Title: Efficacy and Safety of High-Titer Anti-SARS-CoV-2 (COVID 19) Convalescent Plasma for Hospitalized Patients with Infection due to COVID-19 to Decrease Complications: A Phase II Trial.

Short Title: Convalescent Plasma for Treatment of COVID-19 Patients with Pneumonia

Phase of Study: Phase II
IND Number: 20867 Sponsored by L.G. Lum

Study Center: University of Virginia

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1.0 SUMMARY

Background: There are currently no proven treatment options for Coronavirus Disease 2019 (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Human convalescent immune plasma infusion (CIP) is an option for treatment of COVID-19.

Clinical Experience: Passive antibody therapy for infections has been used since the late 1800s and prior to the development of antimicrobial therapy in the 1940s[1,2]. There is some evidence that convalescent plasma can provide neutralizing antibodies to SARS-CoV-2[3]. Convalescent serum was used in the 2013 Ebola epidemic. A small non-randomized study in Sierra Leone revealed a significant increase in survival for patients treated with convalescent whole blood compared to those who received standard treatment[4].

Primary Objective: Evaluate whether CIP can reduce progression of critical illness among patients hospitalized for COVID-19

Primary Endpoints:
1. Transfer to the intensive care unit (ICU)
2. 28 day mortality

Secondary Objectives:
1. Evaluate the safety of CIP.
2. Evaluate cellular and humoral responses before CIP treatment and at CIP treatment.
3. Evaluate the effect of CIP as compared to non-transfused immunologic control patients.

Secondary Endpoints:
- Cumulative incidence of serious adverse events during the study period: transfusion reaction (fever, rash), transfusion related acute lung injury (TRALI), transfusion associated circulatory overload (TACO), transfusion related infection
- Rates and duration of SARS-CoV-2 PCR positivity at days 0, 4, 7, 14 and 21.
- Serum or plasma antibody titer[1] to SARS-CoV-2: Day 0, 7, 14, and 28
- Cellular and humoral immune responses at days 0, 7, 14, and 28 days
- Supplemental oxygen free days
- Ventilator free days
- ICU free days
- Sequential organ failure assessment (SOFA) score at days 0, 1, 2, 17, 14, 21, 28
- Need for vasopressors
- Need for renal replacement therapy
- Need for extracorporeal membrane oxygenation (ECMO)
- Hospital length of stay (LOS)
- ICU LOS
- Grade 3 or 4 adverse events (AEs)

Study Design: This is a single arm phase II trial to assess efficacy and confirm safety of infusions of anti-SARS-CoV-2 convalescent plasma in hospitalized patients with acute respiratory symptoms, with or without confirmed interstitial COVID-19 pneumonia by chest Xray or CT. A total of 29 eligible subjects will be enrolled to receive anti-SARS-CoV-2 plasma. Outcomes will be compared to hospitalized controls

[1] Measured by an anti-SARS-CoV-2 ELISA and serum antibody titer to SARS-CoV-2.

with confirmed COVID-19 disease through retrospective chart review.

In addition, the following Immunologic Controls will be used to meet secondary objectives described above:

1. PBMCs with 5-10 million cryopreserved cells per specimen will be obtained from the prospective COVID 19 Biorepository (2 timepoints per patient t (60 specimens total)and assessed for cellular and humoral immune responses.

2. Serum or plasma 500 Ul each from up to 50 patients in the Covid-19 Biorepository will be collected (2 timepoints from each patient) (100 total specimens) will be evaluated for antibody and cytokine responses.

Study Population:

Inclusion Criteria for Enrollment

1. Patients must be 18 years of age or older
2. Patients hospitalized with COVID-19 respiratory symptoms within 72 hours of admission to a "floor" bed (non-ICU bed) and confirmation via SARS-CoV-2 RT-PCR testing.
3. Patient and/or surrogate is willing and able to provide written informed consent and comply with all protocol requirements.
4. Patients with hematologic malignancies or solid tumors are eligible.
5. Patients with autoimmune disorders are eligible.
6. Patients with immunodeficiency and organ or stem cell transplant recipients are eligible.
7. Patients who have received or are receiving hydroxychloroquine or chloroquine are eligible (but will be taken off the drug)
8. Prior use of IVIG is allowed but the investigator should consider the potential for a hyper-coagulable state.

Exclusion Criteria

1. Patients requiring mechanical ventilation or >6 liters per minute nasal cannula oxygen
2. Patients on other anti-COVID-19 trials being treated with tocilizumab (anti-IL-6 receptor), Siltuximab (anti-IL-2), Remdesivir, or other pharmacological trials that may be initiated hereafter.
3. A pre-existing condition or use of a medication that, in the opinion of the site investigator, may place the individual at a substantially increased risk due to study participation
4. Contraindication to transfusion or history of prior reactions to transfusion blood products.
5. Medical conditions for which receipt of 500-600 mL of intravenous fluid may be dangerous to the subject (e.g., decompensated congestive heart failure).

Clinical Controls (obtained via chart review):

1. Admitted to a "floor" bed (non-ICU bed) at UVAMC on/after March 30, 2020 due to confirmed COVID-19 disease using SARS-CoV-2 RT-PCR Testing
2. Age $\geq$ 18 years of age
3. No receipt of convalescent plasma

Immunologic Controls:

1. PBMCs with 5-10 million cryopreserved cells per specimen present in the prospective COVID 19 Biorepository

2.Serum or plasma 500 Ul each from up to 50 patients present in the COVID-19 Biorepository

Assessment and Data Acquisition:

1. Baseline: Age, sex, comorbidities, date of symptom onset, known COVID-19 contact, type of admission to the floor or ICU, APACHE score (ICU scoring system to classify disease severity and mortality risk), SOFA score (mortality prediction score based on organ function), clinical status, vital signs including temperature, respiratory rate, oxygen saturation, oxygen requirement, neutrophil count, lymphocyte count, CRP, chest x-ray, chest CT, location in hospital.
2. Safety and efficacy: Day 0 (baseline), 1, 2, 3, 4, 7, 14, 21, and 28 and once at day 60.
3. SARS-CoV-2 PCR from OP or NP swabs: Day 0, 4, 7, 14 and at any time when there is clinical suspicion for COVID-19.
4. Outcome measures: increased O_2 requirement (PaO_2/FiO_2 ratio or $SpO2/FIo_2$), supplemental oxygen strategy (nasal cannula, high flow nasal cannula, noninvasive ventilation, intubation and invasive mechanical ventilation, rescue ventilation i.e. neuromuscular blocking agents, prone positioning, corticosteroids, ECMO), vasopressors, renal support, ICU LOS, ICU mortality, hospital LOS, hospital mortality, 28 day mortality.
5. Serum or plasma antibody titer[2] to SARS-CoV-2: Day 0, 7, 14, and 28. Samples from the COVID-19 Biorepository from non-transfused patients will be assayed at two similar timepoints as Immunologic Controls.
6. Blood for immune evaluations of cellular and humoral immunity before CIP infusion (day 0) and 7, 14 and 28 days after CIP infusion. Immune testing may be done at any time if the PI determines there is a need to evaluate acute immune responses (cytokines/chemokines levels). Samples from the Prospective COVID-19 Biorepository from non-transfused patients will be assayed at two similar timepoints as Immunologic Controls.
7. "Clinical controls" will have all relevant datapoints collected via chart review (i.e. biospecimens will not be collected prospectively)

Drug: Pathogen reduced SARS-CoV-2 convalescent plasma (1-2 units; 2 units are preferable but a single unit is allowable in the setting of safety/time/resource/etc concerns; ~200 mL each for a total of upto ~400 mls) given preferably in one day, but allowable to be given over 2 days if clinical circumstances delay infusions in 1 day), with titer to be determined after the unit has been infused.

Directed Donor Plasma Units: Healthy donors who have recovered from COVID-19 infection 21 days after symptom resolution will undergo a donor eligibility questionnaire. If eligible, blood will be drawn for ABO typing, HLA antibodies, and infectious disease panel screening. One additional red top tube will draw and sent to the Lum laboratory at UVA to test for levels of IgG anti-COVID-19 spike protein. No OP or NP PCR testing will be performed at day 21 given that donation will occur at day 28 or after. If the donor is eligible and matched, they will be called back to donate plasma at the ARC on day 28 after symptom resolution or later. The ARC or other local facility will collect blood for ABO, HLA, and infectious disease panel screening obtained via Emergency Use Authorization.

