Safety of Furosemide in Premature Infants at Risk of Bronchopulmonary Dysplasia

Study Protocol

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Protocol Addendum 001

27 September 2019

Safety of Furosemide in Premature Infants at Risk of Bronchopulmonary Dysplasia

Follow-Up Hearing Test Results

NICHD-2014-FUR01

Summary:

This document is an addendum to the **Safety of Furosemide in Premature Infants at Risk of Bronchopulmonary Dysplasia** (NICHD-2014-FUR01) protocol. The purpose of this addendum is to collect additional hearing test results from the subset of FUR01 participants who meet the eligibility criteria outlined below. We will record results from hearing tests that were conducted as part of standard care after completion of final FUR01 study assessments up to and including when the participant passes a hearing test (both ears), or until the participant reached 1 year of age, whichever comes first.

Rationale:

Infants born prematurely with documented failed or equivocal hearing tests typically have follow-up testing as part of their routine medical care through the first year of life. Follow-up testing typically reveals that a substantial number of these infants have normal hearing. Results of these follow-up hearing tests in FUR01 participants with a failed or equivocal hearing test reported at final study assessments prior to discharge, are important to collect to add to the safety profile of furosemide in previously premature infants at risk of bronchopulmonary dysplasia.

Inclusion Criteria:

1. Participated in the FUR01 Study
2. Documented hearing test result (for either ear) was categorized as fail or equivocal at final FUR01 study assessments reported prior to discharge
3. Documented informed consent, or waiver of informed consent, as determined by the ruling IRB

Exclusion Criteria:

1. Informed consent was withdrawn during FUR01 study participation

Events of Interest:

Failed hearing test (in either ear) identified by standard of care hearing screen (e.g. BAER), as determined by clinical care provider.

Follow-Up Hearing Assessment / Data Collection

For eligible participants, the study staff will continue to collect results from standard of care hearing tests, if available, until and including when the participant passes a hearing test (both ears) or until the participant is 1 year of age, whichever came first.

Data collection will occur via medical record review.

Analysis Plan:
The analyses described in the protocol and Statistical Analysis Plan will be conducted using the follow-up hearing test results.

Pediatric Trials Network

Safety of Furosemide in Premature Infants at Risk of Bronchopulmonary Dysplasia

NICHD-2014-FUR01

Phase 2 Trial

Funding Sponsor:

The *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD)

Funding Mechanism: Task Order

Protocol Date:	22-Mar-2017
Protocol Version:	6.0
IND Number:	120,933
Principal Investigator (IND Sponsor):	Matthew M. Laughon, MD, MPH Professor The University of North Carolina at Chapel Hill Chapel Hill, NC 27599-7596 Office: 984-974-7851 E-mail: matt_laughon@med.unc.edu

Statement of Compliance

This trial will be conducted in compliance with the protocol, International Conference on Harmonisation (ICH) guideline E6: Good Clinical Practice (GCP): Consolidated Guideline, and the applicable regulatory requirements from the United States Code of Federal Regulations (CFR), including 45 CFR 46 (human subjects protection), 21 CFR 312 (Investigational New Drug), 21 CFR part 50 (informed consent), and 21 CFR part 56 (institutional review board [IRB]) as well as international regulatory requirements if applicable.

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 effectiveness and safety parameters and the conduct of the study in general. I am aware that, before beginning this study, the institutional review board responsible for such matters must approve this protocol in the clinical facility where it will be conducted.

I agree to provide all participants with informed consent forms, as required by government and International Conference on Harmonisation regulations. I further agree to report to the sponsor or its representative any adverse events in accordance with the terms of this protocol and the U.S. Code of Federal Regulations, Title 21, part 312.64 as well as international regulatory requirements if applicable.

_____
Principal Investigator Name (Print)

_____ _____
Signature Date

STUDY PRINCIPAL INVESTIGATOR / 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 and ICH guidelines.

Matthew M. Laughon, MD, MPH

Pediatric Trials Network Study Principal Investigator Name

Signature

Date

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List of Abbreviations

AE	Adverse Event
AUC_{0-24}	Area Under the Concentration Time Curve 0-24 hours
AUC_{ss}	Area Under the Concentration Time Curve at Steady State
BAER	Brainstem Auditory Evoked Response
BPD	Bronchopulmonary Dysplasia
BUN	Blood Urea Nitrogen
BW	Birth Weight
CA	Calcium
CFR	Code of Federal Regulations
CL	Clearance
C_{max}	Maximum Concentration
CR	Central Review
CRF	Case Report Form
DCC	Data Coordinating Center
DCF	Data Collection Form
DCRI	Duke Clinical Research Institute
DMC	Data Monitoring Committee
eCRF	Electronic Case Report Form
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
IB	Investigator's Brochure
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
K	Potassium
Kg	Kilogram
L	Liter
LPM	Liters per Minute
MedDRA ®	Medical Dictionary for Regulatory Activities
Mg	Milligram
mcg	Microgram
MOP	Manual of Procedures
N	Number (typically refers to participants)
Na	Sodium
NCPAP	Nasal Continuous Positive Airway Pressure
NICU	Neonatal Intensive Care Unit

List of Abbreviations – *continued*

NIH	National Institutes of Health
NRN	Neonatal Research Network
PDA	Patent Ductus Arteriosis
PI	Principal Investigator
PICU	Pediatric Intensive Care Unit
PNA	Postnatal age
PMA	Postmenstrual age (gestational age plus postnatal age)
PK	Pharmacokinetics
PO	By mouth
PTN	Pediatric Trials Network
RANTES	Regulated on Activation, Normal T Cell Expressed & Secreted
SAE	Serious Adverse Event
TORO	Transfer of Regulatory Obligations
$t_{1/2}$	Half-life
V_{ss}	Volume of distribution at steady state
WHO	World Health Organization

