Statistical Analysis Plan

A Multicenter, Randomized, Open-Label, Controlled Trial to Assess the Safety and Tolerability of Lucinactant for Inhalation in Preterm Neonates 26 to 28 Weeks PMA

Protocol Number: 03-CL-1401

SAP Version: Original

13 May 2015

A Multicenter, Randomized, Open-Label, Controlled Trial to Assess the Safety and Tolerability of Lucinactant for Inhalation in Preterm Neonates 26 to 28 Weeks PMA

Statistical Analysis Plan

Author/Project Biostatistician

Eric Ma
Biostatistician
Discovery Laboratories, Inc.

Date

By signing below, I have read through the entire statistical analysis plan and agree to implement the plan.

Phillip D. Simmons
Executive Director, Biostatistics and Data Management
Discovery Laboratories, Inc.

Date

Steven G. Simonson, MD
Senior Vice President, Chief Development Officer
Discovery Laboratories, Inc.

Date

EXECUTIVE SUMMARY

The primary objective of this study is to evaluate the safety and tolerability of lucinactant for inhalation, administered as an aerosol in 4 escalating doses to a preterm, neonatal population receiving nasal continuous airway pressure (nCPAP) for respiratory distress syndrome (RDS), compared to neonates receiving nCPAP alone.

This is a multicenter, randomized, open-label, controlled study comparing lucinactant for inhalation administered with nCPAP to treatment with nCPAP alone for the treatment of RDS in 26 to 28 completed weeks post-menstrual age (PMA) preterm infants. Infants will be randomized into 1 of 4 sequential dose groups, with an allowed repeat dose if the repeat dose criterion is met. Reconstituted lyophilized lucinactant (reconstituted with sterile water for injection) will be aerosolized by the investigational device, ADS, using the capillary aerosol generator and introduced into the nCPAP circuit. Those infants randomized to the control arm will continue to receive nCPAP alone.

Exposure, defined as the emitted dose, is the amount of lucinactant that is delivered at the connection to the patient interface by the ADS at a constant rate of flow. The theoretical inhaled dose – the fraction of the aerosolized lucinactant that the infant is exposed to that is likely to be inhaled – is estimated by product of: 1) the aerosol concentration, 2) the minute ventilation of the infant, and 3) the administration time of the aerosol.

Study Group	Treatment Assignment	Number of Subjects
Dosing Group I	Active Arm: 50 mg TPL/kg Delivered over 30 minutes in conjunction with nCPAP 1 repeat dose allowed if repeat dosing criterion met	n=8
	Control Arm: Continuous nCPAP	n=8
Dosing Group II	Active Arm: 75 mg TPL/kg Delivered over 45 minutes in conjunction with nCPAP 1 repeat dose allowed if repeat dosing criterion met	n=8
	Control Arm: Continuous nCPAP	n=8
Dosing Group III	Active Arm: 100 mg TPL/kg Delivered over 60 minutes in conjunction with nCPAP 1 repeat dose allowed if repeat dosing criterion met	n=8
	Control Arm: Continuous nCPAP	n=8
Dosing Group IV	Active Arm: 150 mg TPL/kg Delivered over 90 minutes in conjunction with nCPAP 1 repeat dose allowed if repeat dosing criterion met	n=8
	Control Arm: Continuous nCPAP	n=8

Note: TPL = Total Phospholipids

Dosing Group I will complete study dosing and all active subjects will complete 72 hour safety assessments and procedures before the Safety Review Committee (SRC) review. The SRC will complete their review of all available Dosing Group I safety data (to include study-related adverse events [AEs], serious adverse events [SAEs], and additional safety endpoints) before recommending advancing enrollment to additional dosing groups. If the SRC concludes there are no safety concerns that would preclude proceeding to the next higher dose, dosing will commence in Dosing Group II. Likewise, the SRC will review the safety and tolerability of this dose and each succeeding dose, with dosing proceeding in the next dosing group if the SRC determines there are no safety concerns.

Repeat dosing will be allowed in each dosing group. Subjects meeting the repeat dosing criterion will receive an additional treatment of the same dose. Repeat dosing will be allowed between 2 and 24 hours after completion of the initial dose.

Subjects will be followed for safety evaluations (including, but not limited to, AEs and concomitant medications) until the subject is 36 weeks PMA or is discharged. Descriptive statistics (number of subjects, percent, mean, standard deviation, median, minimum, maximum) will be presented by treatment group, defined as each of the active doses (n = 8; 32 subjects total) and the all controls combined (n = 32). A final visit will occur at 36 weeks PMA or at the time of discharge or withdrawal (whichever occurs first) for all subjects.

Key safety and tolerability endpoints in the study include:

- AEs and ADEs (including peri-dosing events and nasal excoriations)
- Air leak (especially pneumothorax and pneumomediastinum)
- Survival
- Use of respiratory support and supplemental oxygen
- Complications of prematurity
- Assessments of the following:
    a) Arterial carbon dioxide (PCO_2)
    b) Serum electrolyte measurements
    c) Vital signs
    d) Gastric liquid volume
    e) Oxygen saturations (SpO2)
- Chest radiography prior to intubation, if such a procedure does not delay or compromise the emergent care of the subject

A peri-dosing event is defined as an adverse event with an onset time ≤ 2 hours from the time of initiating administration of lucinactant for inhalation

Efficacy endpoints (exploratory objective) in the study include:

- Incidence of bronchopulmonary dysplasia (BPD) at 36 weeks PMA
- Rate of survival without BPD at 36 weeks PMA
- Technical performance of the ADS
- Worsening respiratory status either during or after exposure to lucinactant for inhalation
- Physiological parameters (eg, FiO_2, PCO_2)

The statistical analysis of both the primary and secondary safety and tolerability objectives will be based on all randomized preterm neonates in the study. In addition, data from all evaluable preterm neonates will be analyzed for efficacy signals as an exploratory endpoint. As this is an open-label study with no hypothesis testing, no adjustments for *p*-values are required or employed.

