

**Treprostinil Sodium Inhalation for Patients At-High Risk for ARDS:
Effect on Oxygenation and Disease-related Biomarkers**

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Treprostinil Sodium Inhalation for Patients At-High Risk for ARDS: Effect on Oxygenation and Disease-related Biomarkers.

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PART 1: Study Description

Title: Treprostinil Sodium Inhalation for Patients At-High Risk for ARDS: Effect on Oxygenation and Disease-related Biomarkers.

Rationale:

ARDS is defined by acute hypoxemia, respiratory failure and the presence of bilateral lung infiltrates. ARDS is a syndrome of inflammation and increased permeability that may coexist with left atrial or pulmonary capillary hypertension. Despite progress in elucidation of underlying mechanisms, and critical care treatment protocols, there is no pharmacological therapy that has had a major impact on disease progression, morbidity or mortality (Adhikari 2004).

Several recent trials in ARDS/ALI (Acute Lung Injury) have generated interest in the use of Prostacyclin (PGI₂) and prostacyclin analogs in improving oxygenation in ARDS/ALI. PGI₂ is an arachidonic acid metabolite naturally produced in the lung by endothelial cells, dendritic cells, smooth muscle cells and fibroblasts. PGI₂ is a potent selective pulmonary vasodilator and inhibitor of platelet aggregation. PGI₂ binds to the IP receptor which activates G_s and increases intracellular cAMP thus activating protein kinase A and subsequent increase in protein phosphorylation. The cellular effects include smooth muscle relaxation and inhibition of cell migration (Dorris 2012). A second IP receptor has been proposed that mediates the inhibition of cytokine release from epithelial cells (Wilson 2011). PGI₂ activated IP receptors can be found on human macrophages, pneumocytes, smooth muscle cells, fibroblasts, platelets, eosinophils and neutrophils (Dorris 2012). PGI₂ can also activate the peroxisome proliferator-activated receptor γ (PPAR γ) resulting in up-regulation or down-regulation of miRNAs potentially playing a role in apoptosis and vascular inflammation (Mohite 2011). A direct comparison of the receptor binding profiles of 2 relatively stable PGI₂ analogs (Iloprost and Treprostinil) identified several differences; treprostinil had a higher affinity for the prostaglandin D receptor and the prostaglandin E₂ receptor with less affinity at the prostaglandin E₁ receptor (Whittle 2012). The prostaglandin E₁ receptor activation is generally associated with vasoconstriction. It is unknown if these differences will translate into differences observed in the clinical activity of both compounds.

PGI₂ has broad anti-inflammatory activity. Treprostinil has been reported to inhibit the production of TNF α , IL-1 β , IL-6 and GM-CSF in human alveolar macrophages by blocking the translocation of activated NF κ B to the cell nucleus (Raychaudhuri 2002). Endothelial cell integrity is also critical to the pathology in ARDS/ALI. Both prostaglandin E₂ and the prostacyclin analog Beraprost increased transendothelial electrical resistance and decreased

dextran permeability in epithelial cell cultures in vitro and decreased high tidal volume mechanical ventilation injury in mice (Birukova 2007). Treprostinil can inhibit fibroblast adhesion and differentiation through increased intracellular cAMP, which could be beneficial in the latter proliferative phases of ALI (Nikam 2011). In vivo, Treprostinil inhibited vascular recruitment of fibrocytes and improved pulmonary hypertension in chronic hypoxia-induced pulmonary hypertension in mice (Nikam 2010).

Several small clinical trials have been conducted with prostacyclin analogues in ALI / ARDS. Zwissler et al. compared inhaled nitric oxide (NO) vs. the prostacyclin analog epoprostenol in 8 patients with ARDS using a cross-over design (Zwissler 1996). Epoprostenol caused a dose-dependent decrease in pulmonary artery pressure (PAP) from 35.1 to 29.6 mm Hg at the highest dose as compared with inhaled NO which decreased PAP from 34.5 to 31.8 mm Hg. INO was only effective at the 2 highest doses. PaO₂ increased 24% after the highest dose of epoprostenol and PaO₂/FiO₂ ratio increased from 105 to 131. There were no changes in Paco₂, mean arterial BP, or heart rate with epoprostenol. A second study comparing INO with aerosolized epoprostenol in 16 patients with ARDS utilized an optimized dose per patient to obtain a maximum increase in arterial oxygen (Walmrath 1996). INO and epoprostenol significantly increased PaO₂/FiO₂ by 29 and 21 mmHg respectively. The increase in PaO₂ was similar for both agents, with no change in Paco₂, or mean systemic arterial pressure. Both agents decreased % shunt, but only epoprostenol decreased pulmonary artery pressure.

In a study similar to that of Walmrath (above), Domenighetti (Domenighetti 2001) also titrated Flolan for optimum oxygenation using an ultrasonic nebulized added to the inspiratory arm of the ventilator. In this study, subjects were separated by their etiology; ARDS originating from pulmonary versus extrapulmonary disease. Patients were considered responders if PGI₂ increased PaO₂ ≥ 7.5 torr or PaO₂/FiO₂ increased ≥ 10%. Eight of the nine subjects with ARDS secondary to pulmonary disease responded, whereas none of the ARDS subjects defined by a pulmonary insult responded. In the pulmonary group, PaO₂/FiO₂ decreased from 146 ± 16 to 135 ± 17, and PaO₂ decreased from 87 to 79. Hemodynamic and other gas exchange variables (mean systemic arterial pressure, cardiac index, oxygen delivery, total systemic resistance, mean right atrial pressure, mixed venous blood gas) were minimally affected.

The effect of Flolan on the PaO₂/FiO₂ ratio is in contrast to the results reported by Walmrath, although in their study subjects were not subset by etiology of the ARDS. One possible explanation is that the pulmonary ARDS subjects in the study reported by Domenighetti had significantly lower mean CT density scores (HU) than the non-pulmonary group indicating more consolidation of the lung and may represent more severe lung pathology. Walmrath had previously demonstrated that aerosolized Flolan was effective in improving gas exchange in

patients with acute respiratory failure linked to pneumonia (Walrath 1995) suggesting that the etiology may not be the only cause of the lack of response in the Domenighetti study.

Aerosolized Iloprost was studied in 20 patients with ARDS and PH (PA > 25 mm Hg). The mean PaO₂/FiO₂ at baseline was 191. The dose of Iloprost was 10 µg administered in 1 ml of saline over 5 minutes for an estimated dose delivered to the lung of 2.5 µg. A second dose of 20 µg was administered 30 minutes after the first dose. Iloprost increased PaO₂ from 82 to 100 mm Hg which lasted for 2 hours. Only 4 subjects increase PaO₂ >10 mm Hg following the second dose. PaO₂/FiO₂ also improved. There were no significant changes in systemic hemodynamics.

A case report of 3 children with ARDS treated with aerosolized Flolan was reported (Pappert 1995). Patients were treated with 3 doses of Flolan (2, 10 and 20 ng/kg/min) for 30 minutes from a nebulizer inserted into the inspiratory arm of the ventilator. In 2 of the 3 children the 10ng/kg/min dose caused an increase in the PaO₂/FiO₂ ratio, but a decrease in the third child. The increase in PaO₂/FiO₂ observed in the 2 children was accompanied with a reduction in intrapulmonary right-to-left shunt (Q_s/Q_t), but an increase in Q_s/Q_t in the third child. Only small changes in mean arterial pressure were reported and no change in PaCO₂. The etiology of ARDS in the children who responded was EBV-pneumonia with aspergillosis and reoperation for an ependymoma of the fourth ventricle. The patient who did not improve oxygenation developed ARDS following drowning and cardiac arrest. All subjects had been on ventilator support for approximately 40 days.

