PROTOCOL SUMMARY:

Long title: Convalescent Plasma to Limit Coronavirus Associated Complications: A Randomized Open label, Phase 1 Study Comparing the Efficacy and Safety of High-Titer Anti-SARS-CoV-2 plasma vs. placebo in hospitalized patients with interstitial pneumonia due to COVID-19

Short title: CSSC-002

Clinical Phase: 1

IND Sponsor: Johns Hopkins via a national IND

Conducted by: Mayo Clinic in collaboration with Johns Hopkins University, Washington University in St. Louis and other interested parties.

Sample Size: 20

Study Population: Hospitalized COVID-19 patients aged 18 years of age with respiratory symptoms within 3 to 7 days from the beginning of illness.

Study Duration: April 1, 2020 to December 31, 2022

Study Design: This randomized placebo-controlled open label phase 1 trial will assess the efficacy and safety of anti-SARS-CoV-2 convalescent plasma in hospitalized patients with acute respiratory symptoms between 3 and 7 days after the onset of symptoms with or without confirmed interstitial COVID-19 pneumonia by chest CT. A total of 20 eligible subjects will be randomized in a 1:1 ratio to receive either high titer anti-SARS-CoV-2 plasma or control (standard thawed plasma)

The following will be assessed in all subjects:

- Age, sex, comorbidities, date of symptoms, source of infection, type of admission, APACHE score, SOFA score, Clinical status, vital signs including temperature, respiratory rate, oxygen saturation, oxygen requirement, CBC with neutrophil counts, lymphocytes count, CRP, chest x-ray, chest CT, location in hospital,
- Safety and efficacy: Day 0 (baseline), 1, 2, 3, 7, 14, and 28 and once monthly at 2-3 months.
- Serum or plasma antibody titer[1] to SARS-CoV-2: Day 0, 1, 3, 7, 14 (additional days 21 and 28 may be included, as available)

[1] Measured via initial anti-SARS-CoV-2 ELISA and serum neutralizing antibody titer to SARS-CoV-2.

- SARS-CoV-2 PCR from nasopharyngeal swabs: Day 0, 3, 7, 14 and at any time when there is clinical suspicion for COVID-19
- Outcome measures: increased O2 requirement (PaO2/FiO2 ratio or SpO2/FIo2), 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

Study Agent:

- Pathogen reduced SARS-CoV-2 convalescent plasma (1-2 units; ~300-600 mL at neutralization antibody titer >1:64 *if possible >1:160 this is a moving target as assays develop*)
- Standard plasma[2]

Primary Efficacy Objective: Reduction in progression of oxygenation and ventilation support

Primary Endpoint: Avoidance of ICU admission.

Secondary endpoints:

1. Cardio-circulatory arrest (at any time)
2. Transfer to ICU
3. Type and duration of respiratory support (and other ICU support)in ICU
4. ICU mortality and LOS
5. Hospital mortality and LOS
6. Ventilator-free days
7. 28 day mortality

Primary Safety Objective: Evaluate the safety of treatment with high-titer anti-SARS-CoV-2 plasma versus control (standard plasma) in hospitalized patients with COVID-19 respiratory symptoms.

Primary Safety Endpoints:

1. Rapid deterioration of respiratory or clinical status on transfusion of SARS-CoV-2 convalescent plasma
2. 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

[2] Solvent detergent treated pooled plasma

Secondary Objectives:

1. Compare the anti-SARS-CoV-2 convalescent plasma and control (standard plasma) groups anti-SARS-CoV-2 titers at days 0, 1, 3, 7 and 14 (additional days 21 and 28 may be included, as available).
2. Compare the rates, levels and duration of SARS-CoV-2 RNA in NP swabs using RT-PCR amongst the anti-SARS-CoV-2 convalescent plasma and control (standard plasma) groups at days 0, 3, 7 and 14. Other specimen types may be tested as available (*eg.,* BAL fluid, tracheal secretions, sputum, etc.) or when RT-PCR assays are validated for additional sources (*i.e.,* stool, blood).

Study population:

Inclusion Criteria for Enrollment

1. Patients must be 18 years of age or older
2. Hospitalized with COVID-19 respiratory symptoms and confirmation via COVID-19 SARS-CoV-2 RT-PCR testing. Patient is willing and able to provide written informed consent and comply with all protocol requirements.
3. Patient agrees to storage of specimens for future testing.
4. If female must not be pregnant and/or breastfeeding.