Immune Evaluations: Immune evaluations will be performed to determine if infusions of immune plasma enhance the development of anti-COVID-19 immune responses: peripheral blood mononuclear cells (PBMC) will be cryopreserved for measurements of specific anti-COVID-19 IFN-γ ELISpots, single cell high-dimensional immunophenotyping by mass cytometry and RNA-Seq; specific serum IgG, IgM and IgA antibodies COVID-19 and levels of Th_1/Th_2 serum cytokines at the designated time points at 0, 14, and 28 days after CIP infusion.

i. List of Abbreviations

ADR Adverse Drug Reaction

[2] Measured by an anti-SARS-CoV-2 ELISA and serum antibody titer to SARS-CoV-2.

ADE	Antibody-mediated enhancement of infection
BP	Blood pressure
CIP	Convalescent Immune Plasma
CTCAE	Common Terminology Criteria for Adverse Events
DBP	Diastolic Blood Pressure
DLT	Dose limiting Toxicity
DSMB	Data and Safety Monitoring Board
ECG/EKG	Electrocardiogram
EUA	Emergency Use Authorization
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HTLV	Human T-cell lymphotropic virus
IB	Investigator's Brochure
ICF	Informed Consent (Informed Consent Form)
ICH	International Conference on Harmonization
ICU	Intensive Care Unit
IEs	Immune Evaluations
IEC	Independent ethics committee
IFN-γ	Interferon-gamma
IND	Investigational New Drug Application
IRB	Institutional review board
ISBT	International Society of Blood Transfusion
LVEF	Left Ventricular Ejection Fraction
MAS	Macrophage Activation Syndrome
MNC	Mononuclear Cells
MERS	Middle East Respiratory Syndrome
NA	Nuclear antibody
NP	Nasopharyngeal
OP	Oropharyngeal
OS	Overall Survival
PBMC	Peripheral Blood Mononuclear Cells
PI	Principal Investigator
Pts	Patients
RT-PCR	Reverse Transcriptase Real-Time Polymerase chain reaction
PK	Pharmacokinetic
SAE	Serious adverse event
SARS	Severe Acute Respiratory Syndrome
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SBP	Systolic Blood Pressure
SOC	Standard of Care
TACO	Transfusion-associated circulatory overload
T. cruzi	*Trypanosoma cruzi*
TRALI	Transfusion-related acute lung injury
UP	Unanticipated Problem
UPnonAE	Unanticipated Problem that is not an Adverse Event
ZIKV	Zika virus

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2.0 OBJECTIVES

2.1 Primary Objective

- Evaluate whether CIP can reduce progression of critical illness among patients hospitalized for COVID-19

2.2 Secondary Objectives

- Evaluate safety of CIP
- Evaluate cellular and humoral responses before CIP treatment and at CIP treatment.
- Evaluate the effect of CIP as compared to non-transfused immunologic control patients.

2.3 Study Design

This is a single arm phase II trial to assess preliminary efficacy and confirm safety of infusions of anti-SARS-CoV-2 convalescent plasma in hospitalized patients with acute respiratory symptoms **with or without** confirmed interstitial COVID-19 pneumonia by CXR or chest CT. A total of 29 eligible subjects will be enrolled to receive high titer anti-SARS-CoV-2 plasma. Participants will be compared to a historical control group via retrospective chart review.

In addition, the following Immunologic Controls will be used to meet secondary objectives described above:

1. PBMCs with 5-10 million cryopreserved cells per specimen will be obtained from the prospective COVID 19 Biorepository (2 timepoints per patient t (60 specimens total)and assessed for cellular and humoral immune responses.

2. Serum or plasma 500 Ul each from up to 50 patients in the Covid-19 Biorepository will be collected (2 timepoints from each patient) (100 total specimens) will be evaluated for antibody and cytokine responses.

3.0 BACKGROUND AND SIGNIFICANCE

3.1 Background and Scientific Rationale

There are no proven treatments for coronavirus disease (COVID-19) and associated pneumonia caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Recent experience in China suggests that convalescent immune plasma (CIP) may be an effective treatment for COVID-19. In the pandemic situation where there are no vaccines for COVID-19, specific antibodies in convalescent plasma induced by infection may provide passive protective immunity. Passive antibody therapy was the first immunotherapy dating back to the 1890s for the treatment of infectious diseases before the development of antibiotics 1940s [1, 2]. Experience from prior outbreaks with other coronaviruses, such as SARS-CoV-1 shows that such convalescent plasma contains neutralizing antibodies to the relevant virus[3]. In SARS-CoV-2, passive antibody therapy from CIP probably provided protection by viral neutralization. CIP was also used in the 2013 Ebola epidemic. A small non-randomized study in Sierra Leone revealed a significant increase in survival for who received CIP[4]. CIP administration is the only approach that provides immediate immunity to patients who have been exposed or who have active disease.

This approach is immediately available from individuals who have recovered, are viral free, and can donate immune plasma (IP) containing high titer neutralizing antibodies. Passive antibody therapy can be given to a patient recently exposed or a patient who is developing an infection with COVID-19 by obtaining plasma units from immune individuals by standard plasmapheresis using FDA-approved blood banking procedures, cross matching the unit(s) to the recipients and infusing the unit(s) using standard

transfusion procedures for blood products. Based on the safety and long-term experience with plasma infusions, plasma exchanges, and other procedures involving plasma or plasma product, this protocol was designed as a phase II single arm trial that involves the administration of antibodies to a given agent to a susceptible individual for the purpose of preventing or treating an infectious disease due to that agent.

The only antibody formulation that is available for emergent use is that found in convalescent plasma. As more individuals contract COVID-19 and recover, the number of potential donors will increase.

The principle of passive antibody therapy is that it is more effective when used for prophylaxis than for treatment of disease. When used for therapy, antibody is most effective when administered shortly after the onset of symptoms. The reason for temporal variation in efficacy is not well understood but could reflect that passive antibody works by neutralizing the initial inoculum, which is likely to be much smaller than that of established disease. Alternatively, antibodies may dampen the early inflammatory response leaving the infected individual asymptomatic[5]. For example, antibody therapy for pneumococcal pneumonia was most effective when given shortly after the onset of symptoms and was of no benefit if antibody therapy was delayed beyond the third day of disease[6]. For passive antibody therapy to be effective, a sufficient amount of antibody must be infused. The antibody will circulate in the blood, reach tissues, and provide protection against infection. Depending on the type of antibody, amount, and composition, the half-life can vary from weeks to months. It is under these circumstances, *we plan to treat patients who are sick enough to be hospitalized before the onset of overwhelming disease involving a systemic inflammatory response, sepsis, and/or ARDS.*

3.2 Experience with the use of convalescent immune plasma against coronavirus diseases

In the 21st century, there were two coronavirus epidemics associated with high mortality. The SARS outbreak in 2003 and MERS outbreak in 2012 had high mortalities and absence of effective therapies which led to the use of convalescent immune plasma. The largest study with SARS using convalescent immune plasma involved the treatment of 80 patients in Hong Kong[7]. **Patients treated before day 14 after onset of symptoms had an improved prognosis as measured by discharge from hospital before day 22.** Furthermore, those who were RT-PCR positive and seronegative for coronavirus at the time of therapy had improved prognosis. Three seriously ill patients recovered from SARS in Taiwan were treated with 500 ml of convalescent plasma which led to a reduction in plasma virus titer[8]. Three patients with MERS in South Korea were treated with convalescent plasma, but only two of the recipients had neutralizing antibody in their plasma[9]. The results suggests that providing passive antibodies may suppress the development of high titers of neutralizing antibodies to the pathogen[10]. The analysis of 99 samples of convalescent sera from patients with MERS showed that 87 had neutralizing antibody with a geometric mean titer of 1:61. These data suggest that antibody declines with time and/or that few patients make high titer responses. There are reports that convalescent plasma was used for therapy of patients with COVID-19 in China during the current outbreak. Although few details are available from the Chinese experience and published studies involved small numbers of patients, the available information suggests that convalescent plasma administration reduces viral load and was safe. Because of these early suggestions of benefit, it is ethically complex to conduct a placebo-controlled trial, therefore we propose to use a comparator group of patients who have been treated at UVA Medical Center prior to the start of this trial.

