Phase II Randomized Study of Convalescent Plasma from Recovered COVID-19 Donors Collected by Plasmapheresis as Treatment for Subjects with Early COVID-19 Infection

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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 from SARS-CoV2

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

HCIP: Human Coronavirus Immune Plasma

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

OP: Oropharyngeal

RT-PCR: Reverse Transcriptase Polymerase chain reaction

PK: Pharmacokinetic

PPE: Personal Protective Equipment

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

PROTOCOL SUMMARY

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Background

SARS-CoV-2

The novel coronavirus, SARS-CoV-2, is currently causing a worldwide pandemic with over 700,000 cases as of March 29th, 2020. There is currently no therapy for SARS-CoV-2, and the need for therapeutic options is urgent. The virus appears to cause multiorgan failure through several mechanisms, including a severe inflammatory response leading to acute respiratory distress syndrome.[1]

Convalescent plasma for novel viruses

Passive antibody therapy has been used for prior virus epidemics.[2-6] Reports from outbreaks of severe acute respiratory syndrome (SARS) from coronavirus (SARS-CoV-1) showed that convalescent plasma from recovered patients contains neutralizing antibodies.[7] In the 2013 Ebola epidemic, there was a significant increase in survival (72% vs 56%) for patients receiving convalescent whole blood compared to those who did not.[8] The proposed mechanism of action by which passive antibody therapy works, is thought to be viral neutralization but may also include antibody-dependent cellular cytotoxicity.

Convalescent plasma in hospitalized patients

We are currently accruing patients on study under IND 19824 of hospitalized patients with COVID-19 infections. With 39/55 patients accrued on this study, we have found that most patients (87%) are showing anti-SARS-CoV2 titer >1:100 by the time of plasma request as very few patients are hospitalized during their first week of symptoms. Conceptually, the use of convalescent plasma is to prevent further viral invasion pending adaptive immunity. Early viral neutralization may prevent the ensuing inflammatory and coagulation cascade.

The purpose of this trial is to intervene in the early phase of viral infection to prevent hospitalization and mortality in the high-risk population.

Study Objectives

Primary Objectives

To compare the hospitalization rate of patients with early COVID-19 infection at high-risk of admission with or without convalescent plasma infusion.

Secondary objectives

- Time to symptoms resolution
- Overall survival
- Rate of virologic clearance by nasopharyngeal swab at 2 and 4 weeks.
- Evaluation of donor parameters including nasopharyngeal swab positivity and titers levels

- Evaluation of the impact of donor titers level on efficacy
- Patients' anti-SARS-CoV2 titer assessment pre-infusion for the Treatment group, +2 weeks (+/- 3 days), +4 weeks (+/-3 days) and +2 months (+/- 5 days). Randomization day will be designated at day 0.

Exploratory Objectives

These are to be performed at Hackensack Meridian Health Center for Discovery and Innovation

- Plasma product analysis for cytokines, mannose-binding lectin (MBL), procalcitonin (PCT), C-reactive protein (CRP), Human neutrophil lipocalin (HNL), Annexin V, Surfactant protein D (SP-D), as well as microRNAs, quantification of IgG, IgM, IgA. It may not be feasible to draw samples on every plasma bag.
- Patients' cytokines and chemokines levels prior to treatment, +2 and +4 weeks (+/- 3 days) post randomization
- Patient's gut microbiota diversity and composition prior to treatment, +3 day, +7 day, +2 weeks, +4 weeks, and +2 months post randomization

Safety endpoints

- Rates of adverse events associated with convalescent plasma infusion.
- Safety assessment will be performed on infusion day for the Treatment group (immediately post infusion), and for all patients on randomization day +3 and +7 days (by telephone, closest business day is acceptable), +2 weeks (+/- 3 days), +4 weeks (+/- 3 days).

Inclusion Criteria

Donor (see Appendix A)

- Age 18-60
- A history of a positive nasopharyngeal swab for COVID-19 or a history of positive antibody titer test.
- At least 14 days from resolution of COVID-19-associated symptoms including fevers.
- A negative nasopharyngeal swab (or similar test) for COVID-19
- anti-SARS-CoV2 titers $\geq$1:500
- Adequate venous access for apheresis
- Meets donor eligibility criteria in accordance to Hackensack University Medical Center (HUMC) Collection Facility at the John Theurer Cancer Center (JTCC) if collecting at the JTCC, and all regulatory agencies as describes in SOP 800 01 (Appendix A).
- Required testing of the donor and product must be performed in accordance to FDA regulations (21 CFR 610.40), and the donation must be found suitable (21 CFR 630.30)

Patient

- Patient age $\geq$30 years old, newly diagnosed with a COVID-19 infection with onset of first symptoms $\leq$ 96 hours.
- And least one other high-risk feature:
    1. Age $\geq$ 65

2. BMI 30 or above
3. Hypertension, defined as SBP above 140 or DBP above 90, or requiring medication for control.
4. Coronary artery disease (history, not ECG changes only)
5. Congestive heart failure
6. Peripheral vascular disease (includes aortic aneurysm >= 6 cm)
7. Cerebrovascular disease (history of CVA or TIA)
8. Dementia
9. Chronic pulmonary disease
10. Liver disease (such as portal hypertension, chronic hepatitis)
11. Diabetes (excludes diet-controlled alone)
12. Moderate or severe renal disease defined as having a GFR < 60 mL/min
13. Cancer (exclude if ≥ 5 year in remission)
14. AIDS (not just HIV positive)

Patient exclusion criteria

- History of severe transfusion reaction to plasma products
- Need for oxygen supplementation
- Positive test for COVID-19 antibodies
- Chemotherapy-induced neutropenia (ANC < 0.5 x 10^3/mcL)
- Immunosuppressive medications except for prednisone (or steroid equivalent) ≥ 10 mg daily.
- Performance status < 50 by KPS
- Pneumonia by radiographic evaluation

Treatment Plan

Plasma collection

Collection of plasma will be performed on eligible and suitable donors meeting regulatory requirements (Appendix A).

SARS-CoV-2 convalescent plasma consisting of 1 unit of approximately 400-500 mL or 2 units of approximately 200 mL will be collected by apheresis from a volunteer donor who recovered from COVID-19 infection and was found to have anti-SARS-CoV2 titers ≥1:500.

Donors may be referred to an outside licensed Blood Center for plasmapheresis to be performed as per the licensed Blood Center SOPs.

Convalescent plasma collected under IND 19824 may be used.

Plasma infusion

Approximately 400-500mL of liquid fresh or fresh frozen plasma will be infused one time.

Statistical Analysis

This is a randomized phase II trial to assess the efficacy and safety of treatment with convalescent plasma infusion (Treatment) versus without convalescent plasma infusion (Control) in patients with early COVID-19 infection at high-risk of admission. Eligible subjects will be stratified randomized by their Charlson Comorbidity Index scoring in a 1:1 ratio to receive either Treatment or Control (8.4). For this trial, we have considered a group sequential design which has more interim analyses and utilized a three-stage O'Brien-Fleming design.[23]

The first stage analysis will be after 51 subjects in each arm, that is after a total of 102 subjects. If the Z statistic is greater than or equal to 0.179 (p-value >= 0.571), the hypothesis H_0 is accepted and the trial stops, we conclude the Treatment is not worth pursuing; if the Z statistic is less than or equal to -2.849 (p-value <= 0.002), the hypothesis H_0 is rejected and early evidence for promising efficacy is established. Otherwise, the trial continues to the next stage.

