TITLE: **A PHASE I/II RANDOMIZED DOUBLE-BLINDED PLACEBO-CONTROLLED CLINICAL TRIAL TO DETERMINE SAFETY AND FEASIBILITY OF USING AN ACELLULAR STERILE FILTERED AMNIOTIC FLUID AS A TREATMENT FOR COVID-19 PATIENTS**

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Principal Investigator

Craig Selzman, MD
Division of Cardiothoracic Surgery
Department of Surgery
University of Utah
Salt Lake City, Utah

Sub-Investigators

Nathan Hatton, MD
Division of Pulmonary
Department of Internal Medicine
University of Utah
Salt Lake City, Utah

Joseph Tonna, MD
Division of Cardiothoracic Surgery
Department of Surgery
University of Utah
Salt Lake City, Utah

Giavonni Lewis, MD
Division of Burn/Wound Surgery
Department of Surgery
University of Utah
Salt Lake City, Utah

John Phillips, PhD
Division of Hematology
Department of Internal Medicine
University of Utah
Salt Lake City, Utah

Jan Pierce, MBA
Cell Therapy and Regenerative Medicine
Department of Internal Medicine
University of Utah
Salt Lake City, Utah

1. SYNOPSIS

Title	A Phase I/II Randomized, Double-Blinded, Placebo-Controlled Clinical Trial to Determine Safety and Feasibility of Using an Acellular Sterile Filtered Amniotic Fluid as a Treatment for COVID-19 Patients.
Short Title	hAF for the Treatment of COVID-19
IRB Number	IRB: 00132922
IND	IND # 23369
Phase	Phase I/II
Design	This is a prospective double-blinded, randomized controlled study to determine the safety and feasibility of using processed sterile filtered amniotic fluid (hAF) to treat and reduce inflammation in COVID-19 patients.
Study Duration	1 year
Study Center(s)	University of Utah
Objectives	Primary Objectives: • To determine the safety of using processed human amniotic fluid (hAF) intravenously (IV) to treat patients with COVID-19. • To determine efficacy of hAF vs. placebo to reduce inflammation in COVID-19 patients. • To determine clinical outcomes in patients treated with hAF vs. placebo.
Number of Subjects	60 patients (30 per treatment arm)
Eligibility Criteria	Inclusion: 1. Age ≥18 2. SARS-CoV-2 positivity by RT-PCR test, obtained within 14 days of enrollment 3. Hospitalized 4. COVID-19 symptomatic (cough, fevers, shortness of breath, and/or sputum production) 5. Has a room air pulse oximetry of ≤94% and requires supplemental oxygen therapy 6. Patients of child bearing potential who agree to use acceptable methods

	of contraception for 90 days after last administration of study IP 7. Patients who are receiving standard of care therapies for COVID-19 that are not FDA approved are eligible for this study 8. Patients must be able to consent to the study (e.g. Glasgow Coma Score of ≥ 14) 9. Patients are required to have controlled blood pressure of < 160/96 and have a pulse of <110 at the time of study drug administration.

	Exclusion: 1. Patients on invasive mechanical ventilation (e.g., endotracheal intubation) 2. Chronic home oxygen utilization 3. Clinically significant home use of immunosuppressive medications, including more than 20mg of steroid (e.g., prednisone) per day, monoclonal antibodies, or chemotherapy medications. Patients who are not chronically immune suppressed are eligible for participation. Patients who are prescribed steroids, including dexamethasone, for the purpose of treating COVID-19 prior to the patient's study hospital admission will not be excluded. 4. Women who are pregnant, breastfeeding or become pregnant during the study 5. Patients on non-invasive positive pressure ventilation 6. Patients on >12 liters per minute via non-rebreather (NRB) or >80% oxygen via high flow nasal cannula 7. Patients who in the opinion of the Investigator has impending respiratory failure, defined as requiring rapidly escalating oxygen supplementation 8. Patients with a hemoglobin < 9 mg/dL 9. Patients diagnosed with Stage 4 or 5 chronic kidney disease (CKD) 10. Patients with diagnosed with Class 3 or 4 congestive heart failure (CHF) 11. Patients with a left ventricular assist device (LVAD) 12. Patients with current thromboembolic phenomena 13. Patients with Type 2 and above heart block

	14. Patients with established positive bacterial blood cultures prior to enrollment 15. Patients with ongoing pericardial effusion, or ascites 16. Patients with clinically significant arrhythmia 17. Patients with liver function tests (ALT or AST) >3x normal 18. Patients with untreated HIV infection 19. Patients diagnosed with end-stage organ disease
Study Product, Dose, Route, Regimen	hAF will be delivered to the clinical setting on the date of application. Dose is 10mL/day for 5 days; patients may be discharged prior to completing regimen. Route: Intravenous (IV) Push
Statistical Methodology	One goal of the analysis is to demonstrate safety, which does not require statistical significance. The other primary goal of the analysis is to assess efficacy of hAF by reducing inflammation in COVID-19 patients, potentially leading to improved clinical outcomes. This will be assessed by measurement of C-reactive protein levels before and after the intervention. Secondary outcomes include: 1. Death within 30 days 2. ICU-free days at 30 days 3. Hospital length of stay 4. Need for invasive mechanical ventilation 5. Biomarker levels (interleukin-6, d-dimer, lactate dehydrogenase) 6. Need for ECMO 7. Major adverse cardiac events 8. Patient-reported functional status