A randomized controlled trial of aerosolized prostacyclin was conducted in children with ALI (Dahlem 2004). This study was the only trial which met the criteria for inclusion in a Cochrane Review of Aerosolized prostacyclin for ALI and ARDS (Afshari 2013). Patients were randomized to either saline or epoprostenol in a cross-over design. Fifteen children with ALI were treated with aerosolized epoprostenol up to a dose of 50ng/kg/min using an ultrasonic nebulizer. Oxygenation index (mean airway pressure x 100x PaO₂/FiO₂) improved by 26% without an effect on hemodynamic measures. Not all children responded; 8/14 had a ≥ 20% improvement in oxygenation index. Long-term outcomes were not reported.

The pharmacologic profile and early beneficial short-term effects of PGI₂ forms the basis for additional clinical trials of PGI₂ in ALI. To date, all studies have focused on short-term improvements in oxygenation. There are no repeat dosing studies of PGI₂ in ALI that utilize additional end points such as longer-term improvement in gas exchange, disease progression or ventilator free days.

Objectives:

- 1) To assess the feasibility of a randomized trial of treprostinil inhalation in patients with acute hypoxemic respiratory failure
- 2) To evaluate the tolerability of inhaled treprostinil for patients with acute hypoxemic respiratory failure
- 3) To assess the effect of treprostinil inhalation on oxygenation in patients with acute hypoxic respiratory failure with, or at risk for, development of ARDS
- 4) To assess the effect of treprostinil inhalation on various biomarkers thought to be related to the pathogenesis and/or clinical course of ARDS.

We propose a proof of concept, double blinded, placebo controlled study of inhaled treprostinil for patients with “at-risk” or early ARDS. Early, or pre-ARDS will be defined as acute hypoxemia and unilateral pulmonary infiltrates in patients presenting with conditions that are conducive to ARDS (e.g. pneumonia, sepsis, aspiration, trauma) but do not yet meet criteria for ARDS: require mechanical ventilation, bilateral infiltrates on CXR, and $\text{PaO}_2/\text{FiO}_2$ ratio < 300 . The primary outcome will be $\text{PaO}_2/\text{FiO}_2$ ratio. We will also measure other physiologic and clinical outcomes and levels of inflammatory and pro-fibrotic biomarkers. This exploratory initial study will enroll 30 patients to assess feasibility and safety in this population.

Hypothesis:

1. Treprostinil inhalation is safe at planned dose titration in patients with acute hypoxemic respiratory failure
2. Treprostinil will improve oxygenation and other secondary outcomes related to mechanical ventilation initiation and duration, as well as exhibit effects on ARDS-related pro-inflammatory and pro-fibrotic biomarkers.

Study Design:

- 1) Single Center, proof of concept, 2:1 active drug to placebo controlled, double-blinded
- 2) Target of 30 patients enrolled
- 3) Patients will be randomized to receive either treprostinil or saline inhalation placebo
- 4) Patients will be followed to hospital discharge or day 28, whichever comes first. Those discharged prior to day 28 will be assumed free of mechanical ventilation and alive unless readmitted in that period. This will be confirmed by phone.

Sample Size/Interim Monitoring:

- 1) This is primarily a feasibility, safety and proof of concept trial.

- 2) The primary endpoint is Change in PaO₂/FiO₂ ratio from Study Day 0 to 2, 7
- 3) Trial progress will be monitored by an independent Data and Safety Monitoring Board to determine if the study should stop for safety reasons. The first interim review will occur 6 months following enrollment of the first patient or after the first 15 patients (whichever occurs first) have been enrolled.

Inclusion Criteria:

- 1) Adults age 18-80 years.
- 2) Acute onset need for 4 LPM or more of supplemental oxygen to maintain PaO₂ > 60 mmHg or arterial O₂ saturation > 90% by pulse oximetry.
- 3) Acute unilateral pulmonary infiltrate/s on chest radiograph with no clinical evidence of left-sided heart failure. Bilateral infiltrates are acceptable as long as all other inclusion/exclusion criteria are met.

“Acute onset” is defined as follows: the duration of the hypoxemia criterion (#2) must be less than 96 hours and the chest radiograph criterion (#3) must be ≤ 28 days at the time of randomization. We acknowledge there is some difficulty determining absolute acuity with a 28 day window for the chest radiograph criterion, but this criterion is consistent with multiple NHLBI ARDSnet studies to date.

Patients must still meet inclusion criteria at the time of informed consent and randomization. Randomization is defined as the time that the IDS is contacted and informed about stratification of non-trauma and trauma patients. Patients that no longer meet eligibility criteria at the time of randomization are eligible to be re-screened if they meet inclusion criteria eligibility again.

Exclusion Criteria:

- 1) No consent/inability to obtain consent
- 2) Presence of pulmonary embolism
- 3) Known diffuse alveolar hemorrhage from vasculitis
- 4) Known pre-existing severe obstructive or restrictive lung disease (FEV₁ < 40% predicted, TLC < 50 % predicted) or need for long-term supplemental oxygen therapy
- 5) Known significant left ventricular systolic dysfunction with LVEF < 40% on echocardiogram.
- 6) Mean arterial pressure < 65 mmHg
- 7) Need for norepinephrine or dopamine dose > 12 mcg to maintain MAP > 65 mmHg
- 8) Severe chronic liver disease (Child-Pugh Score 11-15)

Measure	1 point	2 points	3 points	Units
<i>Bilirubin (total)</i>	<34 (<2)	34-50 (2-3)	>50 (>3)	μmol/l (mg/dl)
<i>Serum Albumin</i>	>35	28-35	<28	g/l
<i>INR</i>	<1.7	1.71-2.20	> 2.20	<i>no unit</i>
<i>Ascites</i>	None	Suppressed with medication	Refractory	<i>no unit</i>
<i>Hepatic Encephalopathy</i>	None	Grade I-II (or suppressed with medication)	Grade III-IV (or refractory)	<i>no unit</i>

- 9) Moribund patient not expected to survive 24 hours
- 10) QTc interval > 550 ms on screening bedside strip or electrocardiogram
- 11) Pregnancy or breast feeding (Women of childbearing potential, defined as < 60 years of age, will require pregnancy testing.)
- 12) Burns > 40% total body surface
- 13) Acute Neurological Disease (that may impair the ability to ventilate without assistance)
- 14) Imminent need for intubation or non-invasive ventilation (patients that are requiring non-invasive ventilation solely for Obstructive Sleep Apnea are not excluded) (A high flow nasal cannula flow rate greater than 50LPM will be considered as an imminent need for positive pressure as well.)
- 15) Patient has comfort care orders written or otherwise will not receive supplemental oxygen including High Flow Nasal Cannula Oxygen ~~Patient is Do Not Resuscitate/Do Not Intubate~~
- 16) Patient has a tracheotomy
- 17) Patient is currently receiving prostacyclin therapy [Epoprostenol (Flolan or Veltri) Iloprost (Ventavis) Treprostinil (Orenitram, oral) (Remodulin, IV or SC)]
- 18) Patient has a language barrier
- 19) Patient is unable to complete the breathing maneuver

Rationale for Exclusions:

Patients less than 18 years old are excluded because of limited clinical trial data with treprostinil in subjects younger than 18 years. Criteria 4, 8, 9, 13, 14 exclude patients unlikely to survive to the primary study endpoint or whose underlying condition or ventilator management complicates

assessment of the endpoint of Ventilator Free Days (VFDs). Criterion 6 and 7 excludes patients that may have worsening of systemic hypotension due to additional effects of treprostinil. Patients with diffuse alveolar hemorrhage (criterion 3) are excluded because the mechanism of lung injury is different from ARDS/ALI due to infection. Patients with left ventricular systolic dysfunction (criterion 5) are excluded because distinguishing ARDS/ALI from pulmonary edema may be difficult and treprostinil may worsen left heart failure. For exclusion criterion 14: The recently updated clinical protocol for high flow nasal cannula use encourages flow rates beginning at 20 LPM up to 50 LPM. Invasive or non-invasive ventilation are the next options for oxygen requirements beyond 50 LPM. For exclusion criterion 15: Randomized patients are withdrawn from treatment if they require positive pressure ventilation in the form of Bi-level Positive Airway Pressure (BiPAP) or Continuous Positive Airway Pressure (CPAP), or intubation and invasive mechanical ventilation. They will receive supplemental oxygen as needed by the study protocol until that point. Unless they have comfort care only orders written, patients with pneumonia or early ARDS who are DNR receive all forms of supplemental oxygen. Therefore there is nothing in the protocol that precludes a patient who is DNR from participating. If the study drug shows a beneficial effect, the benefit would be relevant to patients who are DNR. Patients with a tracheostomy (criterion 16) would be unable to use the inhalation system. Patients that are currently receiving prostacyclin therapy (criterion 17) should be excluded to avoid the possibility of receiving excess prostacyclin therapy. Patients with a language barrier may have difficulty with instructions on how to use the inhalation device and with completing the follow up phone call. For exclusion criterion 19: Some patients with altered mental status may not be alert enough to complete the breathing pattern maneuver.

