAEs within each dose group will be summarized overall as well as by each Medical Dictionary for Regulatory Activities (MedDRA) system organ class and preferred term. Laboratory data will be tabulated by dose groups.

Summary statistics for changes from baseline will be presented.

See Section **8.5** for additional details for halting criteria for safety concerns.

10.4 Analysis Plan

Analyses will be presented by treatment groups. There are 4 planned treatment groups: 1) participants randomized to sildenafil in Cohort 1; 2) participants randomized to sildenafil in Cohort 2; 3) participants randomized to sildenafil in Cohort 3 and 4) participants randomized to placebo.

10.4.1 Descriptive statistics

Descriptive statistics such as number of observations, mean, median, standard deviation, standard error, 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.)

10.4.2 Demographic and baseline characteristics

The number of participants completed and discontinued early from study and the reasons for the discontinuation will be summarized by treatment group. Demographic and baseline characteristics will also be summarized by treatment group. Variables include race, age, sex, and selected clinical variables recorded prior to initiation of study drug. Study drug administration will be summarized in terms of route, number of days of dosing and reasons for final discontinuation of study drug.

10.4.3 Safety

The primary safety endpoint is number and percent of AEs and SAEs and will be summarized by treatment groups. AEs and SAEs will be presented overall and by each Medical Dictionary for Regulatory Activities (MedDRA) system organ class and preferred term. Prior and concomitant medications will be summarized by World Health Organization (WHO) drug class. Laboratory data will be tabulated by treatment groups. Summary statistics for changes from baseline will be presented.

Hypotension, presumed hypotension and presumed low blood pressure (as defined in Section **8.1.4**) will be summarized by treatment group.

10.4.4 Effectiveness analysis

Multivariable mixed-effects model will be used to explore the relationship between the maximum and total dose of sildenafil and change in the risk of moderate BPD, severe BPD or death at 7, 14, 21 and 28 days of study drug. A dose by time interaction term will allow us to estimated change in the risk of BPD within each treatment group and make comparisons between treatment groups.

Death, BPD status, death or moderate/severe BPD, ROP, and pulmonary hypertension will be evaluated at 36 weeks PMA using logistic regression analyses to evaluate the effect of dose. Adjustments will be made for important covariates such as GA, birth weight, sex and maternal race/ethnicity.

10.4.5 PK analysis plan

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

10.4.6 Biomarkers

We will relate the concentration of biomarkers (Section **6.10.1**) to the development of and severity of BPD.

11. PARTICIPANT CONFIDENTIALITY

Participant confidentiality is held strictly in trust by the participating investigators, their staff, and the sponsor(s) 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 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.

This study is covered by a Certificate of Confidentiality (CoC) from the NIH. The CoC prevents U.S. courts and other U.S. agencies from forcing the study team to share information that may identify the participants during a legal or legislative action unless the participant allows this. The CoC does not keep the participants from sharing information about their participation in this study.

For Canadian participants, the NIH CoC may protect data housed in the United States from legal proceedings, but disclosure may still occur if required by Federal, State, or local laws, which may be different from Canadian law. Canadian federal and provincial regulations will govern the confidentiality of data maintained at the Canadian sites.

After the study is completed, information about the study, including study data, will be submitted to the NIH data repository (https://dash.nichd.nih.gov; referred to below as “DASH” (Data and Specimen Hub). With NIH approval, the data submitted to DASH may be used by other researchers for future research. The study data submitted to DASH will be de-identified. When de-identified study data are provided to other researchers for the purposes of future research, it may be done without obtaining additional permission from the participant. Canadian participants must consent to this arrangement before their data is provided.

The principal investigator will ensure that the use and disclosure of personal health information obtained during this research study complies with the Federal Privacy Regulations.