*Enrolled subjects will need to meet blood pressure requirements prior to IP administration, detailed below

2. STUDY OBJECTIVES

The purpose of this study is to explore the effectiveness of processed human amniotic fluid as a treatment for COVID-19. Past use of human amniotic products (i.e., membrane and fluid) has previously been FDA-approved as a human cells, Tissues, and Cellular and Tissue-Based Products (HCT/P) under 21 CFR 1271 for tissue injury; and has been used to reduce inflammation and fibrosis in patients with a variety of ailments. Given this, we hypothesize that intravenously (IV) administered processed sterile filtered amniotic fluid will reduce inflammation in COVID-19 patients, and improve secondary clinical outcomes. Specifically, we hypothesize that patients who receive IV administered hAF will see a 50% reduction in mean C-reactive protein levels following treatment.

3. ENDPOINTS

Primary Endpoint:

- C-reactive protein (CRP) levels measured before and after treatment
- Whether the patient experienced any post-randomization, study-related Adverse Events (AEs) while on study (up to 5 days) of treatment.

Secondary Endpoint:

- Other endpoints include mean days of supplemental oxygen, mean days of mechanical ventilation, mean ICU and hospital length of stay, and patient-reported functional status.
- Survival at days 7, 14, 60, 90 days post randomization.

4. BACKGROUND AND HYPOTHESES

Early data in the COVID-19 pandemic suggests that women who have given birth while actively infected may not transmit the virus to the baby, amniotic fluid, or breast milk.[1-2] Given the use of amniotic products for treatment of inflammation in other situations, described below, our group proposes an experimental and innovative use of hAF as its impact on cardiopulmonary failure has not been previously investigated. We hypothesize that systemic administration of human amniotic fluid (hAF) may assist in decreasing inflammation in patients infected with COVID-19.

Human amniotic membrane and human amniotic fluid have been shown to reduce inflammation,[3-5] have antimicrobial properties,[6-8] and low risk of immunogenicity.[9] hAF is devoid of any cellular products (not to be confused with umbilical cord-derived, AF-derived stem cell products, or AF embolism). Purified hAF is a cell-free, non-antigenic solution of >1000 proteins that mother nature developed. Hence, amniotic products make ideal biocompatible scaffolds for the treatment of diverse conditions, including intractable epithelial defects, burns,

diabetic/peripheral vascular ulcers, partial limbal cell deficiencies, peripheral nerve regeneration, tendon repair, and Stevens-Johnson syndrome.[10-16] The University of Utah is well-poised to perform this investigation, as human amniotic membrane (hAM) is currently being used here as a clinical tissue allograft in burn patients, for digital ulcers in scleroderma patients, as a nerve wrap to protect nerves from adhesions post-surgery, and as trachea-stenosis-tracheal stent covering. Likewise, there is an ongoing study exploring post-cardiac surgery atrial fibrillation, thought to be associated with local and systemic inflammation, and the impact of using hAM as a substitute pericardial patch to decrease post-operative inflammation. While results are not yet available, the six (of twelve) patients who have randomized to treatment have had no ill effects from the placement of the membrane. With regard to fluid, hAF has been successfully injected into >500 burn patients to augment graft survival at the University of Utah. hAF is also currently being used experimentally under IND approval at the University of Utah to treat ocular graft versus host disease and ocular PRK. An IRB application to study hAF to treat chronic wounds is pending. IND applications have been or are being submitted to the FDA to treat osteoarthritis and radicular lower back pain.

In basic science laboratories here at the University of Utah, we have demonstrated that rats undergoing myocardial ischemia reperfusion have a marked decrease in myocardial injury and fibrosis if they receive either intramyocardial or systemic intravenous injection of hAF. Thus, there is conceptual attractiveness in the potential impact of systemic administration of hAF in COVID-19 patients. Many COVID-19 patients, in addition to succumbing to acute hypoxic respiratory failure, are plagued with cardiac manifestations including myocarditis, accelerated heart failure, and arrhythmias.[17] Lung pathology specimens from the SARS-1 epidemic in 2005 demonstrated diffuse alveolar damage with extensive fibrosis.[18] Likewise, lung pathology in COVID-19 patients undergoing lung resection for lung cancer (i.e., non-autopsy pathology) revealed edema and patchy, inflammatory cellular infiltrates, particular with macrophages.[19] As COVID-19 causes an aggressive inflammatory and fibrotic response, this suggests that hAF, by mitigating inflammation and decreasing fibrosis, could impact the natural history of COVID-19 infected patients.

Importantly, the University of Utah is home to the Cell Therapy and Regenerative Medicine (CellReGen) program. CellReGen is unique in the world for being able to process and manufacture hAF for clinical use.[20] hAF has previously been FDA-approved as a human cellular and tissue product (HCT/P) under 21 CFR 1271[21] for tissue injury. It has been generated by CellReGen, utilized widely, and ***safely*** used in thousands of patients without demonstrable toxicity. If hAF proves effective, we are confident that CellReGen could mobilize mass quantities of hAF in a relatively short period of time, which could be used to treat COVID-19 nationally.