Exclusion Criteria

1. Female subjects with positive pregnancy test, breastfeeding, or planning to become pregnant/breastfeed during the study period
2. Receipt of pooled immunoglobulin in past 30 days
3. Contraindication to transfusion or history of prior reactions to transfusion blood products

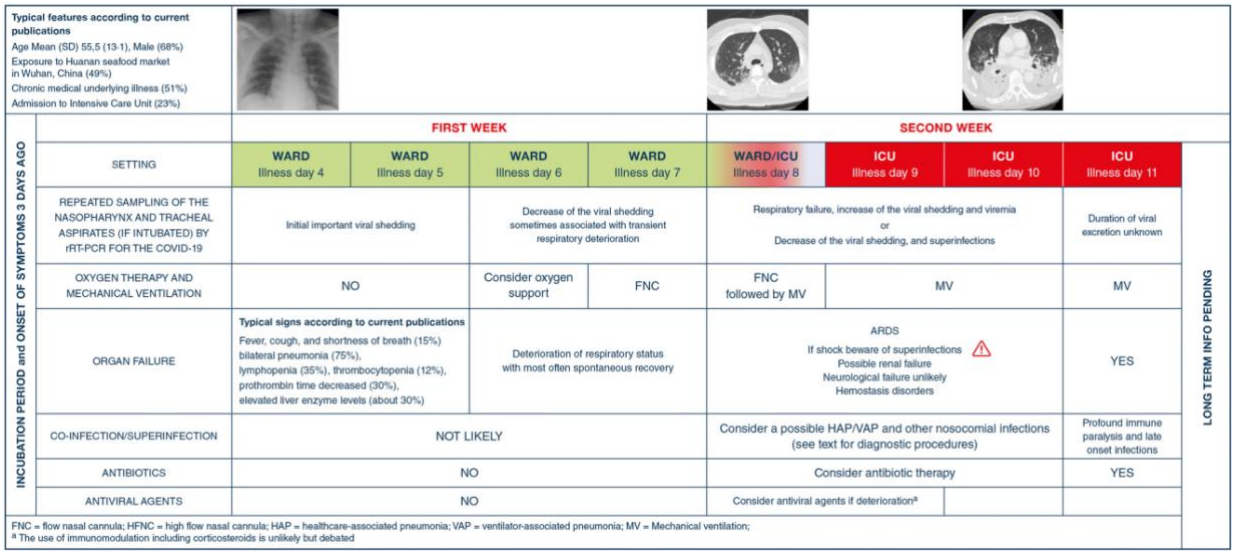

Figure 1 (from Bouadma et al Int Care Med 2020) describes what is known about the potential patient course of COVID-19 pneumonia. Our goal is to intervene in the middle of "green" area and improve patient status and avoid ICU admission.

LIST OF ABBREVIATIONS

ADR: Adverse Drug Reaction
ADE: Antibody-mediated enhancement of infection
AE: Adverse Event/Adverse Experience
CDC: United States Centers for Disease Control and Prevention
CFR: Code of Federal Regulations
CLIA: Clinical Laboratory Improvement Amendment of 1988
COI: Conflict of Interest
COVID-19: Coronavirus Disease
CRF: Case Report Form
DMC: Data Management Center
DSMB: Data and Safety Monitoring Board
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
IEC :Independent ethics committee
IND: Investigational New Drug Application
IRB: Institutional review board
ISBT: International Society of Blood Transfusion
ISM: Independent Safety Monitor
IWRS :Interactive web response system
MERS: Middle East Respiratory Syndrome
NA: Nuclear antibody
NP: Nasopharyngeal
OP: Oropharyngeal
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
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

1. Background and scientific rationale

Beyond supportive care, there are currently no proven treatment options for coronavirus disease (COVID-19) and the related pneumonia, the infection caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Human convalescent plasma is an option for treatment of COVID-19 and could be rapidly available when there are sufficient numbers of people who have recovered and can donate high titer neutralizing immunoglobulin-containing plasma.

Passive antibody therapy 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. In contrast, active vaccination requires the induction of an immune response that takes time to develop and varies depending on the vaccine recipient. Some immunocompromised patients fail to achieve an adequate immune response. Thus, passive antibody administration is the only means of providing immediate immunity to susceptible persons and immunity of any measurable kind for highly immunocompromised patients.