For Canadian sites, privacy requirements are described in several Federal and Provincial laws and regulations. Examples include, but are not limited to the Federal Personal Information Protection and Electronic Documents Act, Genetic Non-Discrimination Act, the Ontario Personal Health Information Protection Act, Quebec’s Act Respecting the Protection of Personal Information in the Private Sector, the BC Personal Information Protection Act, Alberta’s Personal Information Protection Act and Health Information Act. Note that for several provinces more than one act may apply.

In the U.S., the Health Insurance and Portability and Accountability (HIPAA) Privacy Rule applies. The rule provides U.S. federal protection for the privacy of protected health information sent to or collected in the U.S. 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 Canadian Federal and Provincial laws or the HIPAA Privacy rule, whichever applies.

12. 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. Permission forms will be IRB/REB-approved, and the IRBs/REBs will determine whether one parent or both parents must provide permission. 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. For non-English speakers, a fully translated permission document or an oral presentation accompanied by a short form may be used to obtain informed permission. The fully translated permission document and the short form must be approved by the IRB/REB and executed according to IRB/REB requirements.

The IND/CTA sponsor or designee will provide the investigator, in writing, any new information that bears significantly on the participants' risk to receive the study drug. This new information will be communicated by the investigator to participants' parents/legal guardians/LARs in accordance with IRB/REB requirements. The informed permission document will be updated, and, if necessary, the informed permission process will be repeated and participants' parents/legal guardians/LARs asked to sign updated permission documents.

Site staff may employ IRB/REB-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.

13. FUTURE USE OF SPECIMENS

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

After the study is completed, all leftover 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/REB 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 information of the participant. 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, specimens repository, and potential for future research prior to the child's participation in the study.

14. SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

An electronic case report form (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 investigator(s) 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/REB 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 ICH E6 (R2), Section 4.9, 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, which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, 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, and participant files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial. Case report forms will be derived from the eCRF and provided by the Data Coordinating Center (DCC).

15. QUALITY CONTROL AND QUALITY ASSURANCE

The principal investigator will provide direct access to all trial-related sites, source data/documents, case report forms, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The principal 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, Good Clinical Practice, and the applicable regulatory requirements. Clinical monitoring reports will be submitted to the PI, and NIH.

The DCC 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.

16. ETHICS/PROTECTION OF HUMAN PARTICIPANTS

16.1 Ethical Standard

The investigator will ensure that the study will be conducted in accordance with the protocol, the ethical principles of Good Clinical Practice (ICH E6 (R2)) that have their origin in the Declaration of Helsinki, and all applicable national and local regulations. The investigator will ensure that the study is conducted in accordance with the provisions as stated and will comply with the prevailing local laws and customs.

16.2 Institutional Review Board/Research Ethics Board

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

The responsible official for the IRB/REB will sign the letter of approval of the protocol prior to the start of this trial, and a copy will be provided by the site study team to the DCC. Notification of the IRB/REB's composition and the institution’s federal-wide assurance number (if applicable) will be provided to the DCC.

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/REB.

Any refusals by IRBS/REBS must be documented for reporting to Heath Canada.

16.3 Informed Consent

The investigator will choose participants in accordance with the eligibility criteria detailed previously. To prevent bias, the investigator will not exercise selectivity. Since it is not possible to obtain participant consent or assent in this study, parents/legal guardians/legally authorized representatives (LARs) will provide informed permission for study participation.

Before any study procedures or treatments are administered, all parents/legal guardians/LARs must provide informed permission, and sign a permission form that complies with the federal requirements and an authorization form that complies with federal and provincial privacy regulations for the use and disclosure of the participant’s protected health information. The permission form and the authorization form must be approved by the DCC and by the IRB/REB.

For details regarding the informed permission process, see Section **12**.

The study monitor or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the investigator. This documentation includes, but is not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. Clinical study sites will permit access to such records.

16.4 Study Discontinuation

If the study is discontinued, enrolled participants will continue to be followed for safety assessments for 28 days. All adverse events must be followed through resolution. Notification to regulatory authorities, REBs and participants will be carried as required by regulation (e.g. U.S. 21 CFR 312.56, Canadian FDR C.05.012, C.05.015).