Ten patients were enrolled in a pilot study. These patients were diagnosed with COVID-19 by SARS-CoV-2 positivity by RT-PCR test. Patients were 40% female with an average age of 51.9 years old (range 24-76). Patients were 50% white, with two patients identifying as American Indian/Alaskan Native, three patients identifying as Hispanic (one Hispanic/white), and one as unknown or other.

Assessment of treatment efficacy is limited to the last six patients enrolled (the IV-only group), as the administration route and dosage for the (hAF) was adjusted after the first four patients (the nebulization and IV group). Initially, all patients were being treated with 3ml nebulized hAF and only those in the ICU were receiving 3ml IV hAF. However, nebulization was removed after concerns about aerosol exposure were raised by the respiratory therapists. In removing this administration route from the protocol, it was decided that all enrolled patients, regardless of admission location, would receive a standard IV dose of 10ml hAF. The last six enrolled patients received this treatment. Four out of those six patients were considered to be responders, with decreases in inflammatory biomarker levels (C-reactive protein, lactate dehydrogenase, interleukin-6, and d-dimer), while two did only not show reduction in these levels, but saw increases. It is unknown if the increases are an adverse reaction to the hAF administration or an indicator of severity of disease. Nine of the ten patients have been discharged. One patient (in the nebulization and intravenous group) died on hospital day 8. This patient was critically ill and it is unlikely that hAF contributed to her illness severity. There were no other reported safety concerns, other than the nebulization, addressed above. It appears based on this limited pilot sample that patients who responded most favorably to IV hAF were those who were moderately, not critically, ill.

Of the 10 patients enrolled, seven were admitted directly to the intensive care unit (ICU). All of these patients improved and six were moved to the medical floor. Five patients were discharged home and one was discharged to a rehabilitation hospital. One patient was discharged home directly from the ICU. Two patients were initially admitted to the medical floor but were moved to the ICU due to decompensation. One patient improved and was moved back to the medical floor prior to being discharged home, while the other patient died. The final patient was admitted to the medical floor and remained there throughout her hospitalization.

All ten patients required oxygen support during their hospital admission. Three patients required a nasal cannula or simple mask as their highest level of support, while two were placed on a high flow nasal cannula as their highest support. Five patients were intubated during their hospitalization. Two of those patients have received a tracheostomy. Both of these patients had their trach removed prior to discharge. Of the 9 patients who have been discharged alive, 7 required home oxygen use.

Primary outcome measures included ventilator-free days and duration of supplemental oxygen use. Of the three patients who were mechanically ventilated and ultimately extubated and discharged, one was hospitalized for 56 days with 6 days off the ventilator, one was hospitalized for 11 days with 5 days off the ventilator, and one was hospitalized for 44 days, received a tracheostomy, and had 15 days off the ventilator and the final patient was hospitalized for 38 days with 28 days off the ventilator. With regard to supplemental oxygen use, two patients were entirely on room air at the time of discharge. All other patients were requiring continuous (n=7) or intermittent (n=1) supplemental oxygen.
Secondary outcome measures included the following:

- Death within 30 days

  - N=1
- ICU free days at day 30
  - 9 of 10 patients were admitted to the ICU. One has died. One patient was discharged directly from the ICU. Of the remaining eight patients who were in the ICU and moved to the medical floor prior to discharge home, the average ICU-free days/LOS on the medical floor was 5.9 days (range 1-19 days). Average ICU length of stay (n=8) was 17.38 days (range 2-56 days).
- Hospital length of stay
  - Patients (n=10) had an average hospital length of stay (LOS) of 20.4days (range 5-56 days).
- ECMO
  - One patient required treatment with veno-venous ECMO. The patient was on ECMO for 41 days.
- Major adverse cardiac events
  - One patient developed cardiomyopathy, evidenced by worsening tachycardia, shock requiring norepinephrine, and elevated creatine kinase. This patient died on hospital day 8.
- Discharge/death
  - Eight patients have been discharged home. One patient was discharged to acute inpatient rehabilitation. One was readmitted for pain and numbness and subsequently discharged. One patient is deceased.
- Patient-reported outcomes
  - The final six enrolled patients were consented for patient-reported outcomes. These surveys will begin at one-month post-discharge.

Based on the findings of the pilot study, we plan to modify the trial and recruit only patients who are considered moderately ill. Patients who are endotracheally intubated on mechanical ventilation will not be eligible.

Summarily, merging the COVID-19-related observations and our experience with hAF, we hypothesize that systemic administration of hAF may assist in the treatment of this disease in moderately ill patients, specifically by reducing inflammation in the lungs. Ideally, this treatment could decrease the number of infected patients requiring critical care, thereby impacting patient outcomes as well as the socioeconomic burden of this pandemic on precious resources.

5. ELIGIBILITY CRITERIA

Population:
Hospitalized adult patients with confirmed acute COVID-19 infection.

Inclusion:
1. Age ≥18
2. SARS-CoV-2 positivity by RT-PCR test, obtained within 14 days of enrollment
3. Hospitalized

4. COVID-19 symptomatic (cough, fevers, shortness of breath, and/or sputum production)
5. Has a room air pulse oximetry of ≤94% and requires supplemental oxygen therapy
6. Patients of child bearing potential who agree to use acceptable methods of contraception for 90 days after last administration of study IP
7. Patients who are receiving standard of care therapies for COVID-19 that are not FDA approved are eligible for this study
8. Patients must be able to consent to the study (i.e., Glasgow Coma Score of ≥ 14)
9. Patients are required to have controlled blood pressure of < 160/96 and have a pulse of <110 at the time of study drug administration.