TABLE OF CONTENTS

ABBREVIATIONS

Abbreviation	Description
ADE	Adverse device effect
ADS	Aerosurf® Delivery System
AE	Adverse event
BPD	Bronchopulmonary dysplasia
Cl/Cl^-	Chloride/Chloride ion
DMC	Data monitoring committee
FiO_2	Fraction of inspired oxygen
IMV	Intermittent mechanical ventilation
IP	Investigational product
ITT	Intent-to-treat
IVH	Intraventricular hemorrhage
IWRS	Interactive web-response system
K/K^+	Potassium/Potassium ion
MAP	Mean airway pressure
MedDRA	Medical Dictionary for Regulatory Activities
MMAD	Mass median aerodynamic diameter
MV	Mechanical ventilation
Na/Na^+	Sodium/Sodium Ion
nCPAP	Nasal continuous positive airway pressure
NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit
PCO_2	Arterial carbon dioxide
PDA	Patent ductus arteriosus
PMA	Post-menstrual age
PVL	Periventricular leukomalacia
RDS	Respiratory distress syndrome
ROP	Retinopathy of prematurity
SAE	Serious adverse event
SpO_2	Oxygen saturation as measured by pulse oximetry
SAP	Statistical analysis plan
SOC	System Organ Class
SRC	Safety Review Committee
TPL	Total phospholipids
UADE	Unanticipated adverse device effect
WHO	World Health Organization

1 OVERVIEW

This statistical analysis plan (SAP) provides a comprehensive and detailed description of strategy, rationale, and statistical techniques to be used to assess safety and tolerability in the 03-CL-1401 study of lucinactant for inhalation in preterm neonates. The purpose of the SAP is to ensure the credibility of the study findings by pre-specifying the statistical approaches to the analysis of study data prior to database lock. This SAP provides additional details concerning the statistical analyses that are outlined in the protocol.

1.1 BACKGROUND

1.1.1 Treatment of Neonatal Respiratory Distress Syndrome

Surfactant treatment has reduced mortality and morbidity in newborns with respiratory distress syndrome (RDS). Intratracheal[1] instillation of exogenous surfactant is a standard treatment for the treatment of RDS, and the benefits of surfactant treatment, specifically early surfactant treatment, are well established[2]. However, instillation of surfactant into the lung requires endotracheal intubation, an invasive procedure that has potential deleterious effects.

Efforts by neonatologists to avoid endotracheal intubation to reduce the volutrauma and barotrauma associated with airway inflation and deflation have focused on the use of nasal continuous positive airway pressure (nCPAP), reserving intubation and surfactant administration for the most severely ill neonates.

The use of nCPAP in preterm neonates with RDS has become widely accepted[3] and national guidelines (American Association for Respiratory Care)[4] exist for treatment of respiratory distress in preterm newborns. These guidelines outline several patient interface devices that can be used to deliver continuous airway pressure such as nasal masks, short bi-nasal prongs, and nasopharyngeal tubes. It has been demonstrated that nCPAP is effective in increasing functional residual capacity by stabilizing and improving alveolar function[5] and in dilating the larynx[6]. Devices that generate and deliver nCPAP, including patient interfaces such as nasal prongs, are specifically designed, manufactured, and registered for use in neonates.

While the use of nCPAP in preterm infants is common, studies of nCPAP alone, including a meta-analysis of prophylactic nCPAP for preventing morbidity and mortality in very preterm neonates, have not shown a clear benefit of this approach[7]. A large, multicenter, randomized clinical study (N = 610) was performed to compare the effects of nCPAP alone versus intermittent mechanical ventilation (IMV) for very preterm neonates at birth. This study showed that in the nCPAP group, the need for surfactant therapy was half of that compared to subjects in

the intubation group, and death or oxygen treatment at day 28 after birth was less frequent ($p = 0.006$). However, there were no differences in survival or oxygen therapy at 36 weeks, overall mortality, days of respiratory support, oxygen treatment, hospital stay, or other secondary outcomes. Of note, a higher rate of pneumothorax in the nCPAP group compared to the intubation group (9% versus 3%) was observed, and over 50% of infants in the nCPAP group required endotracheal intubation[8]. Other studies comparing nCPAP to endotracheal intubation and surfactant administration have also demonstrated a relatively high rate of endotracheal intubation in the group receiving nCPAP[9].