Enrollment, Randomization, and Study Initiation Time Window:

All inclusion criteria must be present concurrently. Patients must be enrolled within 24 hours of all inclusion criteria being met and identified. Information for determining when these time window criteria were met must be clearly documented.

Screening:

Patients will be identified by reviewing the emergency department (ED), or any UNC Hospitals Medical Service in EPIC. We will review each patient's EPIC ED/hospital encounter to see if they meet inclusion criteria.

Research coordinators will begin screening for eligible patients that come through the ED, or any UNC Hospitals Medical Service. If a patient is consented and enrolled, study drug administration could begin while the patient is still in the ED. However, depending on the patient's status once they are discharged from the ED, patients may go to the ICU or to a floor bed. Therefore, study drug administration could be given on the floor or in the ICU. The admitting diagnosis will determine where a patient will go once discharged from the ED. Therefore we could possibly be conducting this study in any of our adult ICUs or any of our floor beds.

Informed Consent:

Informed consent will be obtained from each patient or surrogate before enrollment in the trial. No study procedures will be conducted before obtaining informed consent. For surrogates that are not present during the screening period of a patient that is not at decision-making capacity, we will contact by phone to obtain verbal consent.

Randomization:

After informed consent is given, a study coordinator will contact the investigational drug service pharmacy and inform them about enrollment and about the stratification. An assignment will be made by a randomization table to administer either treprostinil therapy or placebo. The paper system will be structured to maintain 2:1 active drug to placebo randomization. Randomization will be stratified by trauma and non-trauma diagnosis since the pathophysiology and outcomes of acute lung injury in trauma patients are different than for other etiologies.

Justification for Placebo:

There is currently no approved PGI₂ agent for the prevention of ARDS. The need for a placebo arm in this study is necessary to compare the difference in PaO₂/FiO₂ ratios from patients that receive treprostinil.

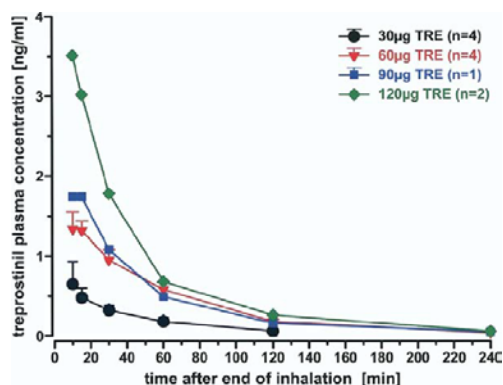
Minorities and Women:

No gender or racial preferences will be utilized in the enrollment of patients. As this is a single-center study, we cannot ensure proportioning of enrollment based on gender or race as would be more likely in a multi-center study. The demographic profile of UNC Hospitals' patients is in general what will be represented. Pregnant women will be excluded because of the lack of significant safety data for treprostinil use during pregnancy.

PART 2: Study Procedures

Dose Rationale

TYVASO (Treprostinil inhalation solution) is approved for the treatment of pulmonary arterial hypertension. The recommended starting treatment regimen is 3 breaths (6 µg/breath, total of 18 µg). If tolerated, doses should be increased by 3 breaths per treatment at 1-2 week intervals until a target maintenance dose of 9 breaths (54 µg) is reached. The TYVASO inhalation system (TD-100) is recommended. The dose and regimen of TYVASO inhalation solution proposed in this study differs in several ways. First the maximum dose is set at 72 µg (12 breaths). Maximum



plasma concentrations (C_{max}) following inhalation of 30, 60, 90 and 120 µg were reported to be 0.65 ± 0.28 ng/ml, 1.59 ± 0.17 ng/ml, 1.74 ng/ml (n=1) and 3.51 ± 1.04 ng/ml respectively (Below; Voswinckel 2006). Plasma concentrations peaked at 10-15 minutes and declined rapidly over the first 60 min. Arterial oxygenation in all 3 subjects was significantly decreased at the 120 µg dose and a severe headache was reported in one of the subjects. All doses had a similar effect on pulmonary vascular resistance, but the duration of effect was longer for the 60 and 90 µg doses. At all doses, treprostinil had minor effects on mean systemic arterial pressure. Gas exchange was unaffected at doses up to 90 µg. Continuous infusion of 15 ng/kg/min resulted in a steady state plasma concentration of 1.56 ng/ml (Wade 2004). Thus the proposed maximum dose for this study (72 µg) was chosen to be below doses known to be associated with adverse effects on gas exchange, and providing a duration of action consistent with Q4 hour dosing to maintain plasma concentrations resulting in a pharmacodynamic effect on pulmonary vascular resistance. Plasma concentrations that could be effective for inhibition of progression of acute lung injury are unknown.

A second difference from the recommended treatment is the dose escalation protocol. We are proposing that the dose be increased from a starting dose of 36 µg (6 breaths) by 3 breaths every 2 doses, such that a maximum of 72 µg (12 breaths) is achieved within the first 20 hours of treatment. Escalation will be dependent on subject tolerability and the absence of significant arterial oxygen desaturation. In long-term open-label studies, 89% of patients achieved a target dose of 9 breaths and 42% achieved a dose of 12 breaths (Package Insert). Because of the rapidly progressing nature of the disease, an accelerated dose escalation protocol was chosen. Voswinckel et al. (2006) administered single doses up to 120 µg over a 6 minute inhalation time period. Doses below 120 µg were without significant systemic effects. Thus a rapid dose escalation protocol appears to be well tolerated.

Limited experience in other diseases exists and the safety in subjects with other lung diseases has not been established. Plasma drug concentrations will be determined to assess changes in pharmacokinetics of Treprostinil in these subjects. To avoid rapid drug withdrawal a step-down protocol will be followed.

Pharmacokinetics and Drug Interactions

Few pharmacodynamics / pharmacokinetic drug interaction studies have been completed. Pharmacokinetic studies with Sildenafil (Viagra) and Bosentan (Tracleer) demonstrated no interactions. Treprostinil is metabolized by the P450 cytochrome CYP2C8. Co-administration of the enzyme inhibitor gemfibrozil (Lopid) increased exposure (C_{max} and AUC) to oral treprostinil and conversely the CYP2C8 induced Rifampin (Rifadin) decreased exposure. Trimethoprim (Septra) is also a modest inhibitor of CYP2C8, although no interactions with Treprostinil have been reported. It is not known if similar effects are observed following inhaled Treprostinil.

Approximately 4% of Treprostinil is excreted unchanged by the kidneys. Therefore patients with hepatic or renal insufficiency are at risk of increased systemic exposure (Peterson 2013).

Plasma drug concentrations will be determined to assess changes in pharmacokinetics of Treprostinil in these subjects.