Exclusion:

1. Patients on invasive mechanical ventilation (e.g., endotracheal intubation)
2. Chronic home oxygen utilization
3. Clinically significant home use of immunosuppressive medications, including more than 20mg of steroid (e.g., prednisone) per day, monoclonal antibodies, or chemotherapy medications. Patients who are not chronically immune suppressed are eligible for participation. Patients who are prescribed steroids, including dexamethasone, for the purpose of treating COVID-19 prior to the patient's study hospital admission will not be excluded.
4. Women who are pregnant, breastfeeding or become pregnant during the study
5. Patients on non-invasive positive pressure ventilation
6. Patients on >12 liters per minute via non-rebreather (NRB) or >80% oxygen via high flow nasal cannula
7. Patients who in the opinion of the Investigator has impending respiratory failure, defined as requiring rapidly escalating oxygen supplementation
8. Patients with a hemoglobin < 9 mg/dL
9. Patients diagnosed with stage 4 or 5 chronic kidney disease (CKD)
10. Patients with diagnosed with NYHA class 3 or 4 congestive heart failure (CHF)
11. Patients with a left ventricular assist device (LVAD)
12. Patients with thromboembolic phenomena
13. Patients with Type 2 and above heart block
14. Patients with established positive bacterial blood cultures prior to enrollment
15. Patients with ongoing pericardial effusion, or ascites
16. Patients with clinically significant arrhythmia
17. Patients with liver function tests (ALT or AST) > 3X normal
18. Patients with untreated HIV infection
19. Patients diagnosed with end-stage organ disease

Consent:

Once a patient has been identified as a confirmed positive case of COVID-19, they will be called for e-consent. A link to REDCap will be sent and the patient will be asked to access it via their personal device to minimize contact between patients and research staff. An outline of the study will be presented and the participant will be free to ask questions and carefully consider whether or not to participate. They will receive a copy of their signed consent form via email.

6. STUDY DESIGN AND PROCEDURES

1. Patients will be screened via the electronic medical system, and/or noted and communicated to the study team by physicians in the units.
2. Patient will be called for e-consent.
3. Patients of childbearing potential will receive a pregnancy test
4. Enrolled patients will be randomized to intervention or control with random permuted blocks of 2-4 via sealed envelope or secured electronic randomization tool that will be selected after consent. Patients or treating clinicians will not be notified of treatment assignment.
5. Amniotic fluid will be procured by the Cell Therapy and Regenerative Medicine group here at the University of Utah. Product will be delivered, either frozen or thawed, by this group to the relevant hospital unit/floor.
6. After consent, but prior to initial intervention, the following measurements will be collected:
    a. Demographics (age, sex, height, weight, race/ethnicity, study hospital admit date and time)
    b. Medical history
    c. Concomitant medications
    d. Confirmation of negative pregnancy test, if applicable
    e. Date of symptom onset
    f. Date of confirmed SARS-CoV-2 positivity by RT-PCR
    g. Vital signs (heart rate, blood pressure, oxygen saturation, respiratory rate, temperature)
        a) Patients must have a blood pressure of <160/96 mmHg prior to receiving study IP
        b) Patients must have a pulse of <110 beats per minute prior to receiving study IP
    h. Glasgow Coma Scale
        a) Patient must have a GCS score of ≥14 prior to consent
    i. Respiratory support modality and settings
    j. Complete blood count with auto differential
    k. Complete metabolic panel
    l. Blood glucose (for patients with diabetes)
    m. Inflammatory biomarkers (interleukin-6, c-reactive protein, d-dimer, lactate dehydrogenase)
    n. Results of chest x-ray and/or chest CT, if available.
7. Patients will receive either 10ml IV hAF or 10ml normal saline (control) each day for 5 consecutive days. hAF and saline with similar lot numbers will be prepared in order to maintain blinding between both clinicians and patients.
8. For 3 hours following IP administration, patients will be placed on continuous monitoring (e.g., pulse oximetry, blood pressure) and monitored for infusion reactions.
9. Assessments on Days 1-5 will include:
    a. Intervention or control date and time

   b. Vital signs (heart rate, blood pressure, oxygen saturation, respiratory rate, temperature)
      a) Patient must have a blood pressure of <160/96 mmHg prior to receiving study IP
      b) Patient must have a pulse of <110 beats per minute prior to receiving study IP
         i. In the event that a patient does not meet blood pressure and/or pulse criteria for IP administration, these vital signs will be rechecked every hour until they have returned to the necessary range.
   c. Glasgow Coma Scale
   d. Respiratory support modality and settings
   e. Blood glucose (for patients with diabetes)
   f. Results of chest x-ray and/or chest CT, if available.
   g. Concomitant medications
10. Day 6 (post-intervention) assessment will include:
   a. Vital signs (heart rate, blood pressure, oxygen saturation, respiratory rate, temperature)
   b. Glasgow Coma Scale
   c. Respiratory support modality and settings
   d. Complete blood count with auto differential*
   e. Complete metabolic panel*
   f. Blood glucose (for patients with diabetes)
   g. Inflammatory biomarkers (interleukin-6, c-reactive protein, d-dimer, lactate dehydrogenase)*
   h. Results of chest x-ray and/or chest CT, if available
   i. Concomitant medications

