DISCOVERY LABORATORIES, INC.

Statistical Analysis Plan

A Multinational, Multicenter, Masked, Randomized, Controlled Study to Assess the Safety and Efficacy of Lucinactant for Inhalation in Preterm Neonates 26 to 32 Weeks Gestational Age with Respiratory Distress Syndrome

Protocol Number: 03-CL-1202

Amendment #2

SAP Version: Final - Revision 2

27 April 2017

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Statistical Analysis Plan

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EXECUTIVE SUMMARY

The objectives of this study are to evaluate the safety and efficacy of lucinactant for inhalation, in comparison to nasal continuous positive airway pressure (nCPAP) alone, in preterm neonates with RDS, as assessed by the time to, and incidence of, respiratory failure and/or death due to RDS, incidence of bronchopulmonary dysplasia (BPD), and change in physiologic parameters (fraction of inspired oxygen [FiO_2] and partial pressure of carbon dioxide [PCO_2]) over the first 72 hours of life.

This is a multinational, multicenter, randomized, 2-part (A, B), double-blind (masked)/open label, controlled study comparing lucinactant for inhalation (AEROSURF®) administered with nCPAP to treatment with nCPAP alone for the treatment of RDS in 26 to 32 completed weeks post-menstrual age (PMA) preterm infants. For Part A (masked), infants 28 to 32 weeks PMA will be randomized into 1 of 3 parallel treatment groups, with 2 repeat doses allowed if the repeat dose criterion is met. For Part B (open-label), infants 26 to 28 weeks PMA will be randomized into 1 of 2 parallel treatment groups, with 4 repeat doses allowed if the repeat dose criterion is met. Reconstituted lyophilized lucinactant (reconstituted with sterile water for injection) will be aerosolized by the investigational device, the Aerosurf Delivery System (ADS), using the capillary aerosol generator and introduced into the nCPAP circuit. Those infants randomized to the control arm will 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.

Treatment Group	Study Assignment
Study Part A[a]	
40 mg/kg	Lucinactant for inhalation 40 mg TPL/kg (administered over 25 minutes) in conjunction with nCPAP (n = up to 80) Up to 2 repeat doses of 40 mg TPL/kg are to be given if repeat dosing criteria are met
80 mg/kg	Lucinactant for inhalation 80 mg TPL/kg (administered over 50 minutes) in conjunction with nCPAP (n = up to 80) Up to 2 repeat doses of 80 mg TPL/kg are to be given if repeat dosing criteria are met
Control	Continuous nCPAP with sham drug treatment (n = up to 80)
Study Part B	
80 mg/kg	Lucinactant for inhalation 80 mg TPL/kg (administered over 50 minutes) in conjunction with nCPAP (n = up to 40) Up to 4 repeat doses of 80 mg TPL/kg are to be given if repeat dosing criteria are met
Control	Continuous nCPAP (n = up to 40)

[a] In Part A of the study, masking procedures will be followed for all doses in all treatment groups.

The data monitoring committee (DMC) will conduct 3 preplanned interim analyses: 1) after approximately 25% of subjects in Part A have been enrolled, 2) after 67% of subjects in Part A have been enrolled, and 3) after approximately 20 subjects in Part B have been enrolled. The DMC will review all available safety data for each interim analysis; if there are safety concerns, a recommendation may be made to suspend study enrollment.

Repeat dosing will be allowed in each dosing group. Subjects meeting the repeat dosing criterion will receive up to 2 (Part A) or 4 (Part B) additional treatments of the same dose. For the control subjects that meet the repeat dosing criteria, all the procedures conducted for the active subjects will be carried out (without delivering a dose) to protect study blinding (Part A).

Primary Study Period:

For the primary study period, subjects will be followed for safety and efficacy evaluations (including, but not limited to, the incidence and timing of intubation, mechanical ventilation and/or surfactant administration; AEs; concomitant medications; physiological assessments; incidence of BPD) until the subject is 36 weeks PMA, is discharged, is transferred, or has died. 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 = 80, 160 subjects total for Part A; $n \leq 80$ subjects for Part B) and the all controls combined (n = 80 for Part A and $n \leq 80$ for Part B). A final visit will occur at 36 weeks PMA or at the time of discharge or transfer (whichever occurs first) for all subjects.

Efficacy endpoints in the study include:

1. Incidence of respiratory failure or death due to RDS within the first 72 hours of life

   Respiratory failure due to RDS will be defined as follows:
   a. any subject receiving intubation for mechanical ventilation (MV) and/or surfactant administration within 72 hours of life or death due to RDS; or
   b. if the subject meets at least 1 of the following criteria, regardless of whether endotracheal intubation is performed:
      - a sustained need for $FiO_2 > 0.45$ to maintain an $SpO_2 > 90\%$ to 95%
      - a sustained (on $\geq$ 2 consecutive observations > 60 minutes apart) transcutaneous $PCO_2 > 65$ mmHgnCPAP > 8 cm H_2O

   Death due to RDS is any death whose primary cause is respiratory failure due to RDS
2. Incidence rate of death due to RDS
3. Time to respiratory failure due to RDS within 72 hours of life
4. Incidence rate of late (> 72 hours of life through Day 7) respiratory failure due to RDS
5. Incidence rate of BPD and rate of survival without BPD at 36 weeks PMA
6. Observed FiO_2 and PCO_2 values and change from baseline values in FiO_2 and PCO_2 over the first 72 hours of life
7. Incidence rate of pulmonary air leak, especially pneumothorax

Safety endpoints in the study include:

1. Survival (all-cause mortality; date and time of death, if applicable)
2. AEs, including adverse device effect (ADEs) and AEs of special interest, including peri-dosing events, complications related to placement of binasal prongs, and air leaks
3. Concomitant medications
4. Use of respiratory support and supplemental O_2
5. Common complications of prematurity
6. Physical examinations
7. Tolerability of lucinactant for inhalation
8. Incidence rate of air leak
9. Assessments of the following:

a) Vital signs
b) O_2 saturation, as determined by pulse oximetry (SpO_2)
c) Serum electrolyte measurements
d) Defecation
e) Chest radiograph prior to intubation

Follow-Up Period:

Neonates may be followed up to 1 year corrected age (based on 40 weeks PMA), at which time a physical examination will be performed, including an abbreviated neurologic assessment. Also, the number of hospitalizations, need for and duration of ventilator support, and use of bronchodilators or steroids will be recorded. An interim 6-month corrected age follow-up (via telephone or visit) will include number and reasons for hospitalizations, need for and duration of ventilator support, and/or oxygen supplementation requirements

Analysis Populations:

The statistical analysis of both the primary and secondary objectives will be based on all enrolled preterm neonates. For the efficacy analysis, the primary analysis will be based on a modified intent-to-treat (mITT) population, defined as subjects who received treatment. In addition, populations of all randomized subjects (ITT), subjects with no treatment interruptions, and subjects with no major protocol deviations (per-protocol) will be evaluated based upon the treatment group to which they were randomized. For the safety analysis, all subjects randomized to the control group or who received any lucinactant for inhalation (including partial doses) will be evaluated based upon the treatment they actually received.

