A Safety Study of Intravenous Infusion of Bone Marrow Mesenchymal Stem Cell-derived Extracellular Vesicles (UNEX-42) in Preterm Neonates at High Risk for Bronchopulmonary Dysplasia

Protocol UNX-BP-101

IND# 18,532

CONFIDENTIAL

UNITED THERAPEUTICS CORPORATION

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Amendment 1:	18 December 2018
Amendment 2:	19 February 2020

LIST OF CONTACTS FOR STUDY

Study Sponsor	**United Therapeutics Corporation** 55 TW Alexander Drive Research Triangle Park, NC 27709 [redacted] Senior Medical Director, Medical Monitoring [redacted]
SAE Reporting	**United Therapeutics Global Drug Safety** [redacted]

INVESTIGATOR'S AGREEMENT

I have read the attached protocol entitled "A Safety Study of Intravenous Infusion of Bone Marrow Mesenchymal Stem Cell-derived Extracellular Vesicles (UNEX-42) in Preterm Neonates at High Risk for Bronchopulmonary Dysplasia," Amendment 2 dated 19 February 2020 and agree to abide by all provisions set forth therein.

I agree to comply with the International Council for Harmonisation Guideline for Good Clinical Practice and applicable Food and Drug Administration regulations/guidelines set forth in 21 Code of Federal Regulations Parts 50, 54, 56, and 312 and any local regulations per country.

I agree to ensure that the confidential information contained in this document will not be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of United Therapeutics Corporation.

I also have read the current Clinical Investigator's Brochure for UNEX-42 and acknowledge that review of the information contained in the Clinical Investigator's Brochure is a requirement for Investigators before using UNEX-42 in a clinical study.

This protocol has been received for information only and must not be implemented before all necessary regulatory agency and Ethics Committee/Institutional Review Board approval documents have been obtained.

Signature of Principal Investigator	Date

Printed Name of Principal Investigator

PROTOCOL SYNOPSIS

Title	A Safety Study of Intravenous Infusion of Bone Marrow Mesenchymal Stem Cell-derived Extracellular Vesicles (UNEX-42) in Preterm Neonates at High Risk for Bronchopulmonary Dysplasia
Study Phase	Phase 1
Indication	Bronchopulmonary dysplasia (BPD) prevention in preterm infants
Primary Objective	To assess the safety and tolerability of intravenous (IV) infusion of UNEX-42 in infants born at <27 weeks of gestational age (GA) and exhibiting respiratory distress.
Secondary Objective(s)	To assess the effect of UNEX-42 on the following: • Incidence and severity (mild, moderate, severe) of BPD or death at 36 weeks postmenstrual age (PMA) • Short-term outcomes including oxygen and ventilation exposure in the first 28 days after treatment. • Long-term outcomes up to 1 year (corrected age).
Study Design	A multicenter, placebo-controlled, randomized, dose escalation, safety, and tolerability study of UNEX-42 in infants born at <27 weeks of GA at high risk for BPD
Sample Size	Target enrollment of 18 total subjects, with 3 cohorts of 6 subjects per cohort, randomly allocated 2:1 (active:placebo)

Summary of Subject Eligibility Criteria	A subject must meet the following criteria to be eligible for the study:
	• Infant whose postnatal age is 3 to 14 days at randomization and meets the following criteria based on GA:
	  • 23 weeks to 24 weeks 6 days: any birth weight, any oxygen requirement
	  • 25 weeks to 26 weeks 6 days: fraction of inspired oxygen (FiO_2) ≥35% (sustained >2 hours) at any point during postnatal Days 1 to 14 AND birth weight ≤750 g
	• Endotracheally intubated and receiving mechanical ventilation
	• Not expected to be extubated within the next 24 hours after randomization
	• The subject has a parent/guardian who gives written informed consent.

Drug Dosage and Formulation	UNEX-42 is a preparation of extracellular vesicles that are secreted from human bone marrow-derived mesenchymal stem cells suspended in phosphate-buffered saline. UNEX-42 is a clear liquid that is provided in appropriately sized vials and stored at -70±10°C prior to use. UNEX-42 will be administered via IV infusion. Three dose cohorts will be evaluated: • Cohort 1: 1 administration of 20 pmol phospholipid/kg body weight • Cohort 2: 1 administration of 60 pmol phospholipid/kg body weight • Cohort 3: 1 administration of 200 pmol phospholipid/kg body weight
Control Group	Placebo (phosphate-buffered saline)
Route of Administration	IV infusion (via umbilical/central/peripheral IV line)

Procedures	Subjects will be assessed during Screening and Baseline (prior to randomization) to determine eligibility for the study. After randomization, subjects will be monitored in the hospital through 40 Weeks PMA or the time of hospital discharge (whichever comes first). The following efficacy and safety assessments will occur during the course of the study: ***Efficacy Assessments*:** incidence and severity of BPD, duration of hospitalization, duration of mechanical ventilation, duration of supplemental oxygen therapy, duration of postnatal steroids, tracheal aspirate, and Respiratory Severity Score. ***Safety Assessments*:** physical examination, vital signs, adverse events, predefined complications of prematurity, clinical laboratory parameters, and chest x-ray. Enrollment between cohorts will pause for data review by a Data Monitoring Committee to evaluate the data available after each of the first 2 cohorts have been enrolled. Dose administration for Cohort 1 will occur such that there is an observational period of 3 days between dosing the first, second, and third subject to assure the opportunity for safety assessments in at least 1 subject on active treatment. This procedure will be followed for each cohort. Subjects that complete the Post-treatment Phase (including those that are discharged from hospital prior to 40 Weeks PMA) will continue into the Long-term Outcome Phase and will be assessed through 1 year of corrected age.
Statistical Considerations	No formal sample size calculations were performed. Categorical assessments (eg, adverse events, death, incidence and severity of BPD) will be summarized with frequencies (number and percentage) by dose cohort and placebo. Descriptive statistics (eg, mean, standard deviation, median, interquartile range) will be calculated to summarize continuous assessments (eg, vital signs, duration of hospitalization, clinical laboratory parameters) by dose cohort and placebo. Kaplan-Meier curves will be constructed for time to event variables (eg, time to discharge from the hospital).
Sponsor	United Therapeutics Corporation 55 TW Alexander Drive Research Triangle Park, NC 27709

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LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	Adverse event
BPD	Bronchopulmonary dysplasia
CSM	Clinical study material
CTCAE	Common Terminology Criteria for Adverse Events
CXR	Chest x-ray
DLT	Dose-limiting toxicity
DMC	Data Monitoring Committee
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
FDA	Food and Drug Administration
FiO_2	Fraction of inspired oxygen
GA	Gestational age
GCP	Good Clinical Practice
ICF	Informed Consent Form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IRT	Interactive Response Technology
IV	Intravenous
MedDRA	Medical Dictionary for Regulatory Activities
MSC	Mesenchymal stem cell
NCI	National Cancer Institute
PDA	Patent ductus arteriosus
PMA	Postmenstrual age
RDS	Respiratory distress syndrome
RSS	Respiratory Severity Score
SAE	Serious adverse event
SAR	Serious adverse reaction
TEAE	Treatment-emergent adverse event

1 BACKGROUND AND RATIONALE

1.1 DEFINITION OF CLINICAL PROBLEM

Respiratory distress syndrome (RDS) is the failure to develop functional residual capacity and the propensity of affected lungs to become atelectatic. Preterm infants born prior to 28 weeks of gestational age (GA), and of very low birthweight, are at an increased risk for developing RDS (Torchin 2016). Surfactant production is low in these infants, and as such, lung recruitment and compliance are compromised. As a result, the atelectatic alveoli are well perfused but poorly ventilated, resulting in hypoxia. The clinical manifestations of RDS appear within minutes of birth and are recognized by respiratory distress associated with cyanosis often requiring respiratory support and/or resuscitation. Symptoms of RDS include low blood oxygen, low blood pH, apnea/tachypnea, atelectasis, and "ground glass" appearance of the lungs on chest x-ray (CXR). These infants are typically treated with supplemental oxygen and continuous positive airway pressure to reduce or reverse the hypoxia and acidosis. If needed, infants are intubated, given surfactant, and mechanical ventilation is employed. While these interventional strategies improve lung compliance and increase blood oxygen level, mechanical ventilation and supplemental oxygen can inadvertently cause additional lung tissue damage (Jobe 2001).