Protocol Synopsis

Protocol Title:	**Safety of Furosemide in Premature Infants at Risk of Bronchopulmonary Dysplasia (BPD)**
Phase:	2
Product:	Furosemide
Objectives:	Primary: Describe the safety of furosemide in premature infants at risk of BPD Secondary: Preliminary effectiveness and pharmacokinetics (PK) of furosemide
Study Design:	Multi-center, randomized, placebo-controlled, dose escalating, double masked, safety study
Study Population:	Inclusion Criteria 1. Documented informed consent from legal guardian, prior to study procedures 2. Receiving positive airway pressure (nasal continuous airway pressure, nasal intermittent positive pressure ventilation, or nasal cannula flow > 1LPM) or mechanical ventilation (high frequency or conventional) at time of randomization. 3. < 29 weeks gestational age at birth 4. 7-28 days postnatal age at time of randomization Exclusion Criteria 1. Exposure to any diuretic ≤ 72 hours prior to randomization 2. Previous enrollment and dosing in current study, "*Safety of Furosemide in Premature Infants at Risk of Bronchopulmonary Dysplasia*" 3. Hemodynamically significant patent ductus arteriosus, as determined by the investigator 4. Major congenital anomaly (e.g. congenital diaphragmatic hernia, congenital pulmonary adenomatoid malformation) 5. Meconium aspiration syndrome 6. Known allergy to any diuretic 7. Serum creatinine >1.7 mg/dl < 24 hours prior to randomization 8. BUN >50 mg/dl < 24 hours prior to randomization 9. Na <125 mmol/L < 24 hours prior to randomization 10. K ≤2.5 mmol/L < 24 hours prior to randomization 11. Ca ≤ 6 mg/dL (or ionized calcium ≤ 3.2 mg/dl) < 24 hours prior to randomization 12. Indirect bilirubin > 10 mg/dl < 24 hours prior to randomization*. 13. Any condition which would make the participant, in the opinion of the investigator, unsuitable for the study. *If total bilirubin is measured without the differentiated (conjugated (direct) and unconjugated (indirect) fractions and is ≤10mg/dl, this value can be accepted for determination of indirect bilirubin ≤10mg/dl.
Number of Participants:	120
Number of Sites:	Approximately 30 sites
Duration of Participation:	Up to 35 days (28 days of study drug plus 7 days of safety monitoring). Information about hospitalization will be collected at 36 weeks post menstrual age and/or at discharge.

Dose Schedule:	**Table. N and dosing scheme**						
			N	**Furosemide (IV)**	**Furosemide (enteral)**	**N**	**Cohort N**
	Cohort 1	Placebo	10	1 mg/kg q 24 hours	2 mg/kg q 24 hours	30	40
	Cohort 2	Placebo	10	1 mg/kg q 6 hours	2 mg/kg q 6 hours	30	40
	Cohort 3	Placebo	10	2 mg/kg q 6 hours	4 mg/kg q 6 hours	30	40

1 KEY ROLES

For questions regarding this protocol, contact:

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

The Best Pharmaceuticals for Children Act (BPCA) mandates the National Institutes of Health (NIH) to prioritize therapeutic areas in critical need for pediatric labeling, sponsor pediatric clinical trials, and submit these data to FDA for consideration for labeling changes. This study will be conducted in accordance with Section 409I of the Public Health Service Act; as such, the results from this research may be submitted to the FDA for review and use in negotiated labeling changes. This research study is contractually supported by the NICHD. The NICHD awarded a contract to Duke University which established a Pediatric Trials Network (PTN) through its Duke Clinical Research Institute (DCRI) in order to facilitate trial design for studies supported by NIH. A separate contract was awarded to The Emmes Corporation (Rockville, MD) 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 (O_2) for ≤28 days and not at 36 weeks PMA
Mild BPD	Receiving > 21% O_2 for ≥28 days but not at 36 weeks PMA
Moderate BPD	Receiving > 21% O_2 for ≥28 days plus treatment with <30% O_2 at 36 weeks PMA
Severe BPD	Receiving > 21% O_2 for ≥28 days plus ≥30% O_2 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]

Approximately 17,500 premature infants develop BPD each year in the US. Although rare in the general population, BPD is the most common pulmonary morbidity associated with prematurity and is increasing. Approximately 50,000 infants are born at ≤ 28 weeks gestational age each year in the US and ~35% 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.[12,13,14] 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.[15,16,17]

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, 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 Glutamine trial identified lower birth weight, lower gestational age, male, 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, patent ductus arteriosus, and late onset sepsis as risk factors for BPD[22] In preterm infants with respiratory failure enrolled in the Neonatal Research Network inhaled nitric oxide trial, the authors found that the risk of BPD was associated with lower birth weight, higher oxygen requirement, male gender, additional surfactant doses, higher oxygenation index and outborn status.[23] Until recently, using these risk factors for prediction of BPD has been challenging.[23]

Accurate prediction of BPD risk is now possible: The NICHD Neonatal Research Network recently developed an online, publicly available BPD prediction tool (https://neonatal.rti.org/index.cfm?fuseaction=BPDCalculator.start) (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, ventilator support, and F_iO_2. 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.[24,25,26] 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.[27,28,29,30,31,32,33,34] 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.

There are no FDA indicated therapies 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.[35,36] Postnatal steroids reduce BPD, but increase the risk of cerebral palsy.[37,38] 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.[39] One problem with the vast majority of trials of drugs to prevent BPD is that they did not establish the safety, preliminary effectiveness, PK, pharmacodynamics, or dose prior to implementation of phase III randomized, controlled trials.[40]

Previous trials of diuretics, including furosemide, are limited to respiratory outcomes after one week of exposure. Two Cochrane reviews examine the use of loop diuretics such as furosemide and diuretics acting on the distal renal tubule.[41,42] They include small studies of single dose or short course therapy and report short-term improvements in extubation rates, lung compliance, and fraction of inspired oxygen. The incidence of BPD was not reported in these studies. Thus, there is biologic plausibility and physiologic studies that demonstrate improvement in lung function, but it is not known if furosemide prevents BPD.[43] Prevention of BPD is an urgent, unmet public health need.

Neonatologists are using furosemide without efficacy or safety data. Neonatologists commonly use diuretics, with furosemide being the most common by far (93% of diuretic use), in premature infants.[44] Despite neonatal medicine's long history of catastrophic adverse events resulting from inadequate study of drugs prior to their widespread use, the majority of drugs used in premature infants have undergone insufficient study to receive FDA labeling, including diuretics. Extrapolating diuretic safety, effectiveness, and dosing from adults underestimates the complicated physiology of premature infants, who have (1) larger extracellular fluid volume per unit body weight, (2) immature renal and hepatic function, and (3) a unique blood-brain barrier, each of which can alter drug disposition and clearance.[45,46,47] Improper use of drugs in these vulnerable patients leads to increased rates of treatment failure, adverse events, mortality, and long-term morbidities.[47] Diuretic studies specifically designed for premature infants are urgently needed.

Furosemide is consistently in the top 10 among all medications reported in all hospitalized infants in the NICU: We reviewed data from the Pediatrix Data Warehouse from 2005-2010 that includes information from ~20% of all infants admitted each year to the NICU in the US. Furosemide was the 7th most commonly used drug overall in the NICU from 2005-2010, and 5th most commonly used in extremely low birth weight infants (<1000 g; **Table 2-2**).[48]
81 out of every 1000 hospitalized infants are exposed to furosemide. (668 infant-days per 1000 infants).