We will also obtain a 4ml K₃EDTA blood sample just prior to the Dose 1 drug administration on Day 3 to assess our trough level and another 4ml K₃EDTA blood sample on Day 3 at 15 min (\pm 5 min) after the Dose 1 drug administration to assess our peak level. In total, for the whole study duration, the maximum amount of blood that could be collected for the purpose of this study and in addition to the usual routine of medical care of care blood drawn is about 3.5 tablespoons. Peak and trough levels can also be obtained +/- one day to accommodate potential weekend blood draws.

Treprostinil or Placebo Administration:

Subjects will be treated using the Tyvaso® TD-100 Inhalation System. Oxygen will be delivered by nasal cannula or high flow nasal oxygen delivery system. Patients will be managed by an Oxygen Titration Protocol (See Appendix 3). Ampules of either active drug or saline (placebo) will be stored in blinded foil packs prepared and dispensed from Investigational Drug Services. Each will be labeled with a specific identifier that correlates with the randomization scheme. Once randomization occurs, the study coordinator will obtain the appropriate foil pack from IDS. The respiratory therapist will load the ampule into the TD-100 system. Remaining ampules will be stored in the foil pack to protect from light. The respiratory therapist will oversee the subject's administration of the drug, including the counting of the number of breaths taken. One ampule of drug will deliver 24 hours of medication or placebo. Ampules will be replaced every 24-hour period. On Study Day 00, during the Dose Escalation Phase, a blood pressure will be checked prior to Dose 1 and within 30 minutes after; prior to Dose 3 and within 30 minutes after; prior to Dose 5 and within 30 minutes after, or after a different dose interval, if the study drug had to be maintained due to an adverse effect. Blood pressure measurements for Study Days 01 through 11 will be captured from the nursing flow-sheets. The coordinators should document the blood pressure that was recorded as close as possible prior to the Dose 1 time frame for each study day. Arterial blood gas, systemic BP measurements, and central venous O₂ saturation (if a CVC is in place) will be checked according to the schedule below (Table 2). Biomarker levels will be checked on Day 0, 3 and 7 as per Table 2.

NOTE: A study day is defined as 24hrs from Randomization, not calendar days. The study calendar window will start with Day 0.

The following dosing schedule will be utilized*:

Dosing Schedule

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Protocol 1.06 (Modification)

Time following Randomization	Number of Breaths
Dose Escalation Phase	
T= 0 Initial Dose	6
T= 4 hours	6
T= 8 hours	9
T= 12 Hours	9
T= 16 hours	12
Maintenance Dose Phase	
T= 20 hours Thru Day 7	12
Taper Down Phase	
Day 8 q 6 hours	12
Day 9 q 6 hours	6
Day 10 q 6 hours	4
Day 11 q 6 hours	2
Day 12 Off Drug	

If an increase in dose (during the Dose Escalation Phase) is not tolerated due to worsening hypoxemia, hypotension, or excessive coughing, the subject will be maintained at the previous dose for an additional 4 hours (unless major adverse events leading to premature withdrawal are noted as defined below). If a dose increase (during the Dose Escalation Phase) is again not tolerated the subject will remain on the highest tolerated dose for the remainder of the 7 day treatment period. During the Dose Escalation Phase, a sustained decrease of mean arterial blood pressure (MAP) by 15% or below MAP 65 will result in a decrease of dose to the previous tolerated level. Transient worsening hypoxemia is defined as a decrease in SPO_2 of 7% or more within 15 minutes of treatment for no longer than 15 minutes not associated with coughing or exertion.

If subject develops worsening oxygenation (as defined by need to increase supplemental oxygen to $> 0.50 FiO_2$) at any point in the Taper Down Phase, then dose should be increased to previously tolerated dose that resulted in acceptable oxygenation. Subject should be maintained at that dose for 24 hours and attempt to taper drug downward again should be made. If subject still cannot be tapered downward, then subject will transition out of the trial and consideration given to transition to inhaled Iloprost (UNC Hospital formulary inhaled prostacyclin) or epoprostenol.

The Taper Down Phase will begin prior to Day 8 for patients who are able to maintain an arterial oxygen saturation of $>90\%$ on room air or for patients for who discharge to home on oxygen is anticipated. If there is a plan to discharge to home, the patient's current dose of inhaled

treprostinil or placebo will be reduced by one-half on the day prior to anticipated discharge, and then discontinued on the day of discharge home.

Cough or pharyngitis may occur with the administration of inhaled treprostinil. It is usually mild. The investigator may choose to utilize conservative measures such as topical throat lozenges/spray or non-narcotic based antitussive medications to improve compliance with drug administration.

All enrolled patients will receive standard medical care for their acute presenting illness regardless of randomization group, including but not limited to: antibiotics, IV fluids, supplemental oxygen, and any other needed diagnostic testing or imaging.

***Premature Withdrawal from Treatment:**

The following adverse effects should prompt cessation of study drug:

1. A drop in oxygen saturation by ≥ 10 percentage points, to an absolute value of $< 90\%$, within 15 minutes of drug administration, regardless of apparent relationship to coughing.
2. Severe, intractable cough resulting in worsening hypoxemia and prolonged increase in O_2 requirement (Defined as an increase of more than 2LPM O_2 that closely correlates temporarily with the cough.)
3. Systemic hypotension requiring increase in vasopressor dose or initiation of vasopressors due solely to the administration of study drug (i.e. not due to underlying illness such as septic shock, as determined per the discretion of the investigator) despite titrating down to previously tolerated level.
4. Intubation and mechanical ventilator support
5. Need for Bi-level Positive Airway Pressure (BiPAP) or Continuous Positive Airway Pressure (CPAP) via face mask for worsening hypoxemia.

These patients will be followed out to study completion as defined by death, 28 days from randomization, or hospital discharge whichever comes first. Discharged subjects will receive a 28 day post randomization phone assessment of disease outcomes and adverse events.

Completion of Study Drug Administration:

Patients will be considered to have completed the study drug administration portion of the study and the study drug will be stopped when one of the following conditions is met, whichever comes first:

1. Up to 12 days after randomization (maximum treatment period)
2. Discharge from study hospital. Subjects will receive a 28 day post randomization phone assessment of disease outcomes and adverse events.

3. Death

The optimal duration of treprostinil therapy for prevention of ARDS is not known. Since this is a prevention trial, a short time course of administration with rapid up titration and subsequent down titration of dose was chosen. Every 6 months or after 15 patients have completed the trial (whichever occurs first), an assessment of adverse events will be conducted by the DSMB, and recommendations for dose adjustments can be made.

Primary Endpoint

- 1) Change in PaO₂/FiO₂ ratio from day 0 to 2, 7

Secondary Endpoints

- 2) 28-day ventilator-free days
- 3) Need for Bi-level Positive Airway Pressure (BiPAP) or Continuous Positive Airway Pressure (CPAP) via face mask for worsening hypoxemia or High flow nasal cannula with a liter flow greater than 50LPM
- 4) Intubation and mechanical ventilation
- 5) Change in SpO₂/FiO₂ from Day 0 to Day 12 and on Day 28 if still hospitalized
- 6) Change in systemic biomarker concentrations from Day 0 to Day 7. Blood samples will also be banked for future analyses.
- 7) Change in SCVO₂ from Day 0 to 3 (if central venous catheter in place)
- 8) Change in CVP from Day 0 to 3 (if central venous catheter in place)
- 9) Change in MAP and Systolic blood pressure from cuff and/or arterial line (if placed) from Day 0 to 7
- 10) Pk studies/Plasma levels of treprostinil
- 11) 28-day mortality
- 12) Hospital mortality

Table 1: Schedule of Endpoint Assessments (See Appendix 2 for complete schedule)

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Biomarkers	X			x				x
PaO ₂ /FiO ₂	X (spot ABG)		X (spot ABG)					X (spot ABG)
SpO ₂ /FiO ₂	X	x	x	X	x	x	x	X to day 12 and Day 28 if still hospitalized
SCVO ₂ (if CVC)	X	x	x	x		x		x

RA/CVP (if CVC)	X	x	x	x				
MAP (cuff)	X	x	x	x	X	x	x	x
MAP (a-line if placed)	X	X	X	x				
Syst. BP (cuff)	X	X	X	x	X	x	x	x
Syst. BP (a-line if placed)	X	x	x	x				

Data Collection

Background Assessments

- 1) Demographic and Admission Data
- 2) Pertinent Medical History and Physical Examination
- 3) Height; gender, measured body weight (MBW); calculated predicted body weight (PBW).
- 4) Type of Admission
 - a. Medical
 - b. Surgical scheduled
 - c. Surgical unscheduled
 - d. Trauma
- 5) Acute or Chronic renal failure and use of dialysis
- 6) Alcohol use
- 7) Survey of smoking history including:
- 8) Ever smoker (> 100 cigarettes in lifetime)?
- 9) If yes, current smoker?
- 10) Estimate of pack years (# packs per day) x (# years smoked)
- 11) If former smoker, when did the subject quit smoking?