Passive antibody therapy has a storied history going back to the 1890s and was the only means of treating certain infectious diseases prior to the development of antimicrobial therapy in the 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 the case of SARS-CoV-2, the anticipated mechanism of action by which passive antibody therapy would mediate protection is viral neutralization. However, other mechanisms may be possible, such as antibody dependent cellular cytotoxicity and/or phagocytosis. Convalescent serum was also used in the 2013 African Ebola epidemic. A small non-randomized study in Sierra Leone revealed a significant increase in survival for those treated with convalescent whole blood relative to those who received standard treatment (4).

The only antibody type that is currently available for immediate use is that found in human convalescent plasma. As more individuals contract COVID-19 and recover, the number of potential donors will continue to increase.

A general 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. Another explanation is that antibody works by modifying the inflammatory response, which is also easier during the initial immune response, which may be asymptomatic (5). As an example, passive antibody therapy for pneumococcal pneumonia was most effective when administered shortly after the onset of symptoms and there was no benefit if antibody administration was delayed past the third day of disease (6). ***In this context, we seek to treat patients who are sick***

enough to warrant hospitalization prior to the onset of overwhelming disease including a systemic inflammatory response, sepsis, and/or ARDS.

For passive antibody therapy to be effective, a sufficient amount of antibody must be administered. When given to a susceptible person, this antibody will circulate in the blood, reach tissues and provide protection against infection. Depending on the antibody amount and composition, the protection conferred by the transferred immunoglobulin can last from weeks to months.

2. Experience with the use of convalescent plasma against coronavirus diseases

In the 21st century, there were two other epidemics with coronaviruses that were associated with high mortality, SARS1 in 2003 and MERS in 2012. In both outbreaks, the high mortality and absence of effective therapies led to the use of convalescent plasma. The largest study involved the treatment of 80 patients in Hong Kong with SARS (7). **Patients treated before day 14 had improved prognosis defined by discharge from hospital before day 22, consistent with the notion that earlier administration is more likely to be effective.** In addition, those who were RT-PCR positive and seronegative for coronavirus at the time of therapy had improved prognosis. There is also some anecdotal information on the use of convalescent plasma in seriously ill individuals. Three patients with SARS in Taiwan were treated with 500 ml of convalescent plasma, resulting in a reduction in plasma virus titer and each survived (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 latter study highlights a challenge in using convalescent plasma, namely, that some who recover from viral disease may not have high titers of neutralizing antibody (10). Consistent with this point, an 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. This suggests that antibody declines with time and/or that few patients make high titer responses. *The plasma product we administer will be collected 14-21 days after symptom resolution and antibody titers will be assessed. We will confirm clearance by at least 1 negative NP swab at the time of collection for NA titer assessment. If NA titers are high enough, at the time of plasma collection, a second NP can be done (potentially) to further confirm that the donor is not viremic. Otherwise the donor will fulfill all of the standard donor criteria.*

It is also possible that other types of non-neutralizing antibodies are made that contribute to protection and recovery as described for other viral diseases (11). There are reports that convalescent plasma was used for therapy of patients with COVID-19 in China during the current outbreak (http://www.xinhuanet.com/english/2020-02/28/c_138828177.htm). 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.

3. Known potential risks

a. 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 (12). 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 neutralizing 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 (13) and anecdotal evidence of its use in patients with COVID-19 (http://www.xinhuanet.com/english/2020-02/28/c_138828177.htm), suggest it is safe. Nevertheless, caution and vigilance will be required in for any evidence of enhanced infection.

b. 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). This concern could be investigated as part of a 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 interventions like mechanical ventilation, ARDS and sepsis.*

c. 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 donor requirements for whole blood donation and frequent apheresis plasma donation with the exception of recent illness, in this case COVID-19 infection.

4. 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 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 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.

Investigational plan

4.1 Study Objectives

Primary Efficacy Objective:

Preliminary evaluation the efficacy of treatment with high-titer Anti- SARS-CoV-2 plasma versus control (standard plasma) in patients with COVID-19 respiratory symptoms.

Primary Safety Objective:

Evaluate the safety of treatment with high-titer anti-SARS-CoV-2 plasma versus control (standard plasma) in patients with COVID-19 respiratory symptoms.