1.1.2 Development of Aerosolized Device for Lucinactant Delivery

Discovery Laboratories, Inc. (Discovery) has developed a capillary aerosol generator (CAG) device to aerosolize lucinactant (Aerosurf®, lucinactant for inhalation). Pre-clinical studies using the CAG technology in spontaneously breathing preterm lambs receiving aerosolized lucinactant have demonstrated significant improvements in lung mechanics and gas exchange compared with lambs receiving CPAP alone. In addition, pilot clinical studies using aerosolized lucinactant in neonates with RDS, as well as in adults with asthma and cystic fibrosis, have preliminarily demonstrated that aerosolized lucinactant is generally safe and well-tolerated. Data from a large neonatal database support the assumption that prophylactic use of aerosolized surfactant and nCPAP may reduce the need for intubation by 36% in neonates with a birth weight of 1001 to 1500 grams.[1]

Because aerosolized surfactant is preferentially delivered to the ventilated parts of the lungs, it is essential to provide appropriate ventilatory support during aerosol delivery. Distribution of aerosol in the lungs has been limited by the technical constraints of currently available aerosol generators and system configurations. Compared with the endotracheal instillation surfactant administration route, efforts to aerosolize surfactants in clinical models have been largely unsuccessful because of the highly inefficient and limited dose delivery compared with the bolus administration route of currently available aerosol generators[10,11]. However, the CAG technology may allow for administration of aerosolized lucinactant to be deposited in the lungs of preterm neonates in potentially sufficient quantities to affect a therapeutic response analogous to that of endotracheal instillation surfactant administration. An investigational device, Aerosurf Delivery System (ADS), utilizing the CAG has been developed and provides a high-density surfactant aerosol output (20 µl/sec), with an appropriate particle size (2 to 3 microns mass median aerodynamic diameter [MMAD]). The ADS will be used in the current study to aerosolize lucinactant.

The purpose of this study is to investigate the safety and tolerability of lucinactant for inhalation, administered as an aerosol, in 4 escalating dose concentrations with a potential repeat dose to a preterm, neonatal population, 26 to 28 completed weeks post-menstrual age (PMA), receiving nCPAP for RDS, in comparison with neonates receiving nCPAP alone. The results of this phase 2a study will provide an assessment of the safety and tolerability of the doses studied, and will assist in the selection of doses for further clinical investigation.

1.2 OBJECTIVES

1.2.1 Primary Objective

The primary objective of this study is to evaluate the safety and tolerability of aerosolized surfactant, specifically lucinactant for inhalation (30 mg TPL/ml), administered in escalating theoretical inhaled doses of 50, 75, 100, and 150 mg TPL/kg to preterm neonates 26 to 28 completed weeks (28 weeks 6 days) PMA who are receiving nCPAP for RDS, compared to neonates receiving nCPAP alone. The primary objective of this study will be assessed based on safety evaluations, including adverse events (AEs) and serious adverse events (SAEs), and tolerability of the device, reported from the time of randomization until 36 weeks PMA.

1.2.2 Secondary Objective

The secondary objective of this study is to determine the maximum tolerated dose of lucinactant for inhalation as determined by the safety evaluations, including AEs and SAEs, obtained within each dosing group.

1.2.3 Safety Evaluations

The following measures are to be documented in the electronic case report form (eCRF) in accordance with timings outlined in study schedule:

1. Survival (date and time of death if applicable)
2. AEs, including ADEs
    - To include: nasal excoriations by examination ($\geq$ 24 hours to $\leq$ 7 days following aerosol exposure) and evidence of lung air leak (especially pneumothorax)
    - To be categorized as a peri-dosing events if onset of event is $\leq$ 24 hours from the time of initiating administration of lucinactant for inhalation
3. Signs consistent with worsening respiratory status
4. Concomitant medications
5. Use of respiratory support and supplemental oxygen to include:

- Need for endotracheal intubation and mechanical ventilation
- Need for intermittent positive pressure breaths using inflating pressures above the set CPAP pressure administered manually or mechanically through any patient interface

6. nCPAP and fraction of inspired oxygen (FiO_2) requirements
7. Complications of prematurity (ie, IVH, periventricular leukomalacia [PVL], pulmonary hemorrhage, NEC, patent ductus arteriosus [PDA], sepsis, ROP, BPD)
8. Physical examinations
9. Tolerability of lucinactant for inhalation
10. Incidence leading to withdrawal from study
11. Incidence of air leak
12. Assessments of the following:
    a) PCO_2
    b) Serum electrolyte measurements
    c) Body weight
    d) Vital signs
    e) Gastric liquid volume
    f) Defecation
    g) Oxygen (O_2) saturation, as determined by pulse oximetry (SpO_2)
    h) Chest radiography, as applicable

1.2.4 Exploratory Efficacy Objective

The objective of exploratory efficacy analysis is to demonstrate the ability to measure clinical effect.

1.2.4.1 Efficacy Endpoints

Exploratory efficacy endpoints will be evaluated from the time of lucinactant for inhalation initiation until the completion of Study Day 7 (non-hypothesis testing) and will include the following:

1. Incidence of BPD
2. Rate of survival without BPD at 36 weeks PMA
3. Worsening of respiratory status

4. Technical performance of the ADS
    - The efficacy of the device delivery of lucinactant for inhalation will be characterized indirectly through the subject's response to treatment and solicited feedback from the principal investigators (PIs) and relevant site-based study staff.
5. Physiological parameters (eg, FiO_2, PCO_2)

1.3 HYPOTHESES

This study has no hypothesis tests.

2 INVESTIGATIONAL PLAN

2.1 STUDY POPULATION

The study population will consist of preterm neonates from 26 to 28 completed weeks PMA with RDS. This study will enroll up to 64 preterm neonates who are candidates for SRT and nCPAP in a neonatal intensive care unit (NICU) setting.

A subject will be enrolled at 1 of approximately 25 study sites in the US. It is anticipated that approximately 640 subjects will be screened to meet the enrollment goal of approximately 64 subjects (10:1 ratio of screened to enrolled).