Baseline Assessments

The following information will be recorded during the 24 hour interval preceding randomization. If more than one value is available for this 24 hour period, the value closest to the time of randomization will be recorded. If no values are available from the 24 hours prior to randomization, then values will be measured post randomization but prior to initiation of study drug.

1. APACHE II Score
2. LIPS Score
3. Vital Signs: Heart rate, systolic and diastolic blood pressure, MAP, body temperature, CVP.

4. Arterial PaO₂, PaCO₂, pH and SpO₂
5. Serum AST and ALT will be documented if the data is available as standard of care
6. Frontal Chest Radiograph – radiographic lung injury score (# of quadrants)
7. Vasopressors or inotropes (epinephrine, norepinephrine, phenylephrine, vasopressin, dopamine > 5 µg/kg/min, dobutamine, phosphodiesterase inhibitors)
8. Suspected or known site of infection (Appendix 1)
9. Blood for banking for future analyses
10. Blood for biomarkers. Plasma obtained from two, 6 ml EDTA anti-coagulated blood samples will be divided immediately after centrifugation into 12 equal 1 ml aliquots in specified tubes and frozen at –80°C. Samples will be temporarily stored in the Pulmonary Division Lab space freezers in Burnett Womack Building, or in the Emergency Room freezer. Specimens will be stored in the Koury Oral Health Sciences Building immediately adjacent to the hospital

Biomarkers: To be measured by the UNC Cytokine & Biomarker Analysis Facility, North Carolina Oral Health Institute.

IL-4

IL-6

IL-8

IL-10

IL-12

RAGE (receptor for advanced glycation end-products)

PCP (procollagen Peptide III)

Ang2 (angiopoietin 2)

MPO (myeloperoxidase)

BNP (brain natriuretic peptide)

VFW (von-Willebrand factor)

PAI-1 (plasminogen activator inhibitor 1)

SP-D (surfactant Protein D)

sICAM (soluble intracellular adhesion molecule)

Protein C

Tissue Factor

KGF (keratinocyte growth factor)

EGF (epithelial growth factor)

Soluble P-selectin

TNF α

MMP9

Procalcitonin

(Others identified from the literature as the study progresses)

Reference Measurements

The following parameters will be measured and recorded using values closest in time to Dose 1 study drug administration on days 0-11 (depending on the specific variable), or until stopping of study drug, whichever comes first. All vascular pressures will be zero-referenced to the mid-axillary line with the patient supine.

- 1) Fluid intake and output (Twenty-four hour net fluid balance)
- 2) Vital signs: Heart rate, systolic and diastolic blood pressure, body temperature, CVP
- 3) Modified Brussels Score data days 0-14:
 - a) Vasopressor use (Y/N), worst systolic BP, creatinine, bilirubin, and platelet count for the day.
- 4) Presence of myopathy
- 5) Frontal Chest Radiograph – Lung Injury Score (Days 0, 3, 7 and 28)
- 6) Concomitant medications, including nebulized and inhaled medications.
- 7) Blood banked for future analyses
- 8) Biomarker samples. Plasma obtained from two, 6 ml EDTA anti-coagulated blood samples will be divided immediately after centrifugation into 12 equal 1 ml aliquots in specified tubes and frozen at -80°C . Samples will be temporarily stored in the Pulmonary Division Lab space freezers in Burnett Womack Building or in the Emergency Room freezer. Specimens will be stored in the Koury Oral Health Sciences Building. Reference biomarker list above (Days 0, 3, and 7).

Statistical Considerations/Data Analysis:

Safety

Safety will be assessed via percentage of patients and number of events recorded for the following outcomes:

- Adverse events, serious adverse events, unanticipated problems, study discontinuation overall and due to adverse event, and deaths.
- Cases of dose intolerance, including cases of worsening oxygenation, intractable cough, and systemic hypotension following dosing
- Cases of intubation or noninvasive ventilation for worsening hypoxemia

Percentages of all outcomes above will be provided to the DSMB for regular unblinded review of safety, to be assessed every 6 months or after 15 participants have completed the 28 day trial or been discharged from the hospital (whichever occurs first).

Efficacy

Analysis of covariance (ANCOVA) will be used to compare trends for the primary outcome PaO₂/FiO₂ ratio between days 0 and 7 between intervention and control patients. PaO₂/FiO₂ ratio is measured at baseline (day 0), and days 2, 7. The difference in treatment groups for change from baseline to day 7 and days 2 will be estimated using an ANCOVA adjusting for baseline value. 95% and 80% confidence intervals for the difference in least squares means will be calculated. Due to small sample sizes, substantial normality departures due to skewness will be assessed. As a supportive exploratory analysis, if a number of patients do not have a day 7 assessment, a mixed linear model for repeated measures (MMRM) of PaO₂/FiO₂ ratio at days 0, 2, and 7 will be explored. The focus of the analysis is to assess preliminary evidence of efficacy. Sample size permitting, trends will be evaluated separately in trauma and non-trauma patients.

The secondary efficacy outcome, 28 day ventilator free days, is defined as days alive and free of mechanical ventilation during the first 28 days after randomization. Patients who die will receive 0 ventilator free days. Patients who are discharged free of mechanical ventilation prior to day 28 will be assumed to remain free of mechanical ventilation unless they are readmitted. Ventilator free days is expected to be skewed. Comparisons between treatment groups will focus on 95% and 80% confidence intervals of the Hodges-Lehman estimator for location shift.

Time until mechanical ventilation and time until death will be described via Kaplan-Meier survival curves.

Remaining efficacy outcomes and biomarkers will be analyzed with similar methods. Categorical outcomes will be characterized by odds ratios and assessed with chi-squared tests or Fisher's exact tests. Biomarker values change from baseline will be assessed comparing treatment groups overall and stratified by whether the acute lung injury was resolved by day 28.

Sample size calculation

Sample size calculations are based on simple t tests. Assuming a change in PaO₂/FiO₂ ratio of 15 in the intervention group and 10 in controls, a standard deviation of 6 (effect size of 0.833), and power of 0.80, the study would require 34 patients in the intervention group and 17 patients in the control group for a 2-sided test with significance level $p < 0.05$. While we will not approach these numbers, they serve to provide a framework as to the sample size needed to observe clinically meaningful changes in oxygenation as statistically significant. This current proposal centers on safety and feasibility, and thus does not meet the statistical criteria needed to observe minimally significant changes in oxygenation. The planned sample size of 30 total participants has 80% power to produce a 2-sided 80% confidence interval with lower confidence limit above 0 if the true effect size is 0.833.