The second stage analysis will be after 102 subjects in each arm, that is after a total of 204 subjects. If the Z statistic is greater than or equal to -0.944 (p-value >= 0.173), the hypothesis H_0 is accepted and the trial stops, we conclude the Treatment is not worth pursuing; and if the Z statistic is less than or equal to -2.015 (p-value <= 0.022), the hypothesis H_0 is rejected and early evidence for promising efficacy is established. Otherwise, the trial continues to the final stage.

At the final stage, a total of 306 subjects will be recruit and 153 of them for each arm. The hypothesis H_0 is rejected for efficacy if the Z statistic is less than -1.64504 (p-value < 0.4498). Otherwise, the H_0 is not rejected.

Study site

This is a single institution study conducted at Hackensack University Medical Center (HUMC)/John Theurer Cancer Center at HUMC, Hackensack, NJ. The exploratory laboratory tests will be performed at the Hackensack Meridian Health Center for Discovery and Innovation (CDI).

PROTOCOL

Phase II Randomized Study of Convalescent Plasma from Recovered COVID-19 Donors Collected by Plasmapheresis as Treatment for Subjects with Early COVID-19 Infection

1. Introduction

1.1 General principals of passive antibody therapy

There is currently no proven effective therapy for coronavirus disease (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Donor convalescent plasma has been successfully used for treatment of other viral infections, and thus may provide an option for the treatment of COVID-19 and could be rapidly available from people who have recovered from the disease and can donate plasma.

Passive antibody therapy involves the administration of antibodies against an infectious agent to an afflicted individual for the purpose of treating their disease. In contrast, active vaccination requires the induction of an immune response, which takes time to develop and varies depending on the recipient. Some immunocompromised patients fail to achieve an adequate immune response. Thus, passive antibody administration may in some instances represent the only way of providing immediate immunity to susceptible persons, and more predictable immunity in immunocompromised patients. It also offers the potential for immediate benefit in hospitalized and ICU patients who are critically ill.

Passive antibody was first described in the 1890s, and was the only way to treat certain infectious diseases prior to the development of antimicrobial therapy in the 1940s.[2-6] Experience from prior outbreaks with other coronaviruses such as SARS-CoV-1 shows that convalescent plasma contains neutralizing antibodies to the relevant virus.[7] 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 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.[8]

The only antibody therapy currently available for immediate use in SARS-CoV-2 is 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 early in the treatment of disease. Antibody therapy 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.[4] 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.[2]

For passive antibody therapy to be effective, enough 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. Therefore, the use of a larger amount of plasma collected by apheresis may be preferable to plasma separated from a unit of whole blood.

1.2 Experience with the use of convalescent plasma to treat coronavirus infections

In recent times there have been 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.[9] 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 PCR positive and seronegative for coronavirus at the time of therapy had improved outcomes. 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 fresh convalescent plasma, resulting in a reduction in plasma virus titer and each survived.[10] Three patients with MERS in South Korea were treated with convalescent plasma, but only two of the recipients had neutralizing antibody in their plasma.[11] 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.[12] Consistent with this point, an analysis of 99 samples of convalescent sera from patients with SARS showed that 87 had neutralizing antibody with a geometric mean titer of 1:61.[7] This suggests that antibody declines with time or that few patients make high titer responses.

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.[13]

Convalescent plasma has been reported in a case series of patients with SARS-CoV-2.[20] Patients received transfusion with 400-500 mL of fresh convalescent plasma with a SARS-CoV-2–specific antibody binding titer greater than 1:1000 and a neutralization titer greater than 40 that had been obtained from 5 patients who recovered from COVID-19. Convalescent plasma was administered between 10 and 22 days after admission. All 5 patients were receiving mechanical ventilation at the time of treatment and all had received antiviral agents and methylprednisolone. ARDS resolved in 4 patients at 12 days after transfusion, and 3 patients were weaned from mechanical ventilation within 2 weeks of treatment. Of the 5 patients, 3 have been discharged from the hospital, and 2 are in stable condition at 37 days after transfusion. Since this is the only available data for SARS-CoV2 available, the infusion of a larger volume (400-500mL) of fresh plasma would be supported.

1.3 Overview of safety

Historical and current anecdotal data on use of convalescent plasma suggest that it is safe in coronavirus infection. The theoretical risk involves the phenomenon of antibody-dependent enhancement of infection (ADE). 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.[14] Since the proposed use of convalescent plasma in the COVID-19 epidemic would rely on preparations with high titers of neutralizing antibody against the specific virus, SARS2-CoV-2, ADE may be unlikely. The available evidence from the use of convalescent plasma in patients with SARS1 and MERS,[15] and the case series evidence of its use in patients with COVID-19[20] suggests it is safe.

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.[16] This concern will be investigated as part of this 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. The antibodies used in this study will be derived from serum obtained from convalescent patients and will be subjected to testing protocols used by blood banks and transfusion services. However, as is the case with any biological product, there is a very small risk of allergy or anaphylaxis, transfusion related acute lung injury (TRALI), and transfusion associated circulatory overload (TACO) or passive transfer of potential unknown infectious agents. Most adverse effects are mild and transient including headaches, flushing, fever, chills, fatigue, nausea, diarrhea, blood pressure changes and tachycardia. Late adverse events are rare.

1.4 Experience at HUMC/JTCC with convalescent plasma in patients with COVID-19

To date 39 patients with COVID-19 hospitalized at HUMC were treated with convalescent plasma under IND 19824. Our review of these patients showed that the treatment is safe with only 1 patient with a plasma infusion-associated rash. Our experience revealed that most patients admitted for COVID-19 were in the second week of their disease and were experiencing immune mediated hypoxemia. 87% of the treated patients were found to have anti-SARS-CoV2 antibody titers prior to the infusion of plasma. This was unexpected at the time of inception of our protocol under IND 19824. The unmet need appears to be in the week preceding admission where patients have unprotected viral infection and tissue invasion. There is precedent of the introduction of antiviral therapy early in the disease process. The oral neuraminidase inhibitor oseltamivir has shown efficacy in reducing duration of symptoms and complication from influenza in patients with less than 36 hours from symptom onset.[21] We believe that a similar strategy needs to be implemented for convalescent plasma in COVID-19 patients.

2. Study Objectives

2.1 Primary objectives

To compare the hospital admission rate of patients with early COVID-19 infection at high-risk of admission with or without convalescent plasma infusion.

2.2 Secondary objectives

- Time to symptoms resolution
- Duration of hospitalization
- Overall survival
- Rate of virologic clearance by nasopharyngeal swab at 2 and 4 weeks.
- Evaluation of donor parameters including nasopharyngeal swab positivity and titers levels
- Evaluation of the impact of donor titers level on efficacy.
- Patients' anti-SARS-CoV2 titer assessment pre-infusion for the Treatment group, +2 weeks (+/- 3 days), +4 weeks (+/-3 days) and +2 months (+/- 5 days). Randomization day will be designated at day 0.