4.0 ELIGIBILITY

4.1 Inclusion Criteria for Enrollment as a Plasma Recipient

- Patients must be 18 years of age or older
- Patients hospitalized with COVID-19 respiratory symptoms and confirmation via SARS-CoV-2 RT-PCR testing.

- Patient positive for COVID-19 with respiratory symptoms within 72 hours of admission (not in ICU or on high flow oxygen)
- Patient or surrogate is willing and able to provide written informed consent and comply with all protocol requirements.
- Patients with hematologic malignancies or solid tumors are eligible.
- Patients with autoimmune disorders are eligible.
- Patients with immunodeficiency or organ or stem cell transplant are eligible.
- Patients who have received or are receiving hydroxychloroquine or chloroquine are eligible (will be taken off the drug at enrollment)
- Prior use of IVIG is allowed but the investigator should consider the potential for a hyper-coagulable state

4.2 Exclusion Criteria for Plasma Recipients

- Patients requiring mechanical ventilation or >6 liters per minute nasal cannula oxygen
- Patients on other anti-COVID-19 treatment trials (for example, those being treated with tocilizumab (anti-IL-6 receptor), Siltuximab (anti-IL-2), or Remdesivir).
- A pre-existing condition or use of a medication that, in the opinion of the site investigator, may place the individual at a substantially increased risk due to study participation
- Contraindication to transfusion or history of prior reactions to transfusion blood products.
- Medical conditions for which receipt of 500-600 mL of intravenous fluid may be dangerous to the subject (e.g., decompensated congestive heart failure).

4.3 Control Group (obtained via chart review and includes control specimens)

Inclusion Criteria:

1. Admitted to a “floor” bed (non-ICU bed) at UVAMC on/after March 30, 2020 due to confirmed COVID-19 disease
2. Age $\geq$ 18 years of age

Exclusion Criteria:

1. Requiring mechanical ventilation or >6 liters per minute nasal cannula upon admission
2. Participation in other interventional anti-COVID-19 treatment trials
3. Medical conditions for which receipt of 200-400mL of intravenous fluid may be dangerous to the subject (e.g. decompensated congestive heart failure)
4. Receipt of convalescent immune plasma for treatment of COVID-19

4.4 Inclusion Criteria for Enrollment as a Plasma Donor

- Age of 18 years or older
- Weight of 110 pounds or greater
- History of confirmed SARS-CoV-2 infection
- At least 21 days post COVID-19 symptom resolution at the time of consent, and at least 28 days post COVID-19 symptom resolution at the time of plasma donation.
- Person has passed the American Red Cross Donor Health Questionnaire
- Person is willing and able to provide written informed consent and comply with all protocol requirements.

4.5 Exclusion Criteria for Plasma Donor

- Female subjects with positive pregnancy test, breastfeeding, or planning to become pregnant/breastfeed during the study period.

- Medical conditions for which plasma donation is not considered safe (presence of a blood borne pathogen, presence of HLA antibodies, etc)
- Other disqualifying condition as set forth by the American Red Cross blood/plasma donation policies.

Inclusion Criteria for Immunologic Controls

1. PBMCs with 5-10 million cryopreserved cells per specimen with 2 timepoints per patient t
2. Serum or plasma 500 Ul each (2 timepoints from each patient)

Exclusion Criteria for Immunologic Controls

Specimens not available through the COVID Repository protocol.

5.0 TREATMENT PLAN

5.1 Treatment Summary

The purpose of this study is to determine whether convalescent immune plasma infusion (CIP) can reduce ICU admissions, level of respirator support (oxygenation and ventilator support), and mortality. This is a single arm phase II trial to assess efficacy and confirm safety of infusions of anti-SARS-CoV-2 convalescent plasma in hospitalized patients with acute respiratory symptoms within 72 hours of admission with or without confirmed interstitial COVID-19 pneumonia by chest imaging. A total of 29 eligible subjects will be enrolled to receive high titer anti-SARS-CoV-2 plasma. We will document adverse events related to respiratory or clinical status on transfusion of SARS-CoV-2 convalescent plasma and serious adverse events during the study period: transfusion reaction (fever, rash), transfusion related acute lung injury (TRALI), transfusion associated circulatory overload (TACO), transfusion related infection, or electrolyte disturbances. Immune testing will be performed to sequentially profile the immune responses of the patients after they receive CIP. COVID-19 patients aged ≥18 years of age and respiratory symptoms within 72 hours of hospitalization will be enrolled in the study. Patients will receive 1-2 units (~200-400 mL) CIP with high titer plasma.

5.2 Study drug administration

- Drug will be administered in the hospital
- Infusion rate ≤ 500 mL/hour
- Pretreatment to minimize transfusion reactions (e.g. acetaminophen, diphenhydramine) may be given per investigator and clinical care team discretion.
- If an AE develops during infusion, the infusion may be slowed or stopped as per investigator's decision.
    - Most reactions to plasma are relatively minor and the infusion can generally be continued at a slower rate. Infusion site burning and non-allergic systemic effects can generally be managed with slowing of the infusion. Infusion can generally be continued in cases of itching or hives after pausing the transfusion, administering antihistamines, and observing the patient for worsening.
    - Severe allergic reactions such as bronchospasm and hypotension may require discontinuation of the infusion.

5.2.1 Concomitant medications will be documented on the Case Report Form (CRF)

Patients will receive full supportive care including transfusion of blood and blood products, antibiotics, and anti-emetics, when appropriate. The reason(s) for treatment, dosage and the dates of treatment will be recorded:

- Prescription medications
- Over the counter medications

- Herbal treatments/nutritional supplements
- Other blood products

5.2.2 Plasma Collection
Donors will be screened using FDA/American Red Cross (ARC) policies, as detailed in section 6.0. Two units of donor-directed plasma units will be collected by ARC or local donation site via FDA Emergency Use Authorization from donors identified to have recovered from COVID-19 28 days or later after symptom resolution. Appropriate donor screening with one lavender top tube for ABO, HLA and infectious disease screening per ARC or local center standard will be performed. If the units are cleared and identified for transfusion, the directed donor will be sent to the ARC or other local donation site for plasmapheresis on day 28 or later after the symptom resolution. The appropriate identifiers will be verified at the blood bank and at the bedside per standard transfusion procedures.

5.2.3 Steroids/other therapy
Steroids for the treatment of reactive airway disease and/or pneumonia are allowed as well as steroids for anti-emetic regimen, adrenal failure, septic shock, pulmonary toxicity or hormones administered for non-disease-related conditions (e.g. insulin for diabetes). High dose steroids are discouraged but steroid use is left to the judgement of the attending physician team.

5.2.4 Acceptable Concomitant Medications
All treatments that the investigator considers necessary for a subject's welfare may be administered the discretion of the investigator. All concomitant medication including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids will be collected from the medical record.

5.2.5 Prohibited Concomitant Medications
Subjects are prohibited from receiving medications or vaccinations that are specified in the exclusion criteria during study treatment.

5.2.6 Subject Removal
Subjects should be removed from the trial if they require >6L/min supplemental oxygen or mechanical ventilation prior to receiving convalescent plasma infusion. Subjects may receive other medications that the investigator deems to be medically necessary.

5.2.7 Enrollment, Screen Failures, and Study Completion
Patients are considered enrolled from time consented to participate until designated as a screen failure or have either been discontinued from the study or completed it. A screen failure is a patient who signed informed consent, but then was determined to be ineligible or withdraws from the study. Subjects are considered to have completed the study when they are followed through day 60 or have had an adverse event or death occurred prior to day 60.

