# A MULTICENTER, SINGLE-TREATMENT STUDY TO ASSESS THE SAFETY AND TOLERABILITY OF LYOPHILIZED LUCINACTANT IN ADULTS WITH COVID-19 ASSOCIATED ACUTE LUNG INJURY

**Protocol Number: 02-CL-2001a**

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| Sponsor Name: | Windtree Therapeutics, Inc. |
| Sponsor's Medical Officer: | Steven G. Simonson, MD, MHS<br>Senior Vice President, Chief Medical Officer |
| Address: | 2600 Kelly Road<br>Suite 100<br>Warrington, PA 18976<br>USA |
| Phone No.: | (215) 488-9300 |
| Fax No.: | (215) 488-9301 |
| Coordinating Investigator: | Yuh-Chin T. Huang, M.D., M.H.S.<br>Professor of Medicine, Pulmonary and Critical Care Medicine<br>Duke University Medical Center |

A Multicenter, Single-Treatment Study to Assess the Safety and Tolerability of Lyophilized Lucinactant in Adults with COVID-19 Associated Acute Lung Injury

| Author | Approver |
|---|---|
| **Name:** Phillip D. Simmons | **Name:** Steven G. Simonson |
| **Title:** Exec. Director, Biostatistics & Data Mgmt. | **Title:** Sr. Vice President, Chief Medical Officer |
| **Signature/Date** | **Signature/Date** |
| | |
| **Approver** | **Approver** |
| **Name:** Carlos Guardia | **Name:** Catherine Kacprzycki |
| **Title:** Senior Medical Director | **Title:** Senior Director, Clinical Operations |
| **Signature/Date** | **Signature/Date** |
| | |

## STATEMENT OF COMPLIANCE

This study will be conducted in accordance with International Conference on Harmonisation (ICH) Guideline for Good Clinical Practice (ICH E6[R2]), applicable United States Code of Federal Regulations (CFR), Title 21, as well as all applicable local, state, and federal regulations and guidelines regarding the conduct of clinical studies. In addition, this study will be conducted in accordance with the ethical principles included in the World Medical Assembly (WMA) Declaration of Helsinki, *Ethical Principles for Medical Research Involving Human Subjects* adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended most recently by the 64th WMA General Assembly, Fortaleza, Brazil, October 2013.

Throughout this protocol, the term "Clinical Investigator" or "Investigator" will be defined, in accordance with 21 CFR 54, as any listed or identified principal investigator (PI) or subinvestigator who is directly involved in study dosing or evaluation of research subjects. PIs or subinvestigators must be listed on Form FDA 1572 and documented as appropriate on the delegation of authority signature log. The PI will assure that no deviation from or changes to the protocol will take place without prior agreement from the Investigational New Drug (IND) sponsor and documented approval from the Institutional Review Board (IRB)/Independent Ethics Committee (IEC), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study will have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB/IEC for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. All changes to the consent form will be IRB/IEC approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent using a previously approved consent form.

**PROTOCOL AGREEMENT**

I have read the protocol specified below and agree that it contains all necessary details for carrying out this study. I will conduct the study as outlined herein and will complete the study within the time designated. I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure they are fully informed regarding the study drug and the conduct of the study.

I will use only the informed consent form approved by the Institutional Review Board (IRB)/ Independent Ethics Committee (IEC) and Windtree Therapeutics, Inc. (Windtree), and will fulfill all responsibilities for submitting pertinent information to the IRB/IEC responsible for the study.

I further agree that regulatory agencies (eg, US Food and Drug Administration), Windtree, or their designees shall have access to any source document from which case report form information may have been generated.

I agree that I and all sub-investigators listed on the delegation of authority form and/or Form FDA 1572 shall inform Windtree of any equity interest in the company prior to participating in this study. I further agree that I and all sub-investigators listed will consult with Windtree before acquiring any financial interest in the company during the study and for one year after the study's completion.

_____ _____

Investigator Signature Date

_____

Print Name and Title

Site #: _____

Site Name: _____

Address: _____

_____

_____

_____

Phone Number: _____

# 1 PROTOCOL 02-CL-2001a

## 1.1 Summary

| | |
|---|---|
| **Protocol Number** | 02-CL-2001a |
| **Title:** | A Multicenter, Single-Treatment Study to Assess the Safety and Tolerability of Lyophilized Lucinactant in Adults with COVID-19 Associated Acute Lung Injury |
| **Phase:** | 2a |
| **US IND Number:** | 149624 |
| **Sponsor:** | Windtree Therapeutics, Inc. (Windtree), Warrington, PA, USA |
| **ClinicalTrials.gov No.:** | NCT04389671 |
| **Committees:** | DMC |
| **Study Drug:** | Lyophilized lucinactant reconstituted with sterile water for injection and administered at a dose of 160 ml (~80 mg total phospholipids (TPL)/kg lean body weight), delivered as a liquid. |
| **Rationale:** | The coronavirus that was first detected in China in 2019 and which has been detected in over 190 countries on every continent, has infected over 150 million people worldwide (including approximately 3 million deaths), including approximately 35 million in the United States (including over 600,000 deaths). The virus has been named "SARS-CoV-2" and the disease it causes has been named "coronavirus disease 2019" (abbreviated "COVID-19").<br><br>Coronaviruses are a large family of viruses that are common in people and many different species of animals, including camels, cattle, cats, and bats. Rarely, animal coronaviruses can infect people and then spread between people such as with MERS-CoV, SARS-CoV, and |

now with this new virus. The SARS-CoV-2 virus is a betacoronavirus, like MERS-CoV and SARS-CoV. All three of these viruses have their origins in bats. The viral sequences from U.S. patients are similar to the one that China initially posted, suggesting a likely single, recent emergence of this virus from an animal reservoir.

Surfactants, specifically lucinactant, have been studied in acute hypoxemic respiratory failure and in acute lung injury. It is hypothesized that lucinactant may improve the respiratory status of patients suffering from COVID-19.

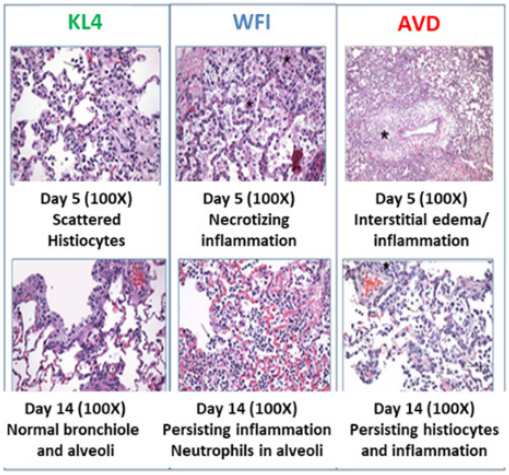
Lucinactant is a synthetic surfactant that is approved by the US FDA (NDA 021746) for the prevention of respiratory distress syndrome (RDS) in premature infants at high risk for RDS. It has been safely administered to over 1000 children and adults. Preliminary data from animal and human adult studies indicate that lucinactant could benefit those with acute respiratory distress syndrome (ARDS) in the context of COVID-19 infection, improving oxygenation and lessening lung damage. When given after intubation, lucinactant could potentially decrease the duration of ventilation or extracorporeal membrane oxygenation (ECMO).

ARDS, as a consequence of COVID-19 infection, seems to have a significant negative impact on surfactant related lung function. SARS-CoV-2 uses the SARS-coronavirus receptor, angiotensin-converting enzyme 2 (ACE2) for entry into the host cells. ACE2 is a surface molecule on alveolar Type 2 cells of lung. Type 2 cells are the source of surfactant in the lung and if they are damaged surfactant production is impaired, resulting in decreased lung compliance. This appears to occur early in the course of COVID-19 lung injury. This increases the likelihood of the need for mechanical ventilation, which may further damage the lungs. Recent publications suggest that lung fibrosis and severe interstitial changes occur in COVID-19 patients who developed ARDS.

In addition to the direct lung injury caused by COVID-19, there is a subsequent damage to the lung through triggering and activation of the inflammatory cascade, similar to that involved in the development of

bronchopulmonary dysplasia (BPD). There is data supporting benefit of lucinactant to decrease the incidence and severity of BPD, supporting the rationale for use of exogenous surfactant in the treatment of pulmonary disease caused by COVID-19.

Lucinactant significantly reduced mortality in a related pre-clinical model of H5N1 in ferrets infected with highly pathogenic avian influenza. Results showed that surfactant treatment significantly improved survival, as well as viral- and inflammation-related lung damage.




Clinical evidence in patients with acute lung injury (ALI) showed potentially beneficial results in children on mechanical ventilation due to severe respiratory syncytial virus (RSV) infection, including pneumonia. Many studies in clinical ARDS have substantiated that damage to surfactant and inadequate quantities of functioning surfactant contribute to the pathophysiology of ARDS. Several studies in adult ARDS have established that lucinactant can be safely administered to critically ill patients and potentially replenish the surfactant pool.

In many animal models, surfactant replacement therapy of pneumonia has been studied showing decreased intrapulmonary bacterial proliferation, decreased bacterial growth, and improved pulmonary function with increased pulmonary compliance. Clinical studies of surfactant in subjects with pneumonia have shown improvements in oxygenation and mean airway pressure, with one study showing a reduced need for ECMO. A series of *in vitro* studies have shown that a synthetic surfactant containing KL4 peptide tends to be more resistant to inhibition by four serum components (fibrinogen, C-

reactive protein, platelet activating factor, lyso-phospatidylcholine) known to inhibit surfactant activity when compared with an animal derived surfactant.

A study on recombinant surfactant protein C–based surfactant, indicated that among patients with ARDS caused by a direct lung injury, those who received surfactant tended to have a higher survival rate than those who received standard therapy. In fact, an oxygenation benefit was found across the entire population of patients who received surfactant.

Lucinactant has an extensive safety profile and has been safely administered to over 1000 subjects in different patient populations (adults, infants, toddlers) for different indications (eg, respiratory distress syndrome of the newborn (RDS), acute hypoxemic respiratory failure (AHRF), ARDS). In almost all cases, there were no safety signals of concern with rates of adverse events (AEs) being similar to control (placebo and active). An increase in AEs was noted in one study when using bronchoalveolar lavage to administer SURFAXIN; however, bronchoalveolar lavage is no longer used and lyophilized lucinactant has a much lower viscosity then SURFAXIN, which also should minimize any temporary airway obstruction.

It is hypothesized that lucinactant may improve the respiratory status of patients suffering from COVID-19.

**Study Description:** This is a multicenter, single-treatment study. Subjects will consist of adults with COVID-19 associated acute lung injury who are being cared for in a critical care environment.

See Section 1.2, Schedule of Activities (SOA).

**Study Objective:** To evaluate the safety and preliminary efficacy of lyophilized lucinactant, as assessed by oxygenation index (OI) through 12 hours post initiation of dosing and other physiological and outcome measurements through 24 hours or through Day 30.

**Endpoints:**

**Primary Endpoints:**

- Safety and Tolerability of reconstituted lyophilized lucinactant given as a bolus to patients with COVID-19.
- Change from baseline in OI at 12 hours post initiation of dosing. OI is defined as $P_{aw} \times FiO_2 \times 100/PaO_2$.