2.1.1 Inclusion criteria

Each subject must meet all of the following inclusion criteria to be enrolled in this study:

1. Signed ICF from legally authorized representative;
2. Gestational age 26 to 28 completed weeks PMA;
3. Successful implementation of controlled nCPAP within 90 minutes after birth;
4. Spontaneous breathing;
5. Chest radiograph consistent with RDS; and
6. Within the first 20 hours after birth, requires an nCPAP of 5 to 6 cm H_2O to maintain SpO_2 of 88% to 95% with an FiO_2 of 0.25 to 0.50 that is clinically indicated for at least 30 minutes. Transient (<10 minutes) FiO_2 excursions below 0.25 or above 0.50 do not reset the 30 minute requirement.

2.1.2 Exclusion Criteria

Subjects meeting any of the following exclusion criteria may not be enrolled in this study:

1. Heart rate that cannot be stabilized above 100 beats/minutes within 5 minutes of birth;
2. Recurrent episodes of apnea occurring after the initial newborn resuscitation period (ie, 10 minutes after birth) requiring intermittent positive pressure breaths using inflating pressures above the set CPAP pressure administered manually or mechanically through any patient interface;
3. A 5-minute Apgar score < 5;

4. Major congenital malformation(s) and cranial/facial abnormalities that preclude nCPAP, diagnosed antenatally or immediately after birth;
5. Other diseases or conditions potentially interfering with cardiopulmonary function (eg, hydrops fetalis or congenital infection such as TORCH);
6. Known or suspected chromosomal abnormality or syndrome;
7. Premature rupture of membranes (PROM) > 2 weeks;
8. Evidence of hemodynamic instability requiring vasopressors or steroids for hemodynamic support and/or presumed clinical sepsis;
9. Need for endotracheal intubation and/or mechanical ventilation; and
10. Has been administered any the following:
    a) Any investigational agent or exposure to a medical device,
    b) Any other surfactant agent, or
    c) Steroid treatment (steroid exposure before birth is acceptable).

2.2 STUDY DESIGN AND RANDOMIZATION

This study is a multicenter, randomized, open-label, controlled study, to evaluate the safety and tolerability of a novel investigational drug-device combination product (lucinactant for inhalation) in conjunction with nCPAP, in preterm neonates with RDS who are under the care and observation of a NICU. The study will explore escalating exposures of lucinactant for inhalation in a sequential manner through 4 unique dosing groups. All subjects randomized to the active study arm will receive the same concentration of study drug to provide the same total dose exposure for that arm. Subjects in all 4 dosing groups are eligible for a repeat dose if the repeat dosing criterion is met (see Section 2.2.2). Subjects randomized to the control arm will receive nCPAP alone.

2.2.1 Treatment Groups

Study Group	Treatment Assignment	Number of Subjects
Dosing Group I	Active Arm: 50 mg TPL/kg Delivered over 30 minutes in conjunction with nCPAP 1 repeat dose allowed if repeat dosing criterion met	n=8
	Control Arm: Continuous nCPAP	n=8
Dosing Group II	Active Arm: 75 mg TPL/kg Delivered over 45 minutes in conjunction with nCPAP 1 repeat dose allowed if repeat dosing criterion met	n=8
	Control Arm: Continuous nCPAP	n=8
Dosing Group III	Active Arm: 100 mg TPL/kg Delivered over 60 minutes in conjunction with nCPAP 1 repeat dose allowed if repeat dosing criterion met	n=8
	Control Arm: Continuous nCPAP	n=8
Dosing Group IV	Active Arm: 150 mg TPL/kg Delivered over 90 minutes in conjunction with nCPAP 1 repeat dose allowed if repeat dosing criterion met	n=8
	Control Arm: Continuous nCPAP	n=8

Note: TPL = Total Phospholipids

The study dosing will initiate in Dosing Group I at the lowest dose. Only after completion of the Safety Review Committee (SRC) review and subsequent approval, will the study progress to the next dose level for subjects in Dosing Group II. After all active subjects within each dosing group have completed assessments through 72 hours; the SRC will review the safety and tolerability of this dose. Following enrollment completion and SRC approval, dose escalation will proceed to the next dosing group.

If there appears to be intolerability with a given dose or operational challenges administering a dose, the remaining subjects may have the dose decreased to a previously administered dose, after consultation with the SRC.

The term “treatment group” will be defined as subjects in each of the active doses and the control arm subjects combined.

2.2.2 Repeat Dosing

In order to ensure the safety of repeat dosing, specifically in regards to AEs and electrolyte changes (such as sodium and potassium levels), and to ensure subjects with a demonstrated need receive treatment, 1 repeat dose will be allowed for all dosing groups. The repeat dose will

consist of repeating the same dose, and will occur between 2 and 24 hours after the initial dose, if the repeat dosing criterion is met. The repeat dosing criterion is defined as: at least 2 hours, but no more than 24 hours, post dosing and a FiO_2 level of at least 0.35.

If, in the opinion of the PI, repeat dosing would compromise the safety of the subject, repeat dosing will not occur.

2.2.3 Sample Size Justification

The number of subjects randomized (8 in each active group and 8 in each control group) is considered sufficient to establish safety and tolerability before proceeding to the next higher dose. A total of approximately 32 subjects assigned to the active arm and approximately 32 subjects for the control arm for the entire study is considered sufficient to assess the overall safety and tolerability and to select safe and well-tolerated doses. Statistical sample size calculations were not performed.