<u>Day -1 to 0 Baseline:</u>
A. Subject informed consent (obtained before performing study related activities)'
B. Baseline Evaluation at screening.
    1. Demographics (Age, sex ethnicity, race)
    2. Medical history (timing of exposure to COVID-19 source patient, acute and chronic medical condition, medications, allergies.) Any medical condition arising after consent should be recorded as AE.
    3. COVID-19 symptom screen (fevers, cough, shortness of breath, diarrhea, anosmia), onset of symptoms, source of contagion
    4. Vital signs

5. COVID-19 testing (RT-PCR) from nasopharyngeal, throat, tracheal aspirate or bronchoalveolar lavage and stool (optional) samples
6. Blood typing, CBC with differential, comprehensive metabolic panel
7. Serological testing: anti-SARS CoV-2 titers
8. Immune testing samples
9. Urine or serum pregnancy test for females of childbearing potential. Results from laboratory tests obtained up to 7 days before enrollment may be used for the pregnancy test.
10. Determination of eligibility as per inclusion/exclusion criteria, age, consent, positive for COVID-19, respiratory symptoms within 72 hours of admission

DAY 0 Baseline and Infusion:

1. Study Plasma Administration: 1-2 units of plasma will be transfused over 1-2 days. Time at start and end of infusion will be recorded and vital signs will be measured immediately prior to infusion, 10-20 minutes after start of infusion, at completion of infusion and 30-60 minutes after the end of the infusion
2. COVID-19 symptom screen (fevers, cough, shortness of breath)
3. Assessment of clinical status (8-point ordinal scale)
4. New medical conditions, concomitant medication, AE evaluation
5. Physical examination
6. COVID-19 testing (RT-PCR) from nasopharyngeal, throat or stool (optional) samples
7. Blood typing, CBC with differential, comprehensive metabolic panel, C-reactive protein
8. Serological testing: anti-SARS CoV-2 titers
9. Blood for Immune evaluations of cellular and humoral immunity (4 green tops, 30-40 milliliters).

Day 1-7 (or for duration of hospitalization)

1. Vital signs daily (including supplemental oxygen requirements)
2. COVID-19 symptom screen (fevers, cough, shortness of breath)
3. Assessment of clinical status (8-point ordinal scale)
4. New medical conditions, AE evaluation
5. Physical examination
6. CBC with differential, comprehensive metabolic panel, CRP daily

Days 0, 4 (± 1 day), 7 (± 2 day), 14 (± 3 days), 21 (± 3 days), and 28 (± 3 days):

1. SARS-CoV-2 PCR from oropharyngeal or nasopharyngeal swabs: Day 0, 4, 7, 14, 21 and at any time when there is clinical suspicion for COVID-19.
2. Serum or plasma antibody titer to SARS-CoV-2: Day 0, 7, 14, and 28
3. Blood for immune evaluations of cellular and humoral immunity before CIP infusion (day 0) and 7, 14, and 28 days after CIP infusion (4 green tops, 30-40 milliliters). Immune testing may be done at any time if the PI determines there is a need to evaluate acute immune responses (cytokines/chemokines levels).

Key issues to consider follow up by phone, alive, at home, in hospital (ICU or not), on supplemental O_2 or not, back to work, fully and partially

- COVID-19 symptom screen (fevers, cough, shortness of breath)
- Assessment of clinical status (8-point ordinal scale)
- New medical conditions, AE evaluation

Day 60 (± 7 days):

1. COVID-19 symptom screen (fevers, cough, shortness of breath)

2. Assessment of clinical status (8-point ordinal scale)
3. New medical conditions, AE evaluation

One year (±1 month) - *optional*

1. **Serum or plasma antibody titer to SARS-CoV-2**
2. **Blood for immune evaluations of cellular and humoral immunity**
3. **CBC with differential, Comprehensive metabolic panel, PT/INR, PTT, Quantitative immunoglobulins**
4. **Interval medical history**
5. **Subject questionnaires regarding health status**

5.3 Subject Withdrawal

1. Subjects can terminate study participation and/or withdraw consent at any time without prejudice.
2. The investigator may withdraw subjects if they are lost to follow up, non-compliant with study procedures or if the investigator determines that continued participation in the study would be harmful to the subject or the integrity of the study data
3. Discontinuation of the study: the study sponsor, FDA and IRB all have the right to terminate this study at any time

5.4 Concomitant medications will be documented on the CRF

- Prescription medications
- Over the counter medications
- Herbal treatments/nutritional supplements
- Blood products

5.5 Known potential benefits

A key potential benefit is treatment for established infection. Convalescent plasma would be administered to those with clinical disease in an effort to reduce their symptoms and mortality. Based on the historical experience with antibody administration, it can be anticipated that *antibody administration relatively early in the course of disease would be more effective in preventing disease progression than in the treatment of established severe disease.*

Given that historical and current anecdotal data on use of convalescent plasma suggest it is safe in coronavirus infection, the high mortality of COVID-19, particularly in elderly and vulnerable persons, suggests that the benefits of its use in those at high risk for poor outcome and or with early disease outweigh the risks. However, for all cases where convalescent plasma administration is considered, a risk-benefit assessment must be conducted to assess individual variables.

5.6 Known potential risks

5.6.1 Antibody-Mediated Enhancement of Infection (ADE)

The theoretical risk involves the phenomenon of antibody-mediated enhancement of infection (ADE). ADE can occur for several viral diseases and involves an enhancement of disease in the presence of certain antibodies. For coronaviruses, several mechanisms for ADE have been described and there is the theoretical concern that antibodies to one type of coronavirus could enhance infection to another viral strain[11]. It may be possible to predict the risk of ADE of SARS-CoV-2 experimentally, as proposed for MERS. Since the proposed use of convalescent plasma in the COVID-19 epidemic would rely on preparations with high titers of antibody against the same virus, SARS2-CoV-2, ADE may be unlikely. The available evidence from the use of convalescent plasma in patients with SARS1 and MERS[12] and anecdotal evidence of its use in patients with

COVID-19[13], suggest it is safe. Nevertheless, caution and vigilance will be required in for any evidence of enhanced infection. Alterations in serum potassium and calcium levels can occur with these patients.

5.6.2 Attenuated Immune Responses

Another theoretical risk is that antibody administration to those exposed to SARS-CoV-2 may avoid disease but modify the immune response such that those individuals mount attenuated immune responses, which would leave them vulnerable to subsequent re-infection. In this regard, passive antibody administration before vaccination with respiratory syncytial virus was reported to attenuate humoral but not cellular immunity[14]. We will investigate this unexpected potential as part of the clinical trial by measuring immune responses in those exposed and treated with convalescent plasma to prevent disease. If the concern proved real these individuals could be vaccinated against COVID-19 when a vaccine becomes available. *These concerns seem modest compared to the possibility of limiting the duration and severity of disease, and avoiding complications like mechanical ventilation, ARDS, sepsis, and death.*

5.6.3 Transfusion Transmitted Diseases

Finally, there are risks associated with any transfusion of plasma including transmission of transfusion transmitted viruses (e.g. HIV, HBV, HCV, etc.), allergic transfusion reactions, anaphylaxis to transfusion, febrile transfusion reaction, transfusion related acute lung injury (TRALI), transfusion-associated cardiac overload (TACO), and hemolysis should ABO incompatible plasma be administered. In order to minimize the risks of disease transmission, pathogen reduction techniques will be utilized to prepare the plasma. In addition, donors will fulfill all donor requirements for whole blood donation and frequent apheresis plasma donation with the exception of recent illness, in this case COVID-19 infection.

6.0 PREPARATION OF CONVALESCENT IMMUNE PLASMA

The plasma product will be collected from **directed-**donors or anonymous donors from the American Red Cross, New York Blood Center, or other local blood donation facility 28 days after symptom resolution. Antibody titers may be assessed by prescreening 21 days after symptom resolution with an anti-SARS-COV-2 titer of preferably >1:64 and/or by measuring titers from the infused plasma. We will use an FDA-approved test to measure antibodies, such as the Ortho Clinical Diagnostic or equivalent test for total IgG, IgM, and IgA directed at COVID-19 spike protein. Donors may be recruited from COIV-19 Screening Clinics, Employee Health Clinics within the health system and by social media advertising. Per current FDA recommendations and ARC practices, it is assumed that by day 28 after symptom resolution, that there will be no viable viral COVID-19 in the circulation. Otherwise the donor will fulfill all of the standard donor criteria for plasma donation.