RDS in preterm infants, if unresolved, is likely to result in bronchopulmonary dysplasia (BPD). BPD is characterized by an inflammatory process causing abnormal lung development and decreased vascular and alveolar development (Collins 2017). Improvements in neonatal care has led to better survival rates of premature infants, yet the cost of this improved survival is an increased risk of developing BPD. While the definition has changed with improvements in assisted ventilation resulting in less barotrauma and oxidative injury, BPD (formerly known as chronic lung disease of prematurity) is currently defined as those infants requiring supplemental oxygen therapy for at least 28 days after birth. Furthermore, the severity of BPD for infants born at less than 32 weeks of gestation is determined by the level of supplemental oxygen required to maintain adequate blood gas oxygen levels at 36 weeks corrected GA. Infants requiring greater than 30% oxygen supplementation and/or continuous positive airway pressure are classified as severe, those requiring less than 30% oxygen are classified as moderate, and those not requiring oxygen supplementation are classified as mild (Jobe 2001).

BPD is associated with increased rates of mortality, pulmonary hypertension, emphysema, and neurodevelopmental abnormalities. Currently employed prevention strategies include the use of antenatal glucocorticoids, treatment of RDS with surfactant, and supportive treatments such as gentler, noninvasive ventilatory modalities and fluid restriction. Additional therapeutic approaches have been evaluated for preventing BPD including postnatal corticosteroids, late surfactant administration, inhaled nitric oxide, and anti-inflammatory agents such as docosahexaenoic acid. However, many of these treatments have been shown to be either relatively ineffective (Collins 2017; Ballard 2014; Donohue 2011) or controversial (Watterberg 2010). An effective therapy to prevent the development of BPD is an unmet medical need.

1.2 UNEX-42 BACKGROUND

UNEX-42 is a preparation of extracellular vesicles that are secreted from primary, nonimmortalized human bone marrow-derived mesenchymal stem cells (MSCs). UNEX-42 contains key molecular components (eg, ribonucleic acid, protein, phospholipids) that drive biological responses in target cells, which regulate immune modulation, angiogenesis, cellular salvage, and cellular metabolism. Activity of UNEX-42 in animal models of BPD, as well as evidence from in vitro analyses, support the continued investigation of UNEX-42 as a potential therapeutic intervention for BPD in neonates.

MSC-derived extracellular vesicles are wholly sufficient in driving improved outcomes in models of acute kidney injury (Bruno 2009; Gatti 2011), myocardial ischemia/reperfusion injury (Lai 2010), and hypoxia-induced pulmonary hypertension (Lee 2012). In a neonatal hyperoxia mouse model, MSC-derived extracellular vesicles were effective in reducing lung inflammation and alveolar simplification, thus attenuating the development of BPD (Willis 2018). These studies and others provide a strong foundation for the continued development of extracellular vesicle-based therapies. Clinically, MSC-derived exosomes have been used in 1 patient to treat therapy-refractory graft-versus-host disease. The patient was administered exosomes every 2 to 3 days over a 2-week period. All applications were tolerated well with no observed side effects (Kordelas 2014). UNEX-42 specifically has not been evaluated clinically.

1.3 RATIONALE FOR DEVELOPMENT OF STUDY DRUG IN DISEASE/CONDITION

There are currently no approved prophylactic interventions for BPD, and the preventative therapies employed are largely ineffective (Beam 2014). The lack of preventative treatment options is in stark contrast to the poor outcomes observed in patients who develop severe BPD, who are at further risk for developing pulmonary hypertension, emphysema, and neurodevelopmental abnormalities. These risks in the population susceptible to developing BPD, and the lack of effective preventative treatment options, make premature infants the population with the most favorable risk-potential benefit profile.

United Therapeutics Corporation believes that safety data available from the most similar comparator under study in a similar population justifies the first-in-human study of UNEX-42 being conducted in premature infants. This comparator study used an intratracheal administration of human umbilical cord blood-derived MSCs to determine safety and inflammatory biomarkers in premature infants at risk for BPD. The study indicated that the treatment was well tolerated by all subjects with no immediate cardiorespiratory compromise or long-term adverse effects after dosing (Chang 2014, Ahn 2017). UNEX-42, which is acellular, should have an improved safety profile over the comparator cell program. United Therapeutics Corporation believes that a product consisting of extracellular vesicles presents less risk of tumorigenesis, less risk of embolus, and less risk of immunological reaction as compared to whole MSCs. As such, United Therapeutics Corporation expects a favorable safety profile.

No adverse reactions to UNEX-42 have been observed in animal studies to date. Toxicity studies conducted in neonatal rats concluded that the no-observed-adverse-effect level for UNEX-42 is 878 pmol phospholipid/kg. This level was the highest feasible dose based on the upper limit of volume that could be administered to the rats. Using the no-observed-adverse-effect level as a benchmark, the starting clinical dose for this study is 20 pmol phospholipid/kg body weight, which takes into account a human equivalent dose conversion factor of 0.22, as well as a 10-fold safety margin.

1.4 CLINICAL HYPOTHESIS

The hypothesis of this study is that UNEX-42 can be administered to preterm infants at high risk for BPD at doses that provide a potential for therapeutic benefit in association with an acceptable safety profile.

2 OBJECTIVES

2.1 PRIMARY OBJECTIVES

To assess the safety and tolerability of intravenous (IV) infusion of UNEX-42 in infants born at <27 weeks of GA and exhibiting respiratory distress.

2.2 SECONDARY OBJECTIVES

To assess the effect of UNEX-42 on the following:

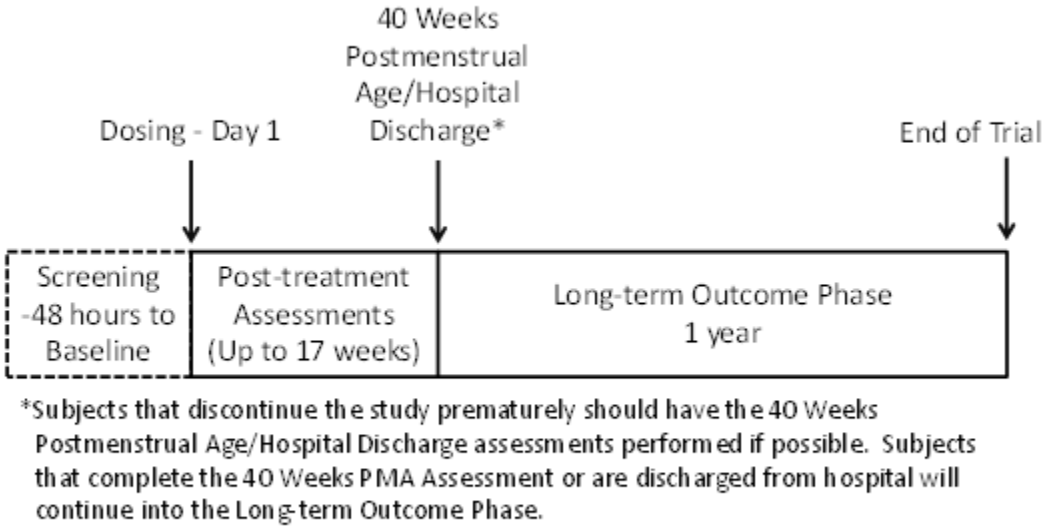
1. Incidence and severity (mild, moderate, severe) of BPD or death at 36 weeks postmenstrual age (PMA)
2. Short-term outcomes including oxygen utilization and ventilation exposure in the first 28 days after treatment.
3. Long-term outcomes up to 1 year corrected age.

3 EXPERIMENTAL PLAN

3.1 STUDY DESIGN

This is a multicenter, placebo-controlled, randomized, dose escalation, safety, and tolerability study of UNEX-42 in infants born at <27 weeks of GA at high risk for BPD. UNEX-42 will be administered via IV infusion to 3 cohorts at 20, 60, and 200 pmol phospholipid/kg body weight. Within each cohort, eligible subjects will be randomly assigned to receive either UNEX-42 or placebo treatment (2:1 allocation ratio). A schematic presentation of the study design is presented in Figure 3-1.

Figure 3-1 Schematic of Study Design for an Individual Subject



*Subjects that discontinue the study prematurely should have the 40 Weeks Postmenstrual Age/Hospital Discharge assessments performed if possible. Subjects that complete the 40 Weeks PMA Assessment or are discharged from hospital will continue into the Long-term Outcome Phase.

