A Phase II/III, Randomized, Double-Blind, Placebo-Controlled Trial of Lisofylline in Patients with Acute Lung Injury and Adult Respiratory Distress Syndrome

ARDS Clinical Network
ARDSNet Study 03, Version I

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Contents

Part I

Study Summary

- **Study Design:** Multicenter, Phase II/III, randomized, double-blind, placebo-controlled study. Patients will be randomly assigned to receive lisofylline (LSF) 3.0 mg/kg or placebo. Patients will be stratified according to center and ventilator strategy.
- **Primary Objective:** To compare the effect of LSF to placebo on the incidence of Day 28 mortality in patients with ALI or ARDS.
- **Secondary Objectives:** To compare the number of days of unassisted breathing during the 28 day study period.
- To examine the effect of LSF on the incidence of serious infections during the 28 day study period.
- To evaluate the effect of LSF on the incidence of infection-related mortality.
- To evaluate the effect of LSF on the number of organ-failure-free days during the 28 day study period.
- To evaluate the safety of LSF in patients with ALI or ARDS.
- **Study Population:** Patients with ALI or ARDS who are eligible and enrolled on ARDSNet Study 01.
- **Number of Subjects:** 800 patients; interim analyses will be performed after approximately 200 and 400 patients have completed treatment.
- **Duration of Patient Participation:** LSF study drug treatment for 21 days or until 2 consecutive calendar days of unassisted breathing, whichever occurs first. Acute evaluation for 28 days and safety follow up for 60 days after final LSF study drug administration.
- **Study Medication:** Lisofylline or placebo.
- **Dosage Form:** Injection solution (60 mg/mL) in 10 mL ampuls.
- **Dosage:** 3.0 mg/kg, patients weighing $\geq$ 100 kg will receive the maximum dose of 300 mg.
- **Dosage Regimen:** Ten minute intravenous infusion every 6 hours.

- **Procedures:** Patients will receive LSF 3.0 mg/kg or placebo every 6 hours beginning on Day 0 and continuing through Day 20 (or when the patient has achieved two consecutive calendar days of unassisted breathing), whichever comes first.
- Blood samples will be drawn to determine surrogate marker levels on Day 0 prior to the first dose of LSF study drug and on Day 3 prior to a dose of LSF study drug and 1 hour after the completion of the LSF study drug dose.
- Blood samples to determine LSF and LSF metabolite levels will be drawn immediately before and immediately after the completion of a LSF study drug dose on Day 0 and Day 3. If a patient develops renal or hepatic impairment (creatinine > 2.5 or bilirubin > 3.0), pre- and post- study drug infusion samples will be drawn daily for three days and then weekly until day 28.
- Assessments including monitoring of vital signs, ventilator settings, laboratory studies and diagnostic testing will be performed during the study period.

1 Study Objectives

1.1 Primary Objective

To compare the effect of LSF to placebo on the incidence of Day 28 mortality in patients with ALI or ARDS.

1.2 Secondary Objectives

To compare the number of days of unassisted breathing during the 28 day study period in patients with ALI or ARDS.

To examine the effect of LSF on the incidence of serious infections during the 28 day study period.

To evaluate the effect of LSF on the incidence of infection-related mortality.

To evaluate the effect of LSF on the number of organ failure-free days during the 28 day study period.

To evaluate the safety of LSF in patients with ALI or ARDS.

Since this study involves the same patients as ARDSnet Study 01 in a factoral design, the data analyses comparing 6ml/kg to 12ml/kg ventilation will be peformed comparing LSF to Placebo. Furthermore it will be a secondary objective to determine if LSF effects mortality or the number of days of unassisted breathing to Day 28, on the two forms of ventilation differently.

Part II

Study Description

A Phase II/III, Randomized, Double-Blind, Placebo-Controlled Trial of Lisofylline in Patients with Acute Lung Injury and Adult Respiratory Distress Syndrome

ARDSNet Study 03, Version I

2 BACKGROUND

2.1 Acute Lung Injury (ALI) and Adult Respiratory Distress Syndrome (ARDS)

The recent American-European Consensus Conference on ARDS ([1]) define ALI according to the following criteria: 1) acute onset, 2) $Pa0_2/Fi0_2 \leq 300$, 3) Bilateral infiltrates on frontal chest radiograph, and 4) no evidence of left atrial hypertension (pulmonary capillary wedge pressure $\leq$ 18 when measured). The definition of ARDS is the same except for $Pa0_2/Fi0_2 \leq 200$.

ALI or ARDS occurs when an event such as sepsis or massive aspiration causes inflammation, increased pulmonary vascular permeability, and extravasation of fluid and inflammatory cells into the pulmonary interstitium and alveolar space ([2]). The inflammatory process leads to inactivation, destruction, and decreased production of surfactant ([3],[4],[5]). This causes increased surface tension at the alveolar air-fluid interface, leading to diffuse microatelectasis. Alveolar flooding and atelectasis cause hypoxemia from shunt. Management of hypoxemic respiratory failure frequently requires positive pressure ventilation. Traditional ventilator management in ALI/ARDS employs positive end-expiratory pressure (PEEP) and generous tidal volumes of 10-15 ml/kg ([6],[7]). Despite aggressive treatments for the conditions that precipitate ARDS, many patients die without resolution of the lung injury.

2.2 Lisofylline

ARDS is now thought to be a consequence of an over-aggressive inflammatory response with associated oxidant injury. Increased release of

cytokines and chemokines, with enhanced expression of adhesion molecules produces an acute inflammatory response in the lungs, involving neutrophil infiltration. These neutrophils migrate across the interstitial space into the intra-alveolar space where they, secondary to activation upon adherence, release proteases and reactive oxygen intermediates.

2.2.1 Lisofylline Mechanism of Action

The biomolecular target for lisofylline (LSF) is unknown. In normal human volunteers, lisofylline causes a prolonged and marked decrease (approximately 70%) in the levels of circulating free fatty acids including levels of the major oxidizable species, linoleic acid (unpublished data). In patients treated with IL-2 or in patients with septic shock, circulating levels of free fatty acids increase by several fold ([8]). This increase is inhibited by therapy with lisofylline. During oxidative stress, linoleic acid is peroxidized to the highly bioactive derivative species, 9 and 13 hydroperoxyoctadecadienoic acids (HPODEs). Exposure of cultured endothelial cells to HPODEs increases their permeability to albumin and causes cellular activation as evidenced by upregulation of vascular cell adhesion molecule (VCAM) expression ([9],[10]). In a prospective study of 50 patients undergoing chemotherapy and radiation for bone marrow transplantation who were on a placebo-controlled trial with lisofylline at either 2.0 mg/kg or 3.0 mg/kg given every 6 hours, lisofylline suppressed formation of serum HPODEs in a dose-dependent manner (unpublished data). HPODE levels in circulation on the day of the transplant were highly predictive for subsequent mortality $p \leq (0.01)$. These data, in conjunction with preclinical data indicating LSF efficacy in protecting against tissue injury mediated by oxidation, suggest that LSF may exert its major effect by decreasing cellular lipid peroxidation and subsequent activation of stress-associated signaling pathways, thereby suppressing production of a number of cytokine mediators that amplify the inflammatory process.

2.3 Pre-clinical Studies with Lisofylline

2.3.1 Hemorrhagic shock induced lung injury (*in vivo*)

Lisofylline was investigated in a murine model of lung injury. Control and treated mice were bled one-third of their blood volume with re-transfusion of stored blood after one hour. Treated animals received lisofylline at the time of transfusion. At clinically achievable concentrations, lisofylline significantly inhibited the release of TNF, gamma interferon (INF), IL-6,

and IL-1 into fluid recovered by bronchoalveolar lavage (BAL). Other than mild accumulation of neutrophils, lisofylline treated animals had no histologic evidence of alveolar edema or hemorrhage, unlike control animals which had extensive air space damage, hemorrhage, edema and neutrophil infiltration ([11]).