The treatment difference (delta) between active and control treatments will be calculated for the primary and key secondary efficacy endpoints for each country and/or region. The delta calculated will be used to plan additional studies and to evaluate regional care contribution variations that may occur in the study.

TABLE OF CONTENTS

ABBREVIATIONS

Abbreviation	Description
ADE	Adverse device effect
ADS	Aerosurf® Delivery System
AE	Adverse event
BPD	Bronchopulmonary dysplasia
CAG	Capillary aerosol generator
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
TEAE	Treatment-emergent adverse event
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 efficacy in the 03-CL-1202 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

Exogenous surfactant treatment reduces mortality and morbidity in preterm infants with respiratory distress syndrome (RDS) [1,2]. Intratracheal SRT has well-established benefits in infants with RDS and has become a standard, recommended therapy for this condition [3,4,5,6]. Early SRT is more effective in reducing morbidity and mortality due to RDS than SRT delivered later [2], and multiple doses are sometimes necessary [1].

Intratracheal instillation of surfactant into the lung requires endotracheal intubation, often with concomitant positive pressure mechanical ventilation (MV). However, endotracheal intubation is an invasive, painful procedure that itself has potential deleterious effects to the infant [7,8], and tracheal injury which may lead to the development of subglottic stenosis [9]. Further, MV is associated with morbidities such as ventilator-associated lung injury and volutrauma/barotrauma resulting in air leak syndromes such as pneumothorax and/or pulmonary interstitial emphysema (PIE), and may also contribute to development of chronic lung disease (CLD) / bronchopulmonary dysplasia (BPD) [10].

In order to avoid endotracheal intubation and MV in preterm neonates with mild-to-moderate RDS, a strategy of using nasal continuous positive airway pressure (nCPAP) as an effective means of providing ventilatory support is now accepted practice [3,4,5,6,11]. nCPAP improves respiratory function in neonates by increasing functional residual capacity, improving lung compliance and dilating upper airway structures, thereby improving gas exchange and reducing work of breathing [11,12]. Devices that generate and deliver nCPAP, as well as patient interfaces such as nasal prongs, have been specifically designed, manufactured, and made commercially available for use in neonates.

Studies in very preterm neonates of initial treatment of RDS with nCPAP alone [13,14,15,16], including meta-analyses [17,18], have shown outcomes with this approach that are similar to traditional, early treatment with intratracheal surfactant. These studies have consistently shown that in neonates treated with nCPAP, the need for surfactant therapy is less than that of neonates treated with intubation and SRT, and that the important outcome of death or bronchopulmonary dysplasia (BPD, defined as the need for oxygen [O_2] treatment at day 28 after birth) is equal or less frequent with nCPAP. In neonates treated with nCPAP, approximately 33-67% of patients required intubation and intratracheal surfactant replacement, meaning that ⅓ to ⅔ of patients were able to avoid intubation altogether. Thus, the strategy of initially supporting neonates with nCPAP and reserving SRT only for those who require intubation appears to be reasonably effective and potentially safer by avoiding intubation. Two meta-analyses [10,19] and a systematic review [20] have suggested that use of nCPAP as the primary respiratory support modality in preterm neonates reduces the need for intubation and the rate of BPD, with a number need to treat (NNT) of 25 for BPD [10].

Older gestational age infants treated with nCPAP may be at higher risk of air leak than younger infants [21]. In a cohort of 297 neonates 25-32 weeks gestation with RDS initially treated with nCPAP alone, 65 (22%) overall required intubation ("nCPAP failure"). The rate of nCPAP failure was 45% in the subgroup 25-28 weeks' gestation, consistent with prior reports, where only 15% of infants 29-32 weeks' gestation had nCPAP failure. Notably, the rate of pneumothorax prior to intubation was 10% in the younger cohort (also consistent with most prior reports), but was 23% in the older gestational age infants as a whole and 47% in those older infants who failed nCPAP.

1.1.2 Development of Aerosolized Device for Lucinactant Delivery

Discovery Laboratories, Inc. (Discovery) has developed a capillary-based aerosol generator (CAG) device to aerosolize lucinactant (lucinactant for inhalation). Nonclinical studies using the CAG technology in spontaneously breathing preterm lambs have demonstrated that lambs receiving aerosolized lucinactant 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 demonstrated that aerosolized lucinactant appears to be 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[3].

Discovery Laboratories, Inc. (Discovery) has developed lucinactant for inhalation, an investigational drug-device combination product, to deliver aerosolized SRT to preterm neonates with RDS who are being supported with nCPAP. Lucinactant for inhalation is comprised of a drug component, lyophilized lucinactant, and a device component referred to as the AEROSURF® Delivery System (ADS). The ADS uses novel capillary-based aerosol generator (CAG) technology to aerosolize lucinactant for inhalation, providing a high-density surfactant aerosol output (1.2 mL/min), with an appropriate particle size (2 to 3 microns mass median aerodynamic diameter [MMAD]) for respiration and deposition within the neonatal lung.

Nonclinical studies using the CAG technology in preterm lambs have demonstrated that aerosolized lucinactant significantly improved lung mechanics and gas exchange compared with preterm lambs receiving CPAP alone [23]. In parallel, pilot clinical studies in neonates with RDS (Study KL4-CPAP-01) [24] as well as in adults with asthma and cystic fibrosis (Study KL4-ASTH-01) have demonstrated that lucinactant aerosolized with commercially available nebulizers appears to be generally well-tolerated. The ADS represents the clinical version of the CAG technology, which is being used in clinical trials, including the current study, to aerosolize lucinactant.

The purpose of this study is to evaluate the safety and efficacy of lucinactant for inhalation used in conjunction with nCPAP, in comparison to nCPAP alone, in preterm neonates 26 to 32 completed weeks postmenstrual age (PMA) with RDS.

1.2 OBJECTIVES

This study is designed to investigate the safety and efficacy of lucinactant for inhalation in preterm neonates 26 to 32 (28 to 32 [Part A]; 26 to 28 [Part B]) completed weeks PMA. Efficacy and safety will be based on clinical evaluations. The endpoints specified are similar to those in Protocols 03-CL-1201 and 03-CL-1401 to allow for potential comparison and pooling of results.

The objectives of this study are to evaluate the safety and efficacy of lucinactant for inhalation in conjunction with nCPAP, compared to nCPAP alone, in preterm neonates with RDS, as assessed by the incidence of, and the time to, respiratory failure and/or death due to RDS over the first 72 hours of life, the incidence of BPD at 36 weeks PMA, and change in physiologic parameters (fraction of inspired oxygen [FiO_2] and partial pressure of carbon dioxide [PCO_2]) over the first 72 hours of life.

In addition, the results of the study should to provide a stable estimate of the size of the treatment effect (treatment delta). The efficacy estimate will be used to calculate the sample size for future studies.

Part A will be unblinded and fully analyzed when enrollment in this part is complete. All relevant data from these subjects will be reviewed, cleaned, and locked. In addition, Part A will not be unblinded until all decisions on site pooling and per-protocol assessments have been completed. An analysis database will be created with the locked data.

Part B will be fully analyzed when enrollment in this part is complete and the database is locked.