Study assessments will occur during the study at the following time points: Screening, Baseline, 24 and 72 hours postdose, 7 days postdose, weekly until 35 weeks PMA, 36 weeks PMA, and 40 weeks PMA/hospital discharge during the Post-treatment Phase. For subjects that complete the Post-treatment Phase, the Long-term Outcome Phase will occur at 6 months (phone contact) and 1 year of corrected age (site visit).

3.2 OVERALL SCHEDULE OF TIMES AND EVENTS

Table 3-1 Schedule of Times and Events – Post-treatment Phase

Study Procedures	Screening[a]	Baseline[a]	Post-treatment Phase					
Assessment Period			**24 Hours Postdose**	**72 Hours Postdose**	**7 Days Postdose**	**Weekly Until 35 Weeks PMA**	**36 Weeks PMA[b]**	**40 Weeks PMA/ Hospital Discharge[b,c]**
Study Day	**-48 Hours to Baseline**	**1**	**2**	**4**	**8**	**15, 22, 29, 36, 43, 50, 57, 64, 71, 78, and 85**	**50 up to 88**	**78 up to 116**
Visit Window			**±4 hours**	**±4 hours**	**±1 day**	**±2 days**	**±2 days**	**±2 days**
Parental Informed Consent	X							
Subject Eligibility	X	X						
Medical History	X							
Demographics	X							
Maternal History	X							
Weight	X	X				X	X	X
Length and Head Circumference		X					X	X
CXR[d]		X					X	
Clinical Laboratory Assessments[e]		X		X			X	
Predefined Complications of Prematurity		X	X	X	X	X	X	X
Vital Signs[f]		X	X	X	X	X	X	X
Physical Examination		X					X	
Mean Airway Pressure[g]		X	X	X	X	X	X	X
Oxygen Saturation[h]		X	X	X	X	X	X	X
Respiratory Severity Score[i]		X	X	X	X	X	X	X
Tracheal Aspirate[j]		X		X	X			
Concomitant Medications		X	X	X	X	X	X	X
Adverse Events[k]	X	X	X	X	X	X	X	X
Randomization		X						
UNEX-42/Placebo Administration		X[l]						
Incidence and Severity of BPD[m]							X	

Study Procedures	**Screening[a]**	**Baseline[a]**	**Post-treatment Phase**					
Assessment Period			**24 Hours Postdose**	**72 Hours Postdose**	**7 Days Postdose**	**Weekly Until 35 Weeks PMA**	**36 Weeks PMA[b]**	**40 Weeks PMA/ Hospital Discharge[b,c]**
Study Day	**-48 Hours to Baseline**	**1**	**2**	**4**	**8**	**15, 22, 29, 36, 43, 50, 57, 64, 71, 78, and 85**	**50 up to 88**	**78 up to 116**
Visit Window			**±4 hours**	**±4 hours**	**±1 day**	**±2 days**	**±2 days**	**±2 days**
Duration of Mechanical Ventilation, Supplemental Oxygen Therapy, and Hospitalization								X^n

Abbreviations: AE, adverse event; CXR, chest x-ray; FiO_2, fraction of inspired oxygen; GA, gestational age; PMA, postmenstrual age; RSS, Respiratory Severity Score

[a] The Screening and Baseline assessments may be combined; however, all entry criteria must be confirmed prior to randomization.

[b] The days of the 36 and 40 Weeks PMA assessments are based on each subject's GA determined during Screening based on local standard practice.

[c] Subjects that discontinue the study prematurely should have the 40 Weeks PMA/Hospital Discharge assessments performed if possible. Subjects that complete the 40 Weeks PMA assessment or are discharged from hospital will continue into the Long-term Outcome Phase shown in Table 3-2.

[d] The most recent CXRs prior to randomization and 36 Weeks PMA can be used.

[e] Clinical laboratory assessments will consist of a complete blood count, differential count, sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase, and total and indirect bilirubin. For Baseline, the clinical laboratory assessments may utilize the laboratory assessments obtained as standard of care treatment within the last 24 hours in lieu of drawing blood for the sole purpose of the study. Any study-required blood tests should be obtained at the same time a blood sample is being collected for clinical purposes, if possible.

[f] On Day 1, vital signs are to be collected just prior to dosing, at the midpoint of dosing, just after the end of dosing, and at least once every hour for the first 4 hours after dosing is completed. Vital signs are to include heart rate, respiratory rate (either spontaneous rate or the sum of spontaneous rate and mechanically delivered rate), and systemic blood pressure (systolic and diastolic).

[g] Mean airway pressure is only to be collected from subjects undergoing mechanical ventilation.

[h] Oxygen saturation should be measured from a post-ductal location and should be consistently obtained post-ductally throughout the study period.

[i] The RSS should be calculated as the mean airway pressure multiplied by FiO_2. Mean airway pressure and FiO_2 will be collected at the same time, and the RSS will only be calculated when the subject has an obtainable mean airway pressure.

[j] A tracheal aspirate will be collected as possible at Baseline (prior to dosing), 72 hours postdose (if still intubated), and 7 days postdose (if still intubated).

[k] All AEs will be documented from the time of informed consent until the time screen failure is documented, or until the subject is either discontinued from the study or all 40 Weeks PMA/Hospital Discharge assessments have been completed and should be followed until either resolution (or return to normal or baseline values), until they are judged by the Investigator to no longer be clinically significant, or for at least 30 days if the AE extends beyond the Post-treatment Phase.

[l] UNEX-42 or placebo will be administered after all other Day 1 assessments are completed, with the exception of vital signs (see footnote f).

[m] This assessment to be performed at 36 Weeks PMA or at Hospital Discharge, whichever comes first (see Section 3.3.1).

[n] For each parameter, the total number of days from Baseline to the 40 Weeks PMA/Hospital Discharge will be calculated.

Table 3-2 Schedule of Times and Events – Long-term Outcome Phase

Study Procedures	**Long-term Outcome Phase**	
Assessment Period	**6 Months (Corrected Age) Phone Contact [a]**	**1 Year (Corrected Age) Visit [b]**
Study Day	**Calculated**	**Calculated**
Visit Window	**±2 weeks**	**±4 weeks**
Weight, Length, and Head Circumference		X
Serious Adverse Reactions [c]	X	X
Assessment of General Health	X	X

Abbreviations: PMA, postmenstrual age

[a] To be performed at 6 months corrected age for all subjects, as possible, including those discharged from the hospital prior to 40 Weeks PMA. See Section 7.5.

[b] To be performed at 1 year corrected age for all subjects, as possible, including those discharged from the hospital prior to 40 Weeks PMA. See Section 7.5.

[c] Refer to Section 9.1.3 for the definition of serious adverse reactions.

3.3 CLINICAL ASSESSMENTS

3.3.1 Efficacy

3.3.1.1 Incidence and Severity of Bronchopulmonary Dysplasia

A clinical diagnosis of BPD for infants born <32 weeks of GA is defined by the requirement for supplemental oxygen (>21%) for at least 28 days. Furthermore, the severity of BPD is classified as mild, moderate, or severe based on the level of supplemental oxygen needed at 36 weeks PMA as shown in Table 3-3. This assessment is performed at the 36 Weeks PMA or Hospital Discharge assessment, whichever comes first (Jobe 2001). Supplemental oxygen percentage will be adjusted for altitude when recorded, as necessary.

Table 3-3 Definition of Bronchopulmonary Dysplasia: Diagnostic Criteria for Gestational Age at Birth <32 Weeks

BPD Severity	Criteria (Treatment with oxygen >21% for at least 28 days plus)
Mild BPD	Breathing room air at 36 weeks PMA or hospital discharge, whichever comes first
Moderate BPD	Need for <30% oxygen at 36 weeks PMA or hospital discharge, whichever comes first
Severe BPD	Need for ≥30% oxygen and/or positive pressure (positive-pressure ventilation or nasal continuous positive airway pressure) at 36 weeks PMA or hospital discharge, whichever comes first

Abbreviations: BPD, bronchopulmonary dysplasia; PMA, postmenstrual age

3.3.1.2 Duration of Hospitalization

The total number of days between Baseline and the 40 Weeks PMA/Hospital Discharge assessment will determine the duration of hospitalization.

3.3.1.3 Duration of Mechanical Ventilation

Duration of mechanical ventilation (invasive or noninvasive) will be determined by the total number of days (consecutive or nonconsecutive) that an infant receives ventilatory support between Baseline and the 40 Weeks PMA/Hospital Discharge assessment.