2.3 Exploratory objectives

These are to be performed at Hackensack Meridian Health Center for Discovery and Innovation

- Plasma product analysis for cytokines, mannose-binding lectin (MBL), procalcitonin (PCT), C-reactive protein (CRP), Human neutrophil lipocalin (HNL), Annexin V, Surfactant protein D (SP-D), as well as microRNAs. It may not be feasible to draw samples on every plasma bag.
- Patients' cytokines and chemokines levels prior to treatment, +2 and +4 weeks (+/- 3 days) post randomization.
- Patient's gut microbiota diversity and composition prior to treatment, +3 day, +7 day, +2 weeks, +4 weeks, and +2 months post randomization.

2.4 Safety endpoints

- Rates of adverse events associated with outpatient convalescent plasma infusion will be determined as defined in section 6.3.
- Safety assessment will be performed on infusion day for the Treatment group (immediately post infusion), and for all patients on randomization day +3 and +7 days (by telephone, closest business day is acceptable), +2 weeks (+/- 3 days), +4 weeks (+/- 3 days) and 2 months (+/- 5 days).

3. Eligibility and Exclusion Criteria

3.1 Donor

Donors and donor products may be collected under IND 19824 as all aspect of donor and product handling is identical.

- Age 18-60
- A history of a positive nasopharyngeal swab for COVID-19 or a history of positive titer test.

- At least 14 days from resolution of COVID-19-associated symptoms including fevers
- One negative nasopharyngeal swab for COVID-19 RNA
- Adequate venous access for apheresis
- Anti-SARS-CoV2 titer titers ≥1:500
- Meets donor eligibility criteria in accordance to regulatory requirements and Hackensack Meridian Health SOP 800 01 "COVID-19 Convalescent Plasma Procurement" (Appendix A).
- Required testing of the donor and product must be performed in accordance to FDA regulation 21 CFR 610.40, and the donation must be found suitable (21 CFR 630.30) (See Appendix A)

3.2 Patient

- Patient age ≥30 years old, newly diagnosed with a COVID-19 infection with onset of first symptoms ≤ 96 hours, and least one other high-risk feature:
    1. Age ≥ 65
    2. BMI 30 or above
    3. Hypertension, defined as SBP above 140 or DBP above 90, or requiring medication for control.
    4. Coronary artery disease (history, not ECG changes only)
    5. Congestive heart failure
    6. Peripheral vascular disease (includes aortic aneurysm >= 6 cm)
    7. Cerebrovascular disease (history of CVA or TIA)
    8. Dementia
    9. Chronic pulmonary disease
    10. Liver disease (such as portal hypertension, chronic hepatitis)
    11. Diabetes (excludes diet-controlled alone)
    12. Moderate or severe renal disease defined as having a GFR <60 mL/min
    13. Cancer (exclude if ≥ 5 year in remission)
    14. AIDS (not just HIV positive)

3.3 Recipient exclusion criteria

- History of severe transfusion reaction to plasma products
- Need for oxygen supplementation
- Positive test for COVID-19 antibodies
- Chemotherapy-induced neutropenia (ANC < 0.5 x 10^3/mcL)
- Immunosuppression medications except for prednisone or steroid equivalent ≤ 10 mg daily.
- Performance status < 50
- Pneumonia by radiographic evaluation

4. Investigational Plan

4.1 Overall study design

- This is a phase II randomized study of convalescent plasma for the treatment of non-immune individuals with COVID-19 infection at high risk of complications.

- Subjects will be considered as having completed the study after 2 months (+/- 5) days, unless consent withdrawal or death occurs first.
- Subjects will be randomized to receiving convalescent plasma or best supportive care.
- Patients randomized to best supportive care may receive plasma should they require hospitalization for progression of COVID-19 disease.
- Statistical analysis will be performed as described in the statistical section 8.
- The final analysis will be conducted once the last subject completes the 2-month visit or withdraws from the study.

4.2 Overall study duration

- The study begins when the first subject (donor or recipient) signs the informed consent. The study will end once the last enrolled subject completes the study (likely a recipient).
- The expected duration of the study is approximately 12 months.

5. Treatment Plan

5.1 Donor procedures and evaluation

All activities pertaining to donor recruitment, enrollment, collection, product handling and processing will take place at Hackensack University Medical Center (HUMC) and John Theurer Cancer Center at HUMC. This facility is FDA-registered and AABB accredited, attesting to robust quality oversight of all operations. All donor-related procedures pertaining to this study are detailed in our SOP 800 01 "COVID-19 Convalescent Plasma Procurement" in Appendix A.

Donors may alternatively be enrolled and collected under IND 19824, as all procedures of evaluation, collection and product handling are identical.

As donor eligibility defined by the FDA may change, donors may be eligible if they follow updated eligibility as per FDA guidelines: https://www.fda.gov/vaccines-blood-biologics/investigational-new-drug-ind-or-device-exemption-ide-process-cber/recommendations-investigational-covid-19-convalescent-plasma

5.1.1 Donor recruitment and screening (see Appendix A)

- Mechanism for recruitment will include advertising in the local community where recent outbreaks have occurred.
- Individuals who agree to participate will do so under full informed consent.
- Individuals who agree to participate will undergo pre-donation screening by a clinical health care provider (visit 1).
- Only those individuals who satisfy all criteria for collection as determined through medical evaluation and laboratory testing described in Appendix A will proceed to a second visit (visit 2) during which the collection will take place. Visit 2 will occur within seven days of visit 1.
    1. Visit 1:
        a. History and targeted physical examination if collecting at JTCC
        b. Donor health questionnaire
        c. Nasopharyngeal swab for COVID-19
        d. Blood for SARS-CoV-2 neutralizing antibody
        e. Blood tests as described in SOP 800.01 section "Donor Evaluation and Eligibility" subsection L (Appendix A), for collections at the JTCC

2. Visit 2:
    a. Donor health questionnaire
    b. Blood tests as described in SOP 800.01 section “Donor Evaluation and Eligibility” subsection L (Appendix A)
    c. Apheresis procedure as described in section 5.1.2 and Appendix A if collecting at the JTCC.