Donors and samples will have been screened for transfusion-transmitted infections (e.g. HIV, HBV, HCV, WNV, HTLV-I/II, T.cruzi, ZIKV) both through the use of the uniform donor questionnaire and FDA mandated blood donor screening tests. Plasma will have been collected by the ARC or local donation site using standard apheresis technology and in accordance with standard FDA and blood bank protocols. The CIP will be in standard plasma unit bags with storage, handling, and release per blood bank guidelines. The patient will receive 1-2 units of anti-SARS-CoV-2 convalescent plasma.

Table I: Treatment Schedule	Screen	Baseline	Transfusion	Follow-up							
Day	-1 to	0	0	1	4±1	7±2	14±3	21±3	28±3	60±7	1 year

	0				day	day	days	days	days	days	±1 month [5]
Eligibility											
Informed consent	x										x
Demographic and Medical history	x										x
COVID-19 symptom screen	x										
SARS-CoV-2 RT-PCR for eligibility	x										
Pregnancy test	x										
ABO	x										
Study Drug Administration											
Drug infusion			X								
Study Procedures											
Vital signs[4]	x	x	Xxxx[3]	x	x	x	x	x	x	X	
Physical examination[4]	x		x	x		x		x		X	
Symptom screen[4]	x	x	x	x	x	x	x	x	x	X	x
Concomitant medications[4]	x	x	x	x	x	x	x	x	x	X	
Assessment with 8-point ordinal scale[4]		x		x	x	x	x	x	x	X	
Adverse event monitoring[4]		x	x	x	x	x	x	x	X	X	
Health Questionnaire											x
Laboratory testing											
CBC and CMP[4]		x		x		x	x	x	X	X	X
SARS-CoV-2 RT-PCR (NP or OP)		x			x	x	x	x			
SARS-CoV-2 antibody		x				x	x		x		x
Blood for immune testing		x				x	x		x		x
PT/INR, PTT, Quantitative Immunoglobulins											x

ABO typing may be historical documentation of blood type [2]Measured by an anti-SARS-CoV-2 ELISA and serum antibody titer to SARS-CoV-2. [3] Vital signs will be performed pre-transfusion, 10-20 mins after the start of transfusion, at completion and 30-60 mins after the end of the transfusion. [4]Done daily through Day 7 or until patient is discharged from the hospital, whichever comes first [5]Optional follow up visit for CIP recipients

7.0 STATISTICAL CONSIDERATIONS

7.1Statistical Analyses

Twenty-nine evaluable patients will have 80% power to declare a significant decrease in ICU admissions from a historical control of 50% of hospitalized patients to 25% with a two-sided type I error of 0.05 (reject ICU admission rate=50% if only 8 or less patients are admitted to ICU among 29 evaluable patients). The historic control group will consist of 29 patients who are gender and age (within 5 years) matched.

7.2 Expected accrual rate, accrual duration, and study duration

Our anticipated accrual rate is 6-8 patients per month. Thus, it should take approximately 3-5 months to accrue the 29 patients needed for the trial. Allowing for 28 days of follow-up to obtain the primary endpoints on the last patient enrolled and 3 months to assemble, analyze and interpret the data the total study duration is projected to be at most 18 months.

7.3 Endpoints

7.3.1 Primary Endpoints

- Transfer to ICU
- 28 day mortality

7.3.2 Secondary Endpoints

- Cumulative incidence of serious adverse events during the study period: transfusion reaction (fever, rash), transfusion related acute lung injury (TRALI), transfusion associated circulatory overload (TACO), transfusion related infection
- Rates and duration of SARS-CoV-2 PCR positivity at days 0, 4, 7, 14 and 21.
- Serum or plasma antibody titer[3] to SARS-CoV-2: Day 0, 7, 14, and 28
- Cellular and humoral immune responses at days 0, 7, 14, and 28 days
- Supplemental oxygen free days
- Ventilator free days
- ICU free days
- Sequential organ failure assessment (SOFA) score at days 0, 1, 4, 7, 14, 21, 28
- WHO 8-point scale for clinical improvement at days 0, 1, 4, 7, 14, 21, 28
- Need for vasopressors
- Need for renal replacement therapy
- Need for extracorporeal membrane oxygenation (ECMO)
- Hospital length of stay (LOS)
- ICU LOS
- Grade 3 or 4 adverse events (AEs)

8.0 EFFICACY, VIROLOGY AND IMMUNE MEASURES

8.1 Clinical Efficacy

1. Transfer to ICU
2. 28 and 60 day mortality
3. Supplemental oxygen free days
4. Ventilator free days
5. ICU free days
6. Sequential organ failure assessment (SOFA) score at days 0, 1, 4, 7, 14, 21, 28
7. Need for vasopressors
8. Need for renal replacement therapy
9. Need for extracorporeal membrane oxygenation (ECMO)
10. Hospital length of stay (LOS)
11. ICU LOS
12. Grade 3 or 4 adverse events (AEs)

8.2 Virologic measures

1. Rates, levels and duration of SARS-CoV-2 RNA in OP or NP swabs and/or subgenomic - messengerRNA (sg-mRNA) from Viral Transport Media (VTM) by RT-PCR at days 0, 4, 7, 14, and 21. Other specimen types may be tested as available (*e.g.* BAL fluid, tracheal secretions, sputum, etc.) or when RT-PCR assays are validated for additional sources (*i.e.,* stool, blood). VTM will be obtained from the original OP or NP swab collection devices as detailed above. Trial patient samples will be compared to control non-transfused hospitalized patient samples stored in the clinical labs.
2. Serologic positivity and neutralization antibody titers for anti-SARS-CoV-2 at days designated time points at 0, 7, 14, and 28.

8.3 Immune Evaluations

Immune evaluations (IEs) will be conducted as delineated in **Table 1**. Baseline serum and cells will be aliquoted from the pheresis. Blood draws (4 green top tubes containing approximately 30-40 milliliters of anti-coagulated blood and 1 red top tube containing 8 milliliters of serum) at each time point. Immunologic Control samples from non-transfused hospitalized patients will be obtained from the UVA COVID-19 Biorepository. Permission has been obtained from the Biorepository Committee.

9.0 STATISTICAL CONSIDERATIONS

9.1 Power calculation

Twenty-nine evaluable patients will have 80% power to declare a significant decrease in ICU admissions from a historical control of 50% of hospitalized patients to 25% with a two-sided type I error of 0.05 (reject ICU admission rate=50% if only 8 or less patients are admitted to ICU among 29 evaluable patients).

Data analysis: For categorical clinical endpoints, frequency with percentage will be summarized. For the quantitative clinical variables and immune response variables measured in this objective, we will produce summary statistics (including means, medians, and standard deviations) for data at pre- and post-CIP infusions. Subsequent analyses will compare the serologic and neutralization antibody titers for anti-SARS-COV-2 antibodies and immune response variables (after a suitable data transformation to improve normal distribution, if necessary) pre- and post-treatment using a paired t-test (or Wilcoxon sign ranked test if the data are not approximately normally distributed). The level of each measured immune biomarker measured at the follow-up time points will be plotted to visually show the change of immune responses over time. To explore whether immune responses at pre- or peak improvement at post-CIP infusions associate with clinical endpoints (such as ICU admission, intubation), logistic regression for binary endpoints and Cox regression for time to event endpoints will be used to analyze each measured immune biomarker respectively. These effects will be compared to biospecimen from non-transfused immunologic controls. Analyses will adjust for other covariates such as age and co-morbidities. No

multiplicity adjustment is planned due to the nature of being exploratory. A two-sided p-value of < 0.05 will be considered significant.

9.2 Expected accrual rate, accrual duration, and study duration
Our anticipated accrual rate is 6-8 patients per month. Thus, it should take approximately 3-5 months to accrue the 29 patients needed for the trial. Allowing for 6 months of follow-up to obtain the primary endpoint on the last patient enrolled and 3 months to assemble, analyze and interpret the data the total study duration is projected to be at most 2 years.