**Secondary Efficacy Variables:**

- Change from baseline through 24 hours post dosing initiation:
  - $FiO_2$;
  - $PaO_2$;
  - $SpO_2$;
  - OI;
  - $PaCO_2$;
  - $ETCO_2$;
  - P/F and or S/F ratios, defined as $PaO_2$ or $SpO_2$ divided by $FiO_2$;
  - Plateau pressure and peak inspiratory pressure (PIP) measured on the ventilator;
  - Ventilation Index (VI). VI is defined as $[RR \times (PIP - PEEP) \times PaCO_2]/1000$;
  - Lung compliance;
  - Pressure-volume loops if ventilator technology permits.
- Through 30 days:
  - Daily lung compliance (static) on ventilator;
  - Ventilator-free days;
  - Days in the intensive care unit (ICU);
  - Days in the hospital;
  - Incidence of all-cause mortality;
  - Organ failure free days.

**Safety Variables:**

- All-cause mortality;
- Peri-dosing adverse events (desaturation, bradycardia, ET reflux, hypotension)
- AEs and serious AEs (SAEs). SAEs that are ongoing at Day 30 will be followed for an additional 30 days;
- Air leaks as AEs of special interest;
- Assessments of vital signs.

**Study Population:** The study population will be comprised of adults 18-75 (inclusive) who are positive for SARS-CoV-2, are exhibiting symptoms of severe COVID-19 infection, who are intubated and are receiving mechanical ventilation in a critical care environment.

The objective of the pilot safety study is to evaluate the safety and feasibility of lucinactant administration in COVID-19 patients. A small number of patients should be sufficient to provide evidence that liquid lucinactant can be safely delivered as an intratracheal bolus. A total of up to 30 study subjects will be enrolled (see "Treatment Group" section).

**Description of Sites:** Approximately 15 study sites in the United States and Argentina.

**Description of Study Intervention:** Reconstituted lyophilized lucinactant will be given at a dose of 160 ml (30 mg TPL/ml), delivered as a liquid in the trachea through the endotracheal tube. Lyophilized lucinactant has the following four active ingredients:

- sinapultide (a synthetic peptide), consisting of amino acid residues lysine (K) and leucine (L), according to the sequence KLLLLKLLLLKLLLLKLLLLK ($KL_4$);
- dipalmitoyl-phosphatidylcholine (DPPC);
- palmitoyloleoyl-phosphatidylglycerol, sodium salt (POPG, Na); and
- palmitic acid (PA).

**Inclusion Criteria:** Each subject must meet all inclusion criteria to be enrolled in this study:

1. Signed and dated ICF (electronically or in ink) by the subject or legally authorized representative;
2. Age 18-75 (inclusive);
3. Assay positive for SARS-CoV-2 virus, preferably by polymerase chain reaction (PCR);
4. Endotracheal intubation and MV within 7 days of initial intubation;
5. In-dwelling arterial line;
6. P/F ratio < 300;
7. Mean blood pressure ≥ 65 mmHg, immediately before enrollment;
8. Bilateral infiltrates seen on frontal chest radiograph.

**Exclusion Criteria:** Subjects meeting any exclusion criteria must not be enrolled in this study:

1. Life expectancy < 48 hours or do not resuscitate orders;
2. Severe lung disease (home $O_2$, $FEV_1$ < 2 liters) not likely to respond to therapy or profound hypoxemia (ie, OI ≥ 25 or P/F < 100);
3. Severe renal impairment (creatinine clearance < 30 mL/min);
4. Within the last 6 months has received, or is currently receiving, immunosuppression therapy (azathioprine, cyclophosphamide or methotrexate) or any transplant recipient;
5. Clinically significant cardiac disease that adversely effects cardiopulmonary function:
    a. Acute coronary syndromes or active ischemic heart disease (as assessed by the PI using troponin and ECG)
    b. Cardiac ejection fraction < 40% (if known);
    c. Need for multiple-dose vasopressors to support blood pressure (single dose vasopressors, such as Levophed™ ≤ 0.1 mcg/kg/min are allowed);
    d. Cardiogenic pulmonary edema as the etiology of the current respiratory distress;
    e. Evidence of myocarditis or pericarditis;
6. Neuromuscular disease;
7. Neutropenia (ANC < 1000);

8. Active malignancy that impacts treatment decisions or life expectancy related to this trial;
9. Suspected concomitant bacterial or other viral lung infection. Bacterial infection defined as WBC > 15k and positive blood/ urine/sputum culture results within 72 hours.

**Treatment Group:** Up to 30 enrolled subjects will be administered an investigational drug product, reconstituted lyophilized lucinactant. Prior to administration, lyophilized lucinactant will be reconstituted with sterile water for injection then delivered as a liquid into the trachea in quarter doses.

**Lucinactant** Lyophilized lucinactant 160 ml (~80 mg TPL/kg lean body weight of 30 mg/ml suspension)

Up to 3 repeat study treatments of the same dose volume may be given if criteria are met.

Enrollment should occur as soon as possible once it has been confirmed that the subject has met all inclusion criteria and has not met any exclusion criteria within 7 days of initial intubation. Treatment initiation is to begin within 6 hours of enrollment. If the subject does not tolerate one of the 40 ml quarter doses, the dosing for that dose may be terminated at that time. It is recommended that the other quarter doses remain 40 ml; however, a lower dose may be administered for the remaining aliquots at the Investigator's discretion.

Up to 3 retreatment doses are allowed no sooner than 6 hours apart (from completion of previous dose). Subjects are eligible for retreatments if they remain on MV, there is no evidence of pneumothorax, the P/F ratio is under 300 (or S/F ratio is under 315, if no arterial line is present), and there are no safety concerns that would prevent retreatment (in the opinion of the investigator). Retreatments are given at the discretion of the investigator.

Retreatment should be performed in the same manner as the initial treatment.

**Early Discontinuation of Treatment:**

The administration of study treatment may be discontinued at any time prior to the completion of dosing based on the clinical judgment of the PI. Reasons for discontinuation of study treatment include, but are not limited to the following:

1. An AE which places subject at risk occurs (eg, severe bradycardia, hypotension, cardiac arrest)
2. If, in the PI's best medical judgment, initiating or continuing the subject's exposure to study treatment is not in the best interest of the subject's safety.

Subjects whose treatment is discontinued based on the PI's judgment will continue in the study. Subjects whose treatment is discontinued based on the subject's request may continue in the study; however, if a subject withdraws consent, the subject will be withdrawn from the study and no further data collection will be done.

**Study Duration:**

Subject participation will be from enrollment to 30 days after enrollment unless they have an ongoing SAE (to be followed for an additional 30 days).

Overall, study enrollment will be completed in approximately 7 to 8 months, with the last subject last visit approximately 8 to 9 months from the time the first subject enrolled.

**Statistical Analysis:**

The statistical analysis will be based on all enrolled subjects.

For the efficacy analysis, populations of all enrolled subjects who received any treatment (modified intent-to-treat [mITT]) will be evaluated. For the safety analysis, all enrolled subjects who received any lucinactant (including partial doses) will be evaluated.

All efficacy and safety parameters will be evaluated using summary statistics. For continuous variables, n, mean, standard deviation, median, min, and max will be presented; for discrete variables, frequency and percent will be presented.

All deaths will be summarized using frequency and percent, overall and by type of death (respiratory or not respiratory) and included as part of the assessment of safety. Also, the deaths will be listed by subject and narratives for each death will be included.

## 1.2 Schedule of Activities (SOA)

| | | Primary Phase Through Day 30 | | |
|---|---|---|---|---|
| **Measurement/Procedure** | **Screening** | **Treatment Period (Day 1)** | **Post-Treatment Period (Days 2-5)** | **Final Period (Days 6 to 30)** |
| Informed consent | X | | | |
| Inclusion/exclusion Criteria | X | | | |
| Demographics | X | | | |
| Medical history | X | | | |
| Physical examination | X | | | |
| Enrollment | | X | | |
| Study treatment administration | | X | | |
| Vital signs | X | X | X[2] | X |
| ABG ($PaO_2$, $PaCO_2$, pH) | | X[1] | X | X |
| $FiO_2$, $SpO_2$, $P_{aw}$, $EtCO_2$ | | X[1] | X | X |
| Ventilator parameters (rate, peak and plateau pressure, lung compliance) | | X[2] | X[2] | |
| Respiratory support parameters | X | X | X | X |
| Pressure volume loops | | X[3] | | |
| AEs/SAEs | | X | X | X[5] |
| Concomitant medications | X | X | X | X |
| Final Visit/Discharge | | | | X[4] |

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

[1] Time 0 (study treatment), 1, 2, 4, 8, 12, 18, 24 hours after treatment

[2] Recorded every 12 hours at 08:00 and 20:00 through Day 5.

[3] Recorded every 2 hours through 24 hours.

[4] Occurs at Day 30, withdrawal from the study, or death (whichever occurs first).

[5] AEs ongoing at Day 30 will be followed for an additional 30 days or until stop date/time (whichever occurs first).

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# 2 INTRODUCTION

## 2.1 Study Rationale

The coronavirus that was first detected in China in 2019 and which has been detected in over 190 countries on every continent, has infected over 150 million people worldwide (including approximately 3 million deaths), including approximately 35 million in the United States (including over 600,000 deaths). The virus has been named "SARS-CoV-2" and the disease it causes has been named "coronavirus disease 2019" (abbreviated "COVID-19").

Coronaviruses are a large family of viruses that are common in people and many different species of animals, including camels, cattle, cats, and bats. Rarely, animal coronaviruses can infect people and then spread between people such as with MERS-CoV, SARS-CoV, and now with this new virus. The SARS-CoV-2 virus is a betacoronavirus, like MERS-CoV and SARS-CoV. All three of these viruses have their origins in bats. The sequences from U.S. patients are similar to the one that China initially posted, suggesting a likely single, recent emergence of this virus from an animal reservoir.

Surfactants, specifically lucinactant, have been studied in acute hypoxemic respiratory failure and in acute lung injury. It is hypothesized that lucinactant may improve the respiratory status of patients suffering from COVID-19.

Lucinactant is a synthetic surfactant that is approved by the US FDA (NDA 021746) for the prevention of respiratory distress syndrome (RDS) in premature infants at high risk for RDS. It has been safely administered to over 1000 children and adults. Preliminary data from animal and human adult studies indicate that lucinactant could benefit those with acute respiratory distress syndrome (ARDS) in the context of COVID-19 infection, improving oxygenation and lessening lung damage. When given after intubation, lucinactant could potentially decrease the duration of ventilation or extracorporeal membrane oxygenation (ECMO).

## 2.2 Background

COVID-19 is the 3rd serious coronavirus outbreak since 2002. At this time, the mortality rate associated with COVID-19 is approximately 5%. This is lower than the 10% and 36% fatality rates seen during the SARS and MERS coronavirus outbreaks, respectively. The characterization of COVID-19 is still evolving. COVID-19 has some similarity to SARS including that it may be able to use the ACE2 receptor in the lung. However, the spread of infection appears to be more rapid with COVID-19 compared to SARS (over 150 million cases in 15 months for COVID-19 vs 8098 cases in 8 months for SARS). Most reported cases have been in adults and most hospitalized

patients have pneumonia. Many patients develop an ARDS-like clinical presentation, some develop a secondary infection, and many require intensive care for respiratory support including mechanical ventilation and ECMO. The fatality rate for COVID-19 patients hospitalized with pneumonia is 7-10% (from CDC June 20, 2020).

COVID-19 uses the SARS-coronavirus receptor, angiotensin-converting enzyme 2 (ACE2) for entry into the host cells. ACE2 is a surface molecule highly expressed in AT2 cells of lung, along with esophageal upper epithelial cells and absorptive enterocytes from ileum and colon which indicated digestive system along with respiratory systems is a potential route for COVID-19 infection (1). AT2 cells are the cells that produce surfactant in the lung. It stands to reason that if they are damaged by COVID-19, that surfactant production would be impaired. Recent publications suggest that lung fibrosis and severe interstitial changes occur in COVID-19 patients who developed ALI/ARDS. These changes resemble those seen in premature infants who are initially ventilated due to RDS and later develop BPD, which also presents with similar lung interstitial and fibrotic changes (2,3,4). These observations may support the of use of exogenous surfactant in the treatment of ALI/ARDS caused by COVID-19.