1.2.1 Efficacy Objective

The efficacy objective is to assess the size of the clinical effect of lucinactant for inhalation in comparison to nCPAP alone, as demonstrated by the incidence of and time to respiratory failure due to RDS, incidence of BPD at 36 weeks PMA, physiologic parameters (FiO_2 and PCO_2), and incidence rate of survival without BPD at 36 weeks PMA.

1.2.2 Primary Endpoint

The primary endpoint for this study is the incidence rate of respiratory failure or death due to RDS within the first 72 hours of life.

A subject will be defined as having respiratory failure due to RDS if either of the following occur:

1. Intubation for MV and/or surfactant administration within 72 hours of life. MV for non-respiratory failure reasons will be excluded (eg, surgery).
2. The subject meets at least 1 of the following criteria, regardless of whether endotracheal intubation is performed:
    a. A sustained (≥ 60 minutes) need for $FiO_2 > 0.45$ to maintain an $SpO_2 > 90\%$ to 95%
    b. A sustained (on ≥ 2 consecutive observations > 60 minutes apart) transcutaneous $PCO_2 > 65$ mmHg
    c. nCPAP > 8 cm H_2O
3. Death due to RDS. Death due to RDS is any death whose primary cause is respiratory failure due to RDS

1.2.3 Secondary Endpoints

The secondary endpoints of this study include the evaluation of the following from the time of initiation of study treatment until study completion:

1. Time to respiratory failure due to RDS

2. Incidence rate of BPD and rate of survival without BPD at 36 weeks PMA
3. All-cause mortality
4. Incidence rate of pulmonary air leak, especially pneumothorax

1.2.4 Tertiary Endpoints

The tertiary endpoints of this study include the evaluation of the following from the time of initiation of study treatment until study completion:

1. Incidence rates of common complications of prematurity other than air leak
2. Change from baseline in FiO_2 and/or transcutaneous PCO_2 over the first 72 hours of life

1.2.5 Safety Evaluations

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

1. Survival (date and time of death, if applicable)
2. AEs, including adverse device effects (ADEs) and AEs of special interest, including peri-dosing events, complications related to placement of bi-nasal prongs, and air leak.
3. Concomitant medications
4. Use of respiratory support and supplemental O_2, including the following:
    a) Need for endotracheal intubation and MV
    b) Mode or respiratory support (including supplemental oxygen) and important parameters for that mode
5. Complications of prematurity (ie, intraventricular hemorrhage [IVH], periventricular leukomalacia [PVL], pulmonary hemorrhage, necrotizing enterocolitis [NEC], patent ductus arteriosus [PDA], acquired sepsis, retinopathy of prematurity [ROP], and BPD)
6. Physical examinations
7. Tolerability of lucinactant for inhalation
8. Incidence of air leak
9. Assessments of the following:
    a) Vital signs
    b) O_2 saturation, as determined by pulse oximetry (SpO_2)
    c) Serum electrolyte measurements
    d) Defecation

e) Chest radiography, at baseline and prior to intubation

1.3 HYPOTHESES

A total of 2 null hypotheses are defined for this study:

1. There is no difference between active treatment and control (40 mg TPL/kg vs. 80 mg TPL/kg vs. Control – Part A; 80 mg TPL/kg vs. control – Part B) in the incidence of respiratory failure or death due to RDS within 72 hours of life. The incidence of respiratory failure or death due to RDS will be compared between both active treatments and control using logistic regression. Pairwise testing between each active treatment and control, and for both active groups combined versus control, will be conducted in Part A.

2. There is no difference between active treatment and control (40 mg TPL/kg vs. control and 80 mg TPL/kg vs. control – Part A; 80 mg TPL/kg vs. control – Part B) for the time to respiratory failure or death due to RDS. The time to respiratory failure or death due to RDS will be compared for both active treatments and for each active treatment separately versus control using the log-rank test.

Part A only: In order to examine the concordance between subjects receiving intubation for MV and/or surfactant administration and subjects who meet respiratory failure criteria (sustained $FiO_2 > 0.45$, sustained $PCO_2 > 65$ mmHg, or nCPAP > 8 cm H_2O), the subjects receiving intubation, the subjects who meet criteria, and the subjects who both received intubation and met criteria will be summarized.

In addition, the treatment difference (delta) between active and control treatments will be calculated for the primary and key secondary efficacy endpoints (eg, incidence of respiratory failure of death due to RDS, incidence of BPD) for each country and/or region. The delta calculated will be used to plan additional studies and to evaluate regional care contribution variations that may occur in the study.

2 INVESTIGATIONAL PLAN

2.1 STUDY POPULATION

The study population will be comprised of preterm neonates 26 to 32 completed weeks PMA who are receiving care in a NICU and are receiving nCPAP as the primary support modality for RDS. Enrollment will be conducted by strata: in Part A, approximately 5-10% of the subjects will be 28 completed weeks PMA and the remainder will be 29 to 32 completed weeks PMA, while in Part B randomization will be stratified by gestational age (26, 27, and 28 completed weeks PMA).

The study population in Part A will be randomized in a 1:1:1 ratio into 1 of 3 treatment groups, and in Part B will be randomized in a 1:1 ratio into 1 of 2 treatment groups.

A subject will be enrolled at 1 of approximately 50 study sites in the US, Canada, Poland, Netherlands, Ireland, Colombia, and Chile. It is anticipated that approximately 2400 subjects will be screened to meet the enrollment goal of approximately 240 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 (consent may be obtained prenatally, where allowed)
2. Gestational age:
   a. Part A: 28 0/7 to 32 6/7 completed weeks' gestation PMA
   b. Part B: 26 0/7 to 28 6/7 completed weeks' gestation PMA
3. Successful implementation of non-invasive support or ventilation (preferably bubble CPAP) within:
   a. Part A: 90 minutes after birth
   b. Part B: 60 minutes after birth
4. Spontaneous breathing
5. Chest radiograph consistent with RDS (may be deferred if in the best medical interest of the subject)
6. Respiratory support:
   a. Part A: Within the first 20 hours after birth, requires an nCPAP of 5 to 7 cm H_2O with an FiO_2 $\geq$0.25 (>0.21 for neonates 28 weeks PMA) to 0.4 that is clinically indicated for at least 30 minutes to maintain SpO_2 of 90% to 95%.

b. Part B: Within the first 12 hours after birth, requires an nCPAP of 5 to 7 cm H_2O with an FiO_2 of >0.21 to 0.4 that is clinically indicated for at least 20 minutes to maintain SpO_2 of 90% to 95%.

Transient (<10 minutes) FiO_2 excursions outside this range do not reset the time requirement.