Table 2-2. Most common drugs in the NICU reported from 887,910 infants

Medication	% exposed	FDA labeling for premature
Ampicillin	68	None
Gentamicin	63	Dosing information
Caffeine	14	None for <28 weeks
Vancomycin	9	Dosing information
Beractant	9	Yes
Cefotaxime	8	Dosing information
Furosemide	***8***	***None***
Fentanyl	6	None
Dopamine	6	None
Midazolam	5	Yes

Furosemide is the most common diuretic used: Among the 39,357 infants < 32 weeks and < 1500 g who received diuretics, 93% (36,759) received furosemide (**Table 2-3**).[44]

Table 2-3. Infants <32 weeks and <1500 g exposed to diuretics by type

Diuretic[1]	N (%)[2]	Exposure[3]	Courses[3]	Days of exposure[4]
	Total N=39,357			
Furosemide	36,759 (93)	342	784	66
Spironolactone	9577 (24)	86	109	3
Chlorothiazide	8309 (21)	77	98	29
Hydrochlorothiazide	2473 (6)	22	26	7
Bumetanide	1443 (4)	13	17	2
Acetazolamide	1131 (3)	11	18	2

[1] metolazone (n=147), hydrochlorothiazide/spironolactone (n=87), diazoxide (n=64), ethacrynic acid (n=61), mannitol (n=8), and amiloride (n=3) exposure was minimal
[2] infants could be exposed to more than one diuretic
[3] per 1000 infants (all infants)
[4] per 1000 infant-days (all infants)

Furosemide clinical pharmacology: Furosemide is a loop diuretic that acts in the proximal and distal tubules, as well as in the loop of Henle; it inhibits reabsorption of sodium and chloride at these sites. Furosemide is indicated in adults and pediatric patients for the treatment of edema associated with congestive heart failure, cirrhosis of the liver, and renal disease, including the nephrotic syndrome. It is also indicated as adjunctive therapy in acute pulmonary edema in adults.

2.2.1 The safety and pharmacokinetics of furosemide in premature infants is limited. There have been 4 studies of the pharmacokinetics of furosemide in premature infants.

Table 2-4. Demographics of premature infants and dosing in furosemide studies

	N	BW	GA	Postnatal age	Dose	Route	Frequency of administration
		g	*weeks*	*weeks*	mg/kg		*hours*
Aranda 1978[49]	8	2391+/-290	35+/-1.8	1.6+/-0.8	1-1.5	IV	Single dose
Peterson 1980[50]	14	1270+/-169	30+/-0.8	1.4+/-0.3	1	IV	Single dose
Vert 1982[51]	8	1326+/-352	29+/-2	3.1+/-3	0.29-1.48	IV	Single and multiple doses
Mirochnick 1988[52]	10	829+/-217	26.6+/-2.9	2.4+/-1	1; 2-3	IV; enteral	12

Table 2-5. Pharmacokinetics of furosemide in premature infants

	Volume of distribution	Clearance	Half-life
	L/kg	*ml/kg/hour*	*hours*
Aranda 1978[49]	0.829+/-0.118	81.6+/-15	7.7+/-1
Peterson 1980[50]	0.239 +/-0.027	10.6+/-2.1	19.9+/-3
Vert 1982[51]	0.204+/-0.075	6.9+/-5.1	26.8+/-12.2
Mirochnick 1988[52]	0.23 +/- 0.04	1.62-73.8 (range)	1.8-67.3 (range)

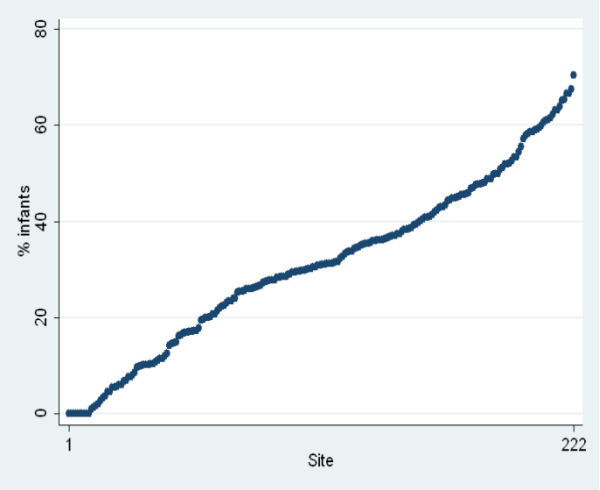
The use of furosemide varies widely among infants (Table 2-6) and among centers (Figure 2-1): Exposure of premature infants to diuretics varied widely across infants and sites. In some centers, the exposure is very high, suggesting that diuretics are used prophylactically, presumably to prevent BPD. Conversely, in other centers exposure is rare. These observations suggest that there is no universally accepted standard of care for when to expose infants to diuretics, and that no exposure is an option exercised in some centers.

Table 2-6. Respiratory support on 1st day of each new diuretic course in infants <32 weeks and <1500 g[44]

	No support	Nasal cannula	HFNC/ NCPAP	Mechanical ventilation	High frequency ventilation	F_iO_2
	n (%)	*n (%)*	*n (%)*	*n (%)*	*n (%)*	*median, IQR*
furosemide	4886 (6)	12,935 (15)	21,324 (25)	32,811 (39)	12,053 (14)	35 (25, 50)
spironolactone	626 (5)	3336 (29)	4073 (35)	3122 (27)	460 (4)	35 (27, 50)
chlorothiazide	463 (4)	2867 (27)	3151 (30)	3418 (33)	613 (6)	36 (28, 50)
hydrochlorothiazide	147 (5)	732 (27)	984 (36)	751 (27)	130 (5)	35 (26, 49)
bumetanide	18 (1)	43 (2)	98 (5)	853 (47)	813 (45)	45 (30, 75)
acetazolamide	40 (2)	178 (9)	636 (33)	917 (48)	153 (8)	35 (27, 48)

HFNC: high flow nasal cannula
NCPAP: nasal continuous positive airway pressure

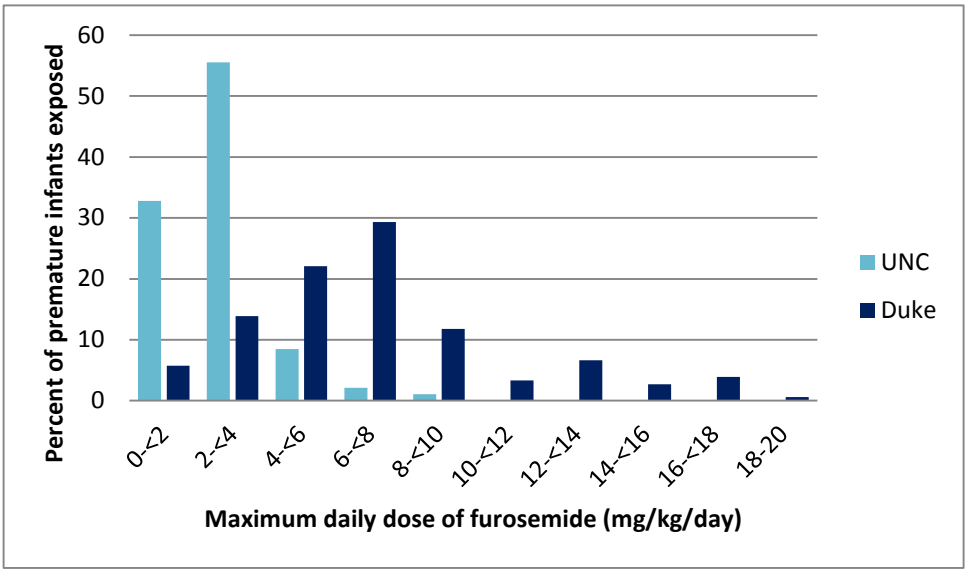
Figure 2-1. Percentage of infants <32 weeks and <1500g exposed to furosemide in 222 sites of the Pediatrix Medical Group[44]