5.1.2 Donor plasma collection (see Appendix A)

- The plasma collection will be performed by apheresis at the John Theurer Cancer Center (JTCC) Collection Facility at HUMC as per “COVID-19 Convalescent Plasma Procurement” SOP (Appendix A) or the donor will be referred to an outside licensed Blood Center for plasmapheresis.
- The JTCC houses a FACT accredited Collection Facility, NJ Blood Bank license 10317, FDA (BMT FEI) Registration 3004726780.
- The plasma collection will be performed using either the Trima Accel® System by Terumo BCT (Appendix A SOP 800 01).
- As per routine practice, samples will be collected at the time of donation for testing for transfusion-transmissible infections (all donors), ABO and red cell antibodies (all donors) and HLA antibodies (female donors), in accordance with FDA regulation 21 CFR 610.40, and the donation must be found suitable (21 CFR 630.30).
- Target collection volume: 400-500mL in a single collection bag or two collection bags of 200mL. The Trima Accel® system considers the total blood volume and will make recommendations to the volume that can be removed safely. Therefore, the collection goal will be either 400mL or the machine recommended maximum volume, whichever is smallest.
- Labeling will be in accordance with FDA regulations 21CFR 606.121.
- The plasma will be maintained in quarantine pending laboratory test results (i.e. infectious screening, ABO and RhD status, Red cell and HLA antibodies).
- If laboratory testing is acceptable (i.e. negative infectious and antibody screening), the products will be appropriately labeled and distributed to hospital blood bank for storage.
- In the event of an abnormal test result, the product will be discarded, and the donor will be notified by the blood center as is standard practice.
- Donors may donate plasma every $\geq$ 7 days. The donor screening procedure described in section 5.1.1 “visit 2” will need to be repeated for each subsequent donation on the day of plasmapheresis, with the addition of blood for SARS-CoV-2 neutralizing antibody

5.1.3 Product handling

Product handling including labeling, testing, transportation, distribution and record keeping including chain of custody recording will be performed in accordance with regulations and HUMC SOP 800.01 "COVID-19 Convalescent Plasma Procurement" (Appendix A).

5.2 Patient procedures and evaluation (see Appendix B)

Patients may be screened, enrolled and randomized remotely. The day of randomization will be designated as day 0 for both Treatment and Control groups. Patients randomized to the treatment arm will need to be seen on the day of infusion which should occur within 48 hours of randomization and be within 96 hours of symptom onset.

5.2.1 Day 0. May be done remotely.

- Screening
- Informed consent must be obtained before performing study related activities (can be from healthcare proxy if subject unable to provide consent due to medical status)
- Baseline Evaluation (at screening)
    1. Demographics (age, sex ethnicity, race)
    2. Medical history
        a. Date of onset of COVID-19 symptoms
        b. Date of positive test
        c. Acute and chronic medical conditions described in the eligibility criteria 3.2.
        d. Charlson Co-morbidity Index calculation
    3. COVID-19 symptom screen (Y/N)
        a. Fever (T Max)
        b. Cough
        c. Shortness of breath
        d. Ageusia and/or anosmia
        e. Myalgias
        f. Headache
        g. Other symptoms that the patient attributes to their COVID-19 infection
    4. Documentation of positive nasopharyngeal COVID-19 testing.
    5. Determination of eligibility as per inclusion/exclusion criteria
    6. Randomization

5.2.2 Plasma infusion day

Patients randomized to the treatment arm will need to be seen on the day of infusion which should occur within 48 hours of randomization and be within 96 hours of symptom onset.

Study plasma administration will be allowed for the Control arm after hospital admission if requested by the patient's medical team.

- On the day of infusion, blood will be drawn for:
    1. Inflammatory markers

a. D-Dimers
b. Ferritin
c. CRP

2. Serological testing: anti-SARS CoV-2 titers
3. Exploratory blood test for genomics (optional)
4. Exploratory blood test for cytokines/chemokines (optional)
5. Rectal swab for gut microbiome analyses, to be sent to the CDI if possible
6. CBC
7. Complete chemistry
8. ABO Rh

- Procedure:

1. A single unit of approximately 400-500 mL of liquid fresh plasma or 2 units of fresh frozen plasma for a total of approximately 400 mL will be transfused at an approximate rate of 50 mL per hour x 30 minutes, then the rate may be increased up to 150 mL per hour until completion. If plasma availability does not allow for 2 units of FFP, a single unit may be administered.
2. If possible, 10mL of plasma will be collected for research from the infusion line and sent to the CDI
3. The infusion may be slowed or stopped as per investigator's decision for minor transfusion reactions only. The transfusion will be discontinued for any transfusion reactions other than minor allergic reactions.
4. An investigation will be initiated when there are signs of a systemic transfusion reaction.
5. Premedication 30 – 60 minutes prior to the infusion will include 100 mg hydrocortisone IV and may include acetaminophen 650 mg PO and diphenhydramine 25 mg IV. Diphenhydramine 25 mg IV and hydrocortisone 100 mg IV may be repeated as needed for an infusion reaction at the discretion of the investigator.
6. Vitals signs should be monitored following Hackensack Meridian Health Blood and Blood Product Administration SOP guidelines:
    a. Temperature, pulse, respirations, and blood pressure pre-infusion (within 30 minutes), 15 minutes after the start, every hour during, if a transfusion reaction is suspected or if there is any change in patient condition, post transfusion (5-15 minutes post)

5.2.3 Post enrollment day +3 and day +7 (or closest working day). May be done remotely.

- COVID-19 symptom screen by telephone (Y/N)

1. Fever (T max)
2. Cough
3. Shortness of breath
4. Ageusia and/or anosmia
5. Myalgias
6. Headaches
7. Other symptoms that the patient attributes to their COVID-19 infection

- New medical conditions, AE evaluation
- Safety assessment
- Rectal swab for microbiome at day +3 and day +7 to be sent to the CDI if possible.

5.2.4 Post enrollment: 2 weeks (+/- 3 days), 4 weeks and 2 months (+/- 5 days). Preferably in person.

- COVID-19 symptom screen (Y/N)
    1. Fever (T max)
    2. Cough
    3. Shortness of breath
    4. Ageusia and/or anosmia
    5. Myalgias
    6. Headaches
    7. Other symptoms that the patient attributes to their COVID-19 infection

- New medical conditions, AE evaluation
- Safety assessment
- Serological testing: anti-SARS CoV-2 titers if possible.
- Swab for COVID-19 testing at 2 weeks, and again at 4 weeks if week 2 is positive.
- Exploratory blood test for cytokines/chemokines at +2 and +4 weeks to be sent to the CDI if possible.
- Rectal swab for microbiome at +2 weeks, +4 weeks, and +2 months to be sent to the CDI if possible.

5.2.5 Patients randomized to the standard best supportive care arm

If a patient in the best supportive care arm requires hospitalization, the patient will be eligible to receive convalescent plasma if requested and/or deemed medically appropriate by the admitting physician. Administration of plasma will be performed as described in 5.2.2.

5.3 Withdrawal criteria

5.3.1 A subject must be withdrawn from study treatment for the following reasons

- Subject has experienced an unacceptable toxicity precluding the completion of plasma infusion.
- The study is terminated by the local health authority, or IRB.

5.3.2 If a subject is withdrawn from study treatment

- The reason(s) for withdrawal must be documented in the subject's medical record and in the CRF.
- The end of treatment (EOT) visit should be performed.
- Subjects must be followed for safety until the time of the follow-up visit or until study treatment–related toxicities resolve, return to baseline, or are deemed irreversible, whichever is longest.
- If the subject discontinues study treatment and actively withdraws consent for collection of follow-up data (safety follow-up or disease assessment), then no additional data collection should occur; however, subjects will have the option of withdrawing consent for study treatment but continuing in the follow-up period of the study.