2.1.2 Exclusion Criteria

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

1. A heart rate that cannot be stabilized above 100 beats per minute (bpm) within 5 minutes of birth
2. Recurrent episodes of apnea requiring positive pressure ventilation (PPV) 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 the use of nCPAP, diagnosed antenatally or immediately after birth
5. Clinically significant diseases or conditions other than RDS which could potentially interfere with cardiopulmonary function (eg, congenital heart disease, hydrops fetalis, or congenital infection)
6. A known or suspected chromosomal abnormality or syndrome
7. Premature rupture of membranes (PROM) > 3 weeks
8. Hemodynamic instability requiring vasopressors or steroids for hemodynamic support and/or presumed clinical sepsis
9. A need for intubation and/or invasive mechanical ventilation at any time before enrollment into the study
10. The administration (or plan for administration) of any the following:
    a) Another investigational agent or investigational medical device
    b) Any other surfactant agent
    c) Systemic corticosteroids (other than antenatal steroids already received)
11. Presence of air leak (pneumothorax, pneumomediastinum, pneumopericardium, subcutaneous emphysema, or definite evidence of pulmonary interstitial emphysema [PIE]) on the baseline chest radiograph

2.2 STUDY DESIGN AND RANDOMIZATION

This study is a multinational, multicenter, 2-part (A, B), masked/open-label, randomized, controlled study to evaluate the safety and efficacy of lucinactant for inhalation in conjunction

with nCPAP compared with nCPAP alone. For Part A (masked), preterm neonates 28 to 32 completed weeks PMA who are being cared for in a neonatal intensive care unit (NICU) and who are within the first 20 hours after birth, who had successful implementation of noninvasive respiratory support within 90 minutes of birth, and who are candidates for SRT will be enrolled. For Part B (open-label), preterm neonates 26-28 completed weeks PMA who are being cared for in a NICU and who are within the first 12 hours after birth, who had successful implementation of noninvasive respiratory support within 60 minutes of birth, and who are candidates for SRT will be enrolled. The preferred initial mode of noninvasive support is study nCPAP; however, other modes are acceptable if the investigator feels it is safe to switch the subject to study nCPAP following consent and screening. There will be 2 phases in the study, a primary phase through 36 weeks PMA and a longer-term follow-up phase through 1-year corrected age. Data analyses and presentations will be conducted after each phase.

For Part A, subjects may be eligible to receive up to 2 repeat doses of study treatment, whether active or control. Repeat doses will be allowed 2 hours from completion of the previous dose up to 36 hours after completion of randomization if subjects meet repeat dosing criteria. Subjects randomized to the control group will be continued on nCPAP alone, but will receive sham treatment as detailed in the Blinding Plan.

For Part B, subjects may be eligible to receive up to 4 repeat doses of study treatment for subjects randomized to the active group. Repeat doses will be allowed 30 minutes from completion of the previous dose up to 36 hours after completion of randomization if subjects meet repeat dosing criteria. Subjects randomized to the control group will be continued on nCPAP alone.

2.2.1 Treatment Groups

Treatment Group	Study Assignment
Study Part A[a]	
40 mg/kg	Lucinactant for inhalation 40 mg TPL/kg (administered over 25 minutes) in conjunction with nCPAP (n = up to 80) Up to 2 repeat doses of 40 mg TPL/kg are to be given if repeat dosing criteria are met
80 mg/kg	Lucinactant for inhalation 80 mg TPL/kg (administered over 50 minutes) in conjunction with nCPAP (n = up to 80) Up to 2 repeat doses of 80 mg TPL/kg are to be given if repeat dosing criteria are met
Control	Continuous nCPAP with sham drug treatment (n = up to 80)
Study Part B	
80 mg/kg	Lucinactant for inhalation 80 mg TPL/kg (administered over 50 minutes) in conjunction with nCPAP (n = up to 40) Up to 4 repeat doses of 80 mg TPL/kg are to be given if repeat dosing criteria are met
Control	Continuous nCPAP (n = up to 40)

[a] In Part A of the study, masking procedures will be followed for all doses in all treatment groups.

To maintain the masking, study treatment for Part A will occur behind movable partitions or curtains (or some equivalent barrier to prevent unblinding); the PI, study staff (eg, site coordinator) (as applicable), and any applicable attending physician as appropriate will not be allowed to observe preparation of study materials, setup of the device, or to observe study treatment unless an emergent condition develops. The sponsor and the parents/legal guardian will also remain masked. Each site will develop and submit a blinding plan that must be approved by Discovery prior to screening and enrollment of subjects.

If at any point during the delivery of lucinactant for inhalation a potential safety risk to the subject is identified, aerosolization must be discontinued by activating the ADS 'Stop Treatment' interface.

2.2.2 Repeat Dosing

Up to 2 (Part A) or 4 (Part B) repeat doses will be allowed for each treatment group. The repeat doses will consist of repeating the same dose and will occur if repeat dosing criteria are met. The repeat dosing criterion is defined as 2 hours (Part A) or 30 minutes (Part B) from completion of the previous dose up to 36 hours after completion of randomization, and the subject must require a sustained need for supplemental oxygen at or above the qualifying FiO_2 for study entry (ie, ≥ 0.25 for neonates 29 to 32 weeks PMA, > 0.21 for neonates 26 to 28 weeks PMA) for at least 30 minutes to maintain SpO_2 of 90% to 95%. Masking procedures for the initial treatment will be followed for repeat doses, including for control patients (Part A).

As with the initial dose, the randomization system will be used to receive the study drug assignments, and all other procedures that were performed for the initial dose will be followed for the administration of the repeat doses.

If, in the opinion of the PI, repeat dosing would compromise the safety of the subject, repeat dosing will not occur. If a subject qualifies for repeat dosing but does not receive repeat dosing, the reason for this will be documented.

2.2.3 Sample Size Justification

A total of approximately 240 study subjects (approximately 80 per treatment group) will be enrolled for Part A. Sample size assumptions were determined by previous data, by medical judgment, and by pragmatic considerations of study feasibility. The sample sizes were calculated separately for incidence of respiratory failure due to RDS and time to intubation. All sample size calculations were two-sided and assumed and an alpha level of 5%.

The sample size for Part A is sufficient at approximately 90% power to detect a reduction of 50% in the incidence of respiratory failure due to RDS within the first 72 hours of life (nQuery Advisor®, version 7.0).

The sample size, with a total number of events required of 81 and an alpha level of 5%, for the log-rank test for equality of event curves, will have approximately 90% power to detect the difference between an event proportion of 0.75 and an event proportion of 0.50 (a constant hazard ratio of 0.415), within the first 72 hours of life.

For Part B, formal sample size calculations have not been conducted. A total of 40 subjects per group are sufficient to provide a stable estimate of the size of the treatment effect.

In addition, the sample size is sufficient to provide a stable estimate of the size of the treatment effect (treatment delta). The efficacy estimate will be used to calculate the sample size for future studies.

2.2.4 Study Schedule

Protocol Event Schedule Summarization

Measurement/Procedure	Phases Through 36 Weeks PMA				Longer-Term Follow-Up
	Screening	Primary Observation (Days 1 to 3)	Extended Observation (Days 4 to 7)	Final Observation (to 36w PMA)	
Informed Consent/HIPAA	X				
Inclusion/Exclusion Criteria	X				
Demographics	X				
Medical/Maternal/Birth History	X				
Birth Weight	X				
Physical Examination	X			X	
Chest Radiograph	X	X	X		
Randomization		X			
Study Drug Administration		X			
Use of Pacifier and/or Chin Strap during Dosing		X			
Use of oro- or nasogastric tube		X	X		
Defecation		X			
Vital Signs		X	X		
Monitoring of SpO_2		X	X		
Monitoring of transcutaneous PCO_2		X			
Resp. support and O_2 delivery	X	X	X	X	X
Serum Electrolytes		X			
Technical Performance of Device		X			
Incidence of BPD				X	
Peri-Dosing Events		X			
Complications of Prematurity				X	
Adverse Events		X	X	X	X
Adverse Device Effects		X			
Survival		X	X	X	
Concomitant Medication		X	X	X	X
Hospitalizations					X
Abbrev. physical and neuro. exam					X

Note: Day 1 for all subjects is the day of study randomization. See protocol for schedule details.