We reviewed the safety and outcomes of infants < 28 weeks born at UNC and Duke from 2006-2012: We reviewed infants < 28 weeks gestation and survived > 7 postnatal days at UNC and Duke. Traditionally, UNC is a low furosemide exposure site with a high rate of BPD and Duke is a high furosemide exposure site with a low rate of BPD. We found that the data confirmed this supposition (**Table 2-7 and Figure 2-2**) — UNC had a 58% furosemide exposure with a 60% incidence of death or BPD while Duke had a 95% furosemide exposure with a 35%% incidence of death or BPD. UNC infants were most often exposed to 0-2 mg/kg/day of furosemide (**Figure 2-2**) while Duke infants were most often exposed to 4-6 mg/kg/day of furosemide, with 25% exposed to >8 mg/kg/day.

Table 2-7. Premature infants <28 weeks from 2006-2012 at Duke or UNC NICU		
Characteristics	**DUKE**	**UNC**
N	350	329
Gestational Age(weeks): Median (IQR)	25 (24, 27)	26 (24, 27)
Birth Weight(grams): Median (IQR)	760 (640, 870)	760 (640, 880)
Antenatal Steroids: %	92.8%	86.8%
Surfactant: %	87.1%	81.7%
Furosemide: %	94.6%	57.8%
Maximum Daily Dose(mg/kg/day): Median (IQR)	6.3 (4.2, 8.3)	2.0 (1.9, 2.9)
Cumulative Dose(mg/kg): Median (IQR)	100.9 (40.3, 191.7)	12.5 (4.3, 39.6)
Duration(days): Median (IQR)	33 (14, 54)	7 (3, 26)
Bronchopulmonary Dysplasia or Death at 36 Weeks: %	35.4%	59.6%
Failed Hearing: %	9.1%	3%
Hypernatremia: %	1.1%	7.3%
Hyponatremia: %	5.4%	3.3%
Hyperchloremia: %	19.7%	36.8%
Hyperkalemia: %	13.1%	15.8%
Hypokalemia: %	21.4%	11.2%
High Bicarbonate: %	9.4%	0%
Low Bicarbonate: %	70.3%	40.7%
Elevated BUN: %	23.4%	4.60%
Elevated Creatinine: %	12.9%	1.5%
Elevated Alkaline Phosphate: %	6.3%	3.6%

Figure 2-2. Maximum daily dose of furosemide at UNC and Duke



[c]Because the bioavailability of enteral furosemide is approximately 0.5 times that of the intravenous formulation, the enteral + intravenous furosemide dose was calculated as:0.5 * enteral dose (mg/kg) + intravenous dose (mg/kg)

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. We will make every effort 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 Furosemide

Furosemide is associated with water loss and electrolyte depletion. Literature reports indicate that premature infants with postmenstrual age < 31 weeks receiving doses exceeding 1 mg/kg/24 hours may develop plasma levels associated with potential toxic effects including ototoxicity. In premature infants, furosemide may precipitate nephrocalcinosis/nephrolithiasis. If furosemide is administered to premature infants during the first weeks of life, it may increase the risk of persistence of patent ductus arteriosus.

2.3.2 Potential Benefits

Furosemide is used to reduce pulmonary edema, improve pulmonary mechanics, reduce exposure to mechanical ventilation, and prevent BPD. Although these improvements are not proven, increase in pulmonary function[42] and decrease risk of BPD are potential benefits for participants enrolled in the study who receive furosemide. Conclusions drawn from this study will benefit infants receiving furosemide in the future through better understanding of dose response and characterization of the safety profile of these drugs.

3 OBJECTIVES

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

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

3.1 Study Outcome Measures

3.1.1 Primary Outcome Measures

Safety as determined by adverse events experienced by the participants.

3.1.2 Secondary Outcome Measures

3.1.2.1 Preliminary effectiveness: Risk of BPD

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

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

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

Note: The 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 %. For this protocol, we will dichotomize the outcome as above (none-mild vs. moderate-severe-death). For example, a 25 week Hispanic female with birth weight of 689 g on postnatal day 14 on mechanical ventilation with a FiO_2 of 0.45 has risk of no BPD of 1.6%, mild 27.9%, moderate 28.9%, severe 30.5%, and death 11%. Thus, none-mild risk is 29.5% and moderate-severe-death risk is 70.5%.

We will collect risk of BPD or death as defined by NICHD NRN BPD estimator on days 7, 14, 21 and 28 of study drug. We will use the day closest to the day used to calculate the BPD estimator. The BPD estimator includes infants up to 28 postnatal days; for infants in this protocol older than that, we will use the 28 day estimates.

3.1.2.2 Pharmacokinetics

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

3.1.3 Other safety and efficacy outcomes

1. **Death:** All infants who died at or before 36 weeks PMA will be included.

2. **BPD:** We will define BPD as it was defined for the NICHD BPD estimator. We will define BPD as a dichotomous (none-mild vs. moderate-severe) variable and as a categorical variable (none, mild, moderate, or severe) among survivors by modifying the NIH consensus definition of BPD[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] We will define BPD as follows:

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

    5. **Death or BPD:** either 1 or 2

    6. **Hearing loss:** Hearing loss will be identified by hearing test (e.g. BAER) prior to discharge.

    7. **Nephrocalcinosis/nephrolithiasis**: Nephrocalcinosis/nephrolithiasis will be determined by renal ultrasounds performed locally and interpreted by the local radiologist. Renal ultrasounds that are positive for nephrocalcinosis or nephrolithiasis will be reviewed by an independent study radiology specialist to verify the positive findings.

4 STUDY DESIGN

See Protocol Synopsis.

This study will be conducted in accordance with current U.S. Food and Drug Administration regulations and guidelines, (or, as applicable, international regulations and associated guidelines), the International Conference on Harmonisation Guidelines on Good Clinical Practice (which incorporate the principles of the Declaration of Helsinki), as well as all other applicable national and local laws and regulations.

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 (furosemide:placebo) into 3 Cohorts with escalating doses of furosemide. There will be 40 randomized and dosed participants in each Cohort for a total of up to 120 participants.