Twenty-eight day ventilator-free days is a continuous variable. The mean 28 day ventilator free

days for the control group of the most recent ARDS network trial was 15 days (95% CI 14.1 – 15.9). The patients enrolled for this trial will have significantly more ventilator free days as a number of them will not require mechanical ventilation at all. A study with 100 patients would have a power of 0.8 to show a 3 day difference in 28 day ventilator free days assuming a value of 22 for controls and 25 for intervention patients, with a SD of 5 days. Since this is an initial, smaller feasibility and proof of concept trial, we will not be able to obtain this level of statistical rigor.

Study feasibility will be assessed by enrollment rate over the first 6 months of the study. If the enrollment rate at that time suggests high feasibility such that the enrollment target of 30 patients can be exceeded easily within the funded enrollment period, we will consider amending the protocol to enroll a small number of additional subjects. This will be discussed and reviewed by the study DSMB. Sonia Davis, PhD, Department of Biostatistics, UNC will provide statistical support.

Part 3. Data Collection and Site Monitoring

Data Collection

Research coordinators will collect data and enter it directly into a computer-based data entry system managed by the TRACS institute or on paper data forms.

Site Monitoring

Since this is single-center study, there is no external site monitoring mechanism. At least 10% of individual case report forms and source documents will be reviewed to confirm that all recorded data is verifiable, accurate and complete. Areas to be assessed include informed consents and process, subject eligibility, case report form and/or source document completion/accuracy, adverse event reporting, drug accountability and the primary efficacy outcome. Data and patient outcomes will be subject to DSMB reviews.

Risk Assessment

Risks of Active Study Drug

The most common (>4% of subjects and >3% above placebo) adverse events reported for inhaled Treprostinil (TYVASO) include cough (54%), headache (41%), throat irritation / pharyngeal pain (25%), nausea (19%), flushing (15%) and syncope (6%). Quantitatively similar adverse events were reported in an open label study in 206 subjects over a mean of 2.3 years. Systemic hemodynamic effects of inhaled Treprostinil are generally minimal. A single inhaled administration of 120µg did result in arterial desaturation. The maximum dose for this study is

set at 72 µg (12 breaths). O₂ saturation will be monitored by pulse oximetry at each escalation of the dose.

Additional adverse events related to treprostinil inhalation in the respiratory tract include epistaxis, hemoptysis and wheezing. One episode of hemoptysis in the open label trial was fatal, and several cases of pneumonia (n=15) were observed. Treprostinil inhibits platelet aggregation, so an increased risk of bleeding may occur in subjects receiving anticoagulants. Similarly concomitant administration of diuretics, antihypertensive agents or vasodilators may increase the risk of symptomatic hypotension.

A retrospective cohort study was conducted from 2004-2006 (Kallen 2008) of patients who received IV formulations of either treprostinil or epoprostenol with documented bloodstream infections (BSI). Adjusted hazard ratios for all BSIs and BSIs due to gram-negative organisms, was higher for treprostinil. There are no reports of BSI following inhaled Treprostinil.

Risks of Blood Draws

All patients will have blood drawn for research purposes. Most blood will be drawn through indwelling catheters if available. Risks of drawing blood percutaneously are uncommon and include bleeding and bruising.

Minimization of Risks

Federal regulations at 45 CFR 46.111(a) (1) requires that risks to subjects are minimized by using procedures which are consistent with sound research design. There are several elements of study design in the present protocol that meets this human subject protection requirement.

First, several of the exclusion criteria prohibit participation of patients who might be at increased risk from the effects of treprostinil. While many of these are considered relative contraindications in clinical care, for this trial they will be absolute contraindications.

Second, there are provisions in the protocol for reduction of the study drug for untoward or adverse effects. Similarly, there are provisions for temporary maintenance of higher doses of treprostinil during scheduled down titration if oxygenation is compromised.

Third, data at each 6 month interval or from the first 15 patients (whichever occurs first) will be evaluated by both the investigators and the DSMB to assure that there are no unrecognized adverse effects. A decision will then be made as to the safety and appropriateness of the protocol dosing regimen.

Potential Benefits

As per the background data presented, there are theoretical reasons to believe that prostacyclins (treprostinil in particular) may improve oxygenation and affect biomarkers

involved or implicated in the pathogenesis of ARDS. While there may not be demonstrable benefit to individual patients in this exploratory trial due to small sample size, we may improve our understanding of the role of prostacyclin in the treatment of ARDS and mitigate safety concerns that would then allow performance of a larger, statistically rigorous trial.

Human Subjects

Each study participant or a legally authorized representative must sign and date an informed consent form. Institutional review board approval will be required before any subject is entered into the study.

Selection of Subjects

Equitable Selection of Subjects

Federal regulations at 45 CFR 46(a) (3) require the equitable selection of subjects. The Emergency Department, hospital floors, and ICUs will be screened to determine if any patient meets the inclusion and exclusion criteria. Data that have been collected as part of the routine management of the subject will be reviewed to determine eligibility. No protocol-specific tests or procedures will be performed as part of the screening process. If any subjects meet criteria for study enrollment, then the attending physician will be asked for permission to approach the patient or his/her surrogate for informed consent. Justifications of exclusion criteria are given in stated in Part 1. These exclusion criteria neither unjustly exclude classes of individuals from participation in the research nor unjustly include classes of individuals from participation in the research. Hence, the recruitment of subjects conforms to the principle of distributive justice.

Justification of Including Vulnerable Subjects

The present research aims to investigate the feasibility, safety and efficacy of a type of treatment for patients with early or at-risk ALI and ARDS. Due to the nature of these illnesses, the vast majority of these patients will have impaired decision-making capabilities. This study cannot be conducted if enrollment is limited to only those subjects with decision-making capacity. Potential benefits to participation in this study are improved oxygenation and VFDs.

Informed Consent

Federal regulations 45 CFR 46.111(a) (5) require that informed consent will be sought from each prospective subject or the subject's legally authorized representative. The investigator is responsible for ensuring that the patient understands the risks and benefits of participating in the study, and answering any questions the patient may have throughout the study and sharing any new information in a timely manner that may be relevant to the patient's willingness to continue his or her participation in the trial. All study participants or their surrogates will be informed of the objectives of the study and the potential risks. The informed consent

document will be used to explain the risks and benefits of study participation to the patient in simple terms before the patient is entered into the study, and to document that the patient is satisfied with his or her understanding of the risks and benefits of participating in the study and desires to participate in the study. The investigator is responsible for ensuring that informed consent is given by each patient or legal representative. This includes obtaining the appropriate signatures and dates on the informed consent document prior to the performance of any protocol procedures and prior to the administration of study agent.

Continuing Consent

For subjects for whom consent was initially obtained from a surrogate, but who subsequently regains decision-making capacity while in hospital, we will attempt to obtain formal consent for continuing participation, inclusive of continuance of data acquisition. The initial consent form signed by the surrogate will reflect that such continuing consent will be obtained when possible.

Identification of Surrogates

Some of the patients approached for participation in this research protocol will have limitations of decision-making abilities due to their critical illness. Hence, some patients will not be able to provide informed consent. Accordingly, informed consent will be sought from the potential subject's legally authorized representative.

Regarding proxy consent, the existing federal research regulations ('the Common Rule') state at 45 CFR 46.116 that "no investigator may involve a human being as a subject in research...unless the investigator has obtained the legally effective informed consent of the subject or the subject's legally authorized representative"; and defines at 45 CFR 46 102 (c) a legally authorized representative (LAR) as "an individual or judicial or other body authorized under applicable law to consent on behalf of a prospective subject to the subject's participation in the procedures(s) involved in the research." OHRP defined examples of "applicable law" as being state statutes, regulations, case law, or formal opinion of a State Attorney General that addresses the issue of surrogate consent to medical procedures. Such "applicable law" could then be considered as empowering the surrogate to provide consent for subject participation in the research. Interpretation of "applicable law" is therefore state specific and hence, will be left to the discretion of the UNC IRB.