3 STUDY SUBJECT CHARACTERISTICS

3.1 SUBJECT DISPOSITION

Subject disposition (overall and by country) will be summarized by treatment group using frequency and percent. The number of subjects screened, randomized (intent-to-treat [ITT] population), randomized and received study medication (modified ITT [mITT] population), administered study medication (safety population), without treatment interruptions, and without protocol violations (per-protocol population) will be described.

The number of subjects who completed the study or died will be summarized using frequency and percent by treatment group and overall. Reasons for early discontinuation from the study include withdrawal of consent, and lost-to-follow up. A subject may withdraw consent (through their legally authorized guardian) at any time without prejudice to further care.

The number of subjects who 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.

3.2 DEMOGRAPHICS AND BASELINE CHARACTERISTICS

3.2.1 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 by treatment group using frequency and percent.

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

3.2.2 Medical, Birth and Maternal History

The mode of delivery (vaginal, c-section), type of birth (single, multiple), and incidence of congenital anomalies will be summarized by treatment group using frequency and percent.

The Apgar score at 1 and 5 minutes and antenatal steroid use will be summarized by treatment group using mean, SD, median, minimum, and maximum.

Incidence of medical history findings, incidence and type of ruptured membranes, incidence of clinical chorioamnionitis, and the incidence and number of doses of antenatal steroids will be summarized by treatment group using frequency and percent.

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. The number of subjects who experienced treatment interruptions for the initial or repeat doses will be summarized by treatment group.

3.3.2 Number of Doses

The number of doses (treatments) received by subjects in the active arms and the number of times a subject qualified for a repeat dose will be summarized by treatment group.

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 does not rise to the level of a protocol violation. All protocol violations/deviations will be summarized by treatment group and listed by subject. Subjects with protocol violations will not be included in the per-protocol population (see Section 4.1.4).

4 EFFICACY ANALYSIS

The efficacy objective is to assess the size of the clinical effect of lucinactant for inhalation in comparison to nCPAP alone, as demonstrated by the incidence of and time to respiratory failure due to RDS, incidence of BPD at 36 weeks PMA, physiologic parameters (FiO_2 and PCO_2), and incidence rate of survival without BPD at 36 weeks PMA.

Hypothesis testing will be done for incidence of respiratory failure due to RDS and time to respiratory failure due to RDS. Due to the preliminary nature of this study and the uncertainty around baseline rates of failure, all analyses, unless otherwise specified, will be conducted at an alpha level of 10%.

4.1 EFFICACY POPULATIONS

For efficacy analyses, subjects will be summarized according to their assigned (randomized) treatment. In addition, for the primary and key secondary endpoints, subjects will be summarized according to the amount of total study therapy received (eg, 25 minutes of aerosol treatment with a repeat would be combined with a 50 minute treatment) for within-group correlations. Within-group correlations will also be used to identify baseline variables to include as independent predictors in the logistic regression (logit) and generalized linear model analyses.

The statistical analysis of both the primary and secondary objectives will be based on all enrolled preterm neonates. For the efficacy analysis, the primary analysis will be based on a modified intent-to-treat population (mITT), defined as subjects who received treatment and completed the 50 minute post initiation of dose vital signs assessments. In addition, populations of all randomized subjects (ITT) and subjects with no major protocol deviations (per-protocol) will be evaluated (as described in Section 3.4), based upon the treatment group to which they were randomized. For the safety analysis, all subjects randomized to the control group or who received any lucinactant for inhalation (including partial doses) will be evaluated, based upon the treatment they actually received.

4.1.1 Modified Intent-to-Treat Population

The mITT population is the primary efficacy analysis population and is defined as subjects who were randomized and received study treatment (any aerosol delivered to infant)

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4.1.2 ITT Population

The ITT population is defined as all subjects who were randomized in the study, regardless of receipt of study treatment.

4.1.3 Without Treatment Interruptions Population

This population is defined as all subjects who received treatment without a treatment interruption (eg, study device shut-down prior to completion of dose).

4.1.4 Per-Protocol Population

The per-protocol population will be used for all efficacy endpoints as a supportive analysis. The per-protocol population is defined as all randomized subjects who received at least one dose of study drug and who do not have a protocol violation which would be considered a reason for exclusion from this population.

Protocol violations which may lead to exclusion from the per-protocol population include, but are not limited to:

- Inclusion and/or exclusion criteria not met.
- Initial dose was not completed.
- Subject was given repeat dose, but did not meet repeat dose criteria.
- Blinded personnel performed unblinded task or activity.
- Subject received the wrong study treatment or incorrect dose.

At the end of the study, but prior to unblinding, the list of protocol violations and deviations will be provided by Data Management. The list will be reviewed by the Medical Monitor and other study personnel, as appropriate, for determination of inclusion into and/or exclusion from the per-protocol population. The complete list of all protocol violations, along with a per-protocol population inclusion indication, will be included in the final data analysis.

4.2 STATISTICAL ANALYSIS

All analyses will be performed for all subjects combined and by country or region.

All continuous variables (eg, birth weight, body temperature, PCO_2) will be summarized using number (n), mean, standard deviation (SD), median, minimum, and maximum. All discrete variables (eg, sex, AEs, common complications of prematurity), will be summarized using frequency (n) and percent.

Sites will be pooled for statistical analyses by geographic region, as described in a separate analysis memo. Site pooling decisions will be made at the end of the study, but prior to unblinding.

The treatment difference (delta) between active and control treatments will be calculated for the primary and secondary efficacy endpoints. The delta calculated will be used to plan additional studies and as supporting evidence of efficacy.

See Section 8, Statistical Technical Issues, for details on statistical methods and calculations.

4.2.1 Primary Efficacy Endpoint

The primary endpoint is the incidence of respiratory failure or death due to RDS, defined as the number of subjects who need intubation for surfactant administration and/or MV or meet the criteria for respiratory failure due to RDS within 72 hours of life, or who die due to RDS-related respiratory failure within 72 hours of life. The incidence of respiratory failure or death due to RDS will be compared between each active treatment and control using logistic regression, controlling for pooled study sites, gender, birth weight, and baseline FiO_2. Additional terms (eg, time to treatment from birth) will be evaluated for appropriateness to include in model. The comparison results will be presented as incidence and percent with associated *p*-value, at an α-level of 10%.

4.2.2 Key Secondary Endpoints

The key secondary endpoint will be based on the log-rank test for time to respiratory failure or death due to RDS, adjusting for pooled study center. The primary endpoint is defined as the time from randomization to when subjects need intubation for surfactant administration and/or MV (for respiratory failure, not for elective procedures), or to when subjects meet the criteria for respiratory failure due to RDS within 72 hours of life, or who die due to RDS-related respiratory failure.