Secondary Objectives:

Compare the anti-SARS-CoV-2 convalescent plasma and control (standard plasma) groups anti-SARS-CoV-2 titers at days 0, 1, 3, 7 and 14 (additional days 21 and 28 may be included, as available).

Compare the rates, levels and duration of SARS-CoV-2 RNA in NP swabs using RT-PCR amongst the anti-SARS-CoV-2 convalescent plasma and control (standard plasma) groups at days 0, 7 and 14. Other specimen types may be tested as available (*eg.,* BAL fluid, tracheal secretions, sputum, etc.) or when RT-PCR assays are validated for additional sources (*i.e.,* stool, blood).

4.2 Definitions

1. Enrolled: From time consented to participate until designated as a screen failure or have either been discontinued from the study or completed it.
2. Randomized: when a randomization number is assigned
3. Screen Failures: signed informed consent, but then determined to be ineligible or withdraws before being randomized
4. Discontinued: randomized, but then withdrawn by investigator or withdraws consent
5. Completed: Subjects are considered to have completed when they are followed through day 60 or have had an adverse event or death occurred prior to day 60.

5.3 Study population

Inclusion Criteria for Enrollment

1. Patients must be 18 years of age or older
2. Hospitalized with COVID-19 respiratory symptoms and confirmation via COVID-19 SARS-CoV-2 RT-PCR testing. Patient is willing and able to provide written informed consent and comply with all protocol requirements.
3. Patient agrees to storage of specimens for future testing.
4. If female must not be pregnant and/or breastfeeding.

Exclusion Criteria

1. Female subjects with positive pregnancy test, breastfeeding, or planning to become pregnant/breastfeed during the study period
2. Receipt of pooled immunoglobulin in past 30 days
3. Contraindication to transfusion or history of prior reactions to transfusion blood products

Table: Schedule of Events

Study period	Screen	Baseline	Transfusion	Follow-up				
Day	-1 to 0	0	0	1	3	7	14	28[4]
Eligibility								
Informed consent	x							
Demographic and Medical history	x							
COVID-19 symptom screen	x							
SARS-CoV-2 RT-PCR for eligibility	x							
Pregnancy test	x							
ABO[3]	x							
Study Drug Administration								
Randomization		x						
Drug infusion			X					
Study Procedures								
Vital signs	x	x	xxxx[4]	x	x	x	x	
Physical examination	x		x	x		x		
Symptom screen	x	x	x	x	x	x	x	x
Concomitant medications	x	x	x					
Assessment with 7-point ordinal scale		x		x	x	x	x	x
Adverse event monitoring		x	x	x	x	x	x	x
Laboratory testing								
CBC and CMP		x		x		x	x	
SARS-CoV-2 RT-PCR[5]		x			x	x	x	
SARS-CoV-2 antibody		x		x	x	x	x	
Blood for future testing		x		x	x	x	x	

5.4 Subject Withdrawal

1. Subjects can terminate study participation and/or withdraw consent at any time without prejudice.
2. Randomized subjects who withdraw from the study will not be replaced.
3. 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
4. Discontinuation of the study: The study sponsor, FDA and IRB all have the right to terminate this study at any time

[3] At least 2 assessments of ABO type on file, with one in the 90 days prior to enrollment
[4] Vital sign testing: 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
[5] Sites could include nasopharyngeal and throat
[4]The assessments performed on day 28 will be repeated on days 60 and 90.

5.5 Treatment

1. Subjects will be randomized in a 1:1 ratio to receive study drug vs standard plasma
2. Study drug: The investigational product is anti-SARS-CoV-2 convalescent plasma obtained from patients identified as having recovered from COVID-19 with neutralizing antibody titers >1:64.
3. 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 using apheresis technology and in accordance with standard FDA and blood bank protocols.
4. Active arm will receive 1-2 units of plasma with an anti-SARS-CoV-2 titer of >1:64
5. Control arm will receive 1-2 units of standard plasma.
6. Both active and control drugs will be in standard plasma unit bags, with a study-specific ISBT label.

5.6 Subject Randomization

1. Subjects enrolled in the study will be randomized using an interactive web response system (IWRS) to receive study drug vs place at a 1:1 ratio.