3.3.1.4 Duration of Supplemental Oxygen Therapy

The number of days of supplemental oxygen therapy use (consecutive or nonconsecutive) will be determined between Baseline and the 40 Weeks PMA/Hospital Discharge assessment.

3.3.1.5 Respiratory Severity Score

The Respiratory Severity Score (RSS) will be calculated as the mean airway pressure multiplied by fraction of inspired oxygen (FiO_2). Mean airway pressure and FiO_2 will be collected at the same time, and the RSS will only be calculated when the subject has a mean airway pressure that can be obtained. The RSS will be monitored throughout the Post-treatment Phase.

3.3.1.6 Duration of Postnatal Steroids

Duration of postnatal steroids for purposes of preventing and/or treating BPD (eg, hydrocortisone, dexamethasone, methylprednisolone) will be monitored through 40 Weeks PMA/Hospital Discharge. Postnatal steroid use will be collected as part of the concomitant medication evaluation (see Section 4.3.1).

3.3.1.7 Tracheal Aspirate Inflammatory Biomarkers

A tracheal aspirate will be collected, as possible, at Baseline (prior to dosing), 72 hours postdose (if still intubated), and 7 days postdose (if still intubated). Tracheal aspirates will be evaluated at the local laboratory, as possible, for cell count and differential.

3.3.2 Safety

During the Post-treatment Phase, treatment emergent changes in physical findings, vital signs, clinical laboratory parameters, and the development of adverse events (AEs) will be the primary assessments of safety. During the Long-term Outcomes Phase, the development of any serious adverse reactions (SARs) will be monitored. Sections 9 and 15.1 provide the guidelines and definitions for AE reporting.

3.3.2.1 Medical History, Maternal History, and Demographics

A complete medical history, including the maternal history parameters shown in Table 3-4, and demographics will be obtained at Screening. Any changes to medical history that occur between the Screening and Baseline assessments should be recorded. Past or present illnesses, current medications, and history of medication allergies should be recorded. Any significant changes to the subject's medical condition must be documented throughout the course of the Post-treatment Phase.

Table 3-4 Maternal History Parameters

Maternal age/race/ethnicity	Reason for delivery (preeclampsia, eclampsia, rupture of membrane, etc)
Diagnosis of chorioamnionitis during pregnancy	Mode of delivery
Medications during pregnancy (corticosteroids, medications used to treat pre-eclampsia)/delivery	Receipt of IV magnesium

3.3.2.2 Physical Examination/Chest X-ray

A complete physical examination, including a CXR, will be conducted at Baseline (prior to randomization) and at 36 Weeks PMA. The most recent CXR prior to randomization and 36 Weeks PMA can be used without being retaken. All treatment emergent clinically significant findings during the Post-treatment Phase will be recorded as AEs.

3.3.2.3 Vital Signs

Vital signs will be collected during all study assessment timepoints from Baseline to 40 Weeks PMA/Hospital Discharge. On Day 1, vital signs are to be collected 7 times: just prior to dosing, at the midpoint of dosing, just after the end of dosing, and at least once every hour for the first 4 hours after the completion of dosing.

Vital signs measured will include systemic blood pressure (systolic and diastolic), heart rate, and respiratory rate (either spontaneous rate or the sum of spontaneous rate and mechanically delivered rate). Except for dose administration, no other measurements or procedures should be performed during measurement of vital signs. Vital signs should also be assessed in the case of abnormal clinical signs and symptoms.

3.3.2.4 Adverse Events

All AEs will be documented from the time of informed consent until the time screen failure is documented, or until the subject is either discontinued from the study or all 40 Weeks PMA/Hospital Discharge assessments have been completed and should be followed until either resolution (or return to normal or baseline values), until they are judged by the Investigator to no longer be clinically significant, or for at least 30 days if the AE extends beyond the Post-treatment Phase.

All AEs meeting the criteria for serious (ie, serious adverse events [SAEs]) should be followed until resolution, death, or the subject is lost to follow-up even if they are ongoing more than 30 days after completion of the Post-treatment Phase

Refer to Section 9.1 for detailed definitions of safety events. All AEs/SAEs that occur during the study will be followed as instructed in Section 9.3.

If, at any time, the parent/guardian withdraws their consent for the subject to remain in the study, any AEs/SAEs that occur from UNEX-42 treatment (including product use errors, product quality problems, and therapeutic failures) will be reported. These events will not be recorded in the electronic Case Report Form (eCRF).

3.3.2.5 Predefined Complications of Prematurity

Complications of prematurity will be assessed from Baseline to 40 Weeks PMA/Hospital Discharge. The diagnosis of the following complications will be recorded: sepsis, pneumothorax, clinically significant patent ductus arteriosus (PDA) requiring treatment or ligation, necrotizing enterocolitis requiring treatment or surgery, intraventricular hemorrhage (including highest grade), periventricular leukomalacia, and retinopathy of prematurity (including stage).

3.3.2.6 Clinical Laboratory Parameters

Blood samples for the clinical chemistry and hematology analyses will be collected at Baseline (randomization) and repeated at the 72 hours postdose and the 36 Weeks PMA assessments to evaluate for study treatment-emergent changes. For Baseline, the clinical laboratory assessments may utilize the laboratory assessments obtained as standard of care treatment within the last 24 hours in lieu of drawing blood for the sole purpose of the study. Any study-required blood tests should be obtained at the same time a blood sample is being collected for clinical purposes, if possible. Values for the following parameters will be obtained:

Table 3-5 Clinical Laboratory Parameters

Electrolyte Panel	Chemistry Panel	Hematology Panel
Bicarbonate	Alanine aminotransferase	Hemoglobin
Chloride	Aspartate aminotransferase	Hematocrit
Potassium	Bilirubin (total and indirect)	Red blood cell count
Sodium	Creatinine	White blood cell count
	Blood urea nitrogen	Platelet count

3.4 NUMBER OF CENTERS

This is a US-based multicenter study with approximately 6 centers participating.

3.5 NUMBER OF SUBJECTS

Approximately 18 subjects will be enrolled in this study. There will be 3 cohorts of 6 subjects per cohort, randomized 2:1 (active:placebo) for a total of 12 subjects receiving UNEX-42 treatment (4 subjects each at 20, 60, and 200 pmol phospholipid/kg body weight) and 6 subjects receiving placebo.

3.6 ESTIMATED STUDY DURATION

The expected duration for a subject to complete the study is up to 69 weeks. Subjects are administered clinical study material (CSM) on Day 1 and the Post-treatment Phase will consist of assessments through 40 Weeks PMA (up to 17 weeks depending on subject GA at birth). Subjects that complete the Post-treatment Phase (including those that are discharged from hospital prior to 40 Weeks PMA) will continue into the Long-term Outcome Phase and will be assessed through 1 year of corrected age. Analysis of data from the Post-treatment Phase will occur after all subjects have completed the Post-treatment Phase.

4 SUBJECT ELIGIBILITY

Inclusion and exclusion criteria are to be assessed at Screening and reconfirmed at Baseline prior to randomization.

4.1 INCLUSION CRITERIA

A subject must meet the following criteria to be eligible for the study:

1. Infant whose postnatal age is 3 to 14 days at randomization.
2. Subject meets the following oxygen and birth weight criteria based on GA:

Gestational Age at Birth	Criteria
23 weeks to 24 weeks 6 days	Any birth weight, any oxygen requirement
25 weeks to 26 weeks 6 days	FiO_2 ≥35% (sustained >2 hours) at any point during postnatal Days 1 to 14 AND birth weight ≤750 g

3. Endotracheally intubated and receiving mechanical ventilation at the time of Screening and randomization.
4. Not expected to be extubated within the next 24 hours after randomization.
5. The subject has a parent/guardian who gives written informed consent.