5.2 Inclusion/Exclusion Criteria

Inclusion Criteria

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

Exclusion Criteria

1. Exposure to any diuretic ≤ 72 hours prior to randomization
2. Previous enrollment and dosing in current study, "*Safety of Furosemide in Premature Infants at Risk of Bronchopulmonary Dysplasia*"
3. Hemodynamically significant patent ductus arteriosus, as determined by the investigator
4. Major congenital anomaly (e.g. congenital diaphragmatic hernia, congenital pulmonary adenomatoid malformation)
5. Meconium aspiration syndrome
6. Known allergy to any diuretic
7. Serum creatinine >1.7 mg/dl < 24 hours prior to randomization
8. BUN >50 mg/dl < 24 hours prior to randomization
9. Na <125 mmol/L < 24 hours prior to randomization
10. K ≤2.5 mmol/L < 24 hours prior to randomization
11. Ca ≤ 6 mg/dL (or ionized calcium ≤3.2 mg/dl) < 24 hours prior to randomization
12. Indirect bilirubin >10 mg/dl < 24 hours prior to randomization*
13. Any condition which would make the participant, in the opinion of the investigator, unsuitable for the study

    *If total bilirubin is measured without the differentiated (conjugated (direct) and unconjugated (indirect)) fractions and is ≤10mg/dl, this value can be accepted for determination of indirect bilirubin ≤10mg/dl.

5.3 Treatment Assignment Procedures

5.3.1 Additional Participants

If a participant is randomized (see 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 (furosemide: placebo). All three Cohorts will use the same randomization scheme. The first 40 subjects will be enrolled in Cohort 1, and dose escalation via enrollment in other Cohorts will occur only after safety criteria are satisfied (see section 8.5). Participants randomized to placebo will receive the standard of care with regards to fluid imbalance; use of similar strategies for fluid and ventilator management will be part of the site selection criteria.

The participant's randomized treatment assignment will be obtained through the AdvantageEDC enrollment module. In the event that AdvantageEDC is not available at the time of randomization, a back-up system specified in the MOP will be used.

Randomization will be stratified by center. 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 CRF.

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, staff assessing participant outcomes will be blinded to treatment.

5.3.4 Reasons for Participant Withdrawal

The clinician may choose to suspend study drug dosing for up to 48 hours for any reason.

The investigator will withdraw a participant from receiving further study interventions if:

1. Any clinically significant adverse event (AE) is deemed by the principal investigator to require discontinuation of investigational product. The participant will continue to be followed for safety for 7 days after the last dose and complete all remaining follow up assessments unless consent has been withdrawn.
2. Suspension of the study drug for >48 hours. If study product is suspended for >48 hours, this is not a protocol deviation. The participant will proceed directly into the follow-up period and be followed for safety for 7 days after the last dose and complete all remaining follow up assessments unless consent has been withdrawn.
3. Intentional unmasking of the subject. All intentional unmasking events should be recorded on a protocol deviation form in EDC within 24 hours of the unmasking. The decision to unmask will be made in consultation with the Medical Monitor if at all possible. The unmasked participant will be followed for safety for 7 days after the last dose and complete all remaining follow up assessments unless consent has been withdrawn.
4. Requested by the NIH, FDA, PTN, or DMC.
5. Participants' parent or legal guardian may withdraw voluntarily from participation in the study at any time. Participants' parent or legal 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 electronic case report form (CRF).

6. Before discontinuing a participant from the investigational product, the local investigator must contact the medical monitor and/or protocol PI/Chair except in emergency situations. If the site cannot wait until the next business day, it will be considered an emergency situation. Participants who are prematurely discontinued from receiving study product for any reason will be followed for 7 days for adverse event monitoring and complete all remaining study assessments unless consent has been withdrawn.

5.3.5 Termination of Study

This study may be terminated at any time by FDA, NIH, the Investigational New Drug Application (IND) sponsor, or the Data Monitoring Committee (DMC).

6 STUDY PROCEDURES

6.1 Summary of Procedures

Table 6-1: Schedule of study procedures

	Screen/ Baseline	Treatment	Follow-up	36 weeks PMA assessment	Final study assessment
Time (Day)	0[1]	1-28[2]	Day 1-7 post last study dose	36 weeks PMA	Discharge/ET/ or Transfer
Informed consent	X				
Demographics	X				
Physical examination	X		X	X	
Medical history	X				
Actual Weight	X		X	X	
Respiratory assessment	X	X (Q WEEK)	X	X	X
Laboratory evaluations	X	X[6] (2X WEEK)	X	X	
Study drug administration		X			
Concomitant medications		X			
Adverse events	X[4]	X	X		
Blood sampling		X (AFTER DAY 7)			
Hearing screen					X
Renal ultrasound		X[5]	X[5]	X[5]	X
Discharge information[3]					X

[1] Refers to time point prior to start of study drug and may be the same calendar date as day 1
[2] While on study drug
[3] Transfer, discharge, or death
[4] AEs will be collected following initial study-specific procedure (e.g., screening blood draws, dosing)
[5] Record results of renal ultrasound if collected as standard of care

6.2 Screening

Infants < 29 weeks GA at birth will be screened for eligibility.

6.3 Enrollment/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. Medical history
3. Physical examination, including weight
4. Respiratory assessment (see 6.8)
5. Laboratory evaluations (see 6.9)
6. Adverse events following initial study-specific procedure (see 6.11)

6.4 Treatment Period

The treatment period will include days 1 to 28 or last day of study drug if early withdrawal of study therapy.

The following information will be collected while the participant is on study:

1. Weight, assessed every 7 (+/- 1) days throughout the treatment period
2. Date, time, and route of each study drug dose will be recorded
3. All concomitant medications
4. Respiratory assessment, weekly (see 6.8)
5. Laboratory evaluations, at least 2x/week minimum (see 6.9).
6. PK sampling (see 6.10)
7. Results of renal ultrasound if performed per SOC; specifically nephrocalcinosis/nephrolithiasis
8. Adverse events (see 6.11)

6.5 Follow-up Period

Follow-up period will include days 1-7 after last study dose, including in cases of early withdrawal of study therapy.