2.2.4 Study Schedule

Protocol Event Schedule Summarization

Measurement/Procedure	Study Period				
	Screening	Primary Observation	Extended Observation	Final Observation	
		Days 1-2	Days 3-7	Day 8 to 36 Weeks PMA	Final Visit (36w PMA)
Informed Consent/HIPAA	X				
Inclusion/Exclusion Criteria	X				
Demographics	X				
Maternal/Birth History[1]	X				
Physical Exam	X				X
Chest Radiograph[2]	X				
Randomization		X			
Study Treatment		X			
Body Weight		X[1]			
Defecation		X			
Vital Signs		X[3]	X[3]		
Monitoring of SpO_2[4]		X	X		
Monitoring of PCO_2[5]		X			
Resp. Support and O_2 Delivery	X	X	X	X[6]	X[6]
Serum Electrolytes		X[7]			
Gastric Liquid Volume[8]		X			
Peri-Dosing Events[9]		X			
Adverse Events		X	X	X	X
Adverse Device Effects		X			
Concomitant Medication		X	X	X	X

Note: Day 1 for all subjects is the day of study randomization.

[1] Birth weight is captured in birth history. The first body weight obtained after randomization is recorded in the eCRF.

[2] A chest radiograph prior to intubation is required if such a procedure does not delay or compromise the care of the subject.

[4] Vital signs will be documented ≤ 15 minutes (± 5 minutes) prior to randomization. For the active arms, vital signs will be documented every 5 minutes (± 2 minutes) during dosing for Dosing Group I and II; every 15 minutes (± 5 minutes) during dosing for Dosing Group III and IV. For the control arms, vital signs will be documented at 15 minutes (± 5 minutes) post randomization. In addition, for all subjects, record at 1, 3 (± 15 minutes), 6, 12 (± 1 hour), 18, 24, 36 and 48 hours (± 2 hours) post randomization and daily at 0800 (± 2 hours) on days 3 to 7.

[5] Continuous monitoring of SpO_2 is initiated at the time of randomization and continued until completion of Study Day 7.

[6] Continuous trancutaneous PCO_2 monitoring for all subjectsis to be initiated following randomization and at least 30 minutes prior to the start of ADS delivery, and continued for 72 hours post randomization.

[7] Only the date and time of the initiation and discontinuation of ventilator and pressure support is to be documented in the study eCRF beyond Study Day 7.

[8] Serum electrolytes are to be evaluated 24 hours from randomization (± 6 hours)

[9] Gastric liquid volume will be assessed 30 minutes (± 15 minutes) following completion of study treatment administration. Enteral feedings are to be held during administration and ≥ 1 hour following completion of study treatment.

[10] Peri-dosing events are defined as any AE with an onset time ≤ 2 hours from the time of initiating study treatment.

3 STUDY SUBJECT CHARACTERISTICS

3.1 DEMOGRAPHICS AND BASELINE CHARACTERISTICS

3.1.1 Demographics

Continuous variables (eg, gestational age, birth weight) will be summarized using mean, standard deviation (SD), median, minimum, and maximum. Categorical variables (eg, sex, race, ethnicity) will be summarized using frequency and percent.

Race categories with small numbers (frequencies of 0 or 1 or percentages of < 10%) will be combined as 'Other' for summary displays.

3.1.2 Birth and Maternal History

Mode of delivery (vaginal, c-section), type of birth (single, multiple), Apgar score at 1 and 5 minutes, and incidence of congenital anomalies will be summarized using frequency and percent.

The incidence and type of ruptured membranes, incidence of clinical chorioamnionitis, and the incidence and number of doses of antenatal steroids will be summarized using frequency and percent.

3.2 SUBJECT DISPOSITION

Subject disposition (overall and by treatment group) will be summarized using frequency and percent by treatment group and overall. The number of subjects screened, randomized (intent-to-treat [ITT] population), and administered study medication (safety population) will be described.

The number of subjects who completed the study or discontinued treatment early will be summarized using frequency and percent by treatment group and overall. Reasons for early discontinuation of treatment include device failure or malfunction, AE or ADE, PI's best medical judgment, respiratory deterioration.

A subject may withdraw consent (through their legally authorized guardian) at any time with prejudice to further care.

If, at the end of the Final Study Period, the status of a subject is unknown, every effort must be made to contact the subject's legally authorized guardian to determine the status of the subject. If the status of the subject has not been established in 28 days following exposure to study treatment, the subject will be considered lost to follow-up.

3.3 STUDY AND CONCOMITANT MEDICATION

3.3.1 Compliance

Compliance summary for this study is not applicable as all subjects will be administered at least one dose by study staff in the NICU.

3.3.2 Number of Doses

The number of doses (treatments) received by subjects in the active arms will be summarized.

3.3.3 Previous and Concomitant Medication

Medications taken since birth and concomitant medications will be coded according to the World Health Organization (WHO) drug dictionary, version DDE September 2013 or later. Medications will be summarized using frequency and percentages for each treatment group by drug category and generic name.

3.4 PROTOCOL VIOLATIONS/DEVIATIONS

A protocol violation occurs when the PI fails to adhere to any significant protocol requirement affecting the inclusion, exclusion, subject safety, and primary endpoint criteria. A protocol deviation is any deviation from the protocol that is not a protocol violation. All protocol violations/deviations will be summarized by treatment group and listed by subject.