17. DATA HANDLING AND RECORD KEEPING

U.S. investigators are obligated to conduct this study in accordance with U.S. Federal Regulation 21 CFR 312.60-69 as specified on the signed form FDA 1572, applicable local laws, and the International Conference on Harmonisation: Good Clinical Practice: Consolidation Guideline.

Canadian investigators must conduct the study in accordance with Canadian Food and Drug Regulations Part C Division 5, and in particular the undertakings and practices described in: C.05.012(3)(f) (Qualified Investigator Undertaking) and C.05.010 concerning Good Clinical Practices.

The investigator is responsible for informing the IRB/REB of any safety issues related to the study and the study drug, including reports of serious adverse events, 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 or reported.

Case report forms will be derived from the eCRF and provided by the DCC to the sites to record and maintain data for each participant enrolled in the study. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Permanent ink is required to data integrity and ensure clarity of reproduced copies. When making a change or correction, the original entry should be crossed out with a single line, and the change should be initialed and dated. Do not erase, overwrite, or use correction fluid or tape on the original.

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

17.1 Data Management Responsibilities

All case report forms 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 principal investigator or designee. Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site principal investigator. During the study, the investigator must maintain complete and accurate documentation for the study.

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

17.2 Data Capture Methods

Clinical data (including AEs 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 the case report forms/source documents.

17.3 Timing/Reports

The DSMB will convene and make recommendations on study continuation based on the safety data collected periodically.

17.4 Study Records Retention

Unless the Sponsor notifies the investigator otherwise, records and source documents pertaining to the conduct of the study, are to be retained by Investigators in the United States for a period of at least

2 years after the date upon which the Sponsor's application to the U.S. FDA is accepted, rejected or withdrawn, or in accordance with institutional requirements, whichever is longer. The disposition date of the Sponsor's FDA application will be posted on the PTN website for the Investigators' reference. In Canada study records will be retained for 25 years after the end of the study.

17.5 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or manual of procedures requirements. 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.

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 Internet Data Entry System (IDES).

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

17.6 Participant Privacy/Authorization

The principal investigator will ensure that the use and disclosure of protected/personal health information obtained during a research study complies with all privacy regulations. For Canadian sites, these requirements are described in several Federal and Provincial laws and regulations (for example the Federal Personal Information Protection and Electronic Documents Act, Genetic Nondiscrimination- Act, the Ontario Personal Health Information Protection Act, Quebec's Act Respecting the Protection of Personal Information in the Private Sector, the BC Personal Information Protection Act, Alberta's Personal Information Protection Act and Health Information Act).

In the U.S., the HIPAA Privacy Rule applies. The HIPAA Privacy Rule applies to all PHI sent or collected in the U.S. for purposes of this research and provides U.S. federal protection for the privacy of protected health information 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 Canadian Federal & Provincial laws and regulations or the HIPAA Privacy Rule whichever applies. Authorization will be combined in the informed consent document (approved by the IRB/REB).

18. PUBLICATION POLICY

Following completion of the study, the investigator may publish the results of this research in a scientific journal under the oversight of the Publication Committee of the Pediatric Trials Network (PTN). The PTN Publication Committee comprises representatives of the network cores, thought-leaders, DCC, and PTN, and is responsible for generation and coordination of the publications that report scientific findings of the network. 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, DCC, and PTN that use PTN data are intended to represent the sponsor or the PTN 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 PTN 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.

The ICMJE defines a clinical trial as any research project that prospectively assigns human participants to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., phase 1 trials), would be exempt from this policy.

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. The NIH Public Access Policy ensures the public has access to the published results of NIH-funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication. Refer to: http://publicaccess.nih.gov/ and http://grants.nih.gov/grants/guide/notice-files/NOT-OD-08-033.html.

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