2.3.2 Cytokine and neutrophil-mediated lung injury (*in vivo*)

A highly fatal and relatively common complication of severe trauma and hemorrhagic shock is the development of acute non-cardiogenic lung injury (ARDS). IL-1, IL-8 and neutrophils are increased in lungs of patients with ARDS and are thought to contribute to lung injury. The effect of lisofylline on cytokines and neutrophils in acute lung injury was evaluated in a series of experiments in intact and isolated rat lung. Lisofylline inhibited lung edema when lungs were perfused with human neutrophils and treated intratracheally with IL-8. Lisofylline also suppressed neutrophil accumulation in lungs treated with intratracheal IL-1. Taken together, these studies suggest that lisofylline may prevent acute lung injury following severe trauma by inhibiting the response to hypoxic injury and the subsequent inflammatory cytokine cascade.

2.3.3 Antibiotic/antimicrobial effects (*in vivo*)

A series of experiments were conducted to evaluate the effect of lisofylline on antimicrobial activity of twelve commonly used antibiotics. Concentrations of lisofylline up to 50 μM (which exceeds planned clinical concentrations), do not appear to antagonize or potentiate the activity of antimicrobial agents in recent blood bacterial isolates. In an *in vivo* model, mice were infected intratracheally with *Pseudomonas* and treated with either sub-therapeutic or therapeutic doses of a cephalosporin antibiotic with or without lisofylline. Lisofylline had no effect on the number of bacterial CFU/g of lung tissue suggesting it had no antagonistic effects on antimicrobial activity *in vivo*. These findings suggest that concomitantly administered lisofylline did not interfere with antimicrobial therapy.

2.3.4 Endotoxic shock (*in vivo*)

Lisofylline was examined in a murine model of endotoxic shock. In these studies, a lipopolysaccharide (LPS) dose sufficient to induce 90-100% lethality in 24-48 hours was utilized. Lisofylline, administered simultaneously or 2 hours after a lethal dose of LPS resulted in 80-100%

survival. Even when lisofylline was administered 4 or 6 hours following an otherwise lethal dose of endotoxin, 37% and 25% of treated animals survived respectively ([12]).

2.3.5 Platelet function (*ex vivo*)

To determine if lisofylline interferes with platelet function and, thus, might increase the hemorrhagic diathesis in patients following trauma, its effect on platelet aggregation was analyzed. Aggregation of normal human donor platelets induced by thrombin, adenosine diphosophate (ADP), ristocetin or collagen, was not affected by lisofylline, at concentrations up to 50 μM. These data along with data indicating that lisofylline had no effect on either a prothrombin time or a partial thromboplastin time assay indicated that it should not increase bleeding tendencies in patients with septic shock.

2.3.6 Wound healing (*in vivo*)

Using a standard wound healing model, incisional wound tensile strength was measured in rats treated BID with 25 mg/kg and 50 mg/kg of lisofylline. On post-operative days 4 and 8 there were no significant differences between the two groups treated with lisofylline and the control group.

2.4 Clinical Studies with Lisofylline

More than 600 subjects have been enrolled in clinical trials with lisofylline, including greater than 350 subjects treated with active drug. In these trials, lisofylline has been studied for its potential ability to reduce the toxicity and to improve the outcome of cytotoxic antineoplastic therapy. Patients undergoing intensive chemotherapy and/or radiation therapy for bone marrow transplantation, for induction therapy in acute myeloid leukemia or biological therapy for other oncologic diseases have been included in clinical trials. LSF has been found to be safe in these trials, compared with the expected background level of adverse events in these patient populations.

Sixty patients with hematological malignancies undergoing bone marrow transplantation from HLA-identical sibling donors were enrolled in a Phase II study of LSF. Patients were randomized to receive placebo, 2.0 mg/kg LSF, or 3.0 mg/kg LSF diluted in 50 mL normal saline delivered as a 10

minute intravenous infusion every 6 hours from the start of conditioning to Day 21 post-transplant or hospital discharge, whichever occurred first. Trial endpoints included hematopoietic recovery, incidence of infections, incidence and severity of mucositis and survival. Eighteen patients received placebo, 23 patients received 2.0 mg/kg LSF, and 19 patients received 3.0 mg/kg LSF. Seventeen of 19 patients (89%) in the 3.0 mg/kg group survived to Day 100 compared to 11 of 23 patients (48%) in the 2.0 mg/kg group and 10 of 18 (56%) in the placebo group. The difference in the total number of patient deaths to Day 100 post-BMT is statistically significantly different ($p=0.022$) between the 3.0 mg/kg group and the placebo group. There was no statistically significant difference in Day 100 survival between the 2.0 mg/kg group and the placebo group.

There were significantly fewer infections ($p<0.01$) in patients who received 3.0 mg/kg LSF compared to placebo. No patients receiving 3.0 mg/kg developed an infection as defined by the study criteria from Day 0 through Day 35 compared to 8 (35%) patients treated with 2.0 mg/kg and 7 (39%) patients treated with placebo. There were 5 (28%) serious infections in the placebo group, 5 (22%) in the 2.0 mg/kg group and 0% in the 3.0 mg/kg group. In addition, no patient receiving 3.0 mg/kg developed a serious or fatal infection through Day 100 compared to 6 (26%) infections in the 2.0 mg/kg group and 7 (39%) in the placebo group.

Overall, mucositis was less severe and occurred in lower frequencies in the 3.0 mg/kg group and the placebo group ($p=0.1$). All placebo treated patients experienced some degree of mucositis whereas 13 (57%) of the 2.0 mg/kg group and 16 (84%) of the 3.0 mg/kg group reported no mucositis. There was a statistically significant benefit observed in the 3.0 mg/kg group for decreased infectious episodes, including life threatening infections and a higher overall Day 100 survival than placebo patients despite the fact that the LSF treatment groups had significantly higher proportion of high risk patients (older age, poorer performance status) than the placebo recipients. The incidence of reported adverse experiences was similar among the treatment groups indicating that LSF was safe and well-tolerated. In a single center, Phase II trial patients with newly diagnosed AML undergoing induction chemotherapy with idarubicin and cytarabine were randomized to receive placebo or LSF 3 mg/kg beginning prior to the first dose of chemotherapy and continuing through the 28 day treatment cycle for a maximum of 2 cycles. Patients were stratified by age and disease (AML or RAEB/RAEBt). Seventy patients ages 19-73 were enrolled and able to be evaluated. The endpoints of this study included the incidence of neutropenic infections (serious and non-serious) and mortality at Day 60. In this Phase II study, compared to treatment with placebo, treatment with lisofylline at 3 mg/kg did not significantly affect the incidence of any neutropenia-related infections ($p=0.337$) but did

result in a statistically significant reduction in serious neutropenia-associated infections (17% vs. 34%; p=0.047) and serious neutropenic fungal infections (0% vs. 14%; p=0.021). All cause mortality at Day 60 was not different between the LSF and placebo groups (p=0.75). Lisofylline's protective effect against serious infections is consistent with its presumed effects on mucosal barrier integrity since pathogenic organisms associated with serious infections are typically associated with breakdown of mucosal barriers in the gastrointestinal tract or in the lung. Lisofylline did not protect against infections from skin or intravenous catheters.

2.5 Rationale for LSF Dose

Recently completed pharmacokinetic studies of lisofylline in human normal volunteers have demonstrated that a dose up to 5.0 mg/kg given by IV infusion over 10 minutes is well tolerated in most subjects. Symptoms that occur at doses higher than 5.0 mg/kg IV infusion over 10 minutes are light headedness or dizziness and mild nausea. Dose progression leads to pronounced nausea and vomiting. Four of 25 subjects in LSF pharmacokinetic studies experienced light headedness, two experienced nausea at both the 3.0 mg/kg and the 6.0 mg/kg oral doses, and two patients experienced hypotension (decreased systolic blood pressure by approximately 25 for baseline) at a dose of 3.0 mg/kg. All symptoms generally resolved within 60 minutes of completion of the LSF study drug administration.

Clinical studies of lisofylline conducted in patients have utilized doses up to 4.8 mg/kg given by IV infusion over 10 minutes every six hours, for up to 28 days post BMT. Of 42 patients in a Phase II BMT study treated with LSF study drug, either 2.0 or 3.0 mg/kg, 6 who received LSF discontinued study drug due to nausea or nausea with vomiting. None of the 18 patients receiving placebo withdrew from the study due to nausea or vomiting. Other adverse events reported in clinical trials with lisofylline have been consistent with the adverse events expected in this patient population. There were no significant hemodynamic effects observed in seven patients with septic shock who received 1.5 mg/kg lisofylline every six hours for up to 5 days. Accumulation of LSF or of two of its primary metabolites was not observed in the six patients treated on this study. Although LSF doses of up to 5.0 mg/kg by 10 minute infusion have been tolerated in both normal volunteers and in patients undergoing bone marrow transplantation, 3.0 mg/kg proved efficacious in Phase II studies in bone marrow transplantation and induction chemotherapy for AML. Because the 3.0 mg/kg lisofylline dose has been well tolerated in clinical studies to date, a 3.0 mg/kg IV infusion over 10 minutes, every six hours has been chosen as the dose for this study.