4.2 EXCLUSION CRITERIA

A subject is not eligible for inclusion in this study if any of the following criteria apply:

1. Has a congenital heart defect, except for PDA, atrial septal defect or a small/moderate, restrictive ventricular septal defect.
2. Has a serious malformation of the lung, such as pulmonary hypoplasia/aplasia, congenital diaphragmatic hernia, or any other congenital lung anomaly.
3. Being treated with inhaled nitric oxide.
4. Has a known chromosomal abnormality (eg, Trisomy 18, Trisomy 13, or Trisomy 21) or a severe congenital malformation (eg, hydrocephalus and encephalocele, trachea-esophageal fistula, abdominal wall defects, and major renal anomalies).
5. Has had a known severe congenital infectious disease (ie, herpes, toxoplasmosis rubella, syphilis, human immunodeficiency virus, cytomegalovirus, etc).
6. High clinical suspicion of active systemic infection, severe sepsis, or septic shock during the Screening period.
7. Underwent a surgical procedure (requiring admission to an operating room) within 72 hours before randomization or who is anticipated to have a surgical procedure (requiring admission to an operating room) within 72 hours before or following randomization.
8. Has had a Grade 3 or 4 intracranial hemorrhage.
9. Has active pulmonary hemorrhage.
10. The subject is currently participating in any other interventional clinical study.
11. The subject is, in the opinion of the Investigator, so ill that death is inevitable, or is considered inappropriate for the study for any reason(s) other than those listed above.

4.3 PRESCRIBED THERAPY

Not applicable.

4.3.1 Concomitant Medications

All concomitant medications taken during the Post-treatment Phase of the study, including those that are ongoing at Baseline or taken for AEs or other medical events, should be recorded in the subject's source documents and transcribed as required to the eCRF.

5 SUBJECT ENROLLMENT

5.1 TREATMENT ASSIGNMENT

Within each cohort, eligible subjects will be randomly allocated to receive either UNEX-42 or placebo at randomization (using a 2:1 allocation ratio). A subject number will be assigned to identify individual subject data. An Interactive Response Technology (IRT) will be responsible for allocation of all study drug.

5.2 RANDOMIZATION

The study will be randomized 2:1 UNEX-42 to placebo. Within each dose cohort, subjects will be randomly allocated to receive UNEX-42 or placebo through the IRT using a centrally administered block randomization.

5.3 BLINDING

The Investigator, study site personnel involved in preparation/administration of CSM, subject's parent/guardian, and Sponsor (except for Global Drug Safety and Regulatory Affairs personnel involved in safety reporting) will not be aware of the treatment allocation. All CSM will be provided as blinded study drug.

6 TREATMENT PROCEDURES

6.1 UNEX-42 DOSAGE AND ADMINISTRATION

UNEX-42 will be administered to 3 cohorts at 20, 60, and 200 pmol phospholid/kg body weight, respectively, in a dose-escalating manner. UNEX-42 is suspended in phosphate-buffered saline. UNEX-42 is a clear liquid that is provided in appropriately sized vials and stored at -70±10°C prior to use. UNEX-42 will be appropriately diluted in normal saline to a achieve a final dosing volume of 1 mL.

Once all entry criteria have been met and random treatment assignment confirmed, the dose of CSM will be prepared by the site pharmacy staff. The filled syringe of CSM will be delivered to the hospital room and administered to the subject by appropriately trained staff. The CSM must be administered within 8 hours after thawing. The CSM should be administered through an existing IV catheter being used for administration of crystalloids, such as an umbilical, central, or peripheral IV line. If any circumstances prevent administration of CSM through an existing IV line, a new IV line may be started for the administration of CSM at the discretion of the Investigator. If necessary, the line for infusion must first be flushed and primed with a sufficient volume of normal saline to clear the line at a controlled rate of 10 mL/hour by a syringe pump, regardless of subject weight. The CSM syringe contents will then be administered at a controlled rate of 10 mL/hour by a syringe pump. Once the CSM has been completely administered, the line will be flushed with a sufficient volume of normal saline to clear the CSM from the line at a controlled rate of 10 mL/hour.

CSM infusions may be stopped at the discretion of the Investigator if any of the following are observed during the course of the infusion or the flush:

- Acute worsening of respiratory function requiring intervention.
- Acute hypotension requiring intervention (including but not limited to fluid bolus, vasopressors, or hydrocortisone).
- Any acute change in vital signs requiring medical intervention.
- Any other medical issue such that, in the opinion of the Investigator, continuation of study drug infusion represents a serious medical risk to the subject.

Subjects that receive any amount of CSM will be followed for the duration of the study, unless the subject is withdrawn from the study as described in Section 8.1 of the protocol.

6.2 SELECTION OF DOSES FOR SUBJECT COHORTS

The starting dose for Cohort 1 of 20 pmol phospholipid/kg body weight was selected as described in Section 1.3. Dose administration for each cohort will occur such that there is an observational period of 3 days between dosing the first, second, and third subject. This observational period will allow for the monitoring of any dose-limiting toxicities (DLTs) (see Section 6.3) in at least 1 subject treated with UNEX-42. The Data Monitoring Committee

(DMC) will make dose escalation recommendations based on review of safety data between cohorts (see Section 10.7).

6.3 DOSE-LIMITING TOXICITY

DLTs will be used to inform dosing decisions; however, no DLTs have been observed during nonclinical development of UNEX-42. Therefore, for the purposes of this study, only an estimate of possible dose-limiting events can be made. As a result, DLTs will be defined as any of the following toxicities assessed by the Investigator and/or Sponsor as at least possibly related to UNEX-42 based on the time to onset following UNEX-42 administration and judged not to be related to the underlying condition or any concomitant medications by the Investigator and/or Sponsor:

- A treatment-emergent adverse event (TEAE) that is of potential clinical significance such that further dose escalation would expose subjects to unacceptable risk.
- Increased liver enzymes (Grade 2 or higher, using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 as guidance) or any evidence of hepatic failure.
- Pulmonary toxicity, worsening respiratory distress, or other acute exacerbation of pulmonary function requiring ventilatory support or escalation of care.

In addition, the DMC may identify as a DLT, any TEAE occurring in subjects treated with UNEX-42 at any time during the study. Each DLT must be recorded in the eCRF as an AE. In addition, any DLT that meets criteria for a SAE must be reported to the Sponsor in an expedited manner as described in Section 9.4.

6.4 ACCESS TO BLINDED TREATMENT ASSIGNMENT

During the study, neither the Investigator, study site personnel involved in preparation/administration of CSM, subject's parent/guardian, nor Sponsor (except for Global Drug Safety and Regulatory Affairs personnel involved in safety reporting) should be unblinded to the treatment assignment of any subject for any reason except in the event of a medical emergency (eg, a life-threatening event), if and when knowledge of the treatment assignment is considered necessary to determine the optimal medical management. Appropriate communications should take place between the site staff and the Sponsor before accessing the IRT to allow unblinding of a subject's treatment assignment. In the event of a medical

emergency that requires immediate unblinding, the IRT may be accessed by site staff or the Sponsor's Medical Monitor to determine treatment assignment.

6.5 COMPLIANCE

All infusions will be administered by site staff as directed in the protocol. Any deviations from planned infusion times will be documented in the subject's source documentation. The Principal Investigator or other site personnel under the direction of the Principal Investigator will be responsible for ensuring the appropriate dose is given to the subject and for recording all dosing information in source documents.

7 EXPERIMENTAL PROCEDURES

Assessments during the study will be performed according to Table 3-1 and Table 3-2. Every effort should be made to perform assessments as close as possible to the scheduled time points, within the visit window noted for each day on study as noted in Table 3-1 and Table 3-2. Details of efficacy assessments and safety assessments are described in Sections 3.3.1 and 3.3.2, respectively. Details of study procedures are provided in Section 7.1 for Screening, Section 7.2 for Baseline, Section 7.3 for the Post-treatment Phase, Section 7.4 for 40 Weeks PMA/Hospital Discharge, and Section 7.5 for the Long-term Outcome Phase.

7.1 SCREENING

Screening can begin up to 48 hours prior to Baseline. Site personnel may conduct Pre-screening assessments prior to a subject's parent/guardian signing an Informed Consent Form (ICF). Pre-screening assessments may include review of the hospital data to identify potential subjects that may be eligible for the study. Screening assessments will be conducted in the hospital as inpatient assessments after informed consent has been obtained according to Table 3-1.

7.2 BASELINE

Following confirmation of eligibility, Baseline assessments will be conducted according to Table 3-1 on Day 1 prior to randomization. Eligible subjects will be randomly allocated to receive either UNEX-42 or placebo as described in Section 5.2.

7.3 POST-TREATMENT PHASE

Following UNEX-42 or placebo treatment on Day 1, subjects will be assessed according to Table 3-1. Post-treatment study assessments will occur during the study at the following time points: 24 and 72 hours postdose, 7 days postdose, weekly until 35 weeks PMA, 36 weeks PMA, and 40 weeks PMA/hospital discharge. The timing of the 36 Weeks PMA and 40 Weeks PMA/Hospital Discharge assessments is based upon each subject's GA, as determined during Screening based on local standard practice.