Lucinactant significantly reduced mortality in a related pre-clinical model of H5N1 in ferrets infected with highly pathogenic avian (H5N1) influenza. Results showed that surfactant treatment significantly improved survival, as well as viral- and inflammation-related lung damage (Figure 2-1).

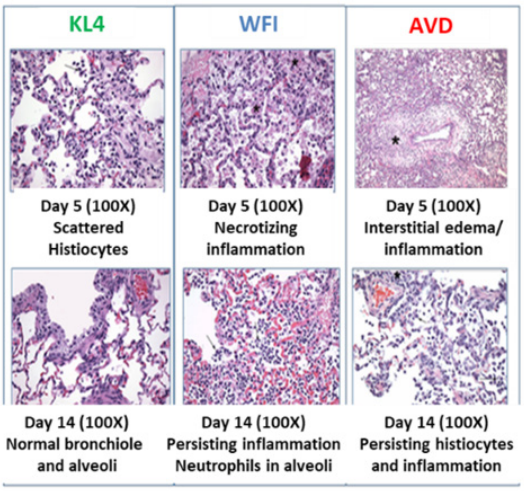


**Figure 2-1. H5N1 in Ferrets**

### 2.2.1 Unmet Medical Need in Treatment of COVID-19 Associated ARDS

Currently, when patients' breathing deteriorates, they are placed on a mechanical ventilator (MV) to help them breathe while they are symptomatic. However, MV cannot correct the underlying issue involved with breathing difficulties, known as acute respiratory distress syndrome (ARDS), and in many cases cannot prevent death.

ARDS is a sudden, progressive pulmonary disorder characterized by lung inflammation and non-cardiogenic pulmonary edema in association with refractory hypoxemia, decreased lung compliance, and diffuse pulmonary infiltrates on the chest radiograph (5). It may occur as a direct result of injury to the lung such as with gastric content aspiration, pneumonia (both bacterial and viral), toxin inhalation or it may be associated with a wide variety of systemic processes such as sepsis, non-thoracic trauma, acute pancreatitis, multiple blood transfusions, fat embolism or shock (6). The pathophysiology of ARDS involves injury to the alveolar-capillary barrier, atelectasis, intrapulmonary shunting of blood flow and surfactant dysfunction. Despite advances in medical management in intensive care units, mortality from ARDS can be as high as 40%, and current therapy remains entirely supportive with no therapies having been demonstrated safe and efficacious and receive regulatory approval (7,8,9,10,11,12,13).

The primary goal of ARDS treatment is to identify and correct the underlying etiology. For coronavirus this is supportive care until the virus is cleared by the immune system. Prevention of potential adverse effects of mechanical ventilation such as barotrauma and volutrauma is also critical. Using mechanical ventilatory support as safely as possible and improving lung compliance can contribute to avoiding these complications.

There is an unmet medical need for a therapy that will improve the patients' respiratory status and potentially reduce the time on mechanical ventilators.

### 2.2.2 Lucinactant

Since the initial report of ARDS by Ashbaugh et al. (14), abnormalities of the surfactant system have been recognized. In subsequent studies, Petty and colleagues reported both qualitative and quantitative alterations in surfactant obtained from patients with ARDS (15,16). It is now widely accepted that surfactant abnormalities play a major role in the pathophysiology of ARDS, favoring alveolar collapse, ventilation/perfusion mismatch, and increased pulmonary shunt fraction (17,18,19,20). Not only have these chemical and functional changes been described in patients

with established ARDS, but similar alterations have been described in patients at-risk to develop ARDS (20,21).

Degradation of native surfactant by inflammatory mediators and reactive oxygen species, and inhibition of surfactant functional activity plasma proteins have been implicated in impairment of alveolar surfactant. One of the hallmarks of ARDS is damaged alveoli and airways that are filled with fluid containing inflammatory cells, protein, inflammatory mediators and other debris. In addition, damage to the epithelial type II cells may result in decreased production and/or release of altered surfactant.

The biophysical change in the surfactant system that occurs with ARDS is an increase in minimum surface tension (20), while the biochemical changes in the surfactant system that occur with ARDS include:

1) a decrease in the total phospholipid content (20),
2) an alteration in the phospholipid profile as demonstrated by a decrease in the percentage of phosphatidylcholine (PC), dipalmitoyl phosphatidylcholine (DPPC), and phosphatidylglycerol (PG) (17,18,19,20),
3) an alteration in the fatty acid composition of the phospholipids (17,22),
4) a reduction in large surfactant aggregates (23,24), and
5) a decrease in the surfactant associated proteins SP-A, SP-B, and SP-D (20,21,23).

Since many of the major pulmonary consequences of ARDS may be directly influenced by surfactant dysfunction, replacement treatment with exogenous surfactant is potentially efficacious in this disorder. In addition, damaged alveoli and airways are filled with fluid containing inflammatory cells, protein, inflammatory mediators and other debris.

In a series of *in vitro* studies, liquid lucinactant for intratracheal instillation (SURFAXIN®) has been shown to be more resistant than natural lung surfactant and exogenous, commercially available animal-derived surfactant to the inhibitory effects of plasma proteins (including fibrinogen (25,26)), and inactivation by hypochlorous acid and reactive oxygen species (27). This is especially relevant in the milieu of the inflamed lung that leads to natural surfactant inactivation and degradation.

Additionally, lucinactant has been shown to modulate the inflammatory response in various models of lung injury. In addition to studies in a murine H1N1 influenza model, we have also generated data showing that lucinactant administration significantly attenuates protein leak and neutrophil migration into the alveolar space in a hyperoxic and LPS lung injury model in mice

(28), decreases cytokine levels (including IL-6 and IL-8) in a preterm mechanically ventilated lamb model of RDS (as well in airway cell culture systems) (29), and ameliorates lung injury in a hydrochloric acid ALI model in spontaneously breathing pigs when delivered both as an intratracheal liquid instillate or as an aerosol (30,31).

### 2.3 Clinical Experience with Lucinactant

Several studies in adult ARDS have established that surfactant can be safely administered to critically ill patients. In addition, clinical evidence in children with acute hypoxic respiratory failure (AHRF) showed potentially beneficial results of lucinactant in a group of patients with mild to moderate RSV infections, including pneumonia.

Windtree has completed 13 clinical studies with lucinactant for intratracheal administration: 4 studies in adult subjects with ARDS (Studies KL4-ARDS-01, KL4-ARDS-02, KL4-ARDS-03, and KL4-ARDS-04), 1 study in children up to 2 years of age with acute hypoxemic respiratory failure (AHRF) (Study KL4-AHRF-01), 1 study in premature neonates with RDS (Study KL4-IRDS-01 [CP-1/CP-2]), 3 studies in premature neonates at risk for developing RDS (Studies KL4-IRDS-02, KL4-IRDS-05, and KL4-IRDS-06), 1 study in very low birth weight (VLBW) premature neonates for the prevention of BPD (Study KL4-BPD-01), and 3 studies in full-term neonates with MAS (Studies KL4-MAS-01, KL4-MAS-02, and KL4-MAS-03). Additionally three clinical studies with lucinactant for inhalation (reconstituted lyophilized lucinactant delivered via an investigational medical device) have been completed in preterm neonates with RDS (Studies 03-CL-1201, 03-CL-1401 and 03-CL-1202). There is 1 ongoing clinical study with lucinactant for inhalation (Study 03-CL-1702) for premature neonates with RDS. Enrollment for this study has been terminated but data analysis is ongoing.

A summary of selected studies is presented below.

**1.** The KL4-ARDS-02 study was a phase 1b safety trial in adult ARDS patients that first attempted to utilize bronchopulmonary segmental surfactant lavage of the entire lung to treat ARDS (32). Bronchoscopic administration of dilute (2.5 mg/mL and 10 mg/mL) concentrations of lucinactant, when administered as two or three 30 mL aliquots followed by suctioning of all 19 bronchopulmonary segments, was safe and well tolerated in all 12 patients tested. A similar safety profile was observed in 4 of the 12 patients who received retreatment of the three 30 mL aliquot dosing regimen of lucinactant 6-24 hours later. A second, similar trial was performed in 14 patients with ARDS. Nine of these

received bronchoscopic administration of lucinactant. In these patients, the therapy was safe and well-tolerated.

**2.** The KL4-ARDS-03 study was a phase 2, open-label, controlled, multi-center, international study of surfactant lavage for the treatment of ARDS in adults. Patients were randomized to receive either surfactant administered in low concentrations and large volumes via a bronchoscopic segmental lavage technique (lung wash), or the current standard of care (SOC), which is MV and other supportive therapies. Surfactant was delivered with a bronchoscope to each of the 19 segments of the lung and was intended to cleanse and remove inflammatory substances and debris from the lung, while leaving sufficient amounts of surfactant behind to help re-establish an adequate lung function and capacity to absorb oxygen. The primary efficacy endpoint was the incidence of patients alive and off mechanical ventilation for ≥ 48 hours at the end of Day 28. Secondary efficacy endpoints included mortality rate at the end of Day 28 and number of days alive and off mechanical ventilation for ≥ 48 hours through the end of Day 28.

Results suggest that a transient increase in oxygenation occurred in both of the lucinactant treatment groups during the dosing period. This increase in oxygenation appeared to be more sustained in the higher lucinactant dose group compared to the lower lucinactant dose group.

**3.** The KL4-ARDS-04 study was a phase 2, multicenter, randomized, open-label controlled study designed to assess the tolerability, safety, and efficacy of lucinactant delivered via bronchopulmonary segmental lavage to adult patients with ARDS. The study population consisted of adult patients between the ages of 16.4 and 76.4 years (inclusive) who were intubated and required mechanical ventilation and who met the criteria for a diagnosis of ARDS.

The study was conducted in 2 parts: Part A was designed to evaluate the safety and tolerability of 4 lucinactant treatment regimens; Part B was designed to evaluate the safety and effectiveness of 2 lucinactant treatment regimens from part A of the study compared to standard of care. An Independent Safety Review Committee evaluated the safety and tolerability data from each treatment regimen in Part A of the study.

The key results of the trial showed that surfactant lavage exhibited a positive pharmacologic effect manifested as improved oxygenation. This was demonstrated by an acute increase in the $PaO_2/FiO_2$ (P/F) ratio after patients received surfactant lavage.

Clinically and statistically significant increases were observed in the P/F ratio at 24 hours after surfactant lavage, compared with SOC.

All-cause mortality at the end of Day 28 was not different between the lucinactant groups and SOC (14.3%; 23.7%; 13.9%, respectively). The mean number of days that subjects were alive and off mechanical ventilation through Day 28 was slightly less for subjects in lucinactant Group B.2 (10.6 days) compared with lucinactant Group B.1 (14.4 days) and SOC (13.9 days).

There were no meaningful differences noted in the clinical outcomes in patients classified as having direct or indirect ARDS.

**4.** The KL4-AHRF-01 study was a phase 2, multicenter, masked, placebo-controlled trial in 165 infants up to 2 years-of-age. Infants were randomized to receive either lucinactant or placebo (33). For the primary outcome, duration of MV through 14 days, there was no difference in the duration of MV between subjects treated with lucinactant and placebo treatment. There were no significant differences in other efficacy endpoints: ventilator free days, duration of intensive care unit (ICU) stay, duration of supplemental oxygen or duration of hospitalization through 14 days post randomization. Similarly, there were no significant differences in fraction of inspired oxygen, P/F ratio and oxygenation index (OI) at any time point. There was a trend for improved oxygenation with lucinactant based on improvement in P/F from eligibility to 48 hours post dose. Subjects with milder disease seemed to respond better to lucinactant treatment, and statistically fewer lucinactant subjects required a second treatment; however, more lucinactant subjects required reintubation compared to placebo.