5.4 Study Completion

5.4.1 A subject will be considered as completing the study if he or she meets any the following criteria:

- Subject completes the 2-month visit
- Subject dies, and a date of death is available
- Subject is known to have died; however, the date of death cannot be obtained (every effort must be made to obtain the date of death)
- Subject has discontinued study treatment and has withdrawn consent for collection of further follow-up data

6. Conduct of study assessment and procedures

6.1 Administration of informed consent form

Valid informed consent must be obtained from the study subjects, donor and patient, before conducting any study-specific procedures pertaining to donor or patient, using an ICF approved by the local IRB/IEC that contains all elements required by ICH E6 and describes the nature, scope, and possible consequences of the study in a form understandable to the study subject. Local and institutional guidelines for ICF content and administration must be followed; the original signed ICF must be retained by the investigator, and a copy of the signed ICF must be provided to the study subject. The informed consent process for each subject must be documented in writing within the subject source documentation. Rescreening of potential subjects is permitted. Recipient consent need not be obtained for a donor to consent and initiate treatment.

6.2 Prior and concomitant medications

The medication record will be maintained after signing the ICF to document concomitant medications.

6.3 Safety assessments

6.3.1 Adverse events

Adverse events for both donors and patients will be monitored from the time the subjects sign the ICF. Subjects will be instructed to report all AEs during the study and will be assessed for the occurrence of AEs throughout the study. In order to avoid bias in eliciting AEs, subjects will be asked general, nonleading questions such as "How are you feeling?" All AEs (serious and nonserious) must be recorded on the source documents and CRFs regardless of the assumption of a causal relationship with study treatment. The definition, reporting, and recording requirements for AEs are described in Section 8.

6.3.2 Performance status

Karnofsky performance status (table 1) will be assessed. Performance status must be assessed by a medically qualified individual and recorded in the CRF.

Table 1. Karnofsky Performance Status Grades:

Grade	Performance Status
100	Normal; no complaints; no evidence of disease.
90	Able to carry on normal activity, minor signs or symptoms of disease.
80	Normal activity with effort; some signs or symptoms of disease.
70	Cares for self; unable to carry on normal activity or to do active work.
60	Requires occasional assistance but can care for most of their personal needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled; requires special care and assistance.
30	Severely disabled; hospital admission is indicated although death not imminent.
20	Very sick; hospital admission necessary; active supportive treatment necessary.
10	Moribund; fatal processes progressing rapidly.
0	Dead.

6.3.4 Infection monitoring

Monitoring for infection other than COVID-19 will be performed at +2 weeks (+/- 3 days), +4 weeks (+/- 3 days) and 2 months (+/- 5 days).

7. Safety Monitoring and Reporting

7.1 Adverse events

7.1.1 Definitions

For the purposes of this Protocol, an AE is defined as any untoward medical occurrence associated with the donation or use of a convalescent plasma for COVID-19 whether or not considered related, that occurs after a subject provides informed consent. Abnormal laboratory values or test results occurring after informed consent constitute AEs only if they induce clinical signs or symptoms, are considered clinically meaningful, or require therapy (eg, hematologic abnormality that requires transfusion).

7.1.2 Reporting

- Adverse events that begin or worsen after initiation of plasma donation or infusion should be recorded on the Adverse Events Form of the CRF. Monitoring for the occurrence of new AEs

should be continued for 1 days after the plasma donation and at least 60 days after the plasma infusion. Adverse events (including laboratory abnormalities that constitute AEs) should be described using a diagnosis whenever possible rather than by individual underlying signs and symptoms. When a clear diagnosis cannot be identified, each sign or symptom should be reported as a separate AE.

- If an adverse event meets the criteria for an SAE (eg, resulted in hospitalization, a life-threatening event, or death), the specific event(s) should be reported as an SAE(s).
- The severity of AEs will be assessed using CTCAE v4.03 Grades 1 through 5. If an event is not classified by CTCAE, the severity of the AE will be graded according to the scale below to estimate the grade of severity:

Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2	Moderate; minimal, local, or noninvasive intervention indicated; limiting age-appropriate activities of daily living.
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living.
Grade 4	Life-threatening consequences, urgent intervention indicated.
Grade 5	Death due to AE

- The occurrence of AEs should be sought by nondirective questioning of the subject during the screening process after signing the ICF and at each visit during the study. Follow-up may occur remotely. This will apply for the donor where a post donation phone call will be placed. For the recipient, occurrence of AEs will be sought on days 0, +3 and +7 days by telephone (closest business day is acceptable), +2 weeks (+/- 3 days), +4 weeks (+/- 3 days). Adverse events may also be detected when they are volunteered by the subject during the screening process or between visits, or through physical examination, laboratory test, or other assessments. To the extent possible, each AE should be evaluated to determine:
    1. The severity grade (CTCAE Grade 1 to 5)
    2. Whether there is at least a reasonable possibility that the AE is related to the study treatment: suspected (yes) or not suspected (no)
    3. The start and end dates, unless unresolved at final follow-up
    4. The action taken about study treatment
    5. The event outcome (eg, not recovered/not resolved, recovered/resolved, recovering/resolving, recovered/resolved with sequelae, fatal, unknown)

6. The seriousness, as per SAE definition provided in section 7.2

- Unlike routine safety assessments, SAEs are monitored continuously and have special reporting requirements described in section 7.2.2.
- All AEs should be treated appropriately. If an AE is treated with a concomitant medication or nondrug therapy, this action should be recorded on the Adverse Event Form and the treatment should be specified on the Prior/Concomitant Medications or Procedures and Non-Drug Therapy Form in the CRF.
- Once an AE is detected, it should be followed until it has resolved or until it is judged to be permanent; assessment should be made at each visit (or more frequently if necessary) of any changes in severity, the suspected relationship to the study drug, the interventions required to treat the event, and the outcome.
- When the severity of an AE changes over time for a reporting period (eg, between visits), each change in severity will be reported as a separate AE until the event resolves.

7.2 Serious adverse events

7.2.1 Definitions

A SAE is defined as an event that meets at least 1 of the following criteria

- Is fatal or life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization, unless hospitalization is a result of:
    1. A routine treatment or monitoring of the studied indication not associated with any deterioration in condition
    2. An elective or preplanned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the ICF
    3. A treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE and not resulting in hospital admission
    4. Any social reasons and respite care, in the absence of any deterioration in the subject's general condition
- Results in persistent or significant disability, incapacity, or a substantial disruption of a person's ability to conduct normal life functions
- Constitutes a congenital anomaly or birth defect
- Is considered to be an important medical event or a medically significant event that may not result in death, be immediately life-threatening, or require hospitalization but may be considered serious when, based upon appropriate medical judgment, the event may jeopardize the subject or may require medical or surgical intervention to prevent 1 of the outcomes listed above.

7.2.2 Reporting

- Information about all SAEs is collected and recorded on the Adverse Event Form of the CRF. The investigator must assess and record the causal relationship of each SAE to the treatment. SEA must be reported in compliance with institutional IRB/DSMD requirements.

- Investigational site personnel must report any new information regarding the SAE within 24 hours of becoming aware of the information in the same manner that the initial SAE Report Form was sent. Follow-up information is recorded on an amended or new SAE Report Form, with an indication that it is follow-up to the previously reported SAE and the date of the original report. The follow-up report should include information that was not provided on the previous SAE Report Form, such as the outcome of the event (eg, resolved or ongoing), treatment provided, action taken with study drug because of the SAE (eg, dose reduced, interrupted, or discontinued), or subject disposition (eg, continued or withdrew from study participation). Each recurrence, complication, or progression of the original event should be reported as follow-up to that event, regardless of when it occurs.