The following information will be reported at Day 7 (or day closest to Day 7, if > 1 assessment is available):

1. Physical examination, including weight
2. Respiratory assessment (see 6.8)
3. Laboratory evaluations (see 6.9)
4. Results of renal ultrasound, if performed per SOC
5. Adverse events (all during the follow-up period) (see 6.11)

6.6 36-week PMA Assessment

The following information will be reported at 36 weeks PMA if available. If the participant is discharged before 36 weeks, record assessment closest to discharge date. (Record latest results within week 36 if > 1 assessment is available):

1. Physical examination, including weight
2. Respiratory assessment (see 6.8)
3. Laboratory evaluations (see 6.9)
4. Results of renal ultrasound (if obtained per SOC)

6.7 Final Study Assessment

Final study assessment will occur at the time of discharge, early termination or transfer and the following information will be collected:

1. Respiratory assessment (see 6.7)
2. Results of hearing test (e.g. BAER; must be performed if not done per SOC after the treatment period). If multiple tests are performed per SOC throughout the study period, all results should be recorded in AdvantageEDC.
3. Results of renal ultrasound (must be performed if not done per SOC after the treatment period). If multiple tests are performed per SOC throughout the study period, all results should be recorded in AdvantageEDC.
4. Discharge information
    a. Home or transfer
    b. Died or alive
    c. Duration of hospitalization

6.8 Respiratory Assessment

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

1. Maximum F_iO_2 (defined as the maximum FiO_2 on day of assessment, unless it is known to be a temporary (<2 hour) increase in FiO_2)
2. Ventilation type:
    a. High frequency ventilator
    b. Conventional mechanical ventilator
    c. NCPAP (or equivalent, see inclusion criteria in protocol synopsis table)
    d. Cannula/Hood
    e. None (room air with no support)

6.9 Laboratory Evaluations

The following laboratory evaluations must be conducted (if not obtained per SOC) prior to randomization (within 24 hours of randomization), and at least 2 times per week (at least 42 hours apart). For cohort 2 and 3, laboratory values must be obtained in the 48 hours prior to increasing the study drug dose. All laboratory values below will be collected from the 1st dose of study drug through 7 days following the last dose of study drug. The values will also be captured at 36 weeks PMA.

1. Indirect bilirubin*
2. Sodium
3. Potassium
4. Bicarbonate
5. BUN
6. Creatinine
7. Calcium and/or ionized calcium

*_If a total bilirubin value is measured without the differentiated (conjugated (direct) and unconjugated (indirect)) fractions and is ≤ 10mg/dl, this value can be reported instead of indirect bilirubin_

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 values for platelets, phosphorus, chloride, alkaline phosphatase, and caffeine levels from 24 hours prior to randomization through 7 days following the last dose of study drug if reported per SOC. The values will also be captured at 36 weeks PMA if reported per SOC.

6.10 PK Sampling

Table 6-2 below provides the optimal 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 below 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; 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: Optimal sampling collection windows (time in relation to end of infusion)

	Dosing interval (hours)	
Sample #	**6 Hour**	**24 Hour**
1	within 30 min	within 30 min
2	2-4 hours	2-4 hours
3	Within 30 min prior to next dose	6-8 hours
4	12-18 hours (elimination)	12-16 hours
5	N/A	20-22 hours
6	N/A	Within 30 min prior to next dose
7	N/A	48-72 hours (elimination)

6.10.1 Minimizing Blood Loss

Plasma samples will be collected in 200 µL blood aliquots. To minimize the amount of blood sampling, a limited sampling scheme will be employed such that no more than 7 timed PK samples (1.4 mL of blood) are obtained from each participant for analysis. Participants in all treatment groups (including placebo) will have samples collected. Infants assigned to the placebo treatment group will have biomarkers of bronchopulmonary dysplasia measured in a central laboratory (details in MOP; biomarkers may include: IL-1, IL-6, IL-8, TNF-α, and RANTES) instead of PK levels.

6.10.2 Specimen Preparation, Handling, and Shipping

Detailed information will be in the MOP.

6.11 Adverse Event

AEs will be collected following initial study-specific procedure (e.g., screening blood draws, drug administration), through 7 days post last study dose. See section 8 for safety information.

7 STUDY PRODUCT DESCRIPTION

7.1 Dosage and Study Drug Information

Table 7-1: Dosing

Table. N and dosing scheme							
		N	**Furosemide (IV)**	**or**	**Furosemide (enteral)**	**N**	**Cohort N**
Cohort 1	Placebo	10	1 mg/kg q 24 hours	**or**	2 mg/kg q 24 hours	30	40
Cohort 2*	Placebo	10	1 mg/kg q 6 hours	**or**	2 mg/kg q 6 hours	30	40
Cohort 3*	Placebo	10	2 mg/kg q 6 hours	**or**	4 mg/kg q 6 hours	30	40
* See dose escalation, below, for Cohorts 2 and 3							

Dosing escalation for participants in Cohort 2 and Cohort 3: Participants enrolled in Cohort 2 and Cohort 3 will initially start at 1 mg/kg q 24 hours of study drug and will increase to the assigned target in "every other day" increments. For example, infants assigned to Cohort 3 who are receiving IV dosing will start at 1 mg/kg q 24, then 1 mg/kg q 12, then 1 mg/kg q 6, and then 2 mg/kg q 6, in this example the assigned target dose would be achieved on treatment day 7. NOTE: Dosing will increase as long as the BUN ≤ 50 mg/dL, the creatinine is ≤ 1.7 mg/dL, the Na is ≥ 125 mmol/L, and the K is > 2.5 mmol/L, Ca >6 mg/dL (or ionized Ca > .8 mmol/L) on surveillance chemistries obtained within 48 hours prior to dose escalation. An infant will meet respiratory criteria for 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.

Weight:

- Actual weight will be recorded at baseline, once during safety follow-up, and at 36 weeks PMA.
- Dosing weight is the weight utilized to determine dosing (which may be different from actual weight), and will be recorded for each study dose. Dosing should be reassessed for adjustment according to any dosing weight changes determined every 7 (+/- 1) days throughout the treatment period. There is no protocol requirement to modify dose based solely on actual weight. Dose may or may not be adjusted based on the weight.

Open label diuretics: Administration of other diuretics during the study period (day 1-28) will be considered a protocol deviation because administration of no diuretics is considered part of standard of care at some centers. Clinicians may choose to administer diuretics after day 28 at their discretion.

Na/K supplementation Na and K supplementation is permitted at the discretion of the clinical team.

Intravenous (IV) versus enteral dosing: 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). Placebo will be administered either enterally or IV as well.

7.2 Formulation, Packaging, and Labeling

All study drugs will be standard intravenous or enteral formulations. Only marketed IV formulation for IV administration and marketed oral solution for the enteral route will be used. Sites must only use the alcohol free enteral formulation. This protocol will not specify the brand of product. Each product will be "off the shelf" as provided by the site's pharmacy. Detailed information will be part of the MOP.

7.3 Preparation and Administration of Study Intervention/Investigational Product

The pharmacy at each site will prepare and distribute the study drug in a masked manner to the NICU/PICU and drug will be administered by bedside nurse. Intravenous doses of study drug will be administered within15 minutes. 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 should follow the institutional policy. If there is no specific institutional policy, study drug will be mixed in 10 mL of feedings to be given at the end of the feed, and if not followed, it will be considered a protocol deviation. If feeds are administered on pump, timing of administration should follow the institutional policy. If there is no specific institutional policy, study drug will be mixed in the last 30 minutes of feeding volume, and if not followed, it will be considered a protocol deviation. The investigational pharmacist will be unmasked, and will prepare masked study drug.