Safety evaluations included assessment of adverse events during dosing (peri-dosing events), adverse events associated with endotracheal intubation and MV, and any other adverse event that occurred throughout the duration of the study. As expected, peri-dosing events were more frequent in the lucinactant-treated subjects, since placebo subjects only received sham air. In general, peri-dosing events were transient and resolved when lucinactant instillations were slowed. There were no significant difference in overall mortality between groups. All deaths were deemed to be not related to study treatment by the Principal Investigators. In summary, in this study in ventilated infants with AHRF, treatment with intratracheal lucinactant appeared to be generally safe and well-tolerated despite the expected transient peri-dosing events observed. An improvement in oxygenation and the observation that significantly less requirement for

retreatment was needed in the lucinactant-treated infants suggests a pharmacologic effect.

**5.** The KL4-IRDS-02 (CP1/CP2) study was a phase 1/2 clinical trial in premature neonates demonstrated that lucinactant is effective in the treatment of RDS in such patients (34). A total of 47 pre-term neonates (750-1750 g birth weight) were enrolled within the first four hours of life. All enrolled patients were treated with a bolus intratracheal injection of lucinactant (133 mg/kg or 200 mg/kg).

Treatment with lucinactant rapidly improved oxygenation, improved lung expansion as demonstrated radiographically, and reduced ventilation support. The fraction of inspired oxygen ($FiO_2$) fell in conjunction with a rise in the ratio of the partial pressure of oxygen in arterial blood compared to the alveoli ($a/A\ O_2$). There were no deaths related to RDS among the study neonates, although two of 47 (4.3%) died of unrelated abnormalities: aortic atresia and sequelae of extreme immaturity.

**6.** The KL4-MAS-01/KL4-MAS-02 study was a multicenter, randomized, controlled phase 1/2 trial comparing the safety and efficacy of lucinactant lavage to standard of care for treatment of MAS in full-term newborn neonates was performed (35). Results indicated that the procedure was well tolerated in the 15 patients treated with lucinactant. There were two serious adverse events (SAEs) related to the drug: one report of hypoxia which was considered by the investigator to be probably related to the drug and one report of hypotension which was considered by the investigator to be possibly related to the drug. The neonates lavaged with lucinactant exhibited more rapid and sustained improvement in oxygenation compared to control neonates. Moreover, the lavaged neonates were disconnected from mechanical ventilation (MV) an average of three days sooner than control neonates.

**7.** The KL4-IRDS-06 study was a phase 3, pivotal, multinational, multicenter, randomized, masked, controlled, event-driven, prophylaxis study in premature neonates that demonstrated lucinactant is significantly superior to colfosceril palmitate (Exosurf®) in preventing and ameliorating RDS, reducing death due to RDS, and keeping neonates alive and without BPD, and lucinactant is generally safe and well tolerated compared with colfosceril palmitate and beractant (Survanta®) (36). In this study, a total of 1294 neonates less than 32 weeks of gestational age and weighing between 600 and 1250 grams at birth were randomized at 50 study centers, 527 to the lucinactant group, 509 to the colfosceril palmitate group**,** and 258 to the beractant group. Efficacy analyses were

based on the intent-to-treat population (all randomized neonates). Safety analyses were based on all treated neonates (n=1288).

Lucinactant was significantly superior to colfosceril palmitate in the primary outcomes and associated clinically important complications of prematurity and mechanical ventilation. Compared with colfosceril palmitate treated neonates, significantly fewer lucinactant treated neonates had RDS at 24 hours (39.1% vs. 47.2% p=0.005) or died from an RDS-related death within 14 days of birth (4.7% vs. 9.4% p=0.002). All-cause mortality rates at 36 weeks post conceptual age (PCA) for lucinactant, colfosceril palmitate and beractant were 21.1%, 23.8% and 26.4% respectively.

The incidence of adverse experiences in the study were those expected in this population and did not differ from those observed with colfosceril palmitate or beractant.

**8.** The KL4-IRDS-02 study was a phase 3, supportive, masked, multicenter, randomized, controlled study comparing lucinactant to poractant alfa (Curosurf®), lucinactant was shown to be non-inferior to poractant alfa for the endpoint of alive and not having BPD at 28 days of age in neonates with birth weight between 600 and 1250 grams, and 24 to < 29 weeks gestational age (37). All-cause mortality rates throughout the course of the study were consistently lower in the lucinactant group but did not reach statistical significance. Lucinactant event rates for other key secondary outcome measures were similar to those in the poractant-treated group.

Adverse events (AEs) and SAEs were reported generally with the same frequency in both treatment groups. Although significantly more instances of transient peri-dosing events were reported with lucinactant compared with poractant alfa, they did not appear to lead to excess deaths, air-leak, or other neonatal complications. Interruption of administration of study drug in those neonates was very infrequent and no neonates discontinued lucinactant because of an AE.

**9.** The KL4-BPD-01 study was a phase 2, multicenter, masked, placebo-controlled trial (38) assessing safety and efficacy of lucinactant, in very low birth weight neonates at risk for developing bronchopulmonary dysplasia (BPD) randomized 136 premature neonates to receive either lucinactant standard dose (175 mg/kg), lucinactant low dose (90 mg/kg), or sham air as a control.

For the primary endpoint, a lower incidence of death or BPD in neonates receiving the standard 175 mg/kg lucinactant dosing compared with controls (57.8% vs. 65.9%, respectively) was observed. This study also showed a higher survival rate through 36 weeks post-menstrual age (PMA) in neonates receiving the standard 175 mg/kg lucinactant dosing compared with controls (88.9% vs. 84.1%, respectively) and a reduction in duration of mechanical ventilation (approximately four less days) and need for supplemental oxygen in neonates receiving the standard 175 mg/kg lucinactant dosing compared with controls.

Lucinactant therapy was generally safe and well tolerated with generally no differences between the lucinactant treatment groups and the control group in common complications of prematurity. There were no differences noted in tolerability of drug between the lucinactant standard dose of 175 mg/kg (given at a volume of 5.8 ml/kg) and the lucinactant low dose of 90 mg/kg (given at a volume of 3.0 ml/kg).

## 2.4 Risk/Benefit Assessment

### 2.4.1 Known Potential Risks

Risks for this study must be considered within the context of treating adults with COVID-19, which carries with it substantial risk of morbidity and mortality. Intratracheal instillation of surfactant into the lung requires endotracheal intubation, often with concomitant positive pressure MV. However, patients in this trial will have been intubated to treat their underlying condition. 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). Surfactant instilled intratracheally has been associated with peri-dosing events such as transient hypoxemia, bradycardia, and hypotension. These are usually not severe, are transient, and typically resolve within 30 minutes.

Studies in adults with ARDS administered lucinactant using bronchopulmonary surfactant lavage of each segment of the lungs to attempt to remove alveolar debris and effectively deposit functional surfactant. Safety results show that this procedure was generally safe and well tolerated; however, this procedure was more invasive than other delivery methods and required more time and skill to complete dosing and may have contributed to the increased treatment-emergent AE in the lucinactant-treated subjects. A total of 44 of 110 lucinactant patients (40%) and 14 of 42 standard of care patients (33%) experienced an SAE. A total of 15 of 88 lucinactant patients (17%) in KL4-ARDS-04 had an SAE that was considered to be related to study treatment (patients receiving

standard of care had all AEs and SAEs defined as not study-treatment related). A total of 28 deaths were observed in the ARDS studies. In the KL4-ARDS-04 Study, all-cause mortality at the end of Day 28 was numerically, but not statistically significantly, higher in lucinactant Group B.2 (24%) compared with lucinactant Group B.1 and SOC (14% in each).

### 2.4.2 Known Potential Benefits

All subjects enrolled in this study will receive surfactant therapy. Since many of the major pulmonary consequences of ARDS may be directly influenced by surfactant dysfunction, replacement treatment with exogenous surfactant is potentially efficacious in this disorder. Surfactant administration in adults with ARDS has been associated with improvements in oxygenation and is known to provide clinical benefits to preterm neonates, including a reduction in the risk of developing RDS or having RDS-related death. There is a potential that lucinactant may reduce lung inflammation and lessen the work of breathing. Nonetheless, the hypothesis that SRT can provide benefit in acute lung injury has not been definitively answered. Windtree believes that with the appropriate dose and delivery technique, there is significant potential benefit for lucinactant to minimize lung injury in COVID-19 and to protect and support the lungs until endogenous surfactant is functional and the underlying pathological process is reversed.

### 2.4.3 Assessment of Potential Risks and Benefits

Adults in this study will be given lyophilized lucinactant, an investigational medicinal product that, once reconstituted with sterile WFI, is chemically and physically comparable to the liquid formulation of the approved product, SURFAXIN. As this is a single-treatment study, all subjects will be subject to the same risks and benefits. Lyophilized lucinactant is expected to generally safe and well tolerated.

Previous results from KL4-ARDS-04 where lucinactant was delivered via bronchopulmonary lavage have been taken into consideration in the design of the proposed study. Dosing will occur by a far less invasive procedure. Close safety review has been incorporated to monitor the safety profile and identify any findings that might merit a change to the study. The program is designed to be a rigorous, iterative process.

We believe this plan provides an appropriate risk/benefit for the 02-CL-2001a clinical trial.

# 3 OBJECTIVES AND ENDPOINTS

| OBJECTIVES | ENDPOINTS | JUSTIFICATION FOR ENDPOINTS |
|---|---|---|
| Primary | | |
| The primary objective of this study is to evaluate the safety and feasibility of lucinactant SRT in treating COVID-19, as assessed by OI at 12 hours post dosing and other physiological and outcome measurements through 24 hours or through Day 30. | Change from baseline in OI at 12 hours post initiation of dosing. OI is defined as $P_{aw}\times FiO_2\times 100/PaO_2$. OI is defined as $P_{aw}\times FiO_2\times 100/PaO_2$ | Improvements in oxygenation should be an indicator of surfactant physiological activity on gas exchange and compliance that should underly improvement in clinical parameters. |
| Secondary Efficacy | | |
| To assess other measures of efficacy to ensure consistency of results across multiple endpoints. | • Change from baseline through 24 hours post dosing initiation:<br>o $FiO_2$;<br>o $PaO_2$;<br>o $SpO_2$;<br>o OI;<br>o $PaCO_2$;<br>o $ETCO_2$;<br>o P/F and or S/F ratios;<br>o Change in plateau pressure and PIP measured on the ventilator;<br>o Ventilation Index (VI). VI is defined as $[RR\times(PIP - PEEP)\times PaCO_2]/1000$;<br>o Lung compliance;<br>o Pressure-volume loops, if ventilator technology permits.<br>• Through 30 days:<br>o Daily lung compliance (static) on ventilator;<br>o Ventilator free days;<br>o Duration of ECMO<br>o Days in the ICU; | These endpoints represent endpoints that are appropriate given the trial design. |

| OBJECTIVES | ENDPOINTS | JUSTIFICATION FOR ENDPOINTS |
|---|---|---|
| | ○ Days in the hospital;<br>○ All-cause mortality;<br>○ Organ failure free days. | |
| Safety | | |
| To ensure that administration of lucinactant does not lead to safety concerns. | • All-cause mortality;<br>• Peri-dosing adverse events (desaturation, bradycardia, ET reflux, hypotension);<br>• AEs and SAEs. SAEs that are ongoing at Day 30 will be followed for an additional 30 days;<br>• Assessments of vital signs. | Standard measurements of safety. |

# 4 STUDY DESIGN

## 4.1 Overall Design

This is a multicenter, single-treatment study in adults with COVID-19, who are being cared for in a critical care environment.