4 EFFICACY ANALYSIS

The efficacy analysis for this study is for exploratory purposes only. No formal hypothesis testing or statistical modeling will be done.

4.1 EFFICACY POPULATIONS

The ITT population is the efficacy analysis population and is defined as all subjects who were randomized in the study.

4.2 EFFICACY ENDPOINTS

Exploratory efficacy endpoints will be evaluated from the time of lucinactant for inhalation initiation until study completion (non-hypothesis testing) and will include the following:

- Intubation and surfactant administration
- Onset/incidence of bronchopulmonary dysplasia (BPD)
- Survival without BPD at 36 weeks PMA
- Worsening of respiratory status
- Technical performance of the ADS

4.3 STATISTICAL ANALYSIS

All statistical analyses will use summary statistics to describe all endpoints; no statistical testing will be done. All time points will be measured from time of randomization unless otherwise specified.

Categorical variables will be summarized using frequency and percent. Continuous variables will be summarized using n, mean, SE, SD, median, minimum, and maximum. Summary statistics will be presented by treatment group.

4.3.1 Onset of BPD and Survival without BPD

The number and percent of subjects who develop BPD by 36 weeks PCA and the number and percent of subjects who are alive and are without BPD at 36 weeks PCA will be summarized.

4.3.2 Worsening of Respiratory Status

The number of subjects with worsening respiratory status will be summarized. A subject is considered to have worsening respiratory status if they meet at least one of: the need for additional surfactant therapy following exposure to lucinactant for inhalation; a sustained

fraction of inspired oxygen (FiO_2) > 0.50 for > 30 minutes to maintain an oxygen saturation (SpO_2) > 90%; arterial CO_2 (PCO_2) > 65 mmHg on ≥ 2 consecutive occasions; persistent, arterial pH < 7.20; any sustained apneic event; intubation for any reason (except for elective surgical procedures); nCPAP > 7 cmH_2O; initiation of intermittent positive pressure breaths using inflating pressures above the set CPAP pressure administered manually or mechanically through any patient interface; death while on study; or the study PI determines that the subject has worsening respiratory status based on their best clinical judgment.

Worsening respiratory status will be categorized as early (within 72 hours after birth) or late (more than 72 hours but within 7 days after birth) worsening.

4.3.3 Intubation and Surfactant Administration

The number of subjects intubated for mechanical ventilation and for all reasons (eg, surfactant administration) will be summarized. In addition, the time to intubation for mechanical ventilation and the time to any intubation will be summarized using n, mean, SE, SD, median, minimum, and maximum.

4.4 MISSING DATA

Missing values represent a potential source of bias in a clinical trial. Hence, every effort will be undertaken to fulfill all the requirements of the protocol concerning the collection and management of data; however, some missing data is inevitable. As the primary objective of this study is safety and tolerability, no imputation or adjustment for missing data will be done.

4.5 SUBGROUP ANALYSES

Due to the small treatment group sizes, subgroup summaries are not planned.

5 TECHNICAL PERFORMANCE OF THE DEVICE

The technical performance of the device will be summarized for each active treatment. The incidence of the following will be summarized: ventilator/CPAP tubing detachments, aerosol tube detachments, aerosol tube condensate obstruction, proximal pressure port obstructions, study drug leakage (either liquid or aerosol), occurrence of alarms signals (before, during, and after dosing), automatic system shutdowns, loss of inspiratory flow, inability to maintain nCPAP, occurrence of error codes.

In addition, the weight and/or volume of liquid in all traps will be summarized as continuous variables.

6 SAFETY ANALYSES

6.1 SAFETY POPULATION

The safety population includes all subjects who received any study medication. All safety assessments will be based on this population.

6.2 SAFETY ENDPOINTS

Safety endpoints include:

- Early termination of therapy
- Peri-dosing events
- AEs and ADEs
- Air Leak (especially pneumothorax)
- Death
- Physical examinations
- Assessments of the following:
  - PCO_2
  - Serum electrolyte measurements (Na^+, Cl^-, K^+)
  - Body weight
  - Vital signs
  - Gastric liquid volume
  - Defecation
  - O_2 saturation, as determined by SpO_2
  - Chest radiograph, as applicable
- Respiratory Support:
  - nCPAP settings (cm H_2O) (or other means of ventilator support)
  - Need for endotracheal intubation and IMV parameters
  - Oxygen support (FiO_2)
- Complications of prematurity (ie, intraventricular hemorrhage [IVH], cystic periventricular leukomalacia [PVL], pulmonary hemorrhage, necrotizing enterocolitis [NEC], patent ductus arteriosus [PDA], sepsis, retinopathy of prematurity [ROP])

6.3 EXTENT OF EXPOSURE

All subjects randomized to active treatment are to receive one dose of study medication. Subjects will receive 30, 45, 60, or 90 minutes of aerosolized lucinactant. The number of subjects who

receive a repeat dose and the number of subjects whose study treatment is terminated early will be summarized.

6.4 ADVERSE EVENTS

All AEs will be coded by preferred term and system organ class (SOC) from the Medical Dictionary for Regulatory Activities (MedDRA), version 16.1 or above, and will be reviewed by the medical monitor or designee. All AEs will be summarized as categorical variables (frequency and percent) by treatment group unless otherwise indicated. AEs that are related to the device (ADEs) will be summarized with all AEs and separately. AEs will not be compared between treatment groups.