Administration of the study drug must occur within:

- +/- 3 hours of the scheduled time of q24h dosing,
- +/- 2 hours of the scheduled time for q12h dosing,
- +/- 1 hour of the scheduled time for q6h dosing.

Further guidance will be included in the MOP.

7.4 Product Storage and Stability

Detailed information will be part of the MOP.

7.5 Concomitant Medications/Treatments

All drugs and/or treatments, other than additional diuretics, are permitted while on study. Use of other or additional diuretics is considered a protocol deviation. All concomitant medications, including intravenous potassium boluses or enteral electrolyte supplements, and treatments (including other diuretics) will be reported during the study drug administration period. We will

collect the dose amount, start time and date for all caffeine doses administered during study drug administration period. Any instances in which additional diuretics, including standard of care furosemide, are administered during the study drug administration period will be considered a protocol deviation.

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 7 days post last study dose and it will be assessed by frequency and incidence of AEs and SAEs. A safety monitoring committee (DMC) will be convened by NIH to review data and safety information from study participants throughout the study and prior to dose escalation into Cohorts 2 and 3.

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

8.1.2 Unexpected Adverse Event

This is defined as any adverse event, the specificity or severity of which is not consistent with the package insert 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 Follow-up of Adverse Events

All events (study-related or not) must be followed until resolution, or if ongoing at the time of last safety contact, will be followed up to adequately evaluate the participant's safety or until the event stabilizes. All serious suspected adverse reactions and serious adverse reactions will be followed until resolution or until the patient is medically stable. All other events that cannot be resolved by 30 days after the safety monitoring period will be considered resolved by convention and entered in the electronic data capture (EDC) system as such. Any event beginning more than 7 days after the last dose of study drug will not be captured.

8.1.5 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

8.1.6 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.

8.1.7 Discontinuation of a Participant Due to Adverse Events

Participants may be withdrawn from the study at any time. Participants withdrawn from the study product 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.

8.1.8 Reporting Procedures

All adverse events will be entered into the safety data system within 7 days of identification. Serious events will be entered into the data system within 24 hours of identification. If there are any technical difficulties, the SAE will be reported by direct communication with the medical monitor. Results of hearing tests (hearing loss / impairment) and renal ultrasound (nephrocalcinosis / nephrolithiasis) are considered adverse events of interest and will be captured on the SPE (Special Testing) form and will not be captured on the AE (Adverse Events) form in the EDC system.

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.

8.3 Regulatory Reporting

Any event that requires expedited reporting based on federal regulations will be forwarded to the IND sponsor. The IND sponsor or its in-country representative will submit expedited 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. Documentation of the submission and receipt by the IRB must be retained for each expedited safety report.

All serious events irrespective of their designation as “related” or “not related” to study product(s) will be reported to the FDA at least annually in a summary format.

8.4 Safety Oversight

The DMC 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 DMC 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 product. 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 DMC will review AEs and SAEs at the regularly scheduled meeting. Additionally, DMC will periodically review interim safety analyses (see Section 10.3). The DMC 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 DMC, and NIH, the IND sponsor will make a decision to terminate or continue the study.

8.5 Dose Escalation and Halting Criteria: Safety Concerns

The trial will be halted (paused) for a safety review by the DMC if there are 4 or more Serious Adverse Reactions within a given cohort (1, 2, or 3). If there are <4 Serious Adverse Reactions

total, the DMC will receive a summary of masked safety data and enrollment will continue in the next highest dose cohort. Enrollment in the next highest dose cohort will begin immediately after the 40th (or 80th) subject is enrolled and the following criteria have been met:

Table 8-5 – Rules for advancing to next highest dose cohort	
Number of Serious Adverse Reactions	**% total evaluable safety days (days of drug dosing + 7 days of safety follow-up for all 40 participants) completed from lower dose cohort**
3	100%
2	95%
1	80%
0	70%

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

9 CLINICAL MONITORING

Site monitoring will be conducted to ensure that human subject 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 sponsor, or as detailed in the Transfer of Regulatory Obligations (TORO), NIH, FDA, or its designee will conduct site-monitoring visits as detailed in the monitoring plan or in the manual of procedures.

Site visits will be made 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 BPD risk, BPD incidence, death rate, rate of death or BPD, incidence of nephrocalcinosis/nephrolithiasis, incidence of hearing loss, and PK parameters.

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 9-1 provides widths for 95% Wilson confidence intervals in the dose groups of size 30 and the total furosemide treatment cohort of 90 with different incidence rates. An event with an incidence rate of 0.05 has a 79% chance of being observed at least once in a dose group and a 99% chance of being observed at least once in the total furosemide cohort.

Table 9-1. Widths for 95% Wilson confidence intervals.

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

Population for Analysis
All participants enrolled, randomized, and dosed will be included in 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

A masked interim safety analysis will be performed after completion of the safety follow up period of Cohort 1 participants, and again after completion of the safety follow up period of Cohort 2 participants to include data entered up to the cutoff time point. Enrollment will not be halted during the analyses. Halting may occur if analysis shows a positive finding. The number and percent of AEs and SAEs within each dose group will be summarized overall as well as by each Medical Dictionary for Regulatory Activities (MedDRA) system organ class and preferred term. Laboratory data will be tabulated by dose groups. Summary statistics for changes from baseline will be presented.

10.4 Analysis Plan

Descriptive statistics
Descriptive statistics such as number of observations, mean, median, standard deviation, standard error, minimum and maximum will be presented by 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.)

Demographic and baseline characteristics
The number of participants completed and discontinued early from study and the reasons for the discontinuation will be summarized. Demographic and baseline characteristics will also be summarized. 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 number of days of dosing and reasons for final discontinuation of study drug.

Effectiveness analysis
We will use multivariable analyses (multinomial, logistic or linear regression, with interactions, as appropriate) to explore the relationship between the maximum and total dose of furosemide and change in risk of BPD (none-mild vs. moderate-severe-death), death, death or BPD, hearing loss, and nephrolithiasis/nephrocalcinosis.

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.

Biomarkers
We will relate the concentration of biomarkers (see 6.10) to the development of and severity of BPD.

Safety
The number and percent of AEs and SAEs within each dose group will be summarized overall and by each Medical Dictionary for Regulatory Activities (MedDRA) system organ class and preferred term. Severity of laboratory values will be assigned according to predetermined definitions (Appendix A). Prior and concomitant medications will be summarized by World Health Organization (WHO) drug class. Laboratory data will be tabulated by dose groups. Summary statistics for changes from baseline will be presented. Incidences of hearing loss and nephrolithiasis/nephrocalcinosis will be compared between dose groups using appropriate statistical hypothesis tests.

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.

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 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.