7.4 40 WEEKS PMA/HOSPITAL DISCHARGE

At the end of the Post-treatment Phase (40 Weeks PMA/Hospital Discharge), final assessments for each subject will be performed according to Table 3-1. If a subject discontinues from the study prematurely, 40 Weeks PMA/Hospital Discharge assessments should occur if possible.

7.5 LONG-TERM OUTCOME PHASE

Subjects that complete the 40 Weeks PMA Assessment or are discharged from the hospital prior to this assessment will continue into the Long-term Outcome Phase. Assessments will occur at 6 months (phone contact) and 1 year (site visit) of corrected age according to Table 3-2. Corrected age should be calculated by the formula below:

> Actual Age (weeks) – Weeks of Prematurity (weeks)* = Corrected Age (weeks)
> *Weeks of prematurity is calculated by subtracting the subject's GA at birth from 40 weeks. For example, if a subject was born at a GA of 28 weeks, they were 12 weeks premature.

Assessment of general health will occur by phone contact at 6 months and by a study site visit at 1 year. The phone contact at 6 months corrected age will be made by trained site staff. The call will follow a script that will facilitate the collection of relevant information. The subject's parent/guardian will be interviewed according to the script, and information relating to SARs and other outcome measures will be recorded. If possible, the site staff should schedule the 1-year visit at the time of the phone call. The same script used at the 6-month assessment will be used at the 1-year visit.

8 STUDY TERMINATION

8.1 CRITERIA FOR SUBJECT WITHDRAWAL

A subject's parent/guardian may voluntarily withdraw the subject, or the subject may be withdrawn from the study and/or study drug by the Investigator at any time for reasons including, but not limited to, the following:

- The subject's parent/guardian wishes to withdraw the subject from further participation.
- Any medically appropriate reason, in the opinion of the Investigator, or significant protocol violation likely to undermine the validity of results.
- The Sponsor terminates the study.

Every effort should be made to follow subjects up to their scheduled long-term follow-up visits. If a subject is discontinued from the study prematurely, the Investigator must provide an explanation in the eCRF and complete the End of Study Record for that subject. The Investigator should make every effort to perform all scheduled assessments prior to hospital discharge. In the event that a subject discontinues study drug infusion prematurely, the Investigator will still attempt to perform all planned assessments as outlined in Table 3-1 and Table 3-2, unless the subject is withdrawn for other reasons as stated in this section of the protocol. If a subject is withdrawn due to an AE, the subject will be followed as outlined in Section 9.3. The date the subject is withdrawn and the primary reason for discontinuation will be recorded on the eCRF.

8.2 CRITERIA FOR TERMINATING THE STUDY

The study may be stopped at any time if, in the opinion of the DMC and/or Sponsor, continuation of the study represents a serious medical risk to the subjects. This may include, but is not limited to, the presence of serious, life-threatening, or fatal AEs or AEs that are unacceptable in nature, severity, or frequency. The Sponsor reserves the right to discontinue the study for any reason at any time.

8.3 CRITERIA FOR DISCONTINUING THE SITE

The study may also be terminated at a given center if:

- The Principal Investigator elects to discontinue the study
- The Sponsor elects to discontinue the study at the site
- US Food and Drug Association (FDA) regulations are not observed
- The protocol is violated
- Changes in personnel or facilities adversely affect performance of the study.

9 ADVERSE EVENT REPORTING

9.1 DEFINITIONS

9.1.1 Adverse Event

An AE is any untoward medical experience occurring to a subject during a clinical study whether or not it is related to study drug. An AE may include an intercurrent illness, injury, or any other concomitant impairment of the subject's health, as well as abnormal laboratory findings if deemed to have clinical significance. AEs may also include worsening of an existing symptom or condition, or post-treatment events that occur as a result of protocol-mandated procedures (eg, exacerbation of a pre-existing illness following the start of the study or an increase in the frequency or intensity of a pre-existing episodic event or condition).

Thus, no causal relationship with the study drug is implied by the use of the term "adverse event."

An AE does not include the following:

- Medical or surgical procedures, as the condition for which the surgery is required may be an AE.
- Planned surgical measures permitted by the study protocol and the condition(s) leading to these measures are not AEs.
- Day-to-day fluctuations of a pre-existing disease or condition that was present or detected at the start of the study that do not worsen.

Specific complications of prematurity will be assessed from Baseline to 40 Weeks PMA/Hospital Discharge as described in Section 3.3.2.5. Therefore, the complications of prematurity should only be recorded as an AE or SAE if the event is unusual with respect to intensity, frequency,

duration as compared with symptoms in the subject's medical history, or if there is a reasonable possibility that the event was caused by the study drug. Congenital disorders should be recorded as medical history, even if diagnosed after the subject has entered the study.

Investigators should use the NCI-CTCAE Version 5.0 as guidance to grade the severity/intensity of all events. If a CTCAE criterion does not exist, the Investigator should grade the severity/intensity according to the following criteria:

- Grade 1 (mild): does not interfere with the patient's usual function
- Grade 2 (moderate): interferes to some extent with patient's usual function
- Grade 3 (severe): interferes significantly with patient's usual function
- Grade 4 (life-threatening): results in a threat to life or in an incapacitating disability
- Grade 5 (death): results in death

9.1.2 Serious Adverse Event

A SAE is an AE occurring at any dose that results in any of the following outcomes:

- Death
- A life-threatening AE
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity

In addition, important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and require medical/surgical intervention to prevent 1 of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in hospitalization, or the development of drug dependency or drug abuse.

Life-threatening in this context means that the subject was, in the view of the Investigator, at immediate risk of death from the event as it occurred. It does not mean that the event, had it occurred in a more severe form, might have caused death.

9.1.3 Serious Adverse Reaction

A SAR is a SAE occurring at any dose that is assessed by the Investigator as possibly or probably related to the CSM. During the Long-term Outcome Phase of the study, only SARs will be reported to United Therapeutics Global Drug Safety according to Section 9.4. Other conditions that will be evaluated as part of the study endpoints are therefore not subject to expedited reporting.

9.2 DOCUMENTATION OF ADVERSE EVENTS

An AE or SAE occurring during the study must be documented in the subject's source documents and in the eCRF. Information relating to the AE such as onset and cessation date, intensity, seriousness, relationship to study drug, and outcome is also to be documented in the eCRF (see Section 15.1 for definitions). Where possible, AEs should be recorded using standard medical terminology. The Investigator should attempt, if possible, to establish a diagnosis based on the presenting signs and symptoms. If several signs or symptoms are clearly related to a medically defined diagnosis or syndrome, the diagnosis or syndrome should be recorded in the eCRF, not the individual signs and symptoms.

9.3 FOLLOW-UP OF ADVERSE EVENTS

All AEs should be followed until either resolution (or return to normal or baseline values), until they are judged by the Investigator to no longer be clinically significant, or for at least 30 days if the AE extends beyond the Post-treatment Phase. All SAEs that occur during the study will be followed until resolution, death, or the subject is lost to follow-up even if they are ongoing more than 30 days after completion of the final assessment. Supplemental measurements and/or evaluations may be necessary to investigate fully the nature and/or causality of an AE or SAE. This may include additional laboratory tests, diagnostic procedures, or consultation with other healthcare professionals. The eCRF should be updated with any new or additional information as appropriate.

9.4 REPORTING RESPONSIBILITIES OF THE INVESTIGATOR

Sites should enter initial or follow-up SAE information regardless of causality or expectedness into the Sponsor's Electronic Data Capture (EDC) system (primary method) within 24 hours of awareness of an SAE. The SAE will be directly transmitted from the EDC system to the Argus

Safety Database. If the site is unable to enter the SAE electronically into EDC system, the alternative for reporting is submitting a scanned copy of the paper SAE Report Form via email to [redacted] within 24 hours of awareness. If the paper SAE Report Form is submitted, the site staff will also be required to enter the SAE data into the EDC system when the EDC becomes available. SAE source documents (eg, hospital discharge summary or death certificate) will be submitted by the site staff via email to [redacted]. The Investigator or Sponsor (if appropriate) must also notify their Institutional Review Board (IRB), Independent Ethics Committee (IEC), and/or other local equivalent body of the reported SAE, including any follow-up information. Copies of each report and documentation of IEC/IRB/local equivalent body notification and receipt will be kept in the Investigator's study file.