   *Patients who have been discharged from the hospital prior to Day 6 will be asked to return to the hospital or an outpatient clinic for Day 6 lab work.
11. At ICU discharge (if applicable) and hospital discharge, the following will be gathered:
   a. Intervention or control date and time
   b. Vital signs (heart rate, blood pressure, oxygen saturation, respiratory rate, temperature)
   c. Glasgow Coma Scale
   d. Respiratory support modality and settings
   e. Blood glucose (for patients with diabetes)
   f. Results of chest x-ray and/or chest CT, if available.
12. The following data will be collected following hospital discharge:
   a. Last available results of chest x-ray(s) and/or chest CT(s), if available.
   b. ICU admit and discharge dates and times (if applicable)
   c. Hospital floor admit and discharge dates and times
   d. Dates and times of intubation and extubation (if applicable)
   e. Tracheostomy (if applicable)
   f. ECMO status (and settings, if applicable)
   g. Major adverse cardiac events

h. Discharge date and time
i. Discharge location
j. Concomitant medications
    a) Including but not limited to current COVID-19 therapies not approved by the FDA, and non-pharmaceutical therapies
k. Date and time of death (if applicable)

13. Safety assessments will be performed at 30, 60, 90 and 180 days (+/- 7 days) post-discharge
    a. Safety assessments will include a defined script inquiring about any new or remaining symptoms (e.g., shortness of breath), sleep quality, if the patient feels they are back to baseline and if they have any positive or negative feedback regarding the IP. Open-ended questions regarding any additional concerns the participant may have as well as a specific question regarding any health events or hospitalizations since the last assessment will also be asked.
    b. These assessments will be performed remotely via telephone by study staff. If the assessment is positive (i.e., if the patient reports AEs that are determined to be related to study drug), a clinician will arrange a telemedicine visit to follow up.
14. At 1, 3, 6, and 12 months post-discharge, patients will be emailed a REDCap survey link in order to complete several surveys: PROMIS measures including Dyspnea Severity, Physical Function, Sleep Disturbance, Anxiety, and Depression, as well as the PTSD Checklist (PCL).
    a. If the patient is still hospitalized at any of these time points, respiratory settings and details of physical therapy progress will be gathered from the chart instead.
    b. Window: 1 month +/- 7 days, 3/6/12 months +/- 14 days
15. If a patient is discharged prior to receiving the 5-days of IP administration the patient will still be followed for the duration of the study

<u>Primary Outcome:</u>

1. C-reactive protein levels as assessed prior to and following intervention.

<u>Secondary Outcomes:</u>

1. Death within 30 days
2. ICU-free days at 30 days
3. Hospital length of stay
4. Need for invasive mechanical ventilation
5. Biomarker levels (interleukin-6, d-dimer, lactate dehydrogenase)
6. Need for ECMO
7. Major adverse cardiac events
8. Patient-reported functional status

7. SAFETY MONITORING

Safety and tolerability will be evaluated by the investigator from the results of reported signs and symptoms, vital sign measurements, and clinical laboratory test results done as standard of care during the duration of hospitalization. More frequent safety evaluations may be performed if clinically indicated or at the discretion of the investigator.

Toxicity will be reported using Common Terminology Criteria for Adverse Events (CTCAE) version 5. All AEs will be collected.

Protocol Deviations

A **protocol deviation** is nonadherence to protocol-specific study procedures or schedules that does not involve inclusion/exclusion criteria, primary objective variable criteria, and/or GCP guidelines. Deviations are considered minor and do not impact the study.

A **protocol violation** is any significant divergence from the protocol, i.e., nonadherence on the part of the patient, the investigator, or the Sponsor to protocol-specific inclusion/exclusion criteria, primary objective variable criteria, and/or GCP guidelines. Protocol violations will be identified and recorded by the study site personnel on the CRF.

Deviations and violations will be reported to the Principal Investigator and the Sponsor and, if necessary (i.e., if the deviation or violation results in an adverse or serious adverse event, the DSMB.

Adverse Events

An AE may consist of the following:

1. A new event which was not pre-existing at initial study drug administration.
2. A pre-existing event which recurs with increased intensity or increased frequency subsequent to study drug administration.
3. An event which is present at the time of study drug administration which is
4. exacerbated following initial study drug administration.

A Serious Adverse Event (SAE) is defined by FDA and NCI as any adverse drug event (experience) occurring at any dose that in the opinion of either the investigator or sponsor results in any of the following outcomes:

1. Death
2. Life-threatening adverse drug experience
3. Inpatient hospitalization or prolongation of existing hospitalization (for > 24 hours)
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5. Congenital anomaly/birth defect
6. Important Medical Event (IME) that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon medical judgment, it may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

IRB Notification: Events meeting the University of Utah IRB reporting requirements (http://www.research.utah.edu/irb/) will be submitted through the IRBs electronic reporting system within 10 working days.

Staggering of patients (first five [5] subjects)
In order to help identify unforeseeable risks, enrollment will be staggered for the first five patients. Each patient must complete IP administration followed by window of at least 7-days before enrollment of the next subject.