Subjects will be enrolled into the lucinactant treatment group if they meet all the screening criteria. Enrolled subjects will receive reconstituted lyophilized lucinactant (30 mg total TPL/mL) as a liquid at 160 ml (~80 mg TPL/kg lean body weight [~2.7 ml/kg lean body weight]). Sites from the US and Argentina will participate in this study.

See Section 1.2, Schedule of Activities, for more details on study measurements and the timing of measurements.

## 4.2 Scientific Rationale for Study Design

Reported illnesses with COVID-19 have ranged from very mild (including some with no reported symptoms) to severe, including illness resulting in death. The lungs appear to be affected in nearly all cases, with typical symptoms including cough and shortness of breath.

Surfactants, specifically lucinactant, have been studied in acute hypoxemic respiratory failure and in acute lung injury. Preliminary data from animal and adult human studies indicate that lucinactant could benefit those with ARDS in the context of COVID-19 infection, improving oxygenation and lessening lung damage. Furthermore, lucinactant, given early in the course of the disease, could potentially decrease the need for endotracheal intubation and mechanical ventilation.

It is hypothesized that lucinactant may provide relief of the respiratory symptoms, allowing patients to recover while suffering from COVID-19.

## 4.3 Justification for Dose

SRT has been proposed to treat ARDS in children and adults. SRT was initially successful in adult ARDS patients (5) and later in pediatric patients (39). These studies used a dose volume of 2–4 ml/kg.

Survanta® (25 mg phospholipid [PL]/ml) was administered to adult ARDS patients by endotracheal instillation (5). Three dose groups were studied: up to 8 doses of 100 mg PL/kg (4 ml/kg), up to 8 doses of 50 mg PL/kg (2 ml/kg), and up to 4 doses of 100 mg PL/kg (4 ml/kg). The

dosing strategies used were chosen on the basis of experience with animal models and the desire to use an incremental approach in applying this untested therapy to patients. The $FiO_2$ at 120 hours after treatment began was significantly decreased only for patients who received up to 4 doses of 100 mg PL/kg as compared with control patients (p =0.011). Mortality in the same group of patients was 18.8%, as compared with 43.8% in the control group (p =0.075). Intratracheal bolus instillation of Survanta was generally well tolerated, and no safety concerns were identified. Data did suggest that after 4 doses of 100 mg PL/kg, further doses were accompanied by greater reflux and transient increases in peak inspiratory pressure than were seen with initial doses and did not result in apparent improvement in measures of gas exchange.

Infasurf® (35 mg PL/ml) was administered to children, 1 week to 21 years of age, with ARDS by endotracheal instillation (39). Two doses of 80 ml/$m^2$ were administered to patients ≥ 10 kg. Infants < 10 kg received 3 ml/kg (equivalent to the premature infant dose). The Infasurf group experienced a decrease in oxygenation index from 20 to 13.9 after 12 hours compared with the placebo group's decrease from 20.5 to 15.1 (p=.01). Mortality was significantly greater in the placebo group compared with the Infasurf group (36% vs 19.5%). No significant decrease in duration of ventilator therapy and ICU or hospital stay was observed. There were no differences in long-term complications.

Future studies in ARDS patients switched to a lower dose volume of approximately 1 mL/kg. This change in dose volume resulted in a failure of SRT in adults (40,41,42).

Venticute® (50 mg PL/ml) was administered to adult ARDS patients by endotracheal instillation (41). Up to 4 doses of 50 mg/kg (1 ml/kg) were administered to patients. Overall survival at 28 days after treatment was 66% and the median number of ventilator-free days was 0. There was no significant difference in mortality or the need for mechanical ventilation between the Venticute and control groups. Patients receiving Venticute had significantly greater, improvement in blood oxygenation during the initial 24 hours of treatment than patients receiving standard of care. However, this improvement was not sustained and was not significantly different by 48 hours. These observations suggest the possible need for a prolonged treatment period, possibly with greater intervals between doses, with a larger number of doses, or with a larger dose volume. Up to 3 treatments (dose = 30 mg [60 mg PL/ml] per centimeter of height) were allowed if retreatment criteria were met. Infasurf administration was not associated with improved survival, length of stay or oxygenation. The authors speculated that the lack of response could be related to insufficient dosing of surfactant, since they choose not to administer a second dose of surfactant if the first was without clear benefit. They also concluded that the lack of oxygenation benefit may

be due to ineffective timing of instillation and/or administration technique rather than lack of efficacy of exogenous surfactant (40).

Based on previous clinical studies, as well as studies in several animal models of ARDS, the proposed dose of lucinactant (30 mg total PL/ml) is 160 ml (80 mg/kg lbw, based on an estimated 60 kg lbw patient similar to those enrolled in the Survanta and Venticute studies). Windtree believes that this dose addresses both the amount of PL required to sufficiently restore alveolar surface tension and the volume of lucinactant required to adequately coat the alveolar surface.

### 4.4 End of Study Definition

A subject is considered to have completed the study if he or she has completed all phases of the study, including the last scheduled procedure at Day 30.

The end of the study is defined as completion of the last visit or procedure for the last subject in the trial (last subject/last visit).

# 5 STUDY POPULATION

## 5.1 Inclusion Criteria

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

1. Signed and dated ICF by the subject or legally authorized representative;
2. Age 18-75 (inclusive);
3. Assay positive for SARS-CoV-2 virus, preferably by polymerase chain reaction (PCR);
4. Endotracheal intubation and MV, within 7 days of initial intubation;
5. In-dwelling arterial line;
6. P/F ratio < 300;
7. Mean blood pressure ≥ 65 mmHg, with or without vasopressor support, immediately before enrollment;
8. Bilateral infiltrates seen on frontal chest radiograph.

## 5.2 Exclusion Criteria

Subjects meeting any exclusion criteria must not be enrolled in this study:

1. Life expectancy < 48 hours or do not resuscitate orders;
2. Severe lung disease (home $O_2$, $FEV_1 < 2$ liters) not likely to respond to therapy or profound hypoxemia (ie, OI ≥ 25 or P/F < 100);
3. Severe renal impairment (creatinine clearance < 30 mL/min);
4. Within the last 6 months has received, or is currently receiving, immunosuppression therapy (azathioprine, cyclophosphamide or methotrexate) or any transplant recipient;
5. Clinically significant cardiac disease that adversely effects cardiopulmonary function:
    a. Acute coronary syndromes or active ischemic heart disease (as assessed by the PI using troponin and ECG);
    b. Cardiac ejection fraction < 40% (if known);
    c. Need for multiple-dose vasopressors to support blood pressure (single dose vasopressors, such as Levophed™ ≤ 0.1 mcg/kg/min are allowed);
    d. Cardiogenic pulmonary edema as the etiology of the current respiratory distress;
    e. Evidence of myocarditis or pericarditis;
6. Neuromuscular disease;
7. Neutropenia (ANC < 1000);
8. Active malignancy that impacts treatment decisions or life expectancy related to this trial;
9. Suspected concomitant bacterial or other viral lung infection. Bacterial infection defined as WBC > 15k and positive blood/urine/sputum culture results within 72 hours.

## 5.3 Lifestyle Considerations

The use of lucinactant in women of childbearing potential not using contraceptive measures should be avoided; women of childbearing potential must have a negative pregnancy test (β-hCG) recorded prior to administration of lucinactant and at the end of the study. Men and premenopausal women will be required to use double-barrier methods of birth control from enrollment through 30 days following discharge from the hospital.

Subjects should not smoke or use illicit drugs, including marijuana.

## 5.4 Screen Failures

Screen failures are defined as patients who consent to participate in the clinical trial but are not subsequently assigned to a study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements, and to respond to queries from regulatory authorities. Minimal information will include the reason for screen failure and may include demography, eligibility criteria, and any serious adverse event (SAE).

## 5.5 Strategies for Recruitment and Retention

Subjects will be recruited from each institution based upon inpatient admission for COVID-19. Patients or their legal guardians who wish to enroll will go through the informed consent process; patients or their legal guardians will be reminded they may withdraw consent at any time. Informed consent will be administered in paper or electronic format.

No overall recruitment strategies are planned; any strategies employed by a site must be approved by Windtree and their IRB/IEC and be included in their site regulatory binder (with a copy sent to Windtree).

# 6 STUDY DOSING AND ADMINISTRATION

## 6.1 Treatments Administered

### 6.1.1 Study Intervention Description

Lucinactant will be provided as a lyophilized powder in a 30 ml vial. Lucinactant will be reconstituted with 10 ml sterile Water for Injection (WFI). Reconstituted lyophilized lucinactant will be given at a volume of 160 ml, delivered as a liquid in the trachea.

Lyophilized lucinactant will be appropriately labeled with the investigational caution statements required by the regional health authority (eg, United States [US] Food and Drug Administration [FDA], the National Administration of Drugs, Foods and Medical Devices [ANMAT]) to ensure that users are aware that the product is limited by federal law to investigational use only. Additional information can be found in the investigator's brochure and in the package insert for SURFAXIN.

### 6.1.2 Dosing and Administration

Eligible patients will be enrolled into the study and receive lucinactant treatment. Treatment initiation is to begin within 6 hours of enrollment. The time of enrollment is defined as the time at which it has been confirmed that the subject has met all inclusion criteria and has not met any exclusion criteria, and a request to the pharmacy to prepare study drug has been made. Enrollment should occur within 7 days of endotracheal intubation and MV.

Initial Dose

Reconstituted lyophilized lucinactant is administered using the following instillation procedure:

a. The ventilator will be set at an $FiO_2$ of 1.00.
b. To prevent cough and throat closure, the patient may receive a short-acting paralytic.
c. Lucinactant will be administered via a number 10 French end-hole suction catheter positioned in the trachea at the end of the endotracheal tube. The suction catheter will be inserted into the ventilator's inspiratory tubing via a side-port connector.
d. The total dose of lucinactant will be divided into four quarter-doses (40 ml). Each quarter-dose will be further sub-divided into aliquots of 20 ml. Each quarter-dose will be instilled in one of the four positions listed below, one aliquot at a time. After each quarter dose, ensure the patient is stable before continuing the dosing.

1. Quarter-dose administered with the patient supine and positioned 45° to the right. Each aliquot will be followed by 1 minute of ventilation.
2. Quarter-dose administered with the patient supine and positioned 45° to the left. Each aliquot will be followed by 1 minute of ventilation.
3. Quarter-dose administered with the patient supine and positioned 45° to the right. Each aliquot will be followed by 1 minute of ventilation.
4. Quarter-dose administered with the patient supine and positioned 45° to the left. Each aliquot will be followed by 1 minute of ventilation.

During the administration of the lucinactant, the investigator should monitor the patient's oxygen saturation by pulse-oximetry. If the subject does not tolerate one of the 40 ml quarter doses, the dosing for that dose may be terminated at that time. It is recommended that the other quarter doses remain 40 ml; however, a lower dose may be administered for the remaining aliquots at the Investigator's discretion. The investigator will remain with the patient during the first 10 minutes post-treatment. No ventilator changes are to be made during this 10-minute period unless cyanosis or bradycardia occur. At the end of this 10-minute period, the patient's $FiO_2$ should be weaned to the patient's pre-treatment $FiO_2$. The patient should not be suctioned for one hour following dosing.