6. Rationale for doses

Provisionally going with 1-2 units; ~300-600 mL at neutralization antibody titer >1:64 *(f possible >1:160 this is a moving target as assays develop*)

Standard plasma[6]

For a 70 Kg person plasma volume is estimated at 2800 mL (40 mL/kg x70 kg) with baseline anti-SARS-CoV-2 titer of 0.

For example, if protective titer was 1:25 and each unit had titer of 1:160, you would need ~500 mL to achieve this [500/(2800+500)] x 1:160=1:25

In practical terms this would be 2 units of FFP per patient, would consider 1 unit if the patient is at high risk of circulatory overload.

6.1 Study drug administration

- Drug will be administered after randomization

[6] Solvent detergent treated pooled plasma

- 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. 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, and may require discontinuation of the infusion.

6.2 Concomitant medications will be documented on the CRF

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

7. Statistical considerations

7.1 Statistical Analysis

Prepared in conjunction with consulting biostastician Dr. Rickey Carter, Ph.D. All analyses will be presented by plasma type. Full factorial univariate analyses of variances will be used to determine differences between the groups in the primary outcome variables. Descriptive summaries of continuous and other numeric variables will at least consist of the following summary statistics: median, minimum and maximum values. Categorical variables will be summarized by the frequency and proportion of subjects falling into each category. Unless otherwise indicated, percentages in tables will be column percentages, using the total number of observations in the population (n=20), grouped by cohort (n=10), as the denominator. Percentages will be rounded to one decimal place, and thus may not always add up to exactly 100%. Clinical laboratory values will be first reported in using International System of Units (SI). Exact confidence intervals for binomial proportions will be derived using the Clopper-Pearson method.

7.1.1 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 3or higher. AE will be compared between randomized arms using Fisher's Exact Test.

7.1.2 Analysis of anti-SARS-CoV-2 titers

Analysis of titers will also primarily be descriptive, comparing the geometric mean titers at days 0,1,3,7 and 14 between the randomized arms.

8. Endpoints

Primary Endpoint: Avoidance of new or ongoing ICU admission.

Secondary endpoints:

1. Cardio-circulatory arrest (at any time)
2. Transfer to ICU
3. Type and duration of respiratory support (and other ICU support)in ICU
4. ICU mortality and LOS
5. Hospital mortality and LOS
6. Ventilator-free days
7. 60 day mortality

Primary Safety Endpoints:

- Rapid deterioration of respiratory or clinical status on transfusion of SARS-CoV-2 convalescent plasma
- 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

Secondary Endpoints

1. Anti-SARS-CoV-2 titers at days 0, 1, 3, 7 and 14.
2. Rates and duration of SARS-CoV-2 PCR positivity (RT-PCR) at days 0, 3, 7, and 14.

9. Study procedures

Day -1 to 0:

A. Screening (must be completed before randomization)
B. Subject informed consent (obtained before performing study related activities)'
C. Baseline Evaluation (at screening) (much of the information will be obtained from the medical record)
    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), onset of symptoms, source of contagion
    4. Vital signs
    5. COVID-19 testing (RT-PCR) from nasopharyngeal, throat, tracheal aspirate or broncho alveolar lavage and stool (optional) samples
    6. Blood typing, CBC, comprehensive metabolic panel
    7. Serological testing: anti-SARS CoV-2 titers
    8. Stored samples for future studies
    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, not already an ICU patient,, between day 3 and 7 of first sign of illness (or within 72 hours of admission)

DAY 0:

1. Randomization of eligible subjects
2. Study Plasma Administration: 1-2 units of plasma will be transfused. 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
3. COVID-19 symptom screen (fevers, cough, shortness of breath)
4. Assessment of clinical status (7-point ordinal scale)
5. New medical conditions, concomitant medication, AE evaluation
6. physical examination
7. COVID-19 testing (RT-PCR) from nasopharyngeal, throat and stool (optional) samples
8. Blood typing, CBC, comprehensive metabolic panel, C-reactive protein
9. Serological testing: anti-SARS CoV-2 titers
10. Stored samples for future studies (cytokines, CD4-CD8, HLA-DR)

Day 1-7 (or for duration of hospitalization)

1. Vital signs daily
2. COVID-19 symptom screen (fevers, cough, shortness of breath)
3. Assessment of clinical status (7-point ordinal scale)
4. New medical conditions, AE evaluation
5. physical examination
6. CBC, comprehensive metabolic panel, CRP daily
7. Serological testing: anti-SARS CoV-2 titers
8. Stored samples for future studies