9.3 Analysis of AE data
Analysis of AE data will primarily be descriptive based on MedDRA coding of events. The proportion of subjects experiencing an SAE and the proportion experiencing a Grade 3 or higher will be recorded. AE will be compared to published data.

9.4 Analysis of anti-SARS-CoV-2 titers
Analysis of titers will be descriptive, comparing the geometric mean titers at days at 0, 7, 14, and 28 days.

10.0 REGISTRATION AND REQUIRED DATA

10.1 Pre-Study
All patients on-study will be evaluated prior to initiation of therapy. Baseline studies will be completed prior to on-study.

10.2 Protocol Registration
Subject must meet all eligibility requirements listed in Section 4.0 prior to enrollment. Subjects who are consented to the study must be registered in the Clinical Trials Office. All subjects who have signed an informed consent should have demographics and date of signed informed consent entered into the database.

10.3 Evaluations and Assessments
All evaluations and assessments should be completed as described in Table 1. Any assessments not described in detail below should be done according to standard of care practices.

10.4 Medical History/Physical Examination
A complete medical history and physical examination will be obtained at the Pre-Study Assessment. The medical history includes clinically significant diseases, surgeries, history of cancer (including date of diagnosis, location of disease, prior cancer therapies and procedures), and all medications (e.g., prescription drugs, over-the-counter drugs, herbal/homeopathic remedies, nutritional supplements). A physical examination documented by the primary physician in the hospital chart will be performed at the re-Study Assessment and prior to the CIP infusion for baseline conditions) only. Physical examinations will include the following body systems: general appearance (including height and body weight), skin, neck, HEENT (head, ears, eyes, nose and throat), heart (auscultation of heart sounds), lungs (auscultation of lung fields), abdomen palpation and auscultation of bowel sounds, lymph nodes, extremities, and nervous system.

10.5 Vital Signs
Vital signs including respiratory rate, pulse rate, systolic blood pressure [SBP], and diastolic blood pressure [DBP] and body temperature, and supplemental oxygen requirements, will be collected at Pre Study Assessment.

10.6 Laboratory Parameters
Blood samples for clinical labs will be obtained as described in the Time and Events table for

adverse event determination and clinical decision making.

10.7 Laboratory and Clinical Data Review

The Investigator, or designee, must review all laboratory values and report any clinically significant change compared to the baseline sample as an AE. When reporting such AEs the Investigator will use the appropriate clinical term, rather than the laboratory test result (e.g. anemia versus low hemoglobin).

10.8 Immune Evaluations

Immune evaluations (IEs) will be conducted as delineated in Table 1. Baseline serum and cells will be aliquoted from the pheresis. Blood draws (4 green top tubes containing approximately 50 mL of anti-coagulated blood and 1 red top tube containing 8 mL of serum) will be collected at designated time points at 0, 7, 14, and 28 days after CIP infusion. All patients receiving any dose of study treatment should be assessed for immune markers. Evaluations will include:

- Serum cytokine/chemokine levels
- Phenotyping of T cells and T cell subpopulations, TCR repertoire analysis, monocyte, B cells, and NK cell by flow cytometry.
- Cytotoxic activity and IFNγ production in PBMC populations. An immune response will be defined as a 30 IFNγ Elispots per million cells plated above the background of unstimulated spontaneous IFNγ Elispots per million cells plated.
- Additional assays may be performed on available samples to evaluate Th_1/Th_2 responses in single cell RNA sequencing on selected samples.

10.9 Subject Status Definitions

- Enrolled: All subjects who sign an informed consent will be considered enrolled on the study. All subjects consented on the study must be entered into Clinical Trials Office Database.
- Screen Failure: A subject who is withdrawn or discontinues from study screening prior to being on study is considered a screen failure. Screen failures are not considered a study accrual and will be replaced. Note: The IRB defines any individual that has signed an informed consent as an enrollment in this study and so screen failures should be reported to the IRB with enrollment numbers.
- On-Study: A subject is considered on-study on the date when the study team has confirmed the subject has met all of the inclusion and none of the exclusion criteria, and the treating physician/surgeon or study PI has signed off on the confirmation.
- On-Treatment: A subject is considered on-treatment on the date that they receive the first study treatment. A subject who is withdrawn or discontinues from the study after receiving at least 1 infusion is considered a discontinuation and will not be replaced.
- On Follow-up: A subject is considered on follow-up on the date that they have met any of the criteria in section 4.0.
- Off-Study: A subject is considered off-study if they are removed from the study for any of the reasons listed in section 4.0 or if they have completed all study assessments through follow-up.
- Immunologic Controls: A subject for whom biospecimen is obtained from the COVID-19 Biorepository.

11.0 RISKS AND BENEFITS

11.1 Potential Benefits of Treatment for the Patient

The benefits of antiviral therapy with anti-COVID-19 plasma in patients with respiratory symptoms consistent with interstitial pneumonia at high risk for requiring ICU admission are not known. It is anticipated that treatment will decrease the risk of disease progression, decrease ICU admissions, and decrease aggressive respiratory support including possible mechanical ventilation and other ICU support.

11.2 Risks of Plasmapheresis for the Donor

There is small risk of breach of privacy and/or confidentiality. The most common risk of blood draw and plasmapheresis is pain. Other risks include tingling of the lips and fingers, bruising at the IV site(s), mild tightness in the chest, a cough, low and/or high blood pressure, a slow pulse rate, and fainting or passing out (not often). There is a rare risk of allergic reactions such as rash, hives, shortness of breath or shock and infection. The risks of citrate include muscle cramps, numbness, cold feeling, tingling sensations in the lips and fingers, feeling anxious, and seizures with abnormal heart rhythms, and allergic reaction including death being rare.

11.3 Potential benefits of clinical monitoring and virologic testing

Subjects enrolled in the study may reduce their chances of disease progression.

11.4 Potential risks

1. Risks of plasma: Fever, chills, rash, headache, serious allergic reactions, TRALI, TACO, transmission of infectious agents
2. Risks of phlebotomy: local discomfort, bruising, hematoma, bleeding, fainting,
3. Total blood draws will not exceed 500 mL
4. Risks of oropharyngeal and throat swab: local discomfort, vomiting

11.5 Alternatives

The alternative to participation in this study is routine care.

12.0 ADVERSE EVENTS AND SAFETY EVALUATION

Adverse events will be evaluated and scored using Common Terminology Criteria for Adverse Events version 5.0 (CTCAE v5.0). ctep.cancer.gov/.../CTCAE_v5_Quick_Reference_5x7.pdf

1. Safety Evaluations will assess for the safety of high titer anti-SARS-CoV-2 plasma
2. Clinical evaluations: Vital signs and symptom screen on days 0-7, 14 and symptom screens on days 28,and 60.
3. Safety Evaluations will assess for the safety of high titer anti-SARS-CoV-2 plasma
4. Clinical evaluations: Vital signs and symptom screen on days 0-7, 14 and symptom screens on days 28,and 60.
5. Laboratory evaluations consistent with ongoing medical care may include radiographic imaging modalities such as chest x-rays and chest CT.
6. Safety laboratory tests (ABO typing, pregnancy testing, CBC, CRP, and comprehensive metabolic panel) will be performed at the local CLIA-certified clinical laboratory on days 0-7 and 14.

12.1 Adverse Event (AE)

Any untoward medical occurrence in a clinical investigation subject who has received a study intervention and that does not necessarily have to have a causal relationship with the study product. An AE can, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of the study product, whether or not considered related to the study product.

12.2 Serious Adverse Event (SAE)

An SAE is any adverse event that results in any of the following outcomes:

1. Death
2. Life-threatening (immediate risk of death)
3. Prolongation of existing hospitalization

4. Persistent or significant disability or incapacity
5. Important medical events that may not result in death, be life threatening, or require intervention or escalation of care may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization.

12.3 Unexpected Adverse event: (UAE)
An adverse reaction, the nature or severity of which is not consistent with the investigator's brochure.