9.5 SAFETY REPORTS

In accordance with USA FDA regulations, the Sponsor or designee will notify the regulatory agency, Investigators, and/or IRBs and IECs of all relevant AEs (usually those that are considered to be possibly attributable to study drug and are both serious and unexpected) in accordance with the applicable regulations. The Investigator must report these AEs to their IRB/IEC in accordance with applicable regulations and guidelines set forth by the IRB/IEC.

10 STATISTICAL CONSIDERATIONS

10.1 DATA PROCESSING

The results of assessments will be transcribed into an eCRF for each subject who has a parent/guardian sign an ICF until study completion or study discontinuation for any reason. A representative from the Sponsor will verify eCRF data fields against source documentation. All data transmitted from the site will be reviewed and entered into a quality assured computerized database. Data clarifications will be generated, and the database will be edited as appropriate. The eCRF screens are to be reviewed by the Investigator for completeness and accuracy. The Investigator must electronically sign each subject's eCRF to signify his/her approval of the data. The Investigator will be required to resign an eCRF if changes are made to a subject's eCRF by the site after the Investigator initially signs the eCRF. The database will be final when all

outstanding queries have been resolved and all data management quality assurance procedures are complete.

10.2 SAMPLE SIZE

No formal sample size calculations have been performed.

10.3 ANALYSIS PLAN

Analysis and unblinding of data from the Post-treatment Phase will occur after all subjects have completed the Post-treatment Phase. All Long-term Outcome Phase data will be analyzed after the last subject completes the 1 Year Visit.

Details of the safety and efficacy analyses are provided below. A separate statistical analysis plan will document further details of the statistical methods to be employed including any changes to planned analyses specified within this protocol. The analysis plan will be finalized prior to any unblinding of study data by the Sponsor. Unless otherwise specified, all statistical tests will be 2-sided at alpha level 0.05 and for exploratory purposes only. All statistical calculations will be completed using the latest version of SAS®. Unless otherwise specified, all summaries will be by dose cohort and the placebo patients from all cohorts will be pooled.

The Intent-to-Treat population will be defined as all subjects randomized into the study who receive any amount of study drug; efficacy analyses will be performed on this Intent-to-Treat population.

The Safety population will be defined as all subjects enrolled into the study who receive any amount of study drug. Safety analyses will be performed on this Safety population.

In general, data collected prior to dosing will serve as Baseline values for the evaluation of data collected during the Post-treatment Phase.

10.3.1 Primary Endpoints

The primary endpoints are the following safety assessments during the Post-treatment Phase:

- Incidence of TEAEs
- Incidence of all-cause mortality through Week 36 PMA
- Incidence of treatment-related AEs
- Absolute and changes from Baseline in laboratory parameters
- Absolute and changes from Baseline in vital signs

All AEs as recorded by the Investigator will be assigned a Medical Dictionary for Regulatory Activities (MedDRA) preferred term and system organ class by the Sponsor for reporting purposes. Summaries of AEs and deaths will include the number and percentage of subjects who experienced these events for each dose cohort and placebo. AEs will be further summarized by preferred term and system organ class for each dose cohort and placebo.

Descriptive statistics (mean, standard deviation, median, interquartile range, minimum, and maximum) will be computed by treatment group and overall for measured values and change from Baseline of clinical laboratory parameters and vital signs.

Summaries of safety data will be descriptive with no inferential analyses planned.

10.3.2 Secondary Endpoints

The number and percentage of subjects will be summarized by dose cohort and placebo for each of the following categorical assessments:

- Incidence of BPD or death at 36 Weeks PMA
- Severity of BPD (mild, moderate, severe) at 36 Weeks PMA
- Complications of prematurity during the Post-treatment Phase (diagnosis of sepsis, pneumothorax, clinically significant PDA requiring treatment or ligation, necrotizing enterocolitis requiring treatment or surgery, intraventricular hemorrhage [including highest grade], periventricular leukomalacia, and retinopathy of prematurity [including stage])

Descriptive statistics (mean, standard deviation, median, interquartile range, minimum, and maximum) will be computed by dose cohort and placebo for each of the following continuous assessments:

- Duration (number of days) of invasive and noninvasive mechanical ventilation during the Post-treatment Phase
- Duration (number of days) of supplemental oxygen during the Post-treatment Phase
- Duration (number of days) of postnatal steroids (eg, hydrocortisone, dexamethasone, methylprednisolone) for the prevention and/or treatment of evolving BPD during the Post-treatment Phase
- Duration (number of days) of hospitalization admission (inclusive of neonatal intensive care unit and step-down unit admission times) during the Post-treatment Phase
- RSS at 36 Weeks PMA and a summary across all time points collected
- Tracheal aspirate cell count and differential at 7 days postdose

Kaplan-Meier curves will be constructed to assess the following time to event variables:

- Time to discharge from the hospital
- Time to end of mechanical ventilation
- Time to end of supplemental oxygen
- Time to end of postnatal steroid use
- Time to all-cause mortality

The appropriate statistical test may be applied to each endpoint to allow for comparison between the dose cohorts and placebo.

10.4 INTERIM ANALYSIS

Enrollment between cohorts will pause for data review by an external DMC to evaluate the data available before proceeding to the next cohort. The constitution and responsibilities of the DMC will be detailed in a DMC charter to be agreed with the members at the inaugural meeting. The endpoints of interest for the DMC review are listed in Section 10.3.1.

10.5 OTHER ANALYSES

Exploratory analyses may be conducted based on available study data.

10.6 DATA LISTINGS AND SUMMARIES

All data gathered in this study will be presented in summary tables and listings as described in the Statistical Analysis Plan.

10.7 DATA MONITORING COMMITTEE

A DMC will be established for the study, comprised of independent members including physicians knowledgeable in the treatment of complications of prematurity. A meeting will occur after each of the first 2 cohorts have been enrolled, as detailed in the DMC charter. The DMC will make dose escalation recommendations based on review of safety data. The criteria for dose escalation are based on the occurrence of AEs and/or DLTs that are unrelated to the underlying conditions or concomitant medications during the initial safety review period for each dosing cohort. The DMC may recommend stopping dose escalation at any point based on available data and sufficient scientific rationale.

All analyses will be prepared by an independent external consultant and reviewed by the DMC as defined in the DMC charter. The Sponsor (with the exception of Global Drug Safety and Regulatory Affairs personnel involved in safety reporting) will only have access to blinded study data until the completion of the study. A separate analysis plan will be prepared for these analyses.

11 PACKAGING AND FORMULATION

11.1 CONTENTS OF STUDY DRUG

United Therapeutics will supply UNEX-42 for administration in the study. UNEX-42 will be provided in 5-mL vials each containing 2 mL of sterile solution. The phospholipid content of the solution will be expected to be 100 to 500 pmol/mL. The exact concentration will be provided at the time of CSM manufacture and release testing.

Placebo will be phosphate-buffered saline provided in vials matching UNEX-42 in color, size, and shape.

11.2 LABELING

Each vial of UNEX-42 or placebo will be labeled in accordance with all applicable regulations and Good Manufacturing Practice guidelines. A description of the pharmaceutical properties and composition of the formulation of UNEX-42 is provided in the Investigator's Brochure.

11.3 STORAGE AND HANDLING OF CSM

UNEX-42 is stored at -70±10°C. Once thawed, UNEX-42 cannot be refrozen for reuse.

11.4 SUPPLY AND RETURN OF CLINICAL STUDY MATERIAL

Study sites will be supplied with a sufficient quantity of CSM to begin enrollment in the study. Appropriate arrangements will be made for resupply as needed based on enrollment rates.

UNEX-42, including both used and unused vials, should be destroyed by the study site and documented according to institutional policy following consultation with the Sponsor and after final drug accountability by Sponsor personnel.

11.5 DRUG ACCOUNTABILITY

The Investigator is responsible for CSM accountability and reconciliation. The Investigator or his/her designee will be responsible for maintaining accurate records of the quantity and dates of all investigational product supplies received, and the amount administered to each subject. The quantity of any investigational product lost, missing, destroyed, etc must also be accounted for and documented.

12 REGULATORY AND ETHICAL OBLIGATION

12.1 UNITED STATES FDA OR APPLICABLE REGULATORY REQUIREMENTS

The study will be conducted in accordance with the International Council for Harmonisation (ICH) and Good Clinical Practice (GCP) guidelines and all applicable national regulations. The Sponsor will obtain the required approval from each national regulatory authority to conduct the study. During the conduct of the study, an annual safety report will be compiled by the Sponsor for submission to those regulatory authorities and IRBs/IECs as required. Any additional national reporting requirements as specified by the applicable regulations, regulatory authorities, or IRB/IEC will also be fulfilled during the conduct of the study.