According to a previous President's Bioethics Committee (National Bioethics Advisory Committee), an investigator should accept as an LAR...a relative or friend of the potential subject who is recognized as an LAR for purposes of clinical decision making under the law of the state where the research takes place. Finally, OHRP has opined in their determination

letters that a surrogate could serve as a LAR for research decision making if such an individual is authorized under applicable state law to provide consent for the “procedures” involved in the research study.

Justification of Surrogate Consent

According to the Belmont Report, respect for persons incorporates at least two ethical convictions; first, that individuals should be treated as autonomous agents, and second, that persons with diminished autonomy are entitled to protection. One method that serves to protect subjects is restrictions on the participation of subjects in research that presents more than minimal risks. Commentators and Research Ethics Commission have held the view that it is permissible to include incapable subjects in research that involves more than minimal risk as long as there is the potential for beneficial effects and if the research presents a balance of risks and expected direct benefits *similar* to that available in the clinical setting. Several U.S. task forces have deemed it is permissible to include incapable subjects in research. For example, the American College of Physicians’ document allows surrogates to consent to research involving incapable subjects only “if the net additional risks of participation are not substantially greater than the risks of standard treatment.” Finally, the National Bioethics Advisory Committee (NBAC) stated that an IRB may approve a protocol that presents greater than minimal risk but offers the prospect of direct medical benefits to the subject, provided that...the potential subject’s LAR gives permission...”

Consistent with the above ethical sensibilities regarding the participation of decisionally incapable subjects in research and the previous assessment of risks and benefits in the previous section, the present trial presents a balance of risks and potential direct benefits that is *similar* to that available in the clinical setting, with the exception of the additional blood draws.

Additional Safeguards for Vulnerable Subjects

The present research will involve subjects who might be vulnerable to coercion or undue influence. As required in 45 CFR 46.111(b), we recommend that additional safeguards be included to protect the rights and welfare of these subjects. Such safeguards might include, but are not limited to: a) assessment of the potential subject’s capacity to provide informed consent, b) requirement for subject’s assent, c) the availability of the LAR to monitor the subject’s subsequent participation and withdrawal from the study, and d) augmented consent processes. The specific nature of the additional safeguards will be left to the discretion of the IRB.

Confidentiality

Federal regulations at 45 CFR 46.111 (a) (7) requires that when appropriate, there are adequate provisions to protect the privacy of subjects and to maintain the confidentiality of data. To

maintain confidentiality, all laboratory specimens, evaluation forms, and reports will be identified only by a coded number. The coded number will be generated at random by a computer, and only the study investigators will have access to the codes. All records will be kept in a locked, password protected computer. All computer entry and networking programs will be done with coded numbers only. All paper case report forms will be maintained in a locked cabinet inside a locked office. Clinical information will not be released without the written permission of the patient, except as necessary for monitoring by the DSMB.

Adverse Event Reporting

Investigators will determine daily if any clinical adverse experiences occur during the period from enrollment through study day 28 or hospital discharge, whichever occurs first. The investigator will evaluate any changes in laboratory values and physical signs and will determine if the change is clinically important and different from what is expected in the course of treatment of patients with ARDS/ALI. If clinically important and unexpected adverse experiences occur, they will be recorded on the adverse event case report form.

For this trial, a *reportable adverse event* is defined as:

1. Any clinically important untoward medical occurrence in a patient receiving study drug or undergoing study procedures which is different from what is expected in the clinical course of a patient with ARDS/ALI, or,
2. Any clinically important, untoward medical occurrence that is thought to be associated with the study drug or procedures, regardless of the “expectedness” of the event for the course of a patient with ARDS/ALI.
3. The following will be reported as adverse events:
 - Systemic hypotension occurring immediately after and clearly a result of the study drug.
 - Worsening of oxygenation immediately after administration of study drug.
 - Cardiac arrhythmia occurring immediately after administration of study drug.

Expected events for ARDS/ALI are untoward clinical occurrences that are perceived by the investigator to occur with reasonable frequency in the day to day care of patients with ARDS treated in an intensive care unit. Examples of adverse events that are expected in the course of ARDS include transient hypoxemia, agitation, delirium, nosocomial infections, skin breakdown, and gastrointestinal bleeding. Such events, which are often the focus of prevention efforts as part of usual ICU care, will not be considered reportable adverse events unless the event is considered by the investigator to be associated with the study drug or procedures, or unexpectedly severe or frequent for an individual patient with ALI. Examples of unexpectedly

frequent adverse events would be repeated episodes of unexplained hypoxemia. This would be in contrast to an isolated episode of transient hypoxemia (e.g. SpO₂ ~85%), related to positioning or movement.

Investigators will report all events to United Therapeutics that are **serious AND unexpected AND study-related** within 24 hours of becoming aware of the event. The local Institutional Review Board must also be notified in a timely manner. The investigator will then submit a detailed written report to the sponsor, the DSMB, and the Institutional Review Board no later than 5 calendar days after the investigator discovers the event. A written report will be sent to the DSMB within 15 calendar days, and this also passed on to the Institutional Review Board. The DSMB will also review all adverse events during scheduled interim analyses. The written summary of the DSMB's periodic review of adverse events will be submitted to the Institutional Review Board in accordance with standards.

The investigators and DSMB will also determine if the serious adverse event is unexpected for an inhaled prostacyclin drug. Unexpected for an inhaled prostacyclin is defined as any event not listed in the inhaled treprostinil package insert. If it is determined that any serious and study-related adverse event is unexpected for inhaled prostacyclin, the FDA will be notified within 7 calendar days. Such events may also meet the definition of Unanticipated Problems as described below.

Investigators must also report Unanticipated Problems, regardless of severity, associated with the study drug or study procedures within 24 hours. An unanticipated problem is defined as follows:

Unanticipated Problem (UP): any incident, experience, or outcome that meets all of the following criteria:⁷⁵

- Unexpected, in terms of nature, severity, or frequency, given the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and the characteristics of the subject population being studied;
 - Related or possibly related to participation in the research, in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research;
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Data Safety Monitoring Board (DSMB)

Version: 02-09-2018

Protocol 1.06 (Modification)

Safety data will be reviewed by an independent DSMB every 6 months or after 15 patients have completed the trial (whichever occurs first), and at the completion of the study prior to database lock. The safety and related data, for placebo and drug-treated groups, will be reviewed by the TraCS DSMB as outlined in the approved TraCS DSMB charter, and includes:

- Enrollment data
- Adverse events, serious adverse events, unanticipated problems, study discontinuation overall and due to adverse event, and deaths.
- Cases of dose intolerance, including cases of worsening oxygenation, intractable cough, and systemic hypotension following dosing
- Cases of intubation or noninvasive ventilation for worsening hypoxemia
- Any other safety supporting data requested by the DSMB

Study Stopping Rules

Patients with ARDS have a high risk of 28-day mortality, which is approximately 45% in general ICU populations. This is because ARDS tends to be part of a syndrome of multi-organ failure that accompanies underlying severe conditions such as septic shock, severe pancreatitis, and multilobar pneumonia in patients with multiple underlying comorbidities. For that reason, 28-day mortality is the most common clinical outcome for large trials of ARDS, and in those large trials, stopping rules for safety or futility are built around 28-day mortality.

This study is a small pilot study of feasibility and safety enrolling patients with early ARDS not yet requiring mechanical ventilation. Due to its small size, a *statistical* stopping rule based on mortality or even Grade IV Adverse events is not possible. This has been confirmed by additional review by the study's statistician. Therefore a *clinical* stopping rule based on Grade 4 events or complete respiratory failure requiring intubation and mechanical ventilation, (an event that is intermediate to possible mortality in ARDS) has served as the stopping rule for this study to date.