6.4.1 Peri-Dosing AEs

The incidence of peri-dosing events (bradycardia, desaturation, and vomiting) will be summarized. In addition, the incidence of complications related to the placement of bi-nasal prongs (bleeding, apparent obstruction to the nares, occlusion of the prongs, nasal irritation, and other) will be summarized. Peri-dosing events and complications related to the placement of the prongs will also be summarized with all adverse events.

6.4.2 Other AEs Related to Surfactant Administration

Individual air leaks (e.g., pneumothorax, pulmonary interstitial emphysema, pneumo-mediastinum, pneumopericardium, and subcutaneous emphysema) will be identified by medical review of all AEs and summarized by treatment group.

6.4.3 Other AEs

AEs, other than those listed above, will be summarized by the MedDRA preferred term and SOC for all AEs, regardless of relationship to study drug, and for AEs at least remotely related. If an AE occurs multiple times for the same subject within the same term or body system, only the most severe occurrence for that term or body system will be counted.

In addition, all AEs will be summarized by severity (mild, moderate, severe), relationship to the study drug (unrelated, unlikely related, possibly related, definitely related), whether or not the AE was device related, gender, race, and ethnic origin.

6.4.4 Serious Adverse Events

All SAEs will be listed and summarized using frequency counts and percentages. If the same SAE occurs multiple times for the same subject, the most severe occurrence will be counted. For this study, unexpected adverse device effects (UADEs) will be analyzed as SAEs.

SAEs, including multiple occurrences, will also be listed, to include severity, relationship to the study drug, gender, race, and ethnic origin.

6.4.5 Deaths

The all-cause mortality during the study will be summarized by treatment group using frequency counts and percentages. Deaths by subject will also be listed and will include primary cause, date and time of death, gender, race, and ethnic origin.

6.5 CLINICAL ASSESSMENTS OF SAFETY

6.5.1 Vital Signs

Vital signs, including body temperature, respiration rate, and heart rate, will be summarized at all pre-specified time points. Clinically significant vital signs will also be recorded as AEs.

6.5.2 Physical Examination

For each body system evaluated at screening and at the final physical examination, frequency counts and percentages of normal and abnormal results will be summarized. In addition, a shift table to describe the changes in normal/abnormal results between screening and the final visit will be presented. Any new abnormal physical examination findings must be documented as AEs.

6.5.3 Electrolytes

Sodium, chloride, potassium, and total carbon dioxide (CO_2) will be summarized at 24 hours post randomization as continuous variables. Clinically significant values will also be recorded as AEs.

6.5.4 Gastric Liquid Volume

Gastric liquid volume will be summarized at 30 minutes following treatment (for active treatments) or randomization as a continuous variable.

6.5.5 Defecation

The number of stools occurring within 24 hours from the time of randomization will be summarized as a discrete variable using frequency and percent.

6.5.6 Respiratory Parameters

Observed and change from baseline values for FiO_2, PCO_2, and SpO_2 over 72 hours will be summarized using n, mean, SD, median, minimum, and maximum. In addition, figures for observed values and change from baseline values will be produced.

6.5.7 FiO_2 and Respiratory Support

nCPAP settings at all pre-specified time points will be summarized as continuous variables. For subjects receiving mechanical ventilation (MV) through Day 7, the initial mean airway pressure (MAP), the mode, the respiratory rate, and FiO_2 will be summarized. For subjects receiving supplemental oxygen through Day 7, the initial mode and the FiO_2 will be summarized. All MV and supplemental oxygen data will be listed by subject.

The number of subjects requiring respiratory support in the delivery room will be summarized using frequency and percent.

6.5.8 Complications of Prematurity

The incidence of IVH, PVL, PDA, NEC, apnea, pulmonary hemorrhage, acquired sepsis, and air leak will be summarized by treatment group. The worst grade of IVH and the worst stage of NEC will also be summarized by treatment group. In addition, the incidence and stage of ROP will be summarized by treatment group.

7 INTERIM ANALYSES AND DATA MONITORING

7.1 SAFETY REVIEW COMMITTEE

Safety is to be monitored by a Safety Review Committee (SRC), also known as a Data Monitoring Committee (DMC). Data analyses will be performed by an independent statistician who is a non-voting member of the SRC. As this is an open-label study, the reports from each interim analysis will be unblinded. The interim analyses are for assessing safety, trial conduct and ethical issues.

7.2 INTERIM ANALYSES

Four interim analyses are planned. Each interim analysis will occur when all subjects from each dosing group have been enrolled and all active subjects have completed 72 hours. In addition to the planned analyses, if more than 2 subjects in any dosing group experience an SAE related to dosing, aerosol delivery will be suspended and further dosing will proceed only if and when the SRC deems it to be appropriate.

In addition, if a treated subject dies due to a related event during or within 12 hours after aerosol administration, then an unscheduled meeting of the SRC will be called. Safety and tolerability data will be presented to the SRC for their immediate review. Study enrollment will be suspended until such time that a safety assessment has been completed and dosing will proceed only if and when the SRC deems it to be appropriate. If the SRC deems it to be appropriate to re-initiate enrollment after an enrollment hold, local IRB approvals will be obtained before the re-initiation of subject screening and enrollment activities. The objective of the interim analyses is to ensure the safety of subjects at a particular dose before proceeding to the next higher dose.

7.3 DATA MONITORING

A system of computerized data validation checks will be implemented and applied to the study database on a regular basis. Queries will be entered, tracked, and resolved through the electronic data capture (EDC) system directly. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented.