If, in the opinion of the investigator, the patient experiences clinically significant desaturations, hypoxia and/or bradycardia, the lucinactant administration should be temporally suspended to allow the patient to recovery. If the patient doesn't not recovery in a reasonable time, the administration should be terminated. If the administration procedure is re-started and the patient experiences a second episode of clinically significant desaturation, hypoxia and/or bradycardia, the lucinactant administration should be immediately terminated.

Retreatment Doses

Up to 3 retreatment doses are allowed no sooner than 6 hours apart (from completion of previous dose). Subjects are eligible for retreatments if they remain on MV, there is no evidence of pneumothorax, the P/F ratio is under 300 (or S/F ratio is under 315 (43), if no arterial line is present), and there are no safety concerns that would prevent retreatment (in the opinion of the investigator). Retreatments are given at the discretion of the investigator, but it is encouraged that retreatments be given at the recommended time interval if the patient is still receiving MV and meets all retreatment criteria.

Retreatment should be performed in the same manner as the initial treatment.

## 6.2 Preparation/Handling/Storage/Accountability

### 6.2.1 Acquisition and Accountability

Lyophilized lucinactant will be shipped to the sites from Windtree or a supply depot. Site pharmacies will be stocked with an initial supply, with re-supply based on pre-set limits.

### 6.2.2 Formulation, Appearance, Packaging and Labeling

Lyophilized lucinactant, is a sterile, white, liposomal powder consisting of a 21-amino acid hydrophobic synthetic peptide, (sinapultide, $KL_4$ peptide), the phospholipids dipalmitoyl-phosphatidylcholine (DPPC) and palmitoyloleoyl-phosphatidylglycerol, sodium salt (POPG, Na), and the fatty acid palmitic acid (PA). Immediately before dosing, the lyophilized product is reconstituted with sterile WFI at a concentration of 30 mg TPL/ml. Once reconstituted with sterile WFI, the product contains the same 4 APIs in the same proportions and concentrations, as SURFAXIN (Table 6-1). Lyophilized lucinactant contains no animal-derived materials or preservatives.

**Table 6-1. Drug Description**

| Ingredient | Amount (per ml) |
|---|---|
| Sinapultide | 0.862 mg |
| DPPC | 22.50 mg |
| POPG, Na | 7.50 mg |
| PA | 4.05 mg |

Note: amounts reflect reconstituted product at 30 mg/ml

### 6.2.3 Product Storage

Lyophilized lucinactant must be stored in refrigerated conditions (5°C ± 3°C ) and protected from light. The refrigerator temperature must be monitored to ensure the viability of the drug product. The refrigerator temperature must be logged at least daily and will be reviewed by the CRA during site visits. The drug product will be shipped in insulated shippers with temperature monitors to ensure that appropriate temperatures were maintained during the shipping process. Instructions for using the temperature monitors will be included with the shipments.

### 6.2.4 Preparation

Details for study drug preparation can be found in the study manual. Briefly, lyophilized lucinactant is reconstituted immediately before use by adding 10 ml of sterile WFI to the vial of lucinactant, after which the vial is gently inverted to mix the suspension; no warming is necessary.

Each vial is drawn up into a single-use syringe. The syringe should be labeled with the subject's identification number.

## 6.3 Measures to Minimize Bias: Randomization and Blinding

For this study, the study drug (lyophilized lucinactant) is being delivered in an open-label manner and no randomization will occur; thus, no procedures to minimize bias relative to randomization or blinding will be employed.

## 6.4 Study Intervention Compliance

Study treatments will be administered in an in-patient setting (ie, ICU) by the PI or a person approved for dosing by Windtree. Completion of drug delivery for each treatment and re-treatment will be monitored; no other compliance measurements will be employed.

## 6.5 Concomitant Therapy

Applicable concomitant medications (sedatives, paralytics, vasopressors, anti-coagulants, COVID-directed antiviral and anti-inflammatory therapy) will be recorded from enrollment until the final subject assessment.

### 6.5.1 Rescue Medicine or Therapy

Not applicable.

# 7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

## 7.1 Discontinuation of Study Intervention

Study treatment may be interrupted at any time as circumstances dictate (eg, peri-dosing event). In all cases, emergent care of the subject takes precedence over study treatment; however, it is important to complete SRT as soon as possible. If study treatment is interrupted or stopped, the reason for doing so must be recorded, especially in regard to peri-dosing events (bradycardia, desaturation, hypotension, pallor, gagging/regurgitation/vomiting).

Discontinuation from study treatment does not mean discontinuation from the study, and the remaining study procedures should be completed as indicated. If a clinically significant finding is identified after enrollment (including, but not limited to changes from baseline), the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an AE.

## 7.2 Participant Discontinuation/Withdrawal from the Study

The subject is free to withdraw consent from subject participation in the study at any time. An investigator may discontinue or withdraw a participant from the study if any clinical AE or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject.

The reason for subject discontinuation or withdrawal from the study will be recorded on the end of study eCRF page. Subjects who are enrolled and subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced.

## 7.3 Lost to Follow-Up

All efforts should be undertaken to ensure that no subject is lost to follow-up. It is expected that subjects will remain in the study site until the end of study; however, if a subject is discharged prior to the final evaluation, the subject should be contacted for the Day 30 visit. If the subject cannot be reached, at least 3 additional attempts must be made (at least one contact using telephone and at least one using email). If the subject still cannot be reached, a certified letter should be sent to the subject. If contact cannot be re-established after the certified letter, the subject will be considered lost to follow-up.

# 8 STUDY ASSESSMENTS AND PROCEDURES

A table of study procedures and activities is presented in Section 1.2.

## 8.1 Screening Process

Before conducting any study-related activities, the ICF must be signed and dated by the subject or their legally authorized guardian and retained by the site and the subject (Section 10.1.1). Evaluation of the entrance criteria are performed during the screening process.

### 8.1.1 Demographics and Baseline Characteristics

The subject's demographic data will be recorded.

### 8.1.2 Medical History

During Screening, medical history, and concomitant mediations will be documented.

### 8.1.3 Physical examination

A physical examination will be conducted during Screening. Any relevant clinically significant abnormalities noted prior to enrollment should be recorded in the eCRF as part of the medical history (and not as an AE).

### 8.1.4 Respiratory Support Parameters

During screening, information on respiratory support parameters will be captured for subjects receiving MV. For the initial intubation, the following must be documented:

- Date and time support started;
- The type of respiratory support and mode.

Lung compliance should be recorded during screening.

### 8.1.5 Enrollment

Eligible patients will be enrolled into the study and receive lucinactant treatment. Treatment initiation is to begin within 6 hours of enrollment. The time of enrollment is defined as the time at which it has been confirmed that the subject has met all inclusion criteria and has not met any

exclusion criteria, and a request to the pharmacy to prepare study drug has been made. Enrollment should occur within 7 days of endotracheal intubation and MV.

Following enrollment, dosing will proceed once the reconstituted product has been prepared (see the Study Manual for reconstitution and preparation instructions). Treatment initiation is to begin within 6 hours of enrollment.

Just prior to dosing, vital signs (including blood pressure, mean airway pressure, weight, and body temperature) will be assessed.

## 8.2 Treatment Period

Reconstituted lyophilized lucinactant is administered as a liquid within 7 days of initial intubation.

### 8.2.1 Gas Exchange Parameters and Arterial Blood Gases

Values for fraction of inspired oxygen ($FiO_2$), oxygenation from pulse oximetry ($SpO_2$), mean airway pressure ($P_{aw}$), end-tidal $CO_2$ ($EtCO_2$), and arterial blood gases (ABGs; $PaO_2$, $PaCO_2$, pH) will be recorded from just prior to dose administration and at 1 (±0.25), 2 (±0.25), 4 (±0.5), 8 (±0.5), 12 (±1.0), 18 (±1.0), and 24 (±1.0) hours after dose administration, daily through Day 5, and at Day 30.

P/F and S/F ratios will be determined.

### 8.2.2 Vital signs

Blood pressure, heart rate, and body temperature will be measured every 12 (±1.0) hours until Day 5 and at Day 30. Systolic and diastolic blood pressure should be measured using either an arterial line or a manual sphygmomanometer with the patient in a supine or semi-supine position.

Weight will be measured at baseline and at Day 30.

### 8.2.3 Respiratory Support Parameters

Information on respiratory support parameters will be captured through Day 30 or discharge, whichever occurs first. For each intubation and change in respiratory support, the following must be documented:

- Date and time

- The type of respiratory support and mode
- The reason for each intubation and change of respiratory support

Lung compliance should be recorded every 12 (±1.0) hours through Day 5. The plateau pressure and peak inspiratory pressure (PIP) will be measured every 12 (±1.0) hours through Day 5. The ventilation index will be calculated.

### 8.2.4 Pressure Volume Loops

Pressure volume loops will be recorded every 2 (±0.25) hours through 24 hours.

### 8.2.5 Readmissions and death

Readmissions and death will be reported through discharge and Day 30. Hospitalization will be defined as any unplanned hospitalization (including admission to a hospital or any attendance in an acute care setting e.g. ED, or in another health care facility) of 24 hours or greater, regardless of whether the patient was admitted to the hospital.

## 8.3 Safety and Other Assessments

#### *8.3.1.1 Concomitant Medications*

Concomitant medications and therapies required for the general care of the subject are permitted, including remdesivir and off-label drugs. Other investigational agents or investigational medical devices should be avoided. Concomitant medications will be documented until the time the subject completes the Day 30, is discharged from the hospital, dies, or is transferred to another hospital. Dose, route, unit, frequency of administration, indication for administration, and dates of medication will be captured on the CRF.

#### *8.3.1.2 Tolerability of drug administration*

During dosing, the incidence of the following will be recorded:

- Oxygen desaturation
- Bradycardia
- Hypotension
- ET tube obstruction

In addition, information on (time, reason) and the incidence of dosing interruptions or dosing discontinuations will be collected.

## 8.4 Adverse Events and Serious Adverse Events

### 8.4.1 Definition of Adverse Events

An adverse event (AE) is defined as any untoward medical occurrence associated with the use of an intervention, whether or not considered intervention related. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product [21 CFR 312.32(a), ICH E6(R2)].

### 8.4.2 Definition of Serious Adverse Events

An AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization.

### 8.4.3 Classification of an Adverse Event

#### *8.4.3.1 Severity of Event*

The severity of an AE is assessed by the PI using the following definitions:

- **Mild** – Events require minimal or no treatment and do not interfere with the subject's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a subject's usual activity level and may require systemic drug therapy or other treatment. Severe events may be potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious."

*8.4.3.2 Relationship to Study Intervention*

All AEs must have their relationship to study intervention assessed by the PI, based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration, follows a known response pattern to the study intervention, and cannot be explained by concurrent disease or other drugs or chemicals. An AE that is determined to be due to the subject's underlying disease state or is common for this patient population generally should not be considered related.
- **Possibly Related** – There is some evidence to suggest a causal relationship (eg, the event occurred within a reasonable time after administration of the trial medication) and/or follows a known response pattern to the study drug. However, other factors may have contributed to the event (eg, the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "related," if appropriate. An AE that is determined to be due to the subject's underlying disease state or is common for this patient population generally should not be considered possibly related.
- **Unlikely Related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (eg, the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (eg, the subject's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There should be an alternative, definitive etiology documented by the clinician.]

*8.4.3.3 Expectedness*

The Windtree Medical Monitor or designee will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention (ie, as per the Investigator's Brochure).

### 8.4.4 Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an AE or SAE may come to the attention of study personnel during the study, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate eCRF. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The site will record all reportable events with start dates occurring any time after informed consent is obtained until 7 days after the last day of study participation or Day 30 (whichever occurs first). Events will be followed for outcome information until resolution or stabilization, or for 30 days after study participation. When possible, information collection should continue for subjects that withdraw or discontinue treatment early.