A Kaplan-Meier graph for time to respiratory failure will be produced. Descriptive statistics of the log-rank test (number of subjects, mean, standard error [SE], and median point estimate) will be presented by treatment group. Both the log-rank test and the Wilcoxon test (another test of time to failure) will be conducted. An α-level of 10% will be considered evidence of clinical effect. The Hochberg modification of the Bonferroni correction will be used to adjust for multiple comparisons. Applying the Hochberg modification means that if both p-values are less than or equal to 0.10, then both comparisons will be considered statistically significant; if one greater than 0.10, the other must be less than 0.05 to be considered statistically significant.

In addition, for Part A, generalized linear models will be used to model occurrence of an event (intubation for surfactant administration and/or MV) against subject, dose number (1, 2, or 3), treatment regimen (study treatment group), time at risk, and any applicable covariates based upon a Poisson distribution. This model will allow for assessing treatment only and treatment with repeats, and determining if there is a treatment by repeat interaction term. See Section 8 for additional details.

Explanations and/or descriptions related to respiratory failure will be listed.

4.2.3 Physiological Assessments

Change in FiO_2 is defined as the change from baseline (initiation of treatment for active subjects or completion of patient interface setup for control subjects) through 72 hours post-randomization. Change in FiO_2 will be compared between each active treatment and control using ANOVA with treatment and pooled study site in the model. The comparison results will be presented as LS mean and standard error, 95% CI, and two-sided *p*-value.

Change in transcutaneous PCO_2 is defined as the change from baseline (initiation of treatment for active subjects or completion of patient interface setup for control subjects) through 72 hours post-randomization. Change in PCO_2 will be compared between each active treatment and control using ANOVA with treatment and pooled study site in the model. The comparison results will be presented as LS mean and standard error, 95% CI, and two-sided *p*-value.

Individual timepoint assessments (eg, 1, 3, 6, 12 hours from randomization) may be performed for change from baseline for both FiO_2 and PCO_2. Comparisons of the individual timepoint assessments will be performed using mixed-model ANOVA with treatment and pooled study center in the model. The comparison results will be presented as LS mean and standard error, 95% CI, and two-sided *p*-value.

Area under the curve (AUC) analyses for FiO_2 and PCO_2 change from baseline will be conducted. The AUC will be calculated using the trapezoidal rule and will be compared between treatment groups using ANOVA with treatment and pooled study site in the model. The comparison results will be presented as LS mean and standard error, 95% CI, and two-sided *p*-value

Line graphs by treatment for observed and change from baseline values will be produced for both PCO_2 and FiO_2.

4.2.4 BPD and Survival without BPD

BPD is defined as the need for supplemental oxygen above room air (21%) at 36 weeks PMA. The number and percent of subjects who develop BPD by 36 weeks PMA will be summarized by treatment group. The number and percent of subjects who are alive and without BPD at 36 weeks PMA will be summarized by treatment group.

Comparisons between treatment groups for the total number of subjects with BPD will be carried out using the CMH test, controlling for pooled study sites.

4.2.5 Concordance between Intubation and Respiratory Failure Criteria

In order to examine the concordance between subjects receiving intubation for MV (for respiratory failure) and/or surfactant administration and subjects who meet respiratory failure criteria (sustained $FiO_2 > 0.45$, sustained $PCO_2 > 65$ mmHg, or nCPAP > 8 cm H_2O), the subjects receiving intubation, the subjects who meet criteria, and the subjects who both received intubation and met criteria will be summarized.

This examination will be done for both the time to intubation or meeting respiratory failure criteria and the incidence of intubation or meeting respiratory failure criteria.

4.2.6 Late Respiratory Failure or Death Due to RDS

Late respiratory failure or death due to RDS is defined as defined as the number of subjects who need intubation or meet the criteria for respiratory failure between 72 hours of life through Day 7, and after Day 7 through Day 28. The incidence of late respiratory failure or death due to RDS will be compared between each active treatment and control using the CMH test, controlling for pooled study sites. The comparison results will be presented as incidence and percent with associated *p*-value.

4.2.7 Complications of Prematurity

Common complications of prematurity (apnea, air leak, IVH, PVL, pulmonary hemorrhage, NEC, PDA, acquired sepsis, and ROP) will be summarized by treatment group using frequency and percent by treatment group and compared using the CMH test, controlling for pooled study sites.

4.3 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. Handling of missing data, as applicable, is described with the analysis for specific parameters (see Section 4.2). No imputation, including last observation carried forward, will be done for the any parameters; only available data will be considered.

4.4 SUBGROUP ANALYSES

Presentations by subgroup (sex, race, ethnic origin, country/region) will be considered for the primary endpoint if sufficient numbers allow.

Analysis of results by gestational age strata may be performed as an exploratory analysis.

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 any alarm signals (before, during, and after dosing), automatic system shutdowns, loss of inspiratory flow, inability to maintain nCPAP, occurrence of any error codes. In addition, the weight and/or volume of liquid in all traps will be summarized as continuous variables. Statistical comparisons between active treatment groups are not planned.

Incomplete treatments with reasons and/or explanations will be listed.

6 SAFETY ANALYSES

6.1 SAFETY POPULATION

The safety population is defined as all subjects who received any study medication, active or sham. Subjects will be included in the treatment group for the treatment actually received (if different than the group that they were randomized). All safety assessments will be based on this population.

Safety analyses will be summarized by all subjects combined and by country or region.

6.2 EXTENT OF EXPOSURE

All subjects randomized to active treatment are to receive at least one dose of study medication. Subjects will receive 25 or 50 minutes of aerosolized lucinactant. The number of subjects who receive a repeat dose, the number of doses received, the amount of treatment received (based on time of treatment), and the number of subjects whose study treatment is terminated early will be summarized.

6.3 ADVERSE EVENTS

All treatment-emergent AEs (TEAEs; AEs occurring at or after randomization) 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 TEAEs will be summarized as categorical variables (frequency and percent) by treatment group unless otherwise indicated. TEAEs that are related to the device (ADEs) will be summarized with all AEs and separately. TEAEs will not be compared between treatment groups.

6.3.1 Peri-Dosing AEs

The incidence of peri-dosing events (ie, bradycardia, desaturation, gagging/regurgitation, apnea, and pallor) 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 by treatment group.

6.3.2 AEs Related to Surfactant Administration (AEs of Special Interest)

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

Occurrences of apnea, bradycardia, and desaturation after the peri-dosing period will be summarized by treatment group.

6.3.3 Other TEAEs

TEAEs, other than those listed above, will be summarized by treatment group by the MedDRA preferred term and SOC for all TEAEs, regardless of relationship to study drug, and for TEAEs at least remotely related. If a TEAE 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 TEAEs will be summarized by severity (mild, moderate, severe), relationship to the study drug (unrelated, unlikely related, possibly related, related), whether or not the TEAE was device related, and, if sufficient number of subjects warrant, by gender, race, and ethnic origin.

6.3.4 Serious Adverse Events

All SAEs will be listed and summarized by treatment group using frequency counts and percentages. If the same SAE occurs multiple times for the same subject, the most severe occurrence will be counted.