A detailed data surveillance plan (DSP) will be produced by the Department of Biometrics. The data surveillance plan (DSP) describes statistical surveillance methods and presentations used to check and evaluate the study conduct and data quality. This is designed to detect anomalous data, data entry errors in the summary outputs, and ensure the quality of the collected data through the use of frequency, trending and other statistical techniques.

Data surveillance activities on frequency monitoring should include, but are not limited to: data anomalies (eg, outliers, potential protocol violators), data entry errors (missing data values, inconsistent data values), criteria violations, and the number of subjects experiencing early termination from the study. Data surveillance activities on trend review include, but are not limited to: the evaluation, documentation, presentation and reporting of (1) recorded endpoint variables, (2) AEs and SAEs, (3) unanticipated adverse device effects (UADEs), and (4) subject deaths.

The DSP will be produced and updated periodically with the periods defined by the Department of Biometrics. Data surveillance procedures described in the plan will take place at least quarterly, depending upon the rate of subject enrollment and the timing of study closure activities.

8 STATISTICAL TECHNICAL ISSUES

8.1 METHODS OF ASSIGNING SUBJECTS TO TREATMENT GROUPS

An overall randomization list will be prepared by Discovery's Biometrics department such that subjects will be randomly assigned to lucinactant or control (nCPAP alone). Each subject's random assignment will be provided to an interactive web response system (IWRS). As soon as possible after a subject meets the entry criteria and is eligible to be enrolled, the PI or authorized designee will log in to the IWRS and complete the randomization procedures. Subjects that discontinue the study prematurely will not be replaced.

8.2 BLINDING/MASKING

This study is open label; thus, no blinding or masking procedures are employed.

8.3 DETAILS ON STATISTICAL METHODS

Not applicable for this study.

8.4 MULTIPLICITY

Since the primary purpose of this study is safety and tolerability, and since no statistical testing will be performed, no adjustment for multiplicity will be done.

8.5 SAMPLE SIZE RE-ESTIMATION

Sample size re-estimation will not be used in this study.

9 GENERAL ANALYSIS DEFINITIONS

All summaries and statistical analyses will be generated using SAS® System for Windows™, version 9.1 or higher.

9.1 BASELINE DEFINITION

Baseline is defined as the last measurement prior to study drug administration for subjects randomized to the active group, and randomization for subjects randomized to the control group.

9.2 WINDOWS FOR VISITS

Accurate clock times will be recorded for each timed event in military time (24-hour clock). Day 1 is the day on which randomization occurs; Day 3 begins at midnight 2 days following randomization and Day 7 will be the sixth day after the day of randomization.

All assessments at pre-specified time points are to be conducted within the windows specified in the protocol. Summary tables will use the pre-specified time points, not the actual times, to summarize the data.

9.3 SITE POOLING METHODS

As no statistical modeling will be performed, pooling of centers will not be done.

10 REFERENCES

1. Soll RF, Hallman M, Merritt TA. Surfactant in the Prevention and Treatment of Respiratory Distress Syndrome. In Boynton B, Carlo W, Jobe A, editors. New Therapies for Neonatal Respiratory Failure. Cambridge: Cambridge University Press; 1994. p. 49-80.
2. Engle W. Surfactant-replacement therapy for respiratory distress in the preterm and term neonate. Pediatrics. 2008; 121.
3. Vermont Oxford Network. Expanded database summary. ; 2003.
4. AARC Clinical Practice Guidelines. Application of continuous positive airway pressure to neonates via nasal prongs, nasopharyngeal tube, or nasal mask. ; 2004.
5. Lewis JF, Ikegami M, Jobe AH, Absolom D. Physiologic responses and distribution of aerosolized surfactant (Surfanta) in a nonuniform pattern of lung injury. Am Rev Respir Dis. 1993; 147: p. 1364-1370.
6. Gaon P, Lee S, Hannan S, Ingram D, Milner AD. Assessment of effect of nasal continuous positive pressure on laryngeal opening using fibre optic laryngoscopy. Arch Dis Child Fetal Neonatal Ed. 1999; 80(3): p. F230-F232.
7. Subramaniam P, Henderson-Smart D, Davis P. Prophylactic nasal continuous positive airways pressure for preventing morbidity and mortality in very preterm infants. Cochrane Database Syst Rev. 2005 Jul 20;(3).
8. Morley, CJ; Davis, PG; Doyle, LW; Brion, LP; Hascoet, JM; Carlin, JB; COIN Trial Investigators. Nasal CPAP or intubation at birth for very preterm infants. N Engl J Med. 2008 Feb 14; 358(7): p. 700-708.
9. Dunn MS, Kaempf J, Klerk Ad, Klerk Rd, Reilly M, Howard D, et al. Randomized trial comparing 3 approaches to the initial respiratory management of preterm neonates. Pediatrics. 2011;128(5).
10. Berggren E, Liljedahl M, Winbladh B, Andreasson B, Curstedt T, Robertson B, et al. Pilot study of nebulized surfactant therapy for neonatal respiratory distress syndrome. Acta Paediatrica. 2000; 89(4): p. 460-464.
11. Finer N, Merritt T, Bernstein G, Job L, Mazela J, Liu G. A multicenter pilot study of Aerosurf delivered via nasal continuous positive airway pressure (nCPAP) to prevent respiratory distress syndrome in preterm neonates. Paper presented at: Pediatric Academic Societies' Annual Meeting. 2006.

11 LIST OF TABLES

Listing Title

Figure Title