12.2 INFORMED CONSENT REQUIREMENTS

Before a subject is enrolled in the study, the Investigator or his/her designees must explain the purpose and nature of the study, including potential benefits and risks and all study procedures to the subject's parent/guardian. The subject's parent/guardian must sign and date an IRB/IEC-approved ICF prior to the conduct of any study-related activities. A copy of the signed consent form will be given to the subject's parent/guardian and the original will be retained in the study site's records.

12.3 INDEPENDENT ETHICS COMMITTEE/INSTITUTIONAL REVIEW BOARD

Prior to study initiation at each site, the Investigator will obtain approval for the study from an appropriate IRB/IEC and provide the Sponsor with a copy of the approval letter. The IRB/IEC must also review and approve the study site's ICF and any other written information provided to the subject's parent/guardian prior to enrollment, as well as any advertising materials used for subject recruitment. Copies of the ICF and advertising materials must be forwarded to the Sponsor for review before submission to the IRB/IEC prior to the start of the study.

If, during the study, it is necessary to amend either the protocol or the ICF, the Investigator is responsible for obtaining IRB/IEC approval of these amended documents prior to implementation. Copies of the IRB/IEC correspondence and approval letters must be sent to the Sponsor.

During the conduct of the study, an annual progress report will be compiled by the Sponsor for submission to IRBs/IECs as required.

A written summary of the study will be provided by the Investigator to the IRB/IEC following study completion or termination according to the IRB or IEC standard procedures. Additional updates will also be provided in accordance with the IRB/IEC's standard procedures.

12.4 PRESTUDY DOCUMENTATION REQUIREMENTS

Before the commencement of the clinical study, the following documents will be provided to the site: Investigators' Brochure, Protocol, ICF, Budget Agreement, and eCRF.

The site will be required to provide the following documents to United Therapeutics Corporation or designee prior to study start: Signature page of the protocol, Form FDA 1572, Financial Disclosure Form, IRB/IEC composition documentation, IRB/IEC protocol and informed consent approval letters, and Curriculum Vitae of study staff listed on the Form FDA 1572.

12.5 SUBJECT CONFIDENTIALITY

Every effort will be made to keep medical information confidential. United Therapeutics Corporation, the FDA or other regulatory bodies, and the IRB/IEC governing this study may inspect the medical records of any subject involved in this study. The Investigator may release the subject's medical records to employees or agents of the Sponsor, the IRB/IEC or the FDA or appropriate local regulatory agencies for purposes of checking the accuracy of the data. A number will be assigned to all subjects and any report published will not identify the subject's name.

13 ADMINISTRATIVE AND LEGAL OBLIGATIONS

13.1 PROTOCOL AMENDMENTS AND STUDY TERMINATION

Protocol amendments that could potentially adversely affect the safety of participating subjects or that alter the scope of the investigation, the scientific quality of the study, the experimental design, dosages, duration of therapy, assessment variables, the number of subjects treated, or subject selection criteria may be made only after consultation between United Therapeutics Corporation or its designee and the Investigator.

All protocol amendments must be submitted to and approved by the appropriate regulatory authorities and IRB/IEC prior to implementation.

A report documenting study termination must also be submitted to and acknowledged by the appropriate IRB/IEC for each study site.

At the end of the study, where applicable, a final report will be provided to the local regulatory agencies.

13.2 STUDY DOCUMENTATION AND STORAGE

In accordance with federal/national regulations, ICH, and GCP guidelines, the Investigator must retain study records for at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. The Investigator must notify United Therapeutics Corporation before any disposal or change in location of study records.

13.3 STUDY MONITORING AND DATA COLLECTION

In accordance with federal/national regulations, ICH, and GCP guidelines, monitors for United Therapeutics Corporation or its designee will periodically contact the sites and conduct on-site visits. During these visits, the monitor will at a minimum: confirm ethical treatment of subjects, assess study progress, review data collected, conduct source document verification, verify drug accountability periodically, and identify any issues requiring resolution.

The Investigator agrees to allow the monitor direct access to all relevant documents and to allocate his/her time and his/her staff to the monitor to discuss any findings or any relevant issues.

14 REFERENCES

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15 APPENDICES

15.1 GUIDELINES AND DEFINITIONS FOR RECORDING ADVERSE EVENTS

The Principal Investigator or a designated member of his/her staff will probe each subject's parent/guardian for any adverse events (AEs) that may have occurred. The Investigator should always ask the same question when conducting the verbal probe in order to ensure uniformity between subjects. The Investigator should ask:

"How is the subject doing (feeling)?"

Based on the parent/guardian's response to this question, the Investigator should ask additional questions relevant to the specific complaint, such as:

"How severe is/was the symptom?"
"How often did the symptom occur?"
"How long did the symptom last?"

It is the Investigator's responsibility to review the results of all diagnostic and laboratory tests as they become available and ascertain if there is a clinically significant change from Baseline. If the results are determined to be a clinically significant change from Baseline, this should be reported as an AE. The Investigator may repeat the diagnostic procedure or laboratory test or request additional tests to verify the results of the original tests. When possible, a diagnosis associated with the abnormality should be used as the reported AE.

Using provided definitions, the Investigator will then:

1. Rate the intensity and seriousness of the AE
2. Estimate the causality of the AE to study drug
3. Note actions taken to counteract the AE.

Definitions of Intensity, Seriousness, Causality, and Outcome

INTENSITY

An assessment of the relative intensity (severity) of an AE should be based on the NCI-CTCAE, Version 5.0. The maximum intensity encountered during the evaluation period should be checked. The assessment of intensity should be independent of the assessment of the seriousness of the AE.

SERIOUSNESS

A serious adverse event (SAE) is one that represents an actual or potential significant hazard. This includes, but is not limited to, an event that is fatal, life-threatening, permanently or severely disabling, requires or prolongs inpatient hospitalization[a], is a congenital abnormality or is medically significant (important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent 1 of the outcomes listed in this definition).

[a]Hospitalizations that would not be considered SAEs include those for:

- Routine treatment or monitoring of the study indication not associated with any deterioration in condition.
- Treatment which was elective or preplanned, for a pre-existing condition not associated with any deterioration in condition (eg, preplanned operation which does not lead to further complications etc).
- Treatment of an emergency, in an outpatient setting for an event not fulfilling any of the definitions of serious as given above and not resulting in hospital admission.

CAUSALITY

An estimate of causality between a specified AE and the study drug is made by the Investigator. Several factors should be considered when determining causality. These factors include temporal relationship to the study drug.

- Definitions of the causality categories are as follows:
  - NOT RELATED – There is not a temporal relationship to study drug administration (too early, or late, or study drug not taken), or there is a reasonable causal relationship between another drug, or concurrent disease and the SAE, or any of the following:

- An event that precedes the first administration of study drug
- An event for which the cause is clearly related to an external event
- Temporal relationship to study drug is atypical
- Is readily explained by an intercurrent illness AND has an expected level of severity, duration, and resolution
- An alternative explanation (concomitant drug, intercurrent illness) is likely.

- POSSIBLE – There is a reasonable causal relationship between the study drug and the SAE, or any of the following:
  - Has a reasonable temporal relationship to study drug
  - The event has a plausible biological link to the activity of the study drug
  - Is unlikely to be related to an intercurrent illness or has an unexpected degree of severity, duration, or complication.
- PROBABLE – There is a reasonable causal relationship between the study drug and the SAE, or any of the following:
  - Has a reasonable temporal relationship to study drug
  - The event has a plausible biologic link to the activity of the study drug
  - Not readily explained by an intercurrent illness
  - Not readily explained by external event.

<u>OUTCOME</u>

- Fatal – the study subject died.
- Not Recovered/Not Resolved – the AE was ongoing at the time of death or at the time the subject was lost to follow-up.
- Recovered/Resolved – the AE resolved.
- Recovered/Resolved with Sequelae – the AE is considered resolved; however, there are residual sequelae. Some events do not return to baseline, such as metastasis or progression of disease; however, once these events are determined by the Investigator to be stable or chronic, the Investigator may consider the event to be resolved or resolved with sequelae.
- Recovering/Resolving – the AE is improving but is not yet completely recovered/resolved.
- Unknown – the outcome of the AE cannot be determined.