3 STUDY OBJECTIVES

3.1 Primary Objective

To compare the effect of LSF to placebo on the incidence of Day 28 mortality in patients with ALI or ARDS.

3.2 Secondary Objectives

To compare the number of days of unassisted breathing during the 28 day study period in patients with ALI or ARDS.
To examine the effect of LSF on the incidence of serious infections during the 28 day study period.
To evaluate the effect of LSF on the incidence of infection-related mortality.
To evaluate the effect of LSF on the number of organ failure-free days during the 28 day study period.
To evaluate the safety of LSF in patients with ALI or ARDS.

Since this study involves the same patients as ARDSnet Study 01 in a factoral design, the data analyses comparing 6ml/kg to 12ml/kg ventilation will be peformed comparing LSF to Placebo. Furthermore it will be a secondary objective to determine if LSF effects mortality or the number of days of unassisted breathing to Day 28, on the two forms of ventilation differently.

4 STUDY DESIGN AND ENDPOINT DEFINTIONS

4.1 Study Design

This is a multi-center, Phase II/III, randomized, double-blind, placebo-controlled study of LSF in patients with acute lung injury or adult respiratory distress syndrome. An estimated 800 patients will be enrolled in the study. Approximately 400 patients will be randomized to be treated with LSF 3.0 mg/kg and approximately 400 patients will be randomized to receive placebo. Patients will be stratified according to the ventilator strategy they have been assigned to receive on ARDSNet Study 01, (12 mL/kg or 6 mL/kg), and by center. After enrollment and study completion of the first 200 patients, the ARDS Network Steering Committee will be provided with an unblinded summary of the primary and secondary

efficacy parameters to determine whether there is enough evidence of lisofylline safety and efficacy to continue the lisofylline randomization.

After approximately 200, 400, 600 and 800 patients, the primary efficacy and safety variables will be reviewed by an independent Data and Safety Monitoring Board to determine whether the randomization between lisofylline and placebo should stop for futility, lack of safety, or proven efficacy. Stopping for futility or efficacy will be based on a formal group sequential stopping boundary.

When study eligibility has been confirmed, patients will be randomized to receive either LSF 3.0 mg/kg or placebo. LSF or placebo study drug administration will begin within 36 hours of the time the final pulmonary inclusion criteria is met. The day of the first dose of LSF or placebo study drug will be Study Day 0. Study drug administration will continue every 6 hours for 21 days, or until the patient has been on unassisted breathing for 2 consecutive calendar days, whichever occurs first. Assessments including vital signs, hemodynamic measurements, ventilator settings, laboratory studies, and diagnostic testing will be performed during the study period. Patients will also be followed beyond Day 28 if serious adverse experiences have occurred prior to the last day of dosing until the event resolves or stabilizes.

4.2 Endpoint Definitions

Days of unassisted breathing (VFD, denoting Vent-Free-Days) to Day 28 is defined as the number of days after initiating unassisted breathing to Day 28 after randomization, assuming a patient survives for at least 2 consecutive calendar days after initiating unassisted breathing and remains free of assisted breathing. If a patient returns to assisted breathing and subsequently achieves unassisted breathing prior to Day 28, VFD will be counted from the end of the last period of assisted breathing to Day 28 unless a period of assisted breathing was $<$ twenty-four hours and the purpose of assisted breathing was for a surgical procedure. If the patient is receiving assisted ventilation at Day 28 or dies prior to Day 28, VFD will be zero.

Unassisted breathing is defined as breathing with face mask or nasal prong oxygen (or room air) following extubation, T tube breathing, breathing with CPAP at $\leq$ 5 cm H_2O pressure, or tracheostomy mask breathing.

Organ failure is defined as present on any date when the most abnormal vital sign/abnormal lab value meets the definition of Clinically Significant Organ Failure (CSOF) according to the Brussels Organ Failure Table

([13]). Patients will be followed for 28 days, with each day scored for the presence of clinically significant organ failure (renal, hepatic, coagulation, pulmonary, cardiovascular). Each day a patient is alive and free of a given clinically significant organ failure will be scored as a failure free day, e.g., a renal failure free day (maximum number is 29 allowing for the day of study entry plus 28 follow-up days, minimum is zero). Any day that a patient is alive and free of ALL five organ failures will represent days alive and free of all organ failure. Central nervous system dysfunction is evaluated using the Glasgow Coma Scale at study entry (Day 0), Day 14 and Day 28.

Microbiologically documented bacterial or fungal infections are those for which a pathogen is isolated or identified from blood, normally sterile tissue or body fluid. If isolated from blood, the pathogen should be present from one or more blood cultures with the exception of coagulase-negative (or thermonuclease-negative) staphylococci or corynebacteria which require the isolation of these organisms from at least two blood cultures drawn within 24 hours of each other containing the same organism in order to be deemed significant.

Culture negative septic shock is defined as sepsis (two or more of the following: T $>$ 38°C or $<$ 36°C, HR $>$ 90 bpm, RR $>$ 20/min, $PaCO_2$ $<$ 32 mmHg, *or* white blood cell count $>$ 12,000/mm^3 or $<$ 40,000/mm^3 or $>$ 10% immature (band) forms.), with hypotension (BP $<$ 90 mmHg systolic or a drop of $\geq$ 40 mmHg) lasting $\geq$ 2 hours despite adequate fluid resuscitation (e.g., 500 ml saline challenge) *or* a requirement for vasopressor support accompanied by the presence of perfusion abnormalities (i.e., lactic acidosis, oliguria, acute alteration in mental status), but without positive blood cultures.

Serious infections and the duration of an infectious episode are defined in Appendix A. Culture negative septic shock episodes are classified as serious infections due to the severity of the clinical symptoms. Both number of patients with infections and number of infectious events will be considered in the analysis. Infections present 72 hours prior to randomization are not considered.

5 PATIENT SELECTION

5.1 Inclusion Criteria

1. Concurrent enrollment in ARDSNet Study 01.
2. $PaO_2/FiO_2 \leq 300$. If altitude $>$ 1000m, then $PaO_2/FiO_2 \leq 300 \times (BP/760)$.

3. Bilateral infiltrates consistent with pulmonary edema on frontal chest radiograph. The infiltrates may be patchy, diffuse, homogeneous, or asymmetric.

4. Requirement for positive pressure ventilation via endotracheal tube.

5. No clinical evidence of left atrial hypertension. (If the patient has a pulmonary artery catheter, the wedge pressure $\leq$ 18 mmHg).

5.2 Exclusion Criteria

1. Age $<$ 18 years.

2. A history of allergy to methylxanthines (e.g., theophylline, pentoxifylline). Treatment with methylxanthines must be discontinued at least 4 hours prior to first dose of LSF study drug. If theophylline was administered within 24 hours of first LSF study dose, theophylline level must be less than 10μmol/L.

3. Participation in other experimental medication or intervention trials within the past 30 days.

4. Planned use of investigational agents or procedures during the 28 day study period.

5. Any other condition which in the investigator's opinion would not make the patient a good candidate for the trial.

6. Positive pregnancy test.

6 STUDY PROCEDURES

6.1 Screening Period

Potential patients will be evaluated in the intensive care units of approximately 24 hospitals that comprise the NIH ARDS Network. Study coordinators will evaluate all patients who meet the eligibility criteria. Informed consent will be obtained from each patient or surrogate. Patients must be enrolled, randomized and receive LSF study drug within 36 hours of meeting all clinical inclusion criteria (section 5.1. Pre-treatment screening procedures including a clinical evaluation, 12-lead ECG, CBC, blood chemistries and pregnancy test (if applicable) will be obtained. After the results of the screening procedures have been reviewed and a patient is determined to meet all eligibility criteria and informed consent

has been signed, the study coordinator will register the patient utilizing telephone randomization systems. Patients will be randomized on the ARDSNet Study 01 first. The ventilator strategy randomization must be known prior to randomization to LSF or placebo. The patient will be assigned a LSF kit number which will correlate to a blinded patient study number in the investigational pharmacy.