Safety Criteria for discontinuation of patient enrollment

- Occurrence of 1 or more Grade 3 allergic reactions
- Occurrence of 1 or more > Grade 3 organ failure (heart, liver, kidney) per CTCAE definitions.

Adverse Event Reporting

The site investigator is responsible for evaluating all adverse events at their Clinical Center and ensuring that they are properly reported. Adverse events that occur during this study will be recorded. The nature of each experience, date and time (where appropriate) of onset, outcome, course, and relationship to treatment will be documented.

Definition of Adverse Event and Serious Adverse Event

Adverse Event (AE) means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)). An event constitutes a disease, a set of related signs or symptoms, or a single sign or symptom.

Serious Adverse Event (SAE): A serious adverse event (SAE) for this population is an adverse event that:

a) results in death; or
b) is life-threatening (the patient was, in the view of the site investigator, in immediate danger of death from the event as it occurred); or
c) requires inpatient hospitalization or prolongs an existing hospitalization; or
d) results in persistent or significant disability or incapacity; or
e) results in congenital anomaly/birth defect; or
f) is any other event that, based upon appropriate medical judgment, may jeopardize the patient's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

Classification of an Adverse Event (Relatedness, Severity, and Expectedness)

Relatedness: The suspected relationship between study interventions and any adverse event will be determined by the site investigator using the criteria below. Relatedness must be assessed by an investigator and may not be assessed by a research coordinator.

a) Not Related: The event is clearly related to other factors, such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.
b) Possibly Related: The event follows compatible temporal sequence from the time of beginning the assigned study intervention, but could have been produced by other factors such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.
c) Probably Related: The event follows a reasonable temporal sequence from the time of beginning the assigned study intervention, and *cannot be reasonably explained* by other factors such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.

Severity: The severity, which is a measure of intensity, of clinical adverse events and laboratory abnormalities will be recorded by the site investigator and categorized. The following guidelines will be used to describe severity.

- Grade 1/Mild: The event is asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2/Moderate: The event is defined as minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental activities of daily living (ADL)
- Grade 3/Severe: The event is medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL
- Grade 4/Life-threatening: The event requires urgent intervention.
- Grade 5/Death related to AE.

Expectedness of the Event: All adverse events, including serious adverse events, will be evaluated as to whether their occurrence was expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information described for the study intervention. There are no expected adverse events for IV

administered hAF.

Treatment or Action Taken: For each adverse event, the site investigator will record whether an intervention was required:

- Medical or surgical procedure
- Concomitant medication: started, changed, or discontinued
- Other, specify
- No action taken

Outcome of Event: Finally, the site investigator will record the clinical outcome of each adverse event as follows:

- Death
- Recovered and the patient returned to baseline status
- Recovered with permanent sequelae
- Symptoms persist

Time Period for Adverse Events
For purposes of this study, events that occur following randomization through the Day 180 will be reported as adverse events. Serious adverse events, unexpected medically attended events, and new onset chronic illnesses will be recorded from randomization through Day 180. Specifically, events that occur following informed consent to participate in the study, but prior to actual randomization are not adverse events. These should be recorded as baseline conditions.

Data Collection Procedures for Adverse Events
After patient randomization all adverse events (including serious adverse events) will be recorded according to relatedness, severity, and expectedness, as well as their duration and any treatment prescribed. Any medical condition present at the time of randomization, recorded in the patient's baseline history at study entry, which remains unchanged or improves (unless the clinician feels it is clinically relevant), will not be recorded as an adverse event at subsequent evaluations. However, worsening of a medical condition that was present at the time of randomization will be considered a new adverse event and reported.

Adverse events will be coded using the MedDRA coding vocabulary. Coding will be done centrally at the University of Utah Pediatric Department Research Enterprise as this requires specific training.

Unanticipated Problems (UP)
Unanticipated problems (UP) are defined as incidents, experiences, or

outcomes that are unexpected, related or possibly related to participation in the study, and suggest that the research places subjects at a greater risk of harm than was previously known or recognized. The site investigator will report unanticipated problems to the Sponsor (CellReGen) within 24 hours of becoming aware of the event. A detailed completed report will be required to be sent to the (CellReGen) within 3 working days of the event.

In accordance with local IRB requirements, the site investigator may be required to report such unanticipated problems to the IRB. In the event that the medical monitor believes that such an event warrants emergent suspension of enrollment in the trial, they will notify the study investigator and all site investigators to cease enrollment in the trial.

Monitoring Serious Adverse Events
A qualified physician will be designated to fulfill the function of the medical monitor for this study. Site investigators and/or research coordinators will report serious adverse events to the DSMB and CellReGen within 24 hours of becoming aware of the event. A detailed completed report will be required to be sent to the DSMB and CellReGen within 3 working days of the event, and the medical monitor will assess all serious adverse events reported from site investigators.

For each of these serious adverse events, the site investigator will provide sufficient medical history and clinical details for a safety assessment to be made with regard to continuation of the trial. The medical monitor will sign off on each SAE report after review. All SAE reports will be retained at the CellReGen. The SAE reporting process may be incorporated into an Electronic Data Capture (EDC) System in use for the study.

In the unlikely event that the medical monitor believes an unexpected and study-related SAE warrants emergent cessation of enrollment in the trial, CellReGen will immediately be consulted. If these individuals concur with the judgment of the medical monitor CellReGen will notify the study investigator and all site investigators to cease enrollment in the trial. Resumption of enrollment will not occur without consent of the CellReGen

In accordance with local IRB requirements, the site investigator may be required to report such events to the IRB.