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

6.3.5 Unexpected Adverse Device Effects

Unexpected adverse device effects (UADEs) will be listed and summarized by treatment group using frequency counts and percentages. If the same UADE occurs multiple times for the same subject, the most severe occurrence will be counted.

6.3.6 Deaths

All-cause mortality and deaths due to RDS 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.

6.4 CLINICAL ASSESSMENTS OF SAFETY

6.4.1 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.

6.4.2 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 by treatment group. 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.

6.4.3 Serum Electrolytes

Serum electrolytes (Na^+, CL^-, K^+) and total serum carbon dioxide (CO_2) will be measured at 24 hours from the time of randomization (± 6 hours) and will be summarized as continuous variables and presented by treatment group. Clinically significant values will also be recorded as AEs.

6.4.4 Defecation

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

6.4.5 Respiratory Support and Supplemental Oxygen

Pressure support, including respiratory rate, CPAP, and FiO_2 (as appropriate) at all time points will be summarized as continuous variables. For subjects receiving mechanical ventilation (MV) through Day 7, the mode, mean airway pressure (MAP), respiratory rate, and FiO_2 will be summarized. For subjects receiving supplemental oxygen through Day 7, the mode and the FiO_2 will be summarized. All MV and supplemental oxygen data, including other settings (eg, tidal volume, PIP, PEEP, flow rate, mode of delivery) will be listed by subject.

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

6.5 LONG-TERM FOLLOW-UP

6.5.1 6-Month Follow-Up

The assessments conducted at the 6-month follow-up (number of hospitalizations, need for and duration of ventilator support, and oxygen supplementation requirements) will be summarized and presented as a listing.

6.5.2 One-Year Follow-Up

Summary tables will be produced for the one-year follow-up to summarize physical examinations, bronchodilator or steroid use, neurologic assessments, the number of hospitalizations, and need for and duration of ventilator support, after all subjects have completed the study.

7 INTERIM ANALYSES AND DATA MONITORING

7.1 DATA MONITORING COMMITTEE

The purpose of the DMC is to evaluate the degree of risk involved in study subject participation within each treatment group and to determine if study continuation in accordance with the current protocol holds the potential to institute any undue harm, or threat to the safety and welfare of study subjects. The DMC will consist of 3 to 5 experts in the field of RDS; at least 2 of the experts must be neonatologists.

The DMC reviews will consist of the evaluation of (1) all AEs, (2) ADEs relevant to potential subject safety issues, (3) case reviews of subjects with reported SAEs, and (4) summary tables of all safety endpoints. Safety and tolerability data will be assessed at the time all active subjects in each dose group completes assessments and procedures through 72 hours. Enrollment will continue throughout each DMC review.

Following a thorough independent review of available study data, the DMC will provide timely recommendations to the Discovery study team.

7.2 INTERIM ANALYSES

The data monitoring committee (DMC) will conduct 2 preplanned interim analyses during Part A of the study: 1) after approximately 25% of subjects have been enrolled and 2) after 66% of subjects have been enrolled. The DMC will be provided full safety listings for review after approximately 20 subjects have been enrolled in Part B, but will not conduct a formal interim analysis. Study enrollment may be suspended if safety concerns are identified during any review.

7.3 DATA MONITORING

The data will be entered into a validated database. Members of Discovery's Data Management department are responsible for data processing, in accordance with procedural documentation. All procedures for the handling and analysis of data will be conducted using good computing practices meeting FDA guidelines for the handling and analysis of data for clinical trials.

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the 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.

8 STATISTICAL TECHNICAL ISSUES

8.1 METHODS OF ASSIGNING SUBJECTS TO TREATMENT GROUPS

For each treatment group, preterm neonates who successfully meet all eligibility criteria within the enrollment period will be randomly assigned to either 40 or 80 mg TPL/kg lucinactant for inhalation (80 mg TPL/kg only in Part B) or control (nCPAP only) within their applicable strata (26-28 completed weeks PMA or 29 to 32 completed weeks PMA). Subjects will be randomized using a web-based centralized allocation. Neonates from multiple births will be randomized independently. In Part A, randomization information will be provided to unblinded site personnel (eg, pharmacist); site staff responsible for care decisions of the neonates will be masked to the treatment assignment as described in the blinding plan. In Part B, open-label randomization information will be provided to designated site personnel. Subjects that discontinue the study (ie, withdrawn consent) or treatment prematurely will not be replaced.

The randomization code list will be generated by Discovery Biometrics personnel. The code list will use a block size of 6 for Part A (40 mg/kg × 2, 80 mg/kg × 2, Sham 40 mg/kg × 1, Sham 80 mg/kg × 1) or a block size of 4 for Part B (80 mg/kg × 2, Sham × 2), stratified by PMA.

8.2 BLINDING/MASKING

In order to minimize bias in subject assessments, study treatment masking will be employed for Part A. Details on blinding can be found in the Blinding Plan for 03-CL-1202.

Part B is open label; no masking will be employed.

8.3 DETAILS ON STATISTICAL METHODS

8.3.1 Logistic Regression for Testing Incidence of Event

Logistic regression is being used to investigate the relationship between the dichotomous primary endpoint and a set of explanatory variables. For binary response models, the response, Y, of an individual or an experimental unit can take on one of two possible values, denoted by 1 and 2 (eg, Y = 1 if respiratory failure has occurred, other Y = 2). Suppose x_i is a vector of explanatory variables and $p = \Pr(Y = 1 \mid x_i)$ is the response probability to be modeled. The linear logistic model has the form

$$\text{logit}(p) \equiv \ln\left(\frac{p}{1-p}\right) = \alpha + \beta' x_i$$

where α is an intercept parameter and β is the vector of slope parameters [14].

The likelihood function expresses the probability of observing the data in hand as a function of the unknown parameters. The likelihood of observing the values of y for all the observations can be written as $L = \Pr(y_1, y_2, \ldots, y_n)$. Because of the assumption that observations are independent, the overall probability of observing all the y_i's can be factored into the product of the individual probabilities:

$$L = \Pr(y_1)\Pr(y_2) \ldots \Pr(y_n) = \prod_{i=1}^{n} \Pr(y_i),$$

where Π indicates repeated multiplication. By definition, $\Pr(y_i = 1) = p_i$ and $\Pr(y_i = 0) = 1 - p_i$.

That implies

$$\Pr(y_i) = p_i^{y_i}(1 - p_i)^{1-y_i}.$$

The logarithm of the equation produces

$$\ln L = \sum_i y_i \ln\left(\frac{p_i}{1 - p_i}\right) + \sum_i \ln(1 - p_i).$$

Substituting this expression in the logit model, the likelihood function can be simplified to

$$\ln L = \sum_i \beta x_i y_i - \sum_i \ln(1 + e^{\beta x_i}).$$

We now choose values of β that make the equation as large as possible [15].

The regression will be performed within the SAS System® using the LOGISTIC procedure; models will be fit by the method of maximum likelihood.