Day 28:

Key issues to consider f/u by phone, alive, at home, in hospital (ICU or not), on supplemental O2 or not, back to work, fully, partially, SNF, nursing home, LTAC0

1. COVID-19 symptom screen (fevers, cough, shortness of breath)
2. Assessment of clinical status (7-point ordinal scale)
3. New medical conditions, AE evaluation

Day 60 and 90:

1. COVID-19 symptom screen (fevers, cough, shortness of breath)
2. Assessment of clinical status (7-point ordinal scale)
3. New medical conditions, AE evaluation

10. Efficacy, virology and PK measures

Clinical Efficacy (ordinal scale)

1. Death/Cardio-circulatory arrest at anytime
2. Transfer to ICU
3. Type and duration of respiratory support (and other ICU support)in ICU
4. ICU mortality and LOS
5. Hospital mortality and LOS
6. Ventilator-free days
7. 28 day mortality

Virologic measures

1. Rates, levels and duration of SARS-CoV-2 RNA in NP swabs by RT-PCR at days 0, 3, 7 and 14. Other specimen types may be tested as available (*eg.,* BAL fluid, tracheal secretions, sputum, etc.) or when RT-PCR assays are validated for additional sources (*i.e.,* stool, blood).

2. Serologic positivity and neutralization antibody titers for anti-SARS-CoV-2 at days 0, 1, 3, 7 and 14 (additional days 21 and 28 may be included, as available).

11. Risks and benefits

Potential Benefits of treatment:

The potential benefits of antiviral treatment with anti-SARS CoV-2 plasma in patients with respiratory symptoms consistent with interstitial pneumonia at high risk for requiring ICU admission are not known. However, it is anticipated that treatment will decrease the risk of disease progression requiring ICU admission and aggressive respiratory support including possible mechanical ventilation (and other ICU support).

Potential benefits of clinical monitoring and virologic testing:

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

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

Alternatives:

The alternative to participation in this study is routine care.

12. Safety measures

1. Safety Evaluations will assess for the safety of high titer anti-SARS-CoV-2 plasma and determine if it is higher, lower or the same as standard plasma
2. Clinical evaluations: Vital signs and symptom screen on days 0-7, 14 and symptom screens on days 28, 60, and 90.
3. Laboratory evaluations consistent with ongoing medical care may include radiographic imaging modalities such as chest x-rays and chest CT.
4. 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.

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.

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.

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

Serious and Unexpected Suspected Adverse Reaction (SUSAR)

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

Reporting Interval

All AEs and SAEs will be documented from the first administration of study product until completion or un-enrollment from the study. 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 completion of the study, if the investigator becomes aware of a SAE that is suspected to be related to study product

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 s.d. increase above baseline.

Assessment of Seriousness

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

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

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. Safety Oversight

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), led by **Dr. David Warner** composed of independent experts without conflict of interests will be established. The Board will review the study before initiation and at least yearly 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.

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.

Halting Criteria for the Study

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, TACO

3. One subject with an SAE associated with study product.

4. Two subjects with a Grade 3 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 responsible physician. 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.

Halting Criteria/Rules for Subject Infusion

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

14. Ethics/Protection of human subjects

1. Ethical Standard

The Mayo Clinic is committed to the integrity and quality of the clinical studies it coordinates and implements. Mayo will ensure that the legal and ethical obligations associated with the conduct of clinical research involving human subjects are met. The information provided in this section relates to all Mayo sites participating in this research study

As the Department of Health and Human Services continues to strengthen procedures for human subjects' protections via new regulations, Mayo will review these evolving standards in relation to the proposed activities and will advise the investigators on those that may apply.

In addition, Mayo has a Federal wide Assurance (FWA) number on file with the Office for Human Research Protections (OHRP).

This assurance commits a research facility to conduct all human subjects' research in accordance with the ethical principles in The Belmont Report and any other ethical standards recognized by OHRP. Finally, per OHRP regulations, the research facility will ensure that the mandatory renewal of this assurance occurs at the times specified in the regulations.

2. Institutional Review Board

The Mayo IRB will review this protocol and all protocol-related documents and procedures as required by OHRP and local requirements before subject enrollment. The Mayo IRB currently holds and will maintain a US FWA issued by OHRP for the entirety of this study.