6.2 Treatment Period

LSF or placebo study drug will be administered as a single agent by a 10 minute intravenous infusion through a central venous catheter every 6 hours. LSF study drug administration will continue for 21 days or until the patient has achieved two consecutive calendar days of unassisted breathing, whichever occurs first.

Patients will be evaluated daily during the 28 day study period. Vital signs, laboratory parameters, ventilator and hemodynamic status and clinical changes will be monitored as outlined in the schedule of events (see Appendix D). On the day that LSF is discontinued, the next scheduled vital signs, CBC, and serum chemistry evaluations will be required. If unassisted breathing is achieved prior to Day 28, the patient should remain hospitalized for a minimum of two consecutive calender days following extubation, if possible, to document the achievement of the endpoint.

If a patient prematurely discontinues LSF study drug administration, study assessments including a clinical evaluation and the next scheduled laboratory evaluation must be performed prior to the institution of other therapy, if possible. If any study drug-related adverse experiences or toxicities are present at Day 20 or at the time of withdrawal from the study, the patient must be re-evaluated for the specific clinical or laboratory abnormality until the abnormality resolves or stabilizes. Patients will be evaluated for the development of serious adverse events for 14 days after study drug discontinuation. All deaths occurring within 60 days of study drug discontinuation will be recorded (see section 10).

7 LABORATORY AND CLINICAL EVALUATIONS

7.1 Screening Evaluations

Pre-treatment evaluations which should be performed within 24 hours of initiating therapy include:

1. Resting 12-lead electrocardiogram (ECG).
2. History and clinical evaluation.
3. Laboratory evaluation: complete blood count (CBC) and platelet count, creatinine, blood urea nitrogen (BUN), albumin, sodium, potassium, chloride, bicarbonate, glucose, total bilirubin, AST, ALT, and alkaline phosphatase.
4. Pregnancy test (serum or urine) for females for child-bearing potential.

7.2 Treatment Period Evaluations

7.2.1 Organ Failure Criteria and Scoring

The Brussels Organ Failure Table (Appendix C) will be utilized to document the development and reversal of multi-system organ failure throughout the 28 day study period. Laboratory values (creatinine, bilirubin, platelets), and vital signs generated as part of the patient's routine care will be used for these evaluations. The most aberrant value for any day for which data are available will be recorded during the first 28 days of the study period. Organ systems followed on a daily basis include cardiovascular (shock), pulmonary, renal, hepatic, and coagulation. Presence of shock will be evaluated throughout the study period on a daily basis using a clinical evaluation process that takes into account the dose of pressors being administered. Central nervous system dysfunction will be evaluated only at study entry, Day 14, Day 28 and study withdrawal.

7.2.2 Laboratory Evaluations

On Day 0 (prior to first dose of LSF study drug), 1, 2, 3, 4, 7, 14 and 21 the following laboratory tests will be performed: CBC and platelets, creatinine, BUN, albumin, sodium, potassium, chloride, bicarbonate, glucose, total bilirubin, AST, ALT, and alkaline phosphatase. If LSF or placebo is discontinued prior to day 20, then the next scheduled laboratory evaluation will be performed.

7.2.3 Surrogate Marker Levels

Blood samples for the determination of surrogate marker levels will be collected at 3 time points; prior to the first dose of LSF study drug on Day

0 and prior to a dose and 1 hour after completion of a dose on Day 3 (see Appendix B).

7.2.4 LSF Study Drug Levels

Blood samples to determine LSF and metabolite levels will be drawn from a port not used for LSF study drug infusion at 4 time points; immediately prior to and immediately upon completion of a LSF study drug infusion on Day 0 and Day 3 (see Appendix B).

If the patient develops a creatinine > 2.5 or bilirubin > 3.0, additional LSF levels will be drawn pre- and post- study drug infusion daily for 3 days and then weekly until Day 28 even if bilirubin or creatinine levels subsequently return to normal.

7.2.5 Infections

Data will be collected on the etiology, site, and day of onset of infectious complications. Investigators will assess infections from Day 0 through Day 28 (see Appendix A for definition of infections).

For all deaths from Day 0 through Day 28, the investigator will be asked to make a determination of the relationship of an episode or episodes of infection to the patient's death. The following is a guide to making that determination.

Not Related:

This category applies to those deaths which after careful consideration are clearly and incontrovertibly due to extraneous causes (underlying disease, complications of therapy, etc.).

Possible

This category applies to those deaths which after careful medical consideration at the time they are evaluated, are judged to be perhaps related to an episode or episodes of infection. A death may be considered possibly related to infection if or when:

1. Death follows a reasonable temporal sequence from the infection.
2. Death could readily have resulted from the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.

3. Death follows a known response pattern to the suspected infection.

Probable

This category applies to those deaths which, after careful medical consideration at the time they are evaluated, are felt with a high degree of certainty to be related to an episode or episodes of infection. A death may be considered probably related to the infection if or when:

1. Death follows a reasonable temporal sequence from the infection.
2. Death could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.
3. Death follows a known response pattern to the infection.

7.2.6 Electrocardiogram (ECG)

ECGs will be performed within one hour after LSF study drug administration on Day 1, Day 7, and Day 21. If LSF or placebo is discontinued prior to day 20, then the next scheduled ECG will be performed.

7.2.7 Vital Signs

Vital signs will be recorded prior to the first dose of LSF study drug on Day 0, at the conclusion of the first dose, and 30 minutes after the completion of the initial dose.

Minimum and maximum blood pressure, heart rate, respiratory rate, and temperature will be recorded on Days 0, 1, 2, 3, 4, 7, 14, and 21. If LSF or placebo is discontinued prior to day 20, then the next scheduled ECG will be performed.

8 LISOFYLLINE STUDY DRUG PREPARATION, PHARMACEUTICAL DATA, LABELING, AND DRUG ACCOUNTABILITY

8.1 Drug Preparation and Administration

Patients meeting eligibility criteria will be randomized to receive either LSF or placebo. Patients will be assigned a seven digit number beginning with "36". The assigned patient number will correspond with a prelabeled patient pack of LSF study drug ampuls at the site investigational pharmacy. The pharmacist will prepare an intravenous solution based on the patient's actual baseline weight to a maximum weight of 100 kg. The dose will be 3 mg/kg to a maximum dose of 300 mg. The dose for LSF or placebo will be prepared from a LSF study drug concentration of 60 mg/mL, and added to 50 mL of 0.9% normal saline.

The intravenous solution of LSF study drug will be administered as a single agent by a central venous catheter, if possible, over 10 minutes every 6 hours. A 5 mL normal saline flush should precede and follow each LSF study drug infusion. LSF administration will continue until Day 20 or until the patient is ventilator-free for 2 consecutive calendar days, whichever comes first.

Prepared LSF study drug should be administered within 72 hours of dilution. All doses should be clearly labeled with an expiration date of not greater than 72 hours after preparation. Diluted LSF study drug may be stored at room temperature prior to administration. There are no known toxic drug interactions.

Vital signs will be recorded prior to, immediately after, and 30 minutes after the first dose of LSF study drug infusion.

8.2 Pharmaceutical Data

8.2.1 Dosage Formulation

Lisofylline Injection is provided as a sterile solution of LSF at a concentration of 60 mg/mL in 10 mL glass ampuls. Note: Solution is hypotonic and is not be administered directly.

Each ampul of the injection solution contains the following:

Active Ingredient:	Lisofylline, 600 mg
Inactive Ingredients:	Citric Acid Monohydrate, USP Sodium Hydroxide, NF Hydrochloric Acid, NF Water for Injection, USP

Placebo, a sterile solution in 10 mL ampuls consists of:

Citric Acid Monohydrate, USP
Sodium Hydroxide, NF
Hydrochloric Acid, NF
Water for Injection, USP

8.3 Labeling

Each ampul will have a two part label. Part two of the ampul label will be considered a critical document and will be retained by the pharmacy. Part one of the ampul label will carry the following information:

ARDSNET STUDY 03

KIT NUMBER

LISOFYLLINE INJECTION, 60MG/ML OR PLACEBO INJECTION 10 ML

FOR INTRAVENOUS USE ONLY

WARNING: Hypotonic solution. Do not administer directly.