Baseline factors, such as gender or gestational age, will be tested to determine if they are correlated with study therapy and thus have a significant impact in the model. Factors that are correlated will be included in the model as covariates. At a minimum, pooled study sites, gender, birth weight, and baseline FiO_2 will be included.

The summary table format for the primary efficacy analysis for Part A is given below.

	Treatment			Logistic Regression		
Respiratory Failure due to RDS	40 mg/kg (N=80)	80 mg/kg (N=80)	nCPAP Only (N=80)	Overall	40 mg/kg vs. Control	80 mg/kg vs. Control
Number of Subjects	xx	xx	xx			
Number of Subjects with Respiratory Failure	xx (yy%)	xx (yy%)	xx (yy%)	x.xxx	x.xxx	x.xxx

8.3.2 Log-Rank Test for Testing Time to Event

The rank statistics used to test homogeneity between the strata (treatments) have the form of a $c \times 1$ vector $\mathbf{v} = (v_1, v_2, \ldots, v_c)'$ with

$$v_j = \sum_{i=1}^{k} w_i \left(d_{ij} - \frac{n_{ij} d_i}{n_i} \right)$$

where c is the number of strata , and the estimated covariance matrix, $\mathbf{V} = (V_{jl})$, is given by

$$V_{jl} = \sum_{i=1}^{k} \frac{w_i^2 d_i s_i \left(n_i n_{il} \delta_{jl} - n_{ij} n_{il} \right)}{n_i^2 (n_i - 1)}$$

where i labels the distinct event times, δ_{jl} is 1 if $j = l$ and 0 otherwise, n_{ij} is the size of the risk set in the jth stratum at the ith event time, d_{ij} is the number of events in the jth stratum at the ith time, and

$$n_i = \sum_{j=1}^{c} n_{ij}$$
$$d_i = \sum_{j=1}^{c} d_{ij}$$
$$s_i = n_i - d_i$$

The term v_j can be interpreted as a weighted sum of observed minus expected numbers of failure under the null hypothesis of identical survival curves. The weight w_i is 1 for the log-rank test and n_i for the Wilcoxon test. The overall test statistic for homogeneity is $\mathbf{v}'\mathbf{V}^{-}\mathbf{v}$, where $\mathbf{V}^{-}$ denotes a generalized inverse of $\mathbf{V}$. This statistic is treated as having a X^2 distribution with degrees of freedom equal to the rank of $\mathbf{V}$ for the purposes of computing an approximate probability level[14].

The summary table format for this efficacy analysis is given below.

		Treatment			Log-Rank/Wilcoxon		
Respiratory Failure or Death due to RDS	Statistics	40 mg/kg (N=80)	80 mg/kg (N=80)	nCPAP Only (N=80)	Overall	40 mg/kg vs. Control	80 mg/kg vs. Control
Time to Respiratory Failure or Death	n	xx	xx	xx			
(hours from treatment initiation)	Mean	xx.x	xx.x	xx.x			
	SD	xx.xx	xx.xx	xx.xx			
	Median	xx.x	xx.x	xx.x			
	Min,Max	xx, xx	xx, xx	xx, xx			
Estimated Time-to-Event	Mean	xx.x	xx.x	xx.x	x.xxx	x.xxx	x.xxx
	SE	xx.xx	xx.xx	xx.xx			
	Median	xx.x	xx.x	xx.x			

If significant differences are noted in baseline factors, such as gender or birth weight, a supportive exploratory analysis will be done to determine if any of these factors have a significant impact in the model using standard step-up and step-down methodology. The variables selected by the two methods are then compared and a new model is constructed with the selected factors included and the primary analysis is evaluated as indicated above.

8.3.3 Generalized Linear Modeling using Poisson Distribution

Occurrence of events can be modeled using generalized linear models with a Poisson distribution. Random variables that have a number of X occurrences of some phenomenon during a fixed period of time have a Poisson distribution. Thus, the Poisson distribution is appropriate for testing the number of events of respiratory failure over 72 hours between study therapies.

Suppose that a response variable Y is distributed as Poisson and has expected value μ. Recall that the variance of a Poisson variable is also μ. If you have a single explanatory variable x, you can write a regression model for μ as

$$g(\mu) = \alpha + x\beta,$$

where g is a link function, in terms of a generalized linear model (GLM). Usually, g is taken to be the log function. If so, you have a loglinear model

$$\ln(\mu) = \alpha + x\beta.$$

You can rewrite this model as

$$\mu = e^{\alpha}e^{x\beta}.$$

If you increase the explanatory variable x by one unit, it has a multiplicative effect of e^{β} on μ. Since this model is specified as a GLM, with a log link and a Poisson distribution, you can fit it with the GENMOD procedure and use the deviance and likelihood ratio tests to assess model fit and use Wald or score statistics to assess the model effects.

Frequently, discrete counts represent information collected over time (days, years), and interest lies in modeling rates. If the exposure time is denoted as N, you write the rate as Y / N and write the expected value as μ / N. Modeling this rate with a loglinear model is written

$$\ln \frac{\mu}{N} = \alpha + x\beta,$$

which can be rearranged as

$$\ln \mu = \alpha + x\beta + \log (N).$$

The term log(N) is called an offset and must be accounted for in the estimation process[15]. In our case, N represents "time at risk." For first dose, time at risk is from treatment initiation to the start of the second dose or time of event or 72 hours; for the second dose, time at risk is from treatment initiation for the second dose to third dose or time of event or 72 hours; for the third dose, time at risk is from treatment initiation for the third dose to time of event or 72 hours.

Baseline factors, such as gender or gestational age, will be tested to determine if they are correlated with study therapy and thus have a significant impact in the model. Factors that are correlated will be included in the model as covariates.

8.4 MULTIPLICITY

Time to respiratory failure or death due to RDS, is a single hypothesis with two comparisons: 40 mg TPL/kg vs. nCPAP Only and 80 mg TPL/kg vs. nCPAP Only. To adjust for multiple comparisons, a Hochburg modification of the Bonferroni correction will be performed. Applying the Hochberg modification means that if both p-values are less than or equal to α, then both comparisons will be considered statistically significant; if one is greater than α, the other must be less than α /2 to be considered statistically significant.

No other adjustments for multiple comparisons will be done.

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 measurement at the start of study drug administration for subjects randomized to the active group, and time of patient interface change for subjects randomized to the control group (both recorded as "initiation of study treatment" on several forms).

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 2 begins at midnight following randomization; Day 3 is the 2nd day following randomization; 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. The windows for the the 6-month and 12-month visits will be ±2 weeks (not specified in protocol). Summary tables will use the pre-specified time points, not the actual times, to summarize the data.

9.3 SITE POOLING METHODS

Pooled centers are used in all analyses where control by center is warranted (eg, CMH tests, ANOVA models). Pooling of centers is done in order to ensure similar sizes of centers and so that testing of treatment by center interaction can be accomplished. Centers will be pooled based on a geographic basis to ensure there is no center that has less than 5 subjects. In order to keep center sizes from being too disparate, pooling will try to balance the enrollment; however, this will be done in a blinded manner. The specific pooling of sites and the rationale will be described prior to unmasking of the study.

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11 LIST OF SUMMARY TABLES, LISTINGS, AND FIGURES

Listing Title

Figure Title