The principle conditions for suspension of study enrollment and review of safety by the DSMB will be:

After enrollment of the first 30 patients:

- An imbalance of greater than 4 to 1 (given 2:1 randomization scheme) of any Grade 4 Adverse event from the NIH Common Terminology Criteria for Adverse Events (CTCAE)
- An imbalance of greater than 4 to 1 (given 2:1 randomization scheme) in the number of patients requiring intubation and mechanical ventilation in favor of intervention.
- A 28-day mortality rate of greater than 60% in either study arm, reflecting a rate measurably higher than expected in ARDS.

Of note: To reduce potential bias, the relationship of any Grade 4 or 5 events to study drug will be adjudicated by an expert in pulmonary hypertension and critical care, Dr. Ashley Henderson, who is not participating in the study and will be blinded for the assessments.

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APPENDIX 1: Guidelines for evidence of infection

1. **Infections of the thorax:**
 - a. Chest x-ray or CT scan showing a new or progressive infiltrate, consolidation, cavitation, collection, or pleural effusion, and a clinical presentation consistent with pneumonia or empyema
 - b. Pneumonia can be defined as the presence of new infiltrate (s), absence of a noninfectious explanation and either signs of SIRS as per protocol or purulent sputum production with an identifiable pathogen.
 - c. Aspiration Pneumonitis in the acute phase is not considered an infection. However, if SIRS persists > 24 hours after aspiration, then an infectious etiology can be presumed.
2. **Abdominal infection:**
 - a. Perforated viscus or ischemic bowel with either localized peritonitis
 - b. Peritoneal fluid with > 250 PMNs
 - c. Clinical signs of cholangitis or appendicitis
 - d. Clostridium difficile toxin positive with evidence of colon dilation
 - e. Suspicion of peritonitis by clinical examination only
3. **Skin or soft tissue infection:** Acute onset infection of the skin, such as erysipelas, or infection involving deeper soft tissue
4. **Bacterial meningitis:** cerebrospinal fluid analyses if available and a clinical presentation consistent with bacterial meningitis
5. **Urinary Tract:**
 - a. Positive test for granulocyte esterase or nitrate in urine, or a positive culture (defined as >10⁵ CFU/mL)
 - b. Urinalysis with increased WBC count or positive Gram stain
6. **Central Line infections:**
 - a. Isolation of a pathogen in one or more blood cultures drawn peripherally, or from a central venous catheter/port in place <48 hours
 - b. Purulence at insertion site
7. **Sinusitis**
 - a. Air fluid levels in sinus seen on CT scan
8. **The following are not considered evidence of infection:**
 - a. Fever of unknown origin
 - b. Blood cultures that are considered positive only because of the isolation of a likely contaminant organism
 - c. Postoperative hypotension within 24 hours of incision and/or fever without a verified infectious focus.
 - d. Leukocytosis alone in the presence of steroid usage is insufficient evidence of infection.
 - e. Leukocytosis alone in the presence of connective tissue disorder is insufficient evidence of infection.

APPENDIX 2:

Schedule of Events

<u>Description of Event</u>	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 28
Inclusion / Exclusion Review	X															
Informed Consent	X															
Screening QTc	X															
Randomization	X															
Physical Examination	X															
Background Assessments	X	Demographic and Admission Data, Medical History, Height; Gender, Measured Body Weight (MBW); Type of Hospital Admission, Dialysis Use, Smoking History														
Baseline Assessments**	**Baseline Assessments will be recorded during the 24 hour interval preceding randomization. If more than one value is available for this 24 hour period, the value closest to the time of randomization will be recorded. If no values are available from the 24 hours prior to randomization, then values will be measured post randomization but prior to initiation of study drug.															
** Scoring (APACHE II and LIPS Scores)	X															
** Vital Signs and PaO ₂ ,PCO ₂ , pH; SpO ₂	X															
Available Labs ** Serum AST, ALT	X															
Required: ABG; Albumin; Bilirubin; BUN; Creat; Glucose; HCT; HGB; K; Na; Plt; WBC	X															
** Vasopressor or Inotropes Use	X															
Reference Measurements++	++ The following parameters will be measured and recorded using values closest in time to Dose 1 on days 0-11 (depending on the specific variable), or until stopping of study drug, whichever comes first. All vascular pressures will be zero-referenced to the mid-axillary line with the patient supine.															
Fluid Intake / Output	X	X	X	X	X	X	X	X	X	X	X	X				
++ Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X				
Concomitant Medication Use (Including Nebulized and Inhaled Medications)	X	X	X	X	X	X	X	X	X	X	X	X				
Lung Injury Score (Chest x-ray)	X			X				X								X
Modified Brussels Score	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Study Drug Administration	X	X	X	X	X	X	X	X	X	X	X	X				
Blood Pressure Assessments (On Study Day 00, blood pressure will be checked prior to Dose 1 and 30 minutes after; prior to Dose 3 and 30 minutes after; prior to Dose 5 and 30 minutes or after a different dose interval, if the study drug had to be maintained due to an adverse effect . Blood pressure measurements for Study Days 01 through 11 will be captured from the nursing flow-sheets.	X	X	X	X	X	X	X	X	X	X	X	X				
Peak / Trough Specimen Collection (Peak Specimen will be obtained 15min [+/-5min] after Dose 1 on Day 3) (Trough Specimen will be obtained just prior to Dose 2 on Day 3)				X												

Biomarkers Specimen Collection (* Baseline specimen collection is prior to study drug administration)	X			X				X								
PaO₂ / FiO₂ Ratio (if the subject is still hospitalized)	X		X					X								
SpO₂ / FiO₂ Ratio	X	X	X	X	X	X	X	X	X	X	X	X				X
SCVO₂ (if central venous catheter is in place)	X	X	X	X		X		X								
CVP (if central venous catheter is in place)	X	X	X	X												
SBP / MAP (if arterial line is in place otherwise cuff)	X	X	X	X	X	X	X	X								
Adverse Events Review	X	X	X	X	X	X	X	X	X	X	X	X				
Hospital Mortality																X
Phone Assessment of Disease Outcomes and Adverse Events																X
Day 28 Mortality																X

Appendix 3

Oxygen Titration Protocol

The goal of the Oxygen Titration Protocol is to maintain a resting oxygen saturation of 90-94%. Brief increases in the research subject's O₂ liter flow for ambulation and exertion can be allowed. However, before each study drug administration, the respiratory therapist should make sure that the research subject is on the lowest amount of oxygenation required to achieve an oxygen saturation of 90-94% before recording their baseline O₂ requirement. Reassessment of oxygen saturation before and after each level should occur. Research subjects will start with a conventional humidified nasal cannula. If oxygen titration is needed, we will titrate from their current nasal cannula flow rate in the following stepwise fashion: 4LPM / 5LPM /6LPM. If there is a need for oxygen titration above 6 LPM, we will utilize a High Flow Nasal Cannula with the flow rate of 20 to 50 LPM. We will titrate the High Flow Nasal Cannula FiO₂ in the following stepwise fashion: 50%, 60%, 70%, 80%, 90%, and 100%. The required liter flow will be titrated per patient need/demand. If a patient requires more than 50LPM via HFNC, they will have met our classification of *requiring positive pressure threshold*. If clinically indicated, clinicians that are managing the care of these research subjects can initiate non-invasive ventilation or proceed to intubate prior to a patient reaching 100% O₂ or 50LPM flow rate, per our stepwise fashion.

FiO₂ Conversion Table

Conventional Humidified Nasal Cannula Flow Rate	FiO ₂
1 LPM	24%
2 LPM	28%
3 LPM	32%
4 LPM	36%
5 LPM	40%
6 LPM	44%
High Flow Nasal Cannula Flow Rate	FiO ₂
20 LPM to 50LPM	50%
20 LPM to 50LPM	60%
20 LPM to 50LPM	70%
20 LPM to 50LPM	80%
20 LPM to 50LPM	90%
20 LPM to 50LPM	100%