### 8.4.5 Adverse Event Reporting

All AEs are to be assessed in all subjects throughout Day 30 (from enrollment to study withdrawal or completion) and documented in the study eCRF. Each AE should be reported spontaneously or in response to general, non-directed discussion with the attending nurse or physician (eg, "has there been any change in subject status since the last assessment period?"). For each AE, the investigator should obtain all information required to complete the AE page of the eCRF, in accordance with eCRF completion guidelines (provided separately by Windtree).

All AEs, regardless of seriousness, severity, or relationship to study participation, must be recorded (using medical terminology) in the source document and on the AE page of the eCRF. Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology.

All AEs must be followed until resolution or until a stable clinical end-point is reached, or for at least 30 days after the subject's last day in the study if an AE is ongoing at the time the subject completes the study.

Documentation of AEs in the eCRF must include the following parameters; (1) duration (time of onset and resolution), (2) severity or grade, (3) outcome, (4) action taken, and (5) relationship to study drug. AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA).

### 8.4.6 Serious Adverse Event Reporting

When an investigator-trained designee becomes aware of an SAE, Windtree must be notified as soon as possible (and no later than 24 hours after the event has occurred) by telephone, regardless of the relationship (or lack thereof) of the SAE to study participation.

SAEs should be reported to Windtree's reporting line. When reporting SAEs, the following information should be provided:

1. Study identifier
2. Study center
3. Subject number
4. A description of the event
5. Date of onset
6. Current status
7. Clarification on whether infusion therapy was discontinued
8. The reason why the event is classified as serious
9. The Investigator's assessment of the association between the event and study participation

All reports of SAEs must be followed up within 24 hours (or sooner at the request of the medical monitor) by the completion of the SAE form and signature by the person who completed the form and the PI.

Safety report distribution is the responsibility of Windtree or designee (ie, CRO), which coordinates distribution of safety reports to applicable regulatory authorities and ECs. Finalized safety reports are distributed by one or more individuals, "Responsible Team," within a department that has responsibility to coordinate the distribution/submission activities, either directly or via a Local Submitting Party on behalf of Windtree.

The Responsible Team receives finalized safety reports (eg, CIOMS-I form/MedWatch 3500A/ E2B file/DSUR/SUSAR Listing) and coordinates distribution to all applicable regulatory

authorities and ECs. Distribution will be done in accordance with project-specific documents, regulatory requirements, other applicable reference sources, and applicable Windtree or designee safety procedural documents.

In addition, Windtree coordinates translation when required for safety reporting. Windtree also coordinates transcription (eg, manual transfer of information from one reporting format to another) when required. Project specific information is used to identify appropriate recipients. Quality checks will be permed on the distribution documents and recipient information for completeness and accuracy prior to distribution.

The names, telephone, and fax numbers of the individuals who should be contacted regarding safety issues are listed below.

FOR QUESTIONS, PLEASE CONTACT THE MEDICAL MONITOR:

Carlos Guardia, MD (Medical Monitor)
WhatsApp: +591 72138559
Office: +1 (717) 300 1415
Email: CGuardia@Windtreetx.com

Steven G. Simonson, MD, MHS (Medical Officer)
Office: +1 (215) 488-9474
Email: SSimonson@Windtreetx.com

FOR REPORTING SAEs, PLEASE CONTACT WINDTREE AT:

Reporting Line (24/7/365): +1 833-4WINDTX

FOR ADDITIONAL ASSISTANCE, PLEASE CONTACT YOUR CRA OR WINDTREE DIRECTOR OF CLINICAL OPERATIONS:

Catherine Kacprzycki
Director of Clinical Operations
Office: +1 (215) 488-9478
Cell: +1 (267) 370-3836
Email: CKacprzycki@Windtreetx.com

### 8.4.7 Reporting Events to Subjects

Occurrences of AEs or SAEs that impact the risk to subjects in the study may result in a change in the informed consent form. Subjects who have not yet completed the study must be reconsented with the amended informed consent form.

### 8.4.8 Events of Special Interest

Air leaks (eg, pneumothorax, pneumomediastinum) will be considered AEs of special interest. Air leaks should be reported using the reporting line within 24 hours of becoming aware of the air leak.

### 8.4.9 Reporting of Pregnancy

The use of lucinactant in women of childbearing potential not using contraceptive measures should be avoided; women of childbearing potential must have a negative pregnancy test (β-hCG) recorded prior to administration of lucinactant and at the end of the study. Men and premenopausal women will be required to use double-barrier methods of birth control for 30 days following discharge from the hospital. Failure to commit to double-barrier methods will be a reason for screen failure.

If a woman becomes pregnant during the study, the pregnancy will be followed until delivery or termination, and pertinent information about the pregnancy will be collected (eg, AEs, concomitant medications).

# 9 STATISTICAL CONSIDERATIONS

## 9.1 Statistical Hypothesis

As this is an open-label study with no comparison group, no hypotheses will be used for this study.

## 9.2 Sample Size Determination

The objective of this safety pilot study is to determine if lyophilized lucinactant administration is safe and tolerable, and to assess the impact on oxygenation for those patients suffering with COVID-19. As a result, no formal sample size calculation was performed but instead enroll a sufficient number of subjects to provide evidence that liquid lucinactant can be safely delivered as an intratracheal bolus.

Thus, up to 30 subjects (out of approximately 90 screened) will be enrolled.

## 9.3 Populations for Analyses

Populations of all enrolled subjects who received any treatment (modified intent-to-treat [mITT]) will be evaluated for safety and efficacy.

## 9.4 Statistical Analyses

### 9.4.1 General Approach

All efficacy and safety parameters will be evaluated using summary statistics. For continuous variables, n, mean, standard deviation, median, min, and max will be presented; for discrete variables, frequency and percent will be presented.

### 9.4.2 Analysis of the Primary Efficacy Endpoint(s)

The primary endpoint is the change from baseline in OI through 12 hours post dosing. OI is define as $(FiO_2 \times P_{aw} \times 100)/PaO_2$. OI will be summarized using mean, standard deviation (SD), median, minimum, and maximum.

### 9.4.3 Analysis of the Secondary Endpoint(s)

*9.4.3.1 Through 24 Hours Post Treatment*

OI, $FiO_2$, $PaO_2$, SpO2, PaCO2, P/F and S/F ratios, change in plateau pressure and PIP measured on the ventilator, VI, and lung compliance will be summarized as change from baseline at each time point measured using mean, SD, median, minimum, and maximum.

*9.4.3.2 Through 30 Days*

Daily lung compliance on ventilator, ventilator free days, days in the ICU, days in the hospital, organ failure free days, and all-cause mortality will be summarized as was done for the primary endpoint.

All deaths will be summarized using frequency and percent, overall and by type of death (respiratory or not respiratory) and included as part of the assessment of safety. Also, the deaths will be listed by subject and narratives for each death will be included. In this study, the secondary endpoint of ventilator-free days (VFD) is a mortality adjusted assessment. This issue will be dealt with in more detail in preparation for the next study.

### 9.4.4 Safety Analyses

Adverse Events will be coded using MedDRA (version 22.0 or later) and will be summarized by treatment group using frequency and percent but will not be compared statistically. Concomitant medications will be classified using the WHODrug dictionary (March 2020 or later) and summarized using frequency and percent. All-cause mortality, physical examination and medical history will similarly be summarized.

### 9.4.5 Baseline Descriptive Statistics

Demographic parameters will be summarized by treatment group and assessed qualitatively for homogeneity of treatment groups. For categorical data (eg, sex, race), data will be summarized using frequency and percent; for continuous (parametric) data, data will be summarized by n, mean, SD, median, minimum, and maximum.

### 9.4.6 Planned Interim Analyses

No interim analyses are planned, other than the safety assessments performed by the DMC.

### 9.4.7 Sub-Group Analyses

No sub-group analyses are planned.

### 9.4.8 Tabulation of Individual Participant Data

Datasets will be provided as CDISC-compliant standard data tabulation modules (SDTM); no separate individual participant data listings will be provided.

### 9.4.9 Exploratory Analyses

Exploratory analyses may be performed based upon subject responses to treatment.

# 10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

## 10.1 Regulatory, Ethical, and Study Oversight Considerations

### 10.1.1 Informed Consent Process

#### *10.1.1.1 Consent/Assent and Other Informational Documents Provided to Participants*

The informed consent form (ICF) describes in detail the study intervention, study procedures, and risks. The ICF (either electronic or paper) is given to the patient and documentation of informed consent is required prior to starting administration of study intervention or any study-specific procedures that are not part of usual care.

#### *10.1.1.2 Consent Procedures and Documentation*

Informed consent is a process that is initiated prior to patient agreement to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB) or Independent Ethics Committee (IEC) approved and the patient will be asked to read and review the paper or electronic document. Within a maximum of 24 hours before administration of study medication, a medical screening will be performed on all prospective patients to assess suitability for the study. The investigator will explain the research study to the patient and answer any questions that may arise. A verbal explanation will be provided in terms suited to the patient's comprehension of the purposes, procedures, and potential risks of the study and of the rights of research participants. Patients will have the opportunity to carefully review the consent form and ask questions prior to signing. The patients should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The patient will sign the informed consent document (in ink or electronically) prior to any procedures being done specifically for the study. Patients must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent will be given to the patient for their records and will be retained in the site source documents. The informed consent process will be documented in the source document (including the date). The rights and welfare of the participants will be protected by emphasizing to the patient that the quality of their medical care will not be adversely affected if they decline to participate in this study. No waivers will be allowed for this study.

### 10.1.2 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the principal investigator and regulatory authorities. If the study is prematurely terminated or suspended, the PI will promptly inform study participants and the IRB/IEC and will provide the reason(s) for the termination or suspension.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Data that are not sufficiently complete and/or evaluable

The study may resume once the reasons for suspension are addressed, and satisfy the sponsor, IRB/IEC and/or Regulatory Agency (eg, FDA or ANMAT).

### 10.1.3 Confidentiality and Privacy

Subject confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and Windtree. The study protocol, documentation, data, and all other information generated will be held in strict confidence, in compliance with the Health Insurance Portability and Accountability Act (HIPAA) and other local privacy laws (such as Law 25326 in Argentina). No information concerning the study or the data will be released to any third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB/IEC, or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant’s contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB/IEC, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be entered into a validated Electronic Data Capture (EDC) system, and will be transmitted to and stored at Windtree. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by Windtree research staff will be secured and password protected and meet the requirements of 21CFR11 and the General Data Protection Regulation. At the end of the study, all study databases will be archived at Windtree.

### 10.1.4 Future Use of Stored Specimens and Data

No specimens will be taken for this study.

### 10.1.5 Key Roles and Study Governance

The coordinating investigator and medical monitor are listed in Table 10-1.

**Table 10-1. Coordinating Investigator and Medical Monitor**

| Coordinating Investigators | Medical Monitor |
|---|---|
| Yuh-Chin T. Huang, MD., MHS.<br>Professor of Medicine, Pulmonary and Critical Care Medicine<br>Duke University Medical Center | Carlos Guardia, MD<br>Senior Medical Director<br>Windtree Therapeutics, Inc.~ |

### 10.1.6 Safety Oversight

Safety oversight will be under the direction of the medical monitor, the principal investigators, and a data monitoring committee (DMC). Given the open-label, single-treatment of this study, real time safety assessments of treated subjects will be done by the sponsor and principal investigators.

#### *10.1.6.1 Data Monitoring Committee*

The DMC, made up of at least 3 experts in the field of infectious diseases, will 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.