3. Informed Consent Process

The informed consent process will be initiated before a volunteer agrees to participate in the study and should continue throughout the individual's study participation. The subject will sign the informed consent document before any procedures are undertaken for the study. A copy of the signed informed consent document will be given to the subject for their records. The consent will explain that subjects may withdraw consent at any time throughout the course of the trial. Extensive explanation and discussion of

risks and possible benefits of this investigation will be provided to the subjects in understandable language. Adequate time will be provided to ensure that the subject has time to consider and discuss participation in the protocol.
The consent will describe in detail the study interventions/products/procedures and risks/benefits associated with participation in the study. The rights and welfare of the subjects will be protected by emphasizing that their access to and the quality of medical care will not be adversely affected if they decline to participate in this study.

4. Subject Confidentiality

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsors and their agents. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor. The results of the research study may be published, but subjects' names or identifiers will not be revealed. Records will remain confidential. To maintain confidentiality, the PI will be responsible for keeping records in a locked area and results of tests coded to prevent association with subjects' names. Data entered into computerized files will be accessible only by authorized personnel directly involved with the study and will be coded. Subjects' records will be available to the FDA, the NIH, the manufacturer of the study product and their representatives, investigators at the site involved with the study, and the IRB.

15. Future Use of Stored Specimens

Subjects will be asked for consent to use their samples for future testing before the sample is obtained. The confidentiality of the subject will be maintained. They will be no plans to re-contact them for consent or to inform them of results. The risk of collection of the sample will be the small risk of bruising or fainting associated with phlebotomy however these samples will be taken at the same time as other protocol required samples.
No human genetic testing will be performed on the samples.
Five ml of blood samples will be collected at 5 time points (See Schedule of Events). Serum will be frozen in 1-ml aliquots. These samples will be used to answer questions that may arise while the study is underway or after it is completed. If for instance, there were unanticipated AEs, serum could be used to run tests that might help determine the reason for the AEs. Cytokines could be measured, for example.

Samples would not be shared with investigators other than investigators at Mayo unless outside investigators had relevant assays or expertise not available to the study investigators. The specimens would remain linked and at Mayo for 5 years. Any use of these specimens not specified in the current protocol will be reviewed by the Mayo IRB.

16. Data management and monitoring

a. Source Documents

The primary source documents for this study will be the subjects' medical records. If the investigators maintain separate research records, both the medical record and the research records will be considered the source documents for the purposes of auditing the study. The investigator will retain a copy of source documents. The investigator will permit monitoring and auditing of these data, and will allow the sponsor, IRB and regulatory authorities access to the original source documents. The investigator is responsible for ensuring that the data collected are complete, accurate, and recorded in a timely manner. Source documentation (the point of initial recording of information) should support the data collected and entered in to the study database and must be signed and dated by the person recording and/or reviewing the data. All data submitted should be reviewed by the site investigator and signed as required with written or electronic signature, as appropriate. Data entered into the study database will be collected directly from subjects during study visits or will be abstracted from subjects' medical records. The subjects' medical records must record their participation in the clinical trial and what medications (with doses and frequency) or other medical interventions or treatments were administered, as well as any AEs experienced during the trial.

Data Management Plan

Study data will be collected at the study site(s) and entered into the study database. Data entry is to be completed on an ongoing basis during the study.

b. Data Capture Methods

Clinical data will be entered into a database which includes password protection and internal quality checks to identify data that appear inconsistent, incomplete, or inaccurate.

c. Study Record Retention

The PI is responsible for retaining all essential documents listed in the ICH GCP Guidelines. The FDA requires study records to be retained for up to 2 years after marketing approval or disapproval (21 CFR 312.62), or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational agent for a specific indication. These records are also to be maintained in compliance with IRB/IEC, state, and federal medical records retention requirements, whichever is longest. All stored records are to be kept confidential to the extent provided by federal, state, and local law. It is the site investigator's responsibility to retain copies of source documents until receipt of written notification to the sponsor.

No study document should be destroyed without prior written agreement between the sponsor and the Principal Investigator. Should the investigator wish to assign the study records to another party and/or move them to another location, the site investigator must provide written notification of such intent to sponsor with the name of the person who will accept responsibility for the transferred records and/or their new location. The

sponsor must be notified in writing and written permission must be received by the site prior to destruction or relocation of research records.

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