Copies of ultrasound images assessed by the site investigator as positive for calcium deposits in the kidneys (nephrocalcinosis) or kidney stones (nephrolithiasis) will be submitted in a secure manner to the Data Coordinating Center. The Data Coordinating Center will remove any PHI that identifies the participant from the images before they are sent to a study radiology specialist for independent review. The study radiologist's assessment of the ultrasounds are for study purposes only, and individual results will not be reported back to investigators.

The principal investigator will ensure that the use and disclosure of protected health information (PHI) obtained during this research study complies with the Federal Privacy Regulation. 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 the applicable Federal Privacy Regulations. The relevant privacy authorization will be combined in the informed consent document (approved by the IRB).

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 participants and their families. Consent forms describing in detail the study procedures and risks are given to the participant's legal guardian, and written documentation of informed consent is required prior to enrolling in the study. Consent forms will be IRB-approved, and the participant's legal guardian will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the participant's legal guardian and answer any questions that may arise. The participant/participant's legal guardian will sign the informed consent document prior to the conduct of any study procedures. The participant's legal guardian should have the opportunity to think about the study prior to agreeing to participate. The participant's legal guardian may withdraw consent at any time throughout the course of the study. A copy of the informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to their legal guardian that the quality of their medical care will not be adversely affected if they decline to participate in this study. For non-English speakers, a fully translated consent or an oral presentation accompanied by a short form may be used to obtain informed consent. The fully translated consent and the short form must be approved by the IRB and executed according to local requirements.

The IND sponsor, or designee will provide the investigator, in writing, any new information that bears significantly on the participants' risk to receive the investigational product. This new information will be communicated by the investigator to participants' legal guardians who consent to participate in the trial in accordance with IRB requirements. The informed consent document will be updated, and participants' legal guardians will be re-consented, if necessary.

Site staff may employ IRB-approved recruitment efforts prior to the participant's legal guardian consenting; however, before any protocol-specific procedures are performed to determine protocol eligibility, informed consent must be obtained and properly executed.

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

13 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, 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
- Final study protocol
- Protocol amendments (if applicable)
- Manual of Procedures
- Informed consent form (blank)
- Signed informed consent form
- Revised informed consent forms and/or all addenda (blank)
- IRB registration or other documentation of IRB compliance with FDA regulations
- Documentation of IRB approval of protocol, consent form, any protocol amendments, and any consent form revisions
- Annual IRB updates and approvals
- All correspondence between the investigator and IRB

Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH E6, 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 eCRFs and provided by the Data Coordinating Center (DCC).

14 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.

DCC-designated 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, PTN, and NIH/NICHD.

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.

15 ETHICS/PROTECTION OF HUMAN PARTICIPANTS

15.1 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) 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.

15.2 Institutional Review Board

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

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

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

15.3 Informed Consent

The investigator will choose participants in accordance with the eligibility criteria detailed previously. The investigator will not exercise selectivity so that bias is prevented. In this study, participant’s parent/legal guardian will sign an informed consent for study participation.
All legal guardians must sign an informed consent form that complies with the federal regulatory and privacy requirements before entering the trial. A consent form that complies with the federal requirements and a separate authorization form that complies with federal privacy regulations for the use and disclosure of the participant’s protected health information may be used instead, per institutional standard operating procedures.

For details regarding the informed consent process, see Section 12.

15.4 Participant Confidentiality

Participant confidentiality is held strictly in trust by the participating investigators, their staff, the sponsor(s), and their agents. This confidentiality extends to genetic and biological sample tests, in addition to the clinical information relating to participating participants.

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 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.

15.5 Study Discontinuation

If the study is discontinued, enrolled participants will continue to be followed for safety assessments for 7 days. All adverse events must be followed through resolution.

16 DATA HANDLING AND RECORD KEEPING

The investigator is 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. The investigator is responsible for informing the IRB 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 the accuracy, completeness, legibility, and timeliness of the data reported.

Case report forms will be derived from the eCRFs 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 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 eCRFs.

16.1 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.

16.2 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.

16.3 Timing/Reports

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

16.4 Study Records Retention

Records and documents pertaining to the conduct of this study, including case report forms, source documents, consent forms, laboratory test results, and medication inventory records, must be retained by the investigator for at least 10 years after the end of the study or per local/state regulations or until participants reach 21 years, or applicable Federal laws, whichever is longer. No study records will be destroyed without prior authorization from the sponsor.

16.5 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.

These practices are consistent with Good Clinical Practice:

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

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

16.6 Participant Privacy/Authorization

The principal investigator will ensure that the use and disclosure of protected health information obtained during a research study complies with the HIPAA Privacy Rule. The rule 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 HIPAA Privacy Rule. Authorization will be combined in the informed consent document (approved by the IRB).

17 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 Trails 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 PTN, or are supported by 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 I 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

18 APPENDIX A: LABORATORY VALUES

Laboratory values	Adverse event	Serious adverse event
Serum electrolytes		
Hypernatremia(Sodium)	150–159 mmol/L	> 159 mmol/L
Hyponatremia(Sodium)	120–124 mmol/L	<120 mmol/L
Hyperchloremia(Chloride)	110-120 mmol/L	> 120 mmol/L
Hypochloremia(Chloride)	80-90 mmol/L	< 80 mmol/L
Hyperkalemia(Potassium)	7.0–7.9 mmol/L	>7.9 mmol/L
Hypokalemia(Potassium)	2.0–2.5 mmol/L	<2.0 mmol/L
High Bicarbonate levels (Bicarbonate)	30–45 mmol/L	> 45 mmol/L
Low Bicarbonate levels(Bicarbonate)	12–14 mmol/L	< 14 mmol/L
Hypercalcemia (iCa)	1.3–1.6 mmol/L	>1.6 mmol/L
Hypocalcemia (iCa)	0.7–1.05 mmol/L	< 0.7 mmol/L
Hypermagnesemia (Magnesium)	3.0-6.0 mg/dL	> 6.0 mg/dL
Hypomagnesemia (Magnesium)	1.0-1.5 mg/dL	< 1.0 mg/dL
Hypophosphatemia(Phosphorus) Hyperphosphatemia(Phosphorus)	1.0-3.0 mg/dL 10.5-12.5 mg/dL	<1.0 mg/dL >12.5 mg/dL
Hyperbilirubinemia (Bilirubin)	20-30 mg/dL	>30 mg/dL
Renal dysfunction (i.e. azotemia)		
Elevated BUN	60–100mg/dL	> 100 mg/dL
Elevated creatinine	1.5–2.5 mg/dL	> 2.5 mg/dL
Gastrointestinal		
Elevated alkaline phosphatase	1000–1400 U/L	>1400 U/L
Hematology		
Thrombocytosis(Platelets)	450–1000 × 10^9/L	>100010 × 10^9/L
Thrombocytopenia(Platelets)	50–100 × 10^9/L	<50 × 10^9/L

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