During the treatment and post-dosing phases, the chair of the DMC will receive regular reports on SAEs and DMC members will be periodically updated on the program and trial status. An ad hoc meeting of the DMC will occur if the chair deems it necessary to address safety in the study. The DMC may recommend that study enrollment be suspended if safety concerns are identified or suspected.

#### *10.1.6.2 Safety Reviews*

The DMC will meet after every 6 subjects have been enrolled and dosed. The DMC will continue to meet after every 6 enrollments and these reviews will continue until enrollment and dosing is complete.

The DMC review may consist of the evaluation of (1) demographics and baseline, (2) all AEs, especially peri-dosing AEs and AEs of special interest, (3) case reviews of subjects with reported SAEs, (4) summary tables of all safety endpoints, and (5) any other available relevant data.

Following a meeting, the DMC will provide timely recommendations to the Windtree study team. Recommendations may consist of, but not be limited to the following:

- Continue the study as planned
- Suspend study enrollment, pending additional information
- Close study enrollment

An independent statistician, not a member of the committee, will be responsible for the statistical analysis of the data.

### 10.1.7 Clinical Monitoring

Windtree or their designee will perform on-site or remote monitoring visits as frequently as it is deemed necessary to ensure quality study data capture and accurate adherence to the study protocol as outlined in the Clinical Monitoring Plan (CMP). It is also necessary to ensure that the rights and well-being of trial participants are protected, and that the conduct of the trial is in compliance with International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) and with applicable regulatory requirements.

Monitoring will be performed as in-person/remote visits and centralized review of records. In-person/remote visits will include source data verification to the eCRF; some source data verification will be performed unless otherwise specified in the CMP.

Before enrollment, a clinical site monitor will complete a site initiation visit (SIV) at each study site. During SIVs, clinical site monitors will provide study training to site staff, ensure study drug storage is in accordance with the study protocol, and validate all other study requirements in accordance with the study monitoring plan.

Clinical site monitors will schedule a study site visit as close as possible to the time of each site's first enrolled study subject; periodic follow-up monitoring visits will ensure on a regularly scheduled basis throughout the study, in accordance with enrollment at the study site. At these visits, the clinical site monitor will compare the data entered into the eCRF with the hospital or clinic records (source documents) and check for protocol compliance. Documentation reviews will include but not be limited to the evaluation and confirmation of the following:

1. A record of informed consent.
2. Adherence to enrollment criteria.
3. Completion of all required study assessments.
4. Accurate and complete data capture of all AEs, concomitant medications, and safety and efficacy observations.
5. Study drug storage and dispensing records maintained in accordance with study and regulatory requirements.

Findings from these reviews will be discussed with the PI and study site staff.

The dates of the monitoring visits will be recorded by the clinical site monitor in a sign-in log to be kept at the site. The study coordinator and PI are expected to be available for questions, have all source documentation readily available, and have a suitable environment provided for review of study-related documents.

In accordance with ICH GCP, Windtree will select (either directly or through a subcontract with a company specifically trained in the monitoring of clinical studies), qualified individuals to monitor study sites to ensure the quality of study progress and the close adherence of study sites to the study protocol and all related governing documents and SOPs.

1. The clinical site monitor(s), before the initiation of each study site, will ensure each investigator and study staff understands the following:
   a) The investigational status of the study drug, placebo and the requirements for its accountability.
   b) The need to uphold all directives within the clinical protocol as it relates to study conduct and subject safety at the study site.
   c) The obligation to obtain informed consent in accordance with the Declaration of Helsinki and ICH GCP guidelines before enrolling each subject in the study.
   d) The obligation to obtain IRB/IEC review and approval of the study before study initiation at his/her clinical site, and ensure timely updates to the IRB/IEC as mandated by local and national regulatory requirements, and to ensure timely communications to Windtree of all IRB/IEC communications (to include reviews and subsequent actions) concerning the study.
2. The clinical site monitor(s) will perform periodic visits to each clinical site during the course of the study to ensure the study protocol is being followed and that:
   a) Drug and placebo inventories are being properly maintained and documentation of vial usage is accurate and complete.
   b) The assignment of responsibilities log is up-to-date and that changes in personnel are reported to Windtree.
   c) The PI is reporting all serious or fatal AEs (Section 8.4.6) as soon as possible, and in no case later than 24 hours after the event, to the medical monitor or designee at Windtree.
3. The clinical site monitor(s) will perform an end-of-study visit to each clinical site to ensure that:
   a) All drug reconciliation forms, as provided in the study manual, are accurate and complete.
   b) All used and unused vials of study drug have been reconciled.
   c) All eCRFs are complete and all monitoring of eCRFs has been completed.

Details of clinical site monitoring are documented in a CMP. The CMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.

### 10.1.8 Quality Assurance and Quality Control

Each clinical site will perform internal quality management of study conduct, data collection, and documentation. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution. Following written SOPs, the monitors will verify that the clinical trial is conducted and data are collected, documented (recorded), and reported in compliance with the protocol, ICH GCP, and applicable regulatory requirements.

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

### 10.1.9 Data Handling and Record Keeping

#### *10.1.9.1 Data Collection and Management Responsibilities*

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets may be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents. Study personnel at each site will enter data from source documents into the protocol-specific eCRF within 5 days of the information becoming available. Subjects will not be identified by name in the study database or on any study documents to be collected by Windtree (or designee) but will instead be identified by a site and subject number.

Clinical data (including AEs, concomitant medications, and expected adverse reactions data) will be entered into a 21 CFR Part 11-compliant data capture system. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate.

Members of Windtree's Data Management department are responsible for data processing, in accordance with procedural documentation and a study specific Data Management Plan (DMP). Windtree's Data Management will also provide eCRF Completion Guidelines; extra training will be provided to the study sites when necessary.

Clinical data will be entered directly from the source documents. 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. Corrections for an existing eCRF record will automatically be recorded by the eCRF system (audit trail capturing the time, date, and the identification of the user who entered or updated eCRF data). Recorded corrections by the eCRF will create an electronic audit trail of study documentation.

Database lock will occur once all quality assurance procedures have been completed; this will include but not be limited to the following: (1) all site-based study data have been entered into the eCRF, (2) all entered data have been reconciled and reviewed by Windtree or designee, and (3) all data related queries have been rendered and resolved.

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.

#### *10.1.9.2 Study Records Retention*

All records that support data entered into the eCRF of each subject must be retained in the files of the PI or the institution/site for a minimum of 2 years (3 years for ICF) following notification by Windtree that all investigations have been discontinued or that the last approval of a marketing application has been obtained. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of Windtree. It is the responsibility of Windtree to inform the investigator when these documents no longer need to be retained.

Supporting documents will include but not be limited to the following:

1. Copies of eCRFs (given to the site on a CD)
2. All original source documents; these may include but not be limited to the following:
    a) ICFs
    b) laboratory reports
    c) progress notes
    d) medical histories
    e) physical and diagnostic findings
    f) diagnoses
    g) dates of therapy before and during this study
    h) drug dispensing/disposition records

If the PI retires, relocates, or for other reason withdraws from the responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility. Windtree must be notified in writing of the name and address of the new custodian.

### 10.1.10 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, ICH GCP, or Study Manual requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

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

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations within 10 working days of identification of the protocol deviation, or within 10 working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents and reported to the site monitor. Protocol deviations must be sent to the reviewing IRB/IEC per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB/EC requirements. Further details about the handling of protocol deviations will be addressed in the protocol deviation process.

### 10.1.11 Publication and Data Sharing Policy

The preparation and submittal for publication of a manuscript containing the study results shall be in accordance with a process determined by a mutual written agreement among Windtree and participating institutions.

The publication or presentation of any study results shall comply with all applicable privacy laws, including but not limited to HIPAA. This trial will be registered at ClinicalTrials.gov and the International Clinical Trial Registration Platform (ICTRP), and results information from this trial will be submitted to both. In addition, every attempt will be made to publish results in peer-reviewed journals.

### 10.1.12 Conflict of Interest Policy

The independence of this study from any actual or perceived influence is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

The FDA has issued regulations (21 CFR Part 54, *Financial Disclosure by Clinical Investigators*) that require sponsors to submit complete and accurate certification or disclosure statements to certify the absence of certain financial interests of clinical investigators, when clinical studies are submitted to FDA in support of marketing approval. These regulations are intended to ensure that financial interests and arrangements of clinical investigators, that could affect reliability of data submitted to FDA in support of marketing approval, are identified and disclosed by the sponsor.

Clinical investigators will be asked to disclose proprietary (eg, patent, licensing agreement) and financial (eg, stock options, royalty) interests as they pertain to Windtree, before participating in the study. In addition, clinical investigators will be required to consult with Windtree before acquiring any financial interest in the company and must disclose any change in their proprietary or financial interests, if it occurs during the course of the study and for 1 year following study completion. The requirement for proprietary and financial disclosure also includes any ownership by the spouse or any dependent subject of the clinical investigator.

If FDA determines that the financial interests of any clinical investigator raise serious questions about the integrity of the data, FDA will take any action it deems necessary to ensure the reliability of the data, including:

1. Initiating agency audits of the data derived from the clinical investigator in question.

2. Requesting that the sponsor submit further data analyses (eg, to evaluate the effect of the clinical investigator's data on overall study outcome).

3. Requesting that the applicant conduct additional independent studies to confirm the results of the questioned study.

4. Refusing to treat the covered clinical study as providing data that can be the basis for an agency action.

If the sponsor does not include certification or disclosure, or both (as required), or does not certify that it was not possible to obtain the information, the FDA may refuse to file the New Drug Application (NDA).

### 10.2 List of Abbreviations

| Abbreviation | Description |
|---|---|
| **β-hCG** | Serum pregnancy test |
| **A** | A wave: peak velocity flow in late diastole caused by contraction |
| **AE** | Adverse event |
| **CFR** | Code of Federal Regulations |
| **CONSORT** | Consolidated standards of reporting trials |
| **CRF/eCRF** | Case report form/electronic CRF |
| **CS** | Cardiogenic shock |
| **DBP** | Diastolic blood pressure |
| **ECG** | Electrocardiogram |
| **FDA** | Food and Drug Administration |
| **GCP** | Good Clinical Practice |
| **HF/AHF** | Heart failure/acute heart failure |
| **HIPAA** | Health Insurance Portability and Accountability Act |
| **E** | E wave: peak velocity blood flow from LV relaxation in early diastole |
| **E/Ea** | Mitral Doppler inflow E velocity to annular tissue Doppler Ea wave velocity |
| **ERO** | Effective regurgitant orifice |
| **eGFR** | Glomerular filtration rate |
| **ICF** | Informed consent form |
| **ICH** | International Conference on Harmonisation |
| **ICU** | Intensive care unit |

| Abbreviation | Description |
|---|---|
| **IEC** | Independent ethics committee |
| **IRB** | Institutional review board |
| **IND** | Investigational new drug |
| **ITT** | Intent-to-Treat |
| **IWRS** | Interactive web response system |
| **LV** | Left ventricular |
| **MAP** | Mean arterial pressure |
| **NDA** | New drug application |
| **PAP** | Pulmonary artery pressure |
| **$P_{aw}$** | Mean airway pressure |
| **PI** | Principal Investigator |
| **PK** | Pharmacokinetics |
| **SAE** | Serious adverse event |
| **SBP** | Systolic blood pressure |
| **SIV** | Site initiation visit |
| **TAPSE** | Tricuspid annular plane systolic excursion |
| **UP** | Unanticipated problem |
| **US** | United States |
| **WHF** | Worsening heart failure |

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