12.4 Serious and Unexpected Suspected Adverse Reaction (SUSAR)

12.5 Unanticipated Problem (UP)
Unanticipated Problem that is not an Adverse Event (e.g. breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.
Protocol Deviation: Deviation from the IRB-approved study procedures. Designated serious and non-serious

1. Serious Protocol Deviation: Protocol deviation that is also an SAE and/or compromises the safety, welfare or rights of subjects or others
2. Safety Reporting Requirements

12.6 Unanticipated Problem that is not an Adverse Event
(e.g. breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.)

12.7 Protocol Deviation
Deviation from the IRB-approved study procedures. Designated serious and non-serious

1. Serious Protocol Deviation: Protocol deviation that is also an SAE and/or compromises the safety, welfare or rights of subjects or others
2. Safety Reporting Requirements

12.8 Reporting Interval
All AEs and SAEs will be documented from the first administration of study product and for the following 7 days or until un-enrollment from the study, whichever comes sooner. All AEs and SAEs will be followed until resolution even if this extends beyond the study-reporting period. Resolution of an adverse event is defined as the return to pre-treatment status or stabilization of the condition with the expectation that it will remain chronic.
At any time after this 7 day period, if the investigator becomes aware of a SAE that is suspected to be related to study product

12.9 Investigator's Assessment of Adverse Events
The determination of seriousness, severity, and causality will be made by an on-site investigator who is qualified (licensed) to diagnose adverse event information, provide a medical evaluation of adverse events, and classify adverse events based upon medical judgment. This includes but is not limited to physicians, physician assistants, and nurse practitioners.

Laboratory abnormalities will be reported as AEs if there is a 2 times standard deviation increase above baseline.

12.10 Assessment of Seriousness

1. Event seriousness will be determined according to the protocol definition of an SAE

2. Assessment of Severity

12.10.1 Event severity

Will be assigned according to the scale below:

1 = Mild: Transient or mild discomfort (<48 hours); no medical intervention/therapy required.)

2 = Moderate: Some worsening of symptoms but no or minimal medical intervention/therapy required)

3 = Severe: Escalation of medical intervention/therapy required

4 = Life-threatening: Marked escalation of medical intervention/therapy required.

5= Death

12.11 Assessment of Association

The association assessment categories that will be used for this study are:

- Associated – The event is temporally related to the administration of the study product and no other etiology explains the event.
- Not Associated – The event is temporally independent of the study product and/or the event appears to be explained by another etiology.

The investigator must provide an assessment of association or relationship of AEs to the study product based on:

- Temporal relationship of the event to the administration of study product;
- Whether an alternative etiology has been identified;
- Biological plausibility;
- Existing therapy and/or concomitant medications

.

13.0 REPORTING ADVERSE REACTIONS

- **Investigators are required to notify the FDA and IRB** of all serious and unexpected adverse drug reactions.
- **All reactions in a 'reportable' category must be reported** unless it is documented in the medical record chart that treatment is definitely **not** responsible for the toxicity.
- **Serious and unexpected adverse reactions** will be reported to the following sources within the stated time frame:
- **All participating investigators**: Written IND safety report within 10 working days.
- **IRB**: Notify IRB in written report within 10 working days.
- **FDA**: Written IND safety report within 10 calendar days. Report by telephone within three working days. Include reports in annual report.

13.1 Procedure for calling the FDA:

The sponsor or designated PI or research nurse will call for ADRs.

The principal investigator or his designee will email or call for regulatory/protocol issues.

The medical reviewer for the FDA is:

[Will be identified by FDA]

Center for Biologics Evaluation and Research

Food and Drug Administration

Division of Biological Investigational New Drugs

1401 Rockville Pike HFM 99

Rockville, MD 20852

Document as completely as possible, and send copies to:

Patient's chart

Protocol binder
UVA Institutional Review Board
FDA correspondence binder
Lawrence G. Lum, MD, DSc, Director of Cellular therapy and Director cGMP Facility
Co-investigators for the protocol

14.0 SAFETY OVERSIGHT

14.1 Monitoring Plan

1. All AE and SAW will be reviewed by protocol team in real time.
2. A medical monitor will be appointed by the sponsor for safety oversight of the clinical study.
3. A data safety monitoring board (DSMB) composed of independent experts without conflict of interests. The Board will review the study before initiation and every 3 months thereafter. The Board will review study data to evaluate the safety, efficacy, study progress, and conduct of the study
4. An Independent Safety Monitor (ISM) will be appointed. The ISM is a physician with expertise in infectious diseases and whose primary responsibility is to provide timely independent safety monitoring. An ISM is in close proximity to the study site and has the authority to readily access study participant records. The ISM reviews any SAE that occurs at the study site in real time and provides a written assessment.

14.2 Study monitoring

1. As per ICH-GCP 5.18 and FDA 21 CFR 312.50, clinical protocols are required to be adequately monitored by the study sponsor. Monitors will verify that
    a. There is documentation of the informed consent process and signed informed consent documents for each subject
    b. There is compliance with recording requirements for data points
    c. All SAEs are reported as required
    d. Individual subjects' study records and source documents align
    e. Investigators are in compliance with the protocol.
    f. Regulatory requirements as per Office for Human Research Protections-OHRP), FDA, and applicable guidelines (ICH-GCP) are being followed.

15.0 STOPPING RULES

The study enrollment and dosing will be stopped and an ad hoc review will be performed if any of the specific following events occur or, if in the judgment of the study physician, subject safety is at risk of being compromised:

1. Death within one hour of plasma infusion
2. Occurrence of a life-threatening allergic/hypersensitivity reaction (anaphylaxis), manifested by bronchospasm with or without urticaria or angioedema requiring hemodynamic support with pressor medications or mechanical ventilation, TRALI, or TACO
3. One subject with a grade 4 or persistent grade 3 (≥72 hours) associated with study product.
4. Two subjects with persistent Grade 3 (≥ 72 hours) or higher lab toxicity for the same parameter associated with study product.
5. An overall pattern of symptomatic, clinical, or laboratory events that the medical monitor, ISM, or SMC consider associated with study product and that may appear minor in terms of individual events but that collectively may represent a serious potential concern for safety.
6. Any other event(s) which is considered to be a serious adverse event in the good clinical judgment of the attending physician team. This will be appropriately documented.

Upon completion of this review and receipt of the advice of the ISM or SMC, DMID will determine if study entry or study dosing should be interrupted or if study entry and study dosing may continue according to the protocol.

15.1 Stopping Rules

Infusion of study drug will be halted if any of the following manifestations of anaphylaxis develop and will not be restarted:

- Skin or mucous membrane manifestations: hives, pruritus, flushing, swollen lips, tongue or uvula
- Respiratory compromise: dyspnea, wheezing, stridor, hypoxemia
- A decrease in systolic blood pressure to < 90 mmHg or >30% decrease from baseline or a diastolic drop of >30% from baseline.
- Tachycardia with an increase in resting heart rate to > 130bpm; or bradycardia <40 that is associated with dizziness, nausea or feeling faint.
- Any other symptom or sign which in the good clinical judgment of the study clinician or supervising physician warrants halting the infusion. For example, the rapid onset of gastrointestinal symptoms, such as nausea, vomiting, diarrhea, and cramps, for instance, may be manifestations of anaphylaxis and may warrant an immediate halt prior to meeting full SAE criteria

16.0 DATA AND SAFETY MONITORING PLAN

16.1 Data Collection/ CRF Completion

The Principal Investigator will provide continuous monitoring of subject safety in this trial with periodic reporting to the UVA Data Safety Monitoring Committee (DSMC).

All data should be entered into the Database in accordance the University of Virginia Clinical Trials Office. Adverse events should be entered into Database. All other study specific data should be entered into Database in the timeframes specified in the SOP.

16.2 Data Safety Monitoring Committee

The University of Virginia Data and Safety Monitoring Committee (DSMC) will provide oversight of the conduct of this study.
The DSMC will review the following:

- All adverse events
- Audit results
- Application of study designed stopping/decision rules
- Whether the study accrual pattern warrants continuation/action
- Protocol violations

The DSMC will meet every 2 months or every other month for aggregate review of AE data. Tracking reports of the meetings are available to the PI for review. Issues of immediate concern by the DSMC are brought to the attention of the PI (and if appropriate to the PRC and IRB) and a formal response from the PI is requested.