Store at Controlled Room Temperature: 20° – 25°C (68°F - 77°F) [See USP]

CAUTION: New Drug-Limited by Federal (or United States) Law to Investigational Use.

Cell Therapeutics, Inc. Seattle, WA 98119

LABEL CODE

8.4 Drug Storage

Drug supplies must be kept in appropriate, limited access and secured area at room temperature: 20° – 25°C (68°F - 77°F).

8.5 Drug Accountability

Accurate records of all drug shipments, ampuls dispensed, and of all drug returned or destroyed must be kept for each LSF study drug. This inventory record must be available for inspection by the Coordinating Center and is subject to FDA inspection at any time. The overall amount of drug shipped to each center and the amount destroyed or returned, with an explanation of any discrepancy must be provided at the conclusion of the study.

Drug supplies are to be used only in accordance with this protocol and under the supervision of the investigator. The investigator agrees not to destroy any labels or unused drug supplies unless otherwise directed by Cti. At the conclusion of the study, the investigator will ship all unused LSF study drug according to the instructions provided.

9 VENTILATOR SUPPORT

All patients treated in this study will be concurrently enrolled on the ARDSNet Study 01. The ARDSNet Study 01 provides detailed ventilator and weaning parameters. Data collection requirements regarding ventilator status are included in ARDSNet Study 01.

If a patient is withdrawn from ARDSNet Study 01, his/her participation in this trial, ARDSNet Study 03, should continue unless the physician determines withdrawal from the study to be in the best interest of the patient.

10 ADVERSE EXPERIENCE

10.1 Definition and Grading Intensity of Adverse Experience

The investigator will determine daily whether any clinical adverse experiences have occurred. The investigator will evaluate any changes in laboratory values and physical signs and will make a determination as to whether the change is clinically important and different from what is expected in the course of treatment for this patient population. If any adverse clinical experiences have occurred they will be recorded on the adverse event form. All adverse experiences that occur from the beginning of the LSF study drug treatment through 14 days after the last dose of

study drug will be recorded on the adverse event case report form.

10.2 Criteria for Determining Relationship of Adverse Experience to Lisofylline

The investigator will be asked to make a determination of the relationship of the clinical adverse experience to the study drug. The following is a guide to making that determination.

Not Related:

This category applies to those adverse events which after careful consideration are clearly and incontrovertibly due to extraneous causes (diseases, environment, etc.).

Possible

This category applies to those adverse events which after careful medical consideration at the time they are evaluated, are judged to be perhaps related to the study drug. An adverse event may be considered possibly related to the study drug if or when:

1. It follows a reasonable temporal sequence from administration of the drug.
2. It could readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.
3. It follows a known response pattern to the suspected drug.

Probable

This category applies to those adverse events which, after careful medical consideration at the time they are evaluated, are felt with a high degree of certainty to be related to the study drug. An adverse event may be considered probably related to the study drug if or when:

1. It follows a reasonable temporal sequence from administration of the drug.
2. It could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.
3. It follows a known response pattern to the infection.

10.3 Reporting

The U.S. Food and Drug Administration (FDA) defines a serious adverse drug experience as one that suggests a significant hazard, contraindication, side effect, or precaution. A serious experience is one considered to be fatal or life-threatening, is permanently disabling, requires inpatient hospitalization, prolongs hospitalization, or is a congenital anomaly, cancer or overdose. An unexpected adverse event is any experience not identified by type, severity, or frequency in the current study protocol or clinical safety updates or an event unexpected in ARDS or more severe or frequent than expected in ARDS patients.

All serious and unexpected adverse experiences that occur from the beginning of study drug treatment through 14 days after the last dose of study drug are immediately reportable to the Coordinating Center. All unexpected patient deaths that occur from the beginning of study drug treatment through 30 days after the last dose of study drug are immediately reportable to the Coordinating Center.

The ARDS Network Clinical Coordinating Center (CCC) must be notified immediately by telephone of any serious and unexpected adverse experience (including death) which occurs during the course of this investigation, whether or not related to study drug.

Clinical Safety Contact
ARDS Network Clinical Coordinating Center
Massachusetts General Hospital- East
149 13th Street, Room 4022
Charlestown, MA 02129
Phone: 800-291-0466
Fax: 617 724-9878

Subsequently, adverse event form must be completed and sent by facsimile or overnight mail to the Coordinating Center. If appropriate, laboratory reports and autopsy reports should be forwarded as they become available. The Coordinating Center will notify Cti who will then determine whether the serious adverse experience requires timely notification of the FDA.

Cti is responsible for timely notification of the FDA. The FDA requires that all serious adverse experiences that are both unexpected and associated with the use of the drug must be reported to the FDA in writing within 10 working days of notification of Cti. If the serious adverse experience is fatal or life-threatening, there is an additional obligation of Cti to notify the FDA by telephone within 3 working days, with a

follow-up written report in 10 working days.

11 PATIENT WITHDRAWAL

The investigator will make every reasonable effort to keep each patient in the study. All results of the evaluations and observations, together with a narrative description of the reason(s) for withdrawal from the study, must be recorded on the case report form (CRF).

Patients who are removed from the study due to adverse experiences (clinical or laboratory) or due to development of acute toxicity will be treated and followed according to established acceptable medical practice. All pertinent information concerning the outcome of such treatment will be entered on the CRF.

The following are justifiable reasons for the investigator to remove a patient from the study:

1. Unacceptable toxicity
2. Withdrawal of consent: The patient's desire to withdraw from the study may occur at any time.
3. Unforseen events: Any event which in the judgment of the principal investigator or principal monitor makes further treatment inadvisable.
4. Withdrawal: Withdrawal by the physician for clinical reasons not related to study drug treatment.
5. Death
6. Adverse Events: Adverse events associated with study drug administration, necessitating discontinuation of treatment, including exacerbation of underlying disease.
7. Violation of study protocol
8. Patient completes the protocol: Regardless of the reason for termination, all data currently available for the patient at the time of discontinuation of follow-up should be recorded on the case report form. All reasons for discontinuation of treatment must be documented.

12 STATISTICAL CONSIDERATIONS

12.1 Study Design and Justification of the Number of Subjects

The primary objective of this trial is to compare the effect of LSF to placebo on the incidence of Day 28 mortality in subjects with ALI and ARDS. From the previous ARDSNet study, the average mortality through Day 28 is expected to be 35%. This study will accrue up to 800 subjects and have an 81% chance of finding a significant benefit in mortality if the true difference is 10% or greater using a one-sided 0.025 significance level test (equivalent to a two-sided p=0.05 significance level). The study will be monitored by a Data Safety Monitoring Board (DSMB). Three interim analyses are planned, after 200, 400, and 600 subjects have completed the study. The study is designed to terminate early for futility and for efficacy.

The trial may be stopped by the DSMB for futility if the mortality for the subjects randomized to LSF is not at least 2% better than that observed in the placebo group. At each interim analysis, prior to evaluation the arm specific mortality, an assessment will be made of the overall mortality. If the combined arm mortality is too low to allow a 2% futility boundary and maintain at least 80% power to detect an absolute 10% difference in mortality, then the futility boundary criteria will be reduced. If there is no difference in mortality between arms the study has a 62% chance of stopping for futility at the first interim analysis, and a 7% chance of stopping early at the third interim analysis.

The DSMB may stop the trial for efficacy. The criteria to be used is based on an O'Brien-Fleming upper boundary with a one-sided 0.025 level. Three interim analyses are planned followed by the final analysis with 800 subjects. The boundary will be lowered slightly to counteract the conservatism of the futility boundary. The boundary is $1.94891\sqrt{4/i}$, where $i = 1, 2, 3, 4$ corresponding to each of the four planned analyses.

12.2 Phase II Evaluation of Lisofylline

At the first interim analysis, after approximately 200 subjects have completed the study, the data will be reviewed by the Steering Committee in addition to the DSMB. The Steering Committee will review the primary and secondary efficacy measures as well as safety data and have the option to stop the study and publish it as a negative Phase II study. Otherwise, the study will continue to accrue subjects and be considered as a Phase III study. The Steering Committee will not review the data at any subsequent

interim analyses. The purpose of the Steering Committee review is to evaluate LSF for safety and efficacy as a Phase II study. For example, the Steering Committee may consider the following:

Futility: There is no indication that LSF improves mortality, duration of ventilation, or other measures of lung function including but not limited to: (P/F ratio, static compliance or oxygenation step calculated as $10 * FiO_2 + 0.4 * PEEP$).