7.3 Data Safety Monitoring Board (DSMB)

This study will be approved and monitored by the HackensackUMC DSMB. The DSMB is responsible for the monitoring of investigator initiated research studies to ensure the safety of participants, the integrity of the data, and the appropriate termination of studies in the event that undue risks have been uncovered, or it appears that trials cannot be conducted successfully. The DSMB provides multidisciplinary, independent oversight of research studies conducted by HackensackUMC staff and or affiliates. The DSMB can require protocol modifications related to participant safety and to recommend suspension of termination to the IRB and institutional official of any research protocols that fall within its jurisdiction. The DSMB may request that HackensackUMC's Corporate Compliance Department conduct periodic audits to assure that data are being collected and recorded according to the protocol.

The investigator is required to submit monthly monitoring reports which include current enrollment data, adverse event summary data and any other data requested by the DSMB. The DSMB will meet monthly to review the trial progress, adverse event data, and any relevant information such as significant amendments or reviews from IRB submitted by the principal investigator. If significant concerns are raised by the DSMB, the concerns will be forwarded to the PI as well as recommendations made to the IRB, and Institutional Official. Recommendations may include modifying, suspending, or terminating the protocol.

8. Study Design and Sample Size Considerations

This is a randomized phase II trial to assess the efficacy and safety of treatment with convalescent plasma infusion (Treatment) versus without convalescent plasma infusion (Control) in patients with early COVID-19 infection at high-risk of admission. Eligible subjects will be stratified randomized by their Charlson Comorbidity Index scoring in a 1:1 ratio to receive either Treatment or Control (8.4). For this trial, we have considered a group sequential design which has more interim analyses and utilized a three-stage O'Brien-Fleming design.[23]

8.1 Hypothesis

The primary objective is the hospitalization rate of patients with early COVID-19 infection at high-risk of admission. Suppose the hospitalization rate by 21 days from therapy initiation is 8% for the Treatment group (P_t) and 18% for the Control group (P_c). This phase II design will aim at the following hypothesis:

The design has a type I error rate at 0.05 with statistical power at least 0.80.

8.2 Study design

This trial will follow a one-sided three-stage O'Brien-Fleming design as follows.[23]

The first stage analysis will be after 51 subjects in each arm, that is after a total of 102 subjects. If the Z statistic is greater than or equal to 0.179 (p-value >= 0.571), the hypothesis H_0 is accepted and the trial stops, we conclude the Treatment is not worth pursuing; if the Z statistic is less than or equal to -2.849 (p-value <= 0.002), the hypothesis H_0 is rejected and early evidence for promising efficacy is established. Otherwise, the trial continues to the next stage.

The second stage analysis will be after 102 subjects in each arm, that is after a total of 204 subjects. If the Z statistic is greater than or equal to -0.944 (p-value >= 0.173), the hypothesis H_0 is accepted and the trial stops, we conclude the Treatment is not worth pursuing; and if the Z statistic is less than or equal to -2.015 (p-value <= 0.022), the hypothesis H_0 is rejected and early evidence for promising efficacy is established. Otherwise, the trial continues to the final stage.

At the final stage, a total of 306 subjects will be recruit and 153 of them for each arm. The hypothesis H_0 is rejected for efficacy if the Z statistic is less than -1.64504 (p-value < 0.4498). Otherwise, the H_0 is not rejected.

Stage	**Cumulative total sample size N**	**Cumulative sample size for treatment group N(Grp 1)**	**Cumulative sample size for control group N(Grp 2)**	**Evidence for undesired rate if Z statistic >= (Accept H0 if Z-statistic >=)**	**Evidence for desired rate if Z statistic <= (Reject H0 if Z-statistic <=)**	**Evidence for undesired rate if p-value >= (Accept H0 if p-value >=)**	**Evidence for desired rate if p-value <= (Reject H0 if p-value <=)**
1	102	51	51	0.17929	-2.84929	0.57115	0.00219
2	204	102	102	-0.94399	-2.01475	0.17259	0.02197
3	306	153	153	-1.64504	-1.64504	0.04998	0.04998

8.3. Analysis Plan

8.3.1 Primary Endpoints

The primary endpoint of the phase II study is the hospitalization rate at 10 days from therapy initiation of patients with early COVID-19 infection at high-risk of admission. The hospitalization rate will be summarized by frequency (%) and compared between the Treatment and Control arms by Mantel-Haenszel test. There will be two interim analyses. Analysis reports will be provided to the DSMB to decide if there is a pronounced treatment effect or futility. A detailed interim analysis plan will be provided before the start of the interim analysis.

8.3.2 Safety Assessment

Safety assessment will be performed on infusion days 0 (post infusion), +3 and +7 days by telephone (closest business day is acceptable), +2 weeks (+/- 3 days), +4 weeks (+/- 3 days), 2 months (+/- 5 days). The safety of the trial will be accessed by evaluating the therapies exposure, adverse events (AE), and

death. Adverse events that begin or worsen after initiation of plasma donation or infusion should be recorded on the Adverse Events Form of the CRF.

A summarization of the number of days and/or cycles subjects were exposed to the therapies will be provided. AE (and serious adverse events, SAE) will be graded and recorded throughout the study and during the follow-up period according to the NCI Common Terminology for Adverse Events (CTCAE) v4.03. Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment. The percentage of subjects experiencing an adverse event at a given severity, an NCI CTCAE toxicity grade, and the relationship to study therapy, will all be provided. And the number of subject deaths throughout the study will be summarized.

8.3.3 Secondary Endpoints

- Time to Symptoms Resolution

  Kaplan-Meier analysis will be used to describe time to symptoms resolution and the median time with 95% CI will be reported. Log-rank test will be utilized to compare the time to symptoms resolution between the Treatment and Control arms.

  The time to symptoms resolution is defined as the time in days from therapies initiation to the first documented symptoms resolution as assessed by a local site. Patients whose symptoms are not resolved, or result in death, or lost follow-up on the designed follow-up date, will be censored on that date.

- Overall Survival (OS)

  Overall survival (OS) will be defined as the time in days from study entry to death. Patients who are alive on the date of closing follow-up will be censored on that date. All events of death will be included, regardless of whether the event occurred while the subject was still taking new therapies, or after the subject discontinued study therapy. If a subject has not died, then the data will be censored according to the following rule: if the subject was lost to follow-up, then data will be censored at the last study visit, or the last contact date, or the date the subject was last known to be alive, whichever is later. If the subject was not lost to follow-up, then data will be censored at the last study visit or the last contact date, whichever is later.

  OS will be estimated by Kaplan-Meier methodology, and median OS with 95% CI will be reported. And the OS between the Treatment and Control arms will be compared by Log-rank test.

- Rate of Virologic Clearance

  The rate of virologic clearance by nasopharyngeal swab at 2 weeks and 4 weeks will be summarized by frequency (%) and compared between two randomized arms by Mantel-Haenszel test. The marginal model will be fitted to the rate of virologic clearance at different time points to evaluate the main effect of randomized arms by using the generalized estimating equations method.