Follow-up of Serious, Unexpected and Related Adverse Events
All serious, unexpected and related adverse events, that are unresolved at the time of the patient's termination from the study, will be followed by the site investigators until the events are resolved, patient is lost to follow-up, the adverse event is otherwise explained or has stabilized, or 12 months have

passed from the time of last study dose.

PATIENT WITHDRAWAL FROM STUDY:
A patient may withdraw their consent at any time without effect to their care.

DISCONTINUATION OF STUDY DRUG:
hAF-specific treatment will be discontinued if any of the following occur:

- Recurrent Grade 1 or Grade 2 allergic reactions will be determined by the PI
- Grade 3 allergic reactions will be cause to discontinue study drug.
- Patient formally requests to withdraw from the study
- Patient is discharged from the hospital prior to completing the study drug dose regimen
- Patient experiences >Grade 3 organ failure (heart, liver, kidney) per CTCAE definitions.

The study PI, treating clinicians and research staff will monitor closely for the above events, as well as any additional adverse events that occur while the patient is receiving treatment. Adverse events will be reported immediately upon discovery of the event to the PI and assessed for seriousness, relatedness, severity, and expectedness.

Any patient who is randomized, and is subsequently withdrawn due either to patient choice and/or an adverse event (such as allergic reaction) will continue to be monitored per study protocol and per hospital protocol for the duration of their hospitalization. As with patients who complete the protocol, these discontinued patients will still have daily hemodynamic and respiratory support data collected in our database during hospitalization. Any patients who are withdrawn from the study will continue to receive all standard of care monitoring and treatments.

Allergic reactions, regardless of severity, will be assessed and treated by the Principal Investigator based on his or her clinical judgment. This may include use of antihistamines, corticosteroids, or albuterol, depending on the patient's symptoms.

The DSMB will meet regularly to review adverse events, safety analyses and study data and will have access to the actual treatment assignments for each group. The DSMB will meet for a kickoff meeting prior to enrollment of the first patient, and again following 5 patients, 20 patients, 40 patients, and 60 patients. They will also meet as needed if any adverse or serious adverse events occur.

If study drug is discontinued, we will continue to follow the patient and monitor for safety and efficacy endpoints.

DISCONTINUATION OF STUDY:

Events used to trigger modification or stopping of the study pending a safety investigation include:

- Death of any patient
- Any SAE as defined in 21 CFR 312.32
- Three (3) or more of the same Grade 3 or higher AEs (judged by the investigator, medical monitor or sponsor), including infusion site reactions
- One (1) Grade 4 AE
- One (1) anaphylaxis event
- Any event which, in the opinion of the investigator, medical monitor or sponsor, contraindicates further dosing of additional patients
- At the discretion of the Data Safety Monitoring Board (DSMB). The DSMB will meet regularly to review adverse events, safety analyses, and study data. The DSMB will have access to the actual treatment assignments for each group.

Any change to the study stopping rules will be reviewed with the FDA. If the study is suspended, it will not be re-opened without FDA approval.

If the study is discontinued (stopped or suspended) all enrolled patients will be followed for the planned duration of the study.

8. STATISTICAL CONSIDERATIONS

Statistical Methods

This is a trial with patients randomized to two groups: (1) treatment group with hAF administered intravenously (n=30), (2) control group with intravenous administration of a saline control (n=30). Both groups will receive standard of care in addition to these treatments.

Descriptive statistics, including mean (SD) and median (interquartile range), will be used to assess patient characteristics, and clinical and biomarker outcomes. Comparisons will be made stratified on ICU vs floor status at enrollment for variables. Categorical characteristics will be compared using chi-square test or Fisher exact test. Continuous characteristics will be compared using independent samples t-test or Wilcoxon-Mann-Whitney test. 95% Confidence intervals and *p*-values will be reported from all comparisons. Statistical analyses will be conducted in R and STATA, significance will be assessed at the 0.05 level and all tests will be two-tailed.

Safety will be assessed by descriptive reporting of the incidence proportion of study-related safety adverse events in each study group, with one-sided 95%

binomial exact confidence intervals. No statistical hypothesis test is planned to assess safety, which is consistent even with large Phase III trials. The table below displays the number of patients, out of 30, experiencing no adverse event and the corresponding lower-bound of the 95% exact confidence interval.

Number of Patients with no Related Adverse Events	Number of Patients Experiencing a Related Adverse Event	Lower-bound of the 95% Exact Binomial Confidence Interval for Safety
30	0	90%
29	1	85%
28	2	80%
27	3	76%

If there are 30 patients in the hAF group and if all patients in the hAF group complete the study without experiencing an adverse event we will be able to state with 95% confidence that the safety of hAF is at least 90%.

Exploratory outcomes for safety include, but are not limited to, the following:

- Whether the patient experienced any post-randomization AEs while on study (regardless of relatedness to study participation)
- Whether the patient experienced any post-randomization serious AEs while on study
- Whether the patient experienced any post-randomization unexpected AEs while on study.