Safety: There are side effects that require a substantial change in the dose or schedule of LSF which makes the drug impractical or unsafe for use in the critically ill.

Wrong Subject Population: It appears that only a subgroup of subjects would benefit from therapy.

This phase II evaluation does not increase the probability of a type I error because of the probability the trial will complete and find a positive result using the phase II evaluation is less than the probability of the same outcome without the phase II evaluation. The type I error rate would only be increased if the Steering Committee evaluation could stop the trial if it were positive at the first interim analysis. Only the DSMB can stop the trial early with a claim of efficacy and the criteria used is based on the O'Brien-Fleming boundary discussed previously.

12.3 Methods of Statistical Analysis

The primary analyses will include all randomized subjects and will compare the treatment groups on the basis of the randomized treatment assignment, regardless of whether this treatment was actually received ("intention-to-treat" approach). As there is a single primary objective, no adjustments will be made for multiple comparisons.

The effect of center and treatment-by-center interaction will be described using the appropriate statistical modeling. However, it is recognized that this evaluation will be of limited value as the number of subjects in each center is small. No subset analyses will be performed for individual centers or subgroup of centers.

12.4 Analysis of Primary and Secondary Endpoints

The primary endpoint is whether or not the subject is alive 28 days after randomization. Secondary endpoints are: 1) Ventilator-free days (VFD) to

Day 28, 2) incidence of serious infections through Day 28, 3) infection-related mortality, and 4) Organ failure-free days to Day 28. The specific definitions of these endpoints are given in section 4.2. In addition, safety will be evaluated by measuring adverse events, laboratory values and subject vital signs.

All subjects will be followed for 28 days after randomization. Comparisons of mortality by treatment will be made using a two-sided stratified Fisher's exact test. Stratification will be by ventilator strategy as defined by the treatment arm of the companion ARDSNet Study 01. A secondary analysis of mortality will be made stratifying by the baseline organ function status. Specifically, subjects will be classified based on the presence of clinically significant organ failure for each of the five sites defined in the Brussels Table (see Appendix C). A Fisher's exact test for a mortality difference by treatment will be performed stratifying by the baseline organ failure status.

For each subject the number of ventilator-free days will be calculated according to the definition in section 4.2. The analysis of ventilator-free days will be made using a two-sided stratified Wilcoxon test ([14]). Stratification will be the same as for the primary analysis.

The definition of serious infections is given in Appendix A. All infectious events meeting the study criteria occurring after randomization through Day 28 will be included in the analysis. For each subject, the number of qualifying infections will be tabulated and compared. A two-sided Wilcoxon test will be used to compare the number of subjects with no infections, one infection, two infections, etc. between treatment groups.

For each subject death in the study period, the investigator will determine if the death was infection related. The analysis of infection-related deaths will be identical to the primary analysis of all cause mortality with the exception that only deaths probably related to infection will be included.

The number of days through Day 28 a subject if free of clinically significant organ failure will be evaluated using the Brussels Table (Appendix C). This endpoint is defined in section 4.2. For each of the five organ systems, the number of days a subject is alive and free of clinically significant organ failure will be calculated. In addition, the number of days a subject is free of all five organ failures will be calculated. If organ failure information is not available for any particular day, data from the previous day will be used in the calculation of organ failure-free days. The analysis of these metrics will be made in the same way as the analysis of ventilator-free days.

This study is a factoral study, with ARDSNet 01. For that reason data analyses performed for that study that compare 6mg/kg to 12mg/kg

ventilation will be performed on this study comparing LSF to placebo as secondary analyses. Furthermore, we will test for interactions of LSF with ventilator strategy on mortality and VFD.

12.5 Safety and Demographic Information

Information which characterizes the subject at entry and during the trial will be summarized and descriptive statistics will be produced. Adverse events will be summarized using the COSTART dictionary by the treatment received at the time of onset of the adverse event. Subject withdrawals will also be summarized by treatment received. ECG, laboratory parameters (chemistry and hematology), and subject vital sign data will also be summarized by treatment received. Changes in laboratory data from baseline will be evaluated. Descriptive statistics will be performed comparing treatment groups. To allow for multiple comparisons, significance levels from these tests will be compared against a critical p-value of 0.01.

13 TERMINATION OF STUDY

For any reasonable cause, the Investigator, the Study Governance, NHLBI or Cti, may terminate this study at any time and all study materials will be removed from the study site. The investigator must inform Cti immediately if the study is terminated. Situations where this might occur include patient enrollment that is unsatisfactory with respect to quality or quantity, data recording is inaccurate or incomplete on a frequent basis, or the FDA terminates the study.

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14 Appendices

A Infection Definitions

A.1 Definitions of Serious Infections

1. Bacteremia due to known pathogen with or without signs or symptoms

   One or more positive blood cultures of a known pathogen. For *Staphylococcus epidermidis* (coagulase-negative staphylococcus), two or more positive cultures drawn on separate occasions within 24 hours are required to define pathogenicity.

   Note: If criteria are met for a primary site of infection and for bacteremia during the same 48 time period, then the combination, e.g., nosocomial pneumonia/bacteremia or empyema/bacteremia, will be counted as one event of serious infection and not as two separate events, for the purposes of assessing frequency of serious infections in both groups. The primary site of infection will be recorded as the serious infection, not the bacteremia.

2. Disseminated fungal infection

   Positive blood cultures or evidence of deep tissue infection (candidal endophthalmitis; multiple small hepatic or splenic nodules with an elevated alkaline phosphatase; cutaneous embolic lesions containing fungal elements) or unexplained fever with three sites of colonization (e.g., sputum, urine, stool, superficial wound cultures).

3. Nosocomial pneumonia

   Suspected or Possible Pneumonia: patient must meet at least one criterion from two categories below (a, b or c).

   Probable Pneumonia: patient must meet at least one criterion from all three categories below (a and b and c).

   (a) Chest radiograph shows new infiltrate corresponding in size (although not necessarily to segmental anatomical boundaries) to at least one segment or cavitation with an air-fluid level within an area of infiltrate (*i.e.*, not a simple subpleural air cyst). The qualifying radiographic abnormality must persist over at least 48 hours with no decrease in its size.

   (b) New onset of or increase in fever ($T \geq 38.3°C$ or increase $\geq 1°C$ over the previous 24 hour T_{max} if T already $\geq 38.3°C$) or new hypothermia ($T \leq 36.0°C$) or increase in WBC (WBC $>$ 10,000

and a 25% increase or an increase in band forms to > 10% of total WBC) or new decrease in WBC to < 4,000.

(c) Bacteriological confirmation of pulmonary infection (can be any of the following):

- quantitative culture of tracheal secretions with $>10^6$ cfu/mm^3.
- quantitative culture of bronchoalveolar lavage with $>10^4$ cfu/mm^3.
- quantitative culture of protected specimen brush with $>10^3$ cfu/mm^3.
- positive Gram stain with $\geq$ 3+ of at least one type of bacteria.
- positive semi-quantitative sputum culture with $\geq$ 3+ growth of at least one type of potentially pathogenic bacteria.
- positive blood culture for bacterial pathogen also identified in sputum or other respiratory specimens.
- positive Gram stain or culture of pleural fluid for bacterial pathogen.

Only one episode will be considered to be present during the 28 day period for the following due to difficulty in defining successful therapy during this time period.

4. Sepsis (ACCP/SCCM Consensus Conference; Bone et al. Chest 1992; 101:1644-55)

Systemic inflammatory response syndrome (SIRS) - at least two of the following:

a. temperature > 38°C or < 36°C.

b. heart rate > 90 beats per minute.

c. respiratory rate > 20 breaths per minute or $PaCO_2$ < 32 mmHg.

d. white blood cell count > 12,000/cu mm < 4000/cu mm, or > 10% immature (band) forms.

Sepsis - the systemic response to infection with at least 2 of the following:

a. temperature > 38°C or < 36°C.

b. heart rate > 90 beats per minute.

c. respiratory rate > 20 breaths per minute or $PaCO_2$ < 32 mmHg.

d. white blood cell count > 12,000/cu mm < 4000/cu mm, or > 10% immature (band) forms.