Viral clearance at a designed follow-up date is defined as the cycle threshold (Ct) of RT-PCT at that designed follow-up date 40.

- Evaluation of Donor parameters

  Donor parameters including nasopharyngeal swab positivity and antibody titer levels. Donor parameters will be summarized by frequency (%) for categorical parameters, and mean (SD) or median [IQR, interquartile range] for continuous parameters based on the data normality. The association between the donor parameters and the hospitalization rate will be check by Mantel-Haenszel test for categorical donor parameters, and t-test or its non-parametric version of the continuous donor parameters. The logistic regression model will be built to evaluate the main effect of donor parameters on the hospitalization rate.

- Patients' Anti-SARS-Cov2 titer Assessment

  Patients' anti-SARS-Cov2 titer will be assessed pre-infusion (for the Treatment group), +2 weeks (+/- 3 days), +4 weeks (+/- 3days), and +2 months (+/- 5 days). The antibody titers at the designed follow-up date will be summarized by mean (SD) or median [IQR] and compared between the Treatment and Control arms by t-test or its non-parametric version based on the data normality. The mixed-effect linear model will be used to assess the association between antibody titers measures with the randomized arms.

- Baseline Characteristics

  All baseline summary statistics and analyses will be based on characteristics prior to the initiation of study drug. Frequency and percentages for categorical variables and mean (SD) or median [IQR] for the continuous variables based on the normalization of the data.

8.3.4 Exploratory

- Descriptive statistics will be used to characterize the patients' cytokines, chemokines, plasma products (mannose-binding lectin (MBL), procalcitonin (PCT), C-reactive protein (CRP), human neutrophil lipocalin (HNL), annexin V, surfactant protein D (SP-D), microRNAs) and gut microbiome at pre-infusion treatment day, + 3 and +7 days (gut microbiome), +2 and +4 weeks (+/- 3 days) and +2 months (gut microbiome) post randomization. Frequency and percentages for categorical variables and mean (SD) or median [IQR] for the continuous variables based on the normalization of the data.

  Univariate test will be performed in terms of identifying the association between exploratory objective and the hospitalization rate, Mantel-Haenszel test for categorical variables, and t-test or its non-parametric version for the continuous variables based on the normalized of the data. Analysis of the exploratory objectives will primarily be descriptive.

  P-value less than 0.05 will be considered significant. All statistical analyses will be performed using RStudio (Version 0.99.902) and SAS (Version 9.4).

8.4 Charlson Co-morbidity Index22

Patients will be stratified in 4 groups based on their Charlson Co-morbidity Index scoring:

Low score ≤3 points; moderate score = 4, 5 points; high score = 6, 7 points; very high score ≥8 points.

One Point for each:

- ❑ Myocardial infarction (history, not ECG changes only)
- ❑ Congestive heart failure
- ❑ Peripheral disease (includes aortic aneurysm >= 6 cm
- ❑ Cerebrovascular disease: CVA with mild or no residua or TIA
- ❑ Dementia
- ❑ Chronic pulmonary disease
- ❑ Connective tissue disease
- ❑ Peptic ulcer disease
- ❑ Mild liver disease (without portal hypertension, includes chronic hepatitis)
- ❑ Diabetes without end-organ damage (excludes diet-controlled alone)

Two Points for each:

- ❑ Hemiplegia
- ❑ Moderate or severe renal disease
- ❑ Diabetes with end-organ damage (retinopathy, neuropathy, nephropathy, or brittle diabetes)
- ❑ Tumor without metastasis (exclude if $\geq$ 5 y from diagnosis)
- ❑ Leukemia (acute or chronic)
- ❑ Lymphoma

Three Points for each:

- ❑ Moderate or severe liver disease

Six Points for each:

- ❑ Metastatic solid tumor
- ❑ AIDS (not just HIV positive)

https://www.mdcalc.com/charlson-comorbidity-index-cci

9. Regulatory consideration

9.1 Good Clinical Practice

The study will be conducted according to the International Conference on Harmonization (ICH), Good Clinical Practice (GCP), the Declaration of Helsinki, Institutional Review Boards (IRB) and in accordance with the U.S. Code of Federal Regulations on Protection of Human Rights (21 CFR 50).

9.2 Institutional Review Board (IRB) Review

- The final study protocol and consent form (and any other appropriate documents as applicable) will be approved by the Institutional Review Board (IRB) at Hackensack Meridian *Health*. Approval will be received in writing before initiation of the study.
- Any changes to the study design will be formally documented in protocol amendments and will be approved by the IRB prior to implementation.
- Any amendment to this protocol must also be approved by the IRB and FDA. The written signed approval from the IRB should specifically reference the investigator name, protocol number, study title and amendment number(s) that is applicable. Amendments that are administrative in nature do not require IRB approval but will be submitted to the IRB for information purposes.
- The investigator must keep a record of all communication with the IRB and, if applicable, between a Coordinating investigator and the IRB. This statement also applies to any communication between the investigator (or Coordinating investigator, if applicable) and regulatory authorities.
- Any advertisements used to recruit subjects for the study must be reviewed by [sponsor] and the IRB prior to use.

9.3 Ongoing Information for IRB Committee

As required by legislation and local regulatory requirements, the investigator must submit to the IRB:

- Information on serious or unexpected adverse events as soon as possible
- Annual reports on the progress of the study
- Deviations from the protocol or anything that may involve added risk to subjects

9.4 Investigator Responsibilities

- Investigator responsibilities are set out in the ICH Guideline for Good Clinical Practice and in the local regulations.
- The investigator should ensure that all persons assisting with the study are adequately informed about the protocol, amendments, study treatments, as well as study-related duties and functions.
- The investigator should maintain a list of Sub-Investigators and other appropriately qualified persons to whom he or she has delegated significant study-related duties. The investigator is responsible for keeping a record of all subjects who sign an informed consent document and are screened for entry into the study. Subjects who fail screening must have the reason(s) recorded in the subject's source documents.
- The investigator, or a designated member of the investigator's staff, must be available during monitoring visits to review data, resolve queries and allow direct access to subject records (eg,

medical records, office charts, hospital charts, and study-related charts) for source data verification. The investigator must ensure timely and accurate completion of CRFs and queries.

9.5 Subject information and informed consent

- The investigator must obtain informed consent of a subject and/or a subject's legal representative prior to any study related procedures.
- Documentation that informed consent occurred prior to the study subject's entry into the study and of the informed consent process should be recorded in the study subject's source documents including the date. The original informed consent document signed and dated by the study subject and by the person consenting the study subject prior to the study subject's entry into the study, must be maintained in the investigator's study files and a copy given to the study subject.
- In addition, if a protocol is amended and it impacts on the content of the informed consent, the informed consent document must be revised. Study subjects participating in the study when the amended protocol is implemented must be re-consented with the revised version of the informed consent document. The revised informed consent document signed and dated by the study subject and by the person consenting the study subject must be maintained in the investigator's study files and a copy given to the study subject.