Sample Size Justification

A sample size of n = 30 patients per group (total n = 60) is expected, though this may differ slightly because of the randomization procedure. If we are unable to enroll 60 patients due to lack of cases, we will expand the study to other hospitals. The IRB application shows an enrollment goal of 65, which is to allow space to continue enrolling and meet the goal of an n = 60 in the event of participant withdrawal.

9. DATA MANAGEMENT

Clinical Site Data Management
The study team has developed a REDCap database for data collection. REDCap is supported by the investigator

Study Monitoring

The investigators recognize the importance of ensuring data of excellent quality. Study monitoring is critical to this process. Monitoring has been a very effective tool for maintaining data quality in previous network studies, and we will utilize this process to ensure excellent quality data in the proposed study. The risk-based approach to monitoring focuses on oversight activities and preventing or mitigating key risks to data quality, as well as to processes critical to human subject protection and integrity of the trial or study.

Study monitors must be provided with appropriate access to study materials and the medical records for study patients. If the medical records are in electronic form, the clinical investigator or an authorized individual must provide any assistance necessary to facilitate the study monitor's review of data in the electronic medical record.

10. PROTECTION OF HUMAN SUBJECTS

Institutional Review Board Approval

Each clinical center must obtain approval from their respective IRBs prior to participating in the study. CellReGen will track IRB approval status at all participating centers and will not permit subject enrollment without documentation of initial IRB approval and maintenance of that approval throughout subsequent years of the project.

Informed Consent

Informed consent is required because all eligible patients are at least 18 years old. Patients who are capable of giving consent and who are alert and competent, will be asked, following an appropriate discussion of risks and benefits, to give consent to the study.

Potential Risks

Infection: The University of Utah Cell Therapy and Regenerative Medicine (CellReGen) screens the amniotic fluid coming from donors for infectious diseases through medical and social history questionnaire, medical record review, and testing of the donor's blood for viruses including HIV, Hepatitis B & C, and others. The CellReGen Medical Director reviews the records and test results to determine if the donor meets eligibility requirements. Although the risk of transmitting infectious disease is minimal, there is a potential for transmission.

Immune Reaction: You could have an immune reaction to the intravenous amniotic fluid. We will monitor you for any signs of such a reaction and provide treatment if needed.

Blood Draw: Minor temporary discomfort may be associated with the removal of blood by venipuncture. There is a risk of bruising and a very small amount of bleeding associated with the blood drawing. There is also a very small risk of infection at the site. Whenever possible, blood samples will be gathered when the participant is scheduled for routine blood testing or procedures.

Clinical Data Collection: There is no significant procedural risks associated with review of medical records. While there is a potential risk of loss of confidentiality, appropriate measures are in place to mitigate this risk.

Protection Against Potential Risks
Regarding loss/breach of privacy and confidentiality, all applicable parties (e.g. clinical site) will be responsible for ensuring that appropriate data security procedures are in place.

Potential Benefits

We are in an unprecedented global crisis with the spread of the COVID-19 pandemic. The data showing that babies born to COVID-19 infected mothers do not have the virus, and neither is it found in breastmilk or amniotic fluid, suggests a possible treatment for this rampant infection. While it would not cure the virus itself, it has the potential to decrease inflammation and fibrosis, thus possibly making critical respiratory treatments less necessary. Considering the possible shortage of ventilators, this could be greatly beneficial to everyone currently battling this pandemic.

It cannot be guaranteed that patients themselves would benefit, as this is an experimental treatment. However, if our hypothesis is correct, it is possible that COVID-19 infected patients would see a reduction of respiratory inflammation and fibrosis. This could result in more effective treatment, shorter hospitalization, and less use of critical resources.

11. REGULATORY CONSIDERATIONS

Food and Drug Administration
This trial is being conducted under an Investigational New Drug application. The clinical investigator at each participating site will complete a Form FDA 1572, “Statement of Investigator."

Health Insurance Portability and Accountability Act
Data elements collected include the medical record number, date of birth, and dates of admission, procedures, discharge and/or death.

Data elements for race, ethnicity, and sex are also being collected. These demographic data are required for Federal reporting purposes to delineate patient accrual by race, ethnicity, and gender.

Inclusion of Women and Minorities

There will be no exclusion of patients based on gender, race, or ethnicity.

12. AMENDMENTS: IRB AND FDA

Any amendments or administrative changes to an IRB-approved protocol will not be initiated without submission of an amendment for IRB review and approval. Any amendments to the protocol that significantly affect the safety of participants, the scope of the investigation, and/or the scientific quality of the study are required to be submitted to the FDA for review.

13. FDA ANNUAL REPORTING

As required by the FDA, annual reports will be submitted on this IND application, so long as it is active. These annual reports will be submitted to the FDA within 60 days of the anniversary of the date that the IND went into effect, per 21 CFR 212.33). Annual reports will include the following information: summary of the status of each project under this IND, including enrollment goals and actual enrollment numbers as well as a summary of any available results from the study; a reporting of adverse events, IND safety reports, participant deaths, and participant withdrawals during the last year; information about the action of the investigational drug, per information from controlled trials; any manufacturing or microbiological changes; the investigational plan for the following year; a revised investigational brochure (if applicable); any protocol amendments; information on foreign marketing developments (if applicable); as well as a log of outstanding FDA communication to which the sponsor expects a reply from the FDA.

12. APPENDIX

APPENDIX A - REFERENCES

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