Severe sepsis

Sepsis associated with organ dysfunction or hypoperfusion. Hypoperfusion and perfusion abnormalities may include, but are not limited to lactic acidosis, oliguria, or an acute alteration in mental status.

Septic shock

Sepsis-induced hypotension (Systolic blood pressure < 90 mmHg or a reduction of ≥ 40 mmHg from baseline or vasopressor use to maintain blood pressure in the absence of other causes for hypotension) of at least 2 hours duration despite at least 500 mL fluid resuscitation along with the presence of perfusion abnormalities which may include, but are not limited to lactic acidosis, oliguria, or an acute alteration in mental status. Patients who are receiving inotropic or vasopressor agents may not be hypotensive at the time that perfusion abnormalities are measured.

Sepsis-induced hypotension

Systolic blood pressure < 90 mmHg or a reduction of ≥ 40 mmHg from baseline or vasopressor use to maintain blood pressure in the absence of other causes for hypotension.

Episode ends when criteria for septic shock are no longer met for a 24 hour period.

5. Peritonitis not associated with peritoneal dialysis

   Positive Gram stain or culture of peritoneal fluid with an elevated PMN count ($>250/mm^3$) in the fluid or free peritoneal air demonstrated radiographically with bacteremia (as defined above).

   Only one episode will be considered to be present during the 28 day period for the following due to difficulty in defining successful therapy during this time period.

6. Wound infection requiring extensive debridement and/or healing by secondary intention.

   Positive culture of normally sterile wound with evidence of infection locally plus debridement of tissue greater or equal in volume to 1 cm thick x 1 cm deep x 2 cm long or removal of sutures to allow wound to dehisce or unexpected wound dehiscence.

   Only one episode will be considered to be present during the 28 day period for the following due to difficulty in defining successful therapy during this time period.

7. Meningitis

Positive CSF Gram stain or culture or antigen detection for a pathogen with WBCs in CSF.

Only one episode will be considered to be present during the 28 day period for the following due to difficulty in defining successful therapy during this time period.

8. Empyema

   Grossly purulent pleural fluid or positive pleural fluid Gram stain or culture for a pathogen associated with an elevated pleural fluid WBC count.

   Only one episode will be considered to be present during the 28 day period for the following due to difficulty in defining successful therapy during this time period.

9. Abdominal or other deep tissue abscess

   Unequivocal radiographic evidence or equivocal radiographic evidence confirmed by positive culture of abscess contents or documentation of a purulent fluid collection by surgical exploration.

   Only one episode will be considered to be present during the 28 day period for the following due to difficulty in defining successful therapy during this time period.

10. Disseminated viral infection

    Skin rash with viral etiology of lesions confirmed by culture or cytology and characteristic lesions present over more than two adjacent dermatomes.

    Only one episode will be considered to be present during the 28 day period for the following due to difficulty in defining successful therapy during this time period.

A.2 Definitions of Other Infections

1. *C. difficile* colitis
   Diarrhea associated with the presence of *C. difficile* toxin or *C. difficile* in the stool.

2. Indwelling vascular line infection

   Central venous, peripheral venous, or peripheral arterial infection is defined as catheter-related bloodstream infection (bacteremia) or catheter colonization in the presence of local site inflamation.

   Catheter-related bloodstream infection

- isolation of the same organism from the blood and one or both catheter segments ($\geq$ 15 colony-forming units), with no other identified source.

  Catheter colonization

- growth of 15 or more colony-forming units of an organism on semi-quantitative culture of the tip or subcutaneous segment.

  Local site inflammation

- presence of purulence alone, or erythema with one of the following: tenderness, increased warmth, induration, lymphangitis, or a palpable thrombosed vein.

3. Oral or mucosal candidiasis

   Presence of characteristic lesions on a mucosal surface (e.g., oral or vaginal mucosa) with a positive fungal stain, KOH preparation, or culture.

4. Peritonitis associated with peritoneal dialysis

   Positive Gram stain or culture of peritoneal fluid with an elevated PMN count ($> 500/mm^3$) in the fluid.

   Episode ends when criteria for definition are not longer met.

5. Sinus infection

   Endoscopic observation of purulent drainage from maxillary, frontal, or sphenoid sinuses or purulent nasal drainage with moderate to abundant growth of pathogenic bacteria and a sinus CT scan demonstrating an air-fluid level or a completely opacified sinus.

6. Skin infection including non-disseminated viral infection

   Cellulitis, cutaneous abscess not requiring extensive debridement, wound infections not requiring extensive debridement or resulting in wound dehiscence.
   or
   Skin rash with viral etiology of lesions suggested by characteristic lesions present two or less adjacent dermatomes with or without confirmation by culture or cytology. This includes localized labial HSV infections.

7. Septic arthritis

   Clinical evidence of joint inflammation (erythema, warmth, pain, or presence of an effusion) with an elevated PMN count in joint fluid and a positive Gram stain or culture for a pathogen.

8. Urinary tract infection

   Greater than or equal to 100,000 colony forming units of a bacteria on culture of the urine associated with evidence of infection on urinalysis based on one of the following criteria: $\geq$ 2+ WBC, presence of leukocyte esterase, or presence of nitrites. The presence of perinephric or renal abscess should be classified as a serious infection under "abdominal or other deep tissue abscess".

B Specimen Collection and Handling Procedures

B.1 Surrogate Marker Levels

Blood samples for the determination of surrogate marker levels are to be drawn at three time points:

1. Within 30 minutes of the first dose of LSF study drug administration on Day 0
2. Within 30 minutes of a dose of LSF study drug on Day 3
3. One hour after a dose of LSF study drug on Day 3

Procedures

Each 12.0 mL blood sample is to be collected in a 12.0 mL green top vacutainer tube (sodium heparin). The sample should be placed on ice immediately and refrigerated until further processing. Each sample should be processed within 2 hours of collection. Plasma should be prepared by centrifugation and aliquoted into the 1.5 mL polypropylene tubes. Plasma samples should be stored in a freezer set to maintain a temperature of -20°C or colder.

B.2 LSF Study Drug Levels

Blood samples for the determination of peak and trough LSF levels are to be drawn at four time points:

1. Up to 30 minutes before a dose of LSF study drug on Day 0
2. Within one minute after the completion of LSF study drug on Day 0
3. Up to 30 minutes before a dose of LSF study drug on Day 3
4. Within one minute after the completion of LSF study drug on Day 3

Additional samples will be collected if a patient's creatinine is >2.5 or bilirubin is >3.0.

1. First calendar day of creatinine >2.5 or bilirubin >3.0

2. Second calendar day (drawn regardless of lab value)
3. Third calendar day (drawn regardless of lab value)
4. Ten calendar days after first day of lab evaluations (if prior to Day 28)
5. Seventeen calendar days after first day of lab evaluation (if prior to Day 28)
6. Twenty-four calendar days after first day of lab evaluation (if prior to Day 28)

Only one pre- and post- LSF level is required each day.

Procedures

A 4.5 mL sample is to be collected in a 4.5 mL blue top tube (containing buffered sodium citrate). The sample should be drawn precisely at the end of the infusion but should not be drawn through the same line through which the LSF study drug was infused. Samples should be placed on ice and stored in the refrigerator no longer than 2 hours before the sample is processed. Plasma should be prepared by centrifugation and aliquoted into a 4.5 mL polypropylene tube. Pre-labeled tubes and collection records will be provided by the repository. Plasma samples should be stored in a freezer set to maintain a temperature of -20°C.

B.3 Documentation of Blood Sample Collection and Processing

The repository (McKesson BioServices) will provided pre-printed labels, tubes and sample collection records. The following information will be documented in the sample collection records:

- Patient identification number
- Start and completion time of LSF study drug administration
- Date of collection
- Time of sample collection
- Label identification number

B.4 Shipment of Plasma Samples

The plasma samples will be shipped to McKesson BioServices for storage. Shipping containers, cryotubes, barcoded labels and instructions will be provided by the repository. All samples must be shipped on dry ice. Notify McKesson BioServices and the Coordinating Center in advance by faxing the requested forms to each location.