9.6 Confidentiality

- The patient charts, collected data, and all analysis of the data will adhere to HIPAA & institutional patient confidentiality requirements.
- More specifically, a coding system will be used for which a unique identifier (study ID number) will be assigned to each patient name and contact details. Only the study number will be included in the data collection tool, data analysis software and potential publications. The list with the direct identifiers (for the purposes of linking data and keeping track of patients) will be stored separately in a secure server at each site.
- Analytical datasets will be stored on secure servers that also limit access to the investigator team. Should results of the study be published or reported, individual names or other identifying information will not be used.

9.7 Retention of records

Records will be retained in accordance with regulatory, organizational and sponsor requirements, but no less than six (6) years following the completion of the research. Disposal of records will be done in such a manner that no identifying information can be linked to research data.

10. Data handling and recordkeeping

10.1 Data/documents

The investigator must ensure that the records and documents pertaining to the conduct of the study and the distribution of the investigational product are complete, accurate, filed and retained.

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 into the study database/case report form 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.

10.2 Data management

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. Clinical data will be entered into an electronic case report form. The data system includes password protection and internal quality checks to identify data that appear inconsistent, incomplete, or inaccurate.

APPENDIX A

COVID-19 Convalescent Plasma Procurement SOP BMT 800 01

- BMT 800 01, appendices A-L
- BMT 800 01, referenced SOPs

APPENDIX B

Recipient schedule of assessment

REFERENCES

1. Mehta P, McAuley DF, Brown M, Sanches E, Tattersall RS, Manson JJ. COVID-19: Consider cytokine storm syndromes and immunosuppression. Lancet March 13, 2020.
2. Casadevall A, and Scharff MD. "Serum Therapy" revisited: Animal models of infection and the development of passive antibody therapy. Antimicrob Agents Chemotherap. 1994;38(1695-702).
3. Casadevall A, and Scharff MD. Return to the past: the case for antibody-based therapies in infectious diseases. Clin Infect Dis. 1995;21(150-61).
4. Casadevall A, and Pirofski LA. Antibody-mediated regulation of cellular immunity and the inflammatory response. Trends Immunol. 2003;24(9):474-8.
5. Casadevall A, Dadachova E, and Pirofski L. Passive antibody therapy for infectious diseases. Nature Microbiol Rev. 2004;2(695-703).
6. Casadevall A, Pirofski, L. The Convalescent Sera Option for Containing COVID-19. Journal of Clinical Investigation. 2020; (1-4).
7. Zhang JS, Chen JT, Liu YX, Zhang ZS, Gao H, Liu Y, Wang X, Ning Y, Liu YF, Gao Q, et al. A serological survey on neutralizing antibody titer of SARS convalescent sera. Journal of medical virology. 2005;77(2):147-50.
8. Sahr F, Ansumana R, Massaquoi TA, Idriss BR, Sesay FR, Lamin JM, Baker S, Nicol S, Conton B, 256 Johnson W, et al. Evaluation of convalescent whole blood for treating Ebola Virus Disease in 257 Freetown, Sierra Leone. The Journal of infection. 2017;74(3):302-9.
9. Cheng Y, Wong R, Soo YO, Wong WS, Lee CK, Ng MH, Chan P, Wong KC, Leung CB, and Cheng G. Use of convalescent plasma therapy in SARS patients in Hong Kong. European journal of clinical microbiology & infectious diseases: official publication of the European Society of Clinical Microbiology. 2005; 24(1):44-6.].
10. Yeh KM, Chiueh TS, Siu LK, Lin JC, Chan PK, Peng MY, Wan HL, Chen JH, Hu BS, Perng CL, et al. 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):919-22.
11. Ko JH, Seok H, Cho SY, Ha YE, Baek JY, Kim SH, Kim YJ, Park JK, Chung CR, Kang ES, et al. Challenges of convalescent plasma infusion therapy in Middle East respiratory coronavirus infection: a single centre experience. Antiviral therapy. 2018; 23(7):617-22.
12. Arabi YM, Hajeer AH, Luke T, Raviprakash K, Balkhy H, Johani S, Al-Dawood A, Al-Qahtani S, Al- Omari A, Al-Hameed F, et al. Feasibility of Using Convalescent Plasma Immunotherapy for MERS-283 CoV Infection, Saudi Arabia. Emerging infectious diseases. 2016; 22(9):1554-61.
13. Gunn BM, Yu WH, Karim MM, Brannan JM, Herbert AS, Wec AZ, Halfmann PJ, Fusco ML, Schendel SL, Gangavarapu K, et al. A Role for Fc Function in Therapeutic Monoclonal Antibody-Mediated Protection against Ebola Virus. Cell host & microbe. 2018; 24(2):221-33. e5.
14. Wan Y, Shang J, Sun S, Tai W, Chen J, Geng Q, He L, Chen Y, Wu J, and Shi Z. Molecular mechanism for antibody-dependent enhancement of coronavirus entry. Journal of Virology. 2020; 94(5)
15. Mair-Jenkins J, Saavedra-Campos M, Baillie JK, Cleary P, Khaw FM, Lim WS, Makki S, Rooney KD, Nguyen-Van-Tam JS, and Beck CR. 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.)
16. Crowe JE, Firestone C-Y, and Murphy BR. Passively acquired antibodies suppress humoral but not cell-mediated immunity in mice immunized with live attenuated respiratory syncytial virus vaccines. The Journal of Immunology. 2001; 167(7):3910-8.].

17. Tan, M and Xiong, X. "Continuous and group sequential conditional probability ratio tests for phase II clinical trials". Statistics in medicine 15, no.19 (15 Oct.1996): 2037-2051. doi:10.1002/(SICI)1097-0258(19961015)15:19.
18. Tan, Ming T. and Xiong, Xiaoping. "A flexible multi-stage design for phase II oncology trials". Pharmaceutical statistics 10, no.4 (Jul.2011): 369-373. doi:10.1002/pst.478.
19. Yeh KM, Chiueh TS, Siu LK, Lin JC, Chan PK, Peng MY, Wan HL, Chen JH, Hu BS, Perng CL, et al. 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):919-22.
20. Chenguang Shen, PhD[1]; Zhaoqin Wang, PhD[1]; Fang Zhao, PhD[1]; et al. Treatment of 5 Critically Ill Patients With COVID-19 With Convalescent Plasma. JAMA. Published online March 27, 2020. doi:10.1001/jama.2020.4783.
21. John J. Treanor, MD; Frederick G. Hayden, MD; Peter S. Vrooman, MD; et al . Efficacy and Safety of the Oral Neuraminidase Inhibitor Oseltamivir in Treating Acute InfluenzaA Randomized Controlled Trial. *JAMA.* 2000;283(8):1016-1024. doi:10.1001/jama.283.8.1016
22. Austin SR, Wong YN, Uzzo RG, Beck JR, Egleston BL. Why Summary Comorbidity Measures Such As the Charlson Comorbidity Index and Elixhauser Score Work. *Med Care*. 2015;53(9):e65-e72. doi:10.1097/MLR.0b013e318297429c
23. O'Brien, P.C., & Fleming, T.R. (1979, September). A multiple testing procedure for clinical trials. *Biometrics.*, *35*(3), 549-556. doi:10.2307/2530245