16.3 Independent Safety Review Committee

This study will accrue subjects sequentially, with the accrual of each new subject dependent upon review of cumulative data by the Safety Review Committee. The Safety Review Committee will be comprised of clinicians with relevant experience and at least one statistician. Following completion of all assessments for a subject, the Safety Review Committee will review the following information, cumulative for all subjects accrued in the study:

- Adverse events
- ECGs
- Clinical labs
- Other

The minutes of these meetings and final recommendations will be maintained and provided to the University of Virginia Data and Safety Monitoring Committee (DSMC).

17.0 STUDY MANAGEMENT

17.1 Institutional Review Board (IRB) Approval and Consent

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to all ICH E6 principles and Good Clinical Practice (GCP), to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and confirmation of eligibility for this study, the subject will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the subject and the investigator is assured that the subject understands the implications of participating in the study, the subject will be asked to give consent to participate in the study by signing an IRB-approved consent form.

Prior to a subject's participation in the trial, the written informed consent form should be signed and personally dated by the subject or subject's surrogate and by the person who conducted the informed consent discussion.

17.2 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study subject requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

17.2.1 Emergency Modifications

Investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB-HSR approval/favorable opinion.
For any such emergency modification implemented, a UVA IRB modification form must be completed by study Personnel within five (5) business days of making the change.

17.2.2 Other Protocol Deviations/Violations

Protocol Deviations: A protocol deviation is any unplanned variance from an IRB approved protocol that:

- Is generally noted or recognized after it occurs
- Has no substantive effect on the risks to research participants
- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected
- Did not result from willful or knowing misconduct on the part of the investigator(s).

Study personnel will record the deviation, and report to any sponsor or data and safety monitoring committee in accordance with their policies. Deviations should be summarized and reported to

the IRB at the time of continuing review.

Protocol Violations: An unplanned protocol variance is considered a violation if the variance:

- Has harmed or increased the risk of harm to one or more research participants.
- Has damaged the scientific integrity of the data collected for the study.
- Results from willful or knowing misconduct on the part of the investigator(s).
- Demonstrates serious or continuing noncompliance with federal regulations, State laws, or University policies.

Violations should be reported by study personnel to the IRB within one (1) week of the investigator becoming aware of the event.

17.3 Retention of Records

Study documentation includes all Case Report Forms, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed subject consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the study investigator for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until six years after the completion and final study report of this investigational study.

17.4 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations, all applicable local regulatory laws and regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study subjects. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

It is the responsibility of the Principal Investigator to ensure that all study site personnel are aware that the study protocol and all data generated is confidential and should not be disclosed to third parties (with the exception of local and national regulatory bodies which require access for oversight purposes).

The Principal Investigator will be responsible for assuring that all the required data will be collected and entered onto the Case Report Forms. Periodically, monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all case report forms will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

18.0 REFERENCES

1. Casadevall A, Scharff, M.D. Return to the past: the case for antibody-based therapies in infectious diseases. *Clinical Infectious Diseases* 1995; **21**(1)**:** 150-161.

2. Casadevall A, Dadachova, E., Pirofski, L. Passive antibody therapy for infectious diseases. *Nature Reviews Microbiology* 2004; **2**(9)**:** 695-703. doi: 10.1038/nrmicro974

3. Zhang JS, Chen, J. T., Liu, Y. X., Zhang, Z. S., Gao, H., Liu, Y., Wang, X., Ning, Y., Liu, Y. F., Gao, Q., Xu, J. G., Qin, C., Dong, X. P., Yin, W. D. A serological survey on neutralizing antibody titer of SARS convalescent sera. *Journal of medical virology* 2005; **77**(2)**:** 147-150. e-pub ahead of print 2005/08/27; doi: 10.1002/jmv.20431

4. Sahr F, Ansumana, R., Massaquoi, T. A., Idriss, B. R., Sesay, F. R., Lamin, J. M., Baker, S., Nicol, S., Conton, B., Johnson, W., Abiri, O. T., Kargbo, O., Kamara, P., Goba, A., Russell, J. B., Gevao, S. M. Evaluation of convalescent whole blood for treating Ebola Virus Disease in Freetown, Sierra Leone. *The Journal of infection* 2017; **74**(3)**:** 302-309. e-pub ahead of print 2016/11/22; doi: 10.1016/j.jinf.2016.11.009

5. Casadevall A, Pirofski, L. A. Antibody-mediated regulation of cellular immunity and the inflammatory response. *Trends in immunology* 2003; **24**(9)**:** 474-478. e-pub ahead of print 2003/09/12; doi: 10.1016/s1471-4906(03)00228-x

6. Casadevall A, Scharff, M. D. Serum therapy revisited: animal models of infection and development of passive antibody therapy. *Antimicrobial agents and chemotherapy* 1994; **38**(8)**:** 1695-1702. e-pub ahead of print 1994/08/01; doi: 10.1128/aac.38.8.1695

7. Cheng Y, Wong, R., Soo, Y. O., Wong, W. S., Lee, C. K., Ng, M. H., Chan, P., Wong, K. C., Leung, C. B., Cheng, G. Use of convalescent plasma therapy in SARS patients in Hong Kong. *European journal of clinical microbiology & infectious diseases* 2005; **24**(1)**:** 44-46. e-pub ahead of print 2004/12/24; doi: 10.1007/s10096-004-1271-9

8. Yeh KM, Chiueh, T. S., Siu, L. K., Lin, J. C., Chan, P. K., Peng, M. Y., Wan, H. L., Chen, J. H., Hu, B. S., Perng, C. L., Lu, J. J., Chang, F. Y. Experience of using convalescent plasma for severe acute respiratory syndrome among healthcare workers in a Taiwan hospital. *The Journal of antimicrobial chemotherapy* 2005; **56**(5)**:** 919-922. e-pub ahead of print 2005/09/27; doi: 10.1093/jac/dki346

9. Ko JH, Seok, H., Cho, S. Y., Ha, Y. E., Baek, J. Y., Kim, S. H., Kim, Y. J., Park, J. K., Chung, C. R., Kang, E. S., Cho, D., Muller, M. A., Drosten, C., Kang, C. I., Chung, D. R., Song, J. H., Peck, K. R. Challenges of convalescent plasma infusion therapy in Middle East respiratory coronavirus infection: a single centre experience. *Antiviral therapy* 2018; **23**(7)**:** 617-622. e-pub ahead of print 2018/06/21; doi: 10.3851/imp3243

10. Arabi YM, Hajeer, A.H., Luke, T., Raviprakash, K., Balkhy, H., Johani, S., et al. Feasibility of using convalescent plasma immunotherapy for MERS-CoV infection, Saudi Arabia. *Emerging infectious diseases.* 2016; **22**(9)**:** 1554.

11. Wan Y, Shang, J., Sun, S., Tai, W., Chen, J., Geng, Q., He, L., Chen, Y., Wu, J., Shi, Z., Zhou, Y., Du, L., Li, F. Molecular Mechanism for Antibody-Dependent Enhancement of Coronavirus Entry. *Journal of Virology* 2020; **94**(5). e-pub ahead of print 2019/12/13; doi: 10.1128/jvi.02015-19

12. Mair-Jenkins J, Saavedra-Campos, M., Baillie, J.K., Cleary, P., Khaw, F-M., Lim, W.S., et al. The effectiveness of convalescent plasma and hyperimmune immunoglobulin for the treatment of severe acute respiratory infections of viral etiology: a systematic review and exploratory meta-analysis. *The Journal of infectious diseases.* 2015; **211**(1)**:** 80-90.

13. China puts 245 COVID-19 patients on convalescent plasma therapy. In: Huaxia, (ed): XinhuaNet, 2020.

14. Crowe JE, Jr., Firestone, C. Y., Murphy, B. R. Passively acquired antibodies suppress humoral

but not cell-mediated immunity in mice immunized with live attenuated respiratory syncytial virus vaccines. *Journal of immunology* 2001; **167**(7)**:** 3910-3918. e-pub ahead of print 2001/09/21; doi: 10.4049/jimmunol.167.7.3910