C Brussels Table

ORGANS		ABNORMAL			
			CLINICALLY SIGNIFICANT ORGAN DYSFUNCTION		
	Normal	Mild	Moderate	Severe	Extreme
Cardiovascular systolic BP (mmHg)	> 90	≤ 90 Responsive to fluid	≤ 90 Unresponsive to fluid	≤ 90 plus pH ≤7.3	≤ 90 plus pH ≤ 7.2
Pulmonary PaO_2/FiO_2	>400	400-301	300-201 Acute Lung Injury	200-101 ARDS	≤100 Severe ARDS
CNS (Glasgow Score)	15	14-13	12-10	9-6	≤5
Coagulation Platelets ($\times 10^3/mm^3$)	> 120	120-81	80-51	50-21	≤ 20
Renal Creatinine (mg/dL)	< 1.5	1.5-1.9	2.0-3.4	3.5-4.9	≥ 5.0
Hepatic Bilirubin (mg/dL)	< 1.2	1.2-1.9	2.0-5.9	6.0-11.9	≥12
Round Table Conference on Clinical Trials for the Treatment of Sepsis Brussels, March 12-14, 1994					

Schedule of Events for Study Procedures

ARDSNet Study 03

	Pre-	Treatment Period																					Post-TX		Follow
Study Day	RX	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	28	up(s)
Randomization [a]		X																								
LSF or placebo (IV)[f]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
Informed Consent	X																									
Vital Signs [b,g]		X	X	X	X	X			X							X							X			
CBC [g]	X	[b]	X	X	X	X			X							X							X			
Serum Chemistry [g]	X		X	X	X	X			X							X							X			
Pregnancy Test [c]	X																									
12-lead ECG	X	X							X														X			
Glasgow Coma Score	X															X									X	
Pre and Post LSF		X			X																					
Surrogate Marker [d,e]	X				X																					
Mortality Assessment																									X	X

a ***Randomization:*** Patients must be randomized on the ARDSNet Study 01 prior to being randomized to ARDSNet Study 03.

b ***Vital Signs:*** Initial LSF study drug infusion prior to, immediately post, and 30 minutes post.

c ***Pregnancy Test***: Females with child bearing potential.

d ***Pre & Post LSF Levels:*** Immediately prior to and immediately upon completion of LSF study drug infusion.

e ***Pre & Post LSF Levels:*** If Creatinine > 2.5 or Bilirubin > 3.0 draw pre & post levels daily for 3 days and then weekly until Day 28.

f ***LSF or placebo:*** LSF or placebo to be discontinued when the patient achieves two consecutive calendar days of unassisted breathing.

g When LSF or placebo is discontinued, the next scheduled vitals signs, CBC and serum chemistry are required.

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Amendment to the ARDSNet Study 03, A Phase II/III, Randomized, Double-Blind, Placebo-Controlled Trial of Lisofylline in Patients with Acute Lung Injury and Adult Respiratory Distress Syndrome.

Page 41, ARDSNet Study 03, version I, January 5, 1998

B. Specimen Collection and Handling Procedures

B.1. Surrogate Marker Levels

Blood samples, serum and plasma, for the determination of surrogate marker levels are to be drawn at three time points. Samples should be drawn in both green top and red top tubes at the three time points.

1. Within 30 minutes prior to the first dose of LSF study drug administration on Day 0.

2. Within 30 minutes prior to a dose of LSF study drug on Day 3.

3. One hour after a dose of LSF study drug on Day 3.

Procedures:

Each 12.0 mL blood sample is to be collected in a 12.0 mL green top vacutainer tube (sodium heparin). The sample should be placed on ice immediately and refrigerated until further processing. Each sample should be processed within 2 hours of collection. Plasma should be prepared by centrifugation and aliquoted into the 1.5 mL polypropylene tubes. Plasma samples should be stored in a freezer set to maintain a temperature of -20°C or colder.

An additional 12.0 mL blood sample is to be collected in a 12.0 mL red top vacutainer tube. The sample should be placed on ice immediately and refrigerated until further processing. Each sample should be processed within 2 hours of collection. Serum should be prepared by centrifugation and aliquoted into the 1.5 mL polypropylene tubes. Serum samples should be stored in a freezer set to maintain a temperature of -20°C or colder.

Clarifications to the January 5, 1998 version I, Lisofylline and Respiratory Management in ALI/ARDS (ARDSNet Study 03)

Page 6:
Part I, Study Summary

"**Number of subjects**: 800 patients; interim analyses will be performed after approximately 200 and 400 patients have completed treatment."

"**Number of subjects**: 800 patients; interim analyses will be performed after approximately 200, 400, and [600] patients have completed treatment."

"**Duration of Patient Participation**: ...Acute evaluation for 28 days and safety follow up for 60 days after final LSF study drug administration."

"**Duration of Patient Participation**: ...Acute evaluation for 28 days and safety follow up for 60 days after [study entry]."

Page 19:

First paragraph, delete the last 4 words, [in the investigational pharmacy].

"The patient will be assigned a LSF kit number which will correlate to a blinded patient study number in the investigational pharmacy."

The last sentence should now read: "The patient will be assigned a LSF kit number which will correlate to a blinded patient study number."

Section 6.2, third paragraph, last line, change "drug discontinuation" to "entry".

"All deaths occurring within 60 days of study drug discontinuation will be recorded."

"All deaths occurring within 60 days of study [entry] will be recorded."

Page 23:

Section 8.1, first paragraph, second line, change "seven" to "six". On the third line, change "patient number" to "kit number".

"Patients will be assigned a seven digit number beginning with "36". The assigned patient number will correspond with a prelabeled patient pack of LSF study drug ampuls at the site investigational pharmacy."

"Patients will be assigned a [six] digit number beginning with "36". The assigned [kit] number will correspond with a prelabeled patient pack of LSF study drug ampuls at the site investigational pharmacy."

Section 8.2.1, first paragraph, second line, add the word "to".

"Note: Solution is hypotonic and is not [to] be administered directly."

Page 24:

Section 8.3, fourth line, change ARDSNET STUDY 03 to [CTI1036]

Page 26, last line, change the word "infection" to "drug".

"3. It follows a known response pattern to the infection."

"3. It follows a known response pattern to the [drug]."

Page 27:

Section 10.3, second paragraph, fifth line, change "30 days after the last dose of study drug" to "60 days after study entry."

"All unexpected patient deaths that occur from the beginning of study drug treatment through 30 days after the last dose of study drug are immediately reportable to the Coordinating Center."

"All unexpected patient deaths that occur from the beginning of study drug treatment through [60] days after [study entry] are immediately reportable to the Coordinating Center."

Page 32:

section 12.5 second sentence, delete [using the COSTART dictionary by the treatment received as the time on onset of the adverse event]. Change "treatment received" to "treatment assignment".

"Adverse events will be summarized using the COSTART dictionary by the treatment received at the time of onset of the adverse event. Subject withdrawals will also be summarized by treatment received. ECG, laboratory parameters (chemistry and hematology), and subject vital sign data will also be summarized by treatment received."

"Adverse events will be summarized by treatment [assignment]. Subject withdrawals will also be summarized by treatment [assignment]. ECG, laboratory parameters (chemistry and hematology), and subject vital sign data will also be summarized by treatment [assignment]."

Section 13: first sentence,

"For any reasonable cause, the Investigator, the Study Governance, NHBLI or Cti, may terminate this study at any time ....."

"For any reasonable cause, the Investigator, the [ARDS Network Steering Committee], NHBLI or Cti, may terminate this study at any time ....."

Page 41, section B.1, items 1 and 2,

Blood samples, [serum and plasma], for the determination of surrogate marker levels are to be drawn at three time points. [Samples should be drawn in both green top and red top tubes at the three time points.]

"1. Within 30 minutes of the first dose of LSF study drug administration on Day 0.

2. Within 30 minutes of a dose of LSF study drug on Day 3."

"1. Within 30 minutes [prior to] the first dose of LSF study drug administration on Day 0.

2. Within 30 minutes [prior to] a dose of LSF study drug on Day 3."

Add additional paragraph after first paragraph in the Procedures section:

An additional 12.0 mL blood sample is to be collected in a 12.0 mL red top vacutainer tube. The sample should be placed on ice immediately and refrigerated until further processing. Each sample should be processed within 2 hours of collection. Serum should be prepared by centrifugation and aliquoted into the 1.5 mL polypropylene tubes. Serum samples should be stored in a freezer set to maintain a temperature of -20°C or colder.