Title: Defibrotide therapy for SARS-CoV2 Acute Respiratory Distress Syndrome (ARDS)

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Defibrotide therapy for SARS-CoV2 Acute Respiratory Distress Syndrome (ARDS)

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1.0 STUDY SYNOPSIS

This is an investigator initiated, open label trial of defibrotide for therapy of patients with SARS-CoV2 related acute respiratory distress syndrome (ARDS). The primary endpoint is safety and tolerability. Secondary endpoints will assess response and ventilator free survival.

Eligibility**:** Patients ≥ 18 years in age with evidence of SARS-CoV2 related ARDS. All patients must exhibit signs of coagulopathy evidenced by a D-dimer ≥ 2X ULN.

Study therapy**: Defibrotide will be administered intravenously (IV) every 6 hours (Q6H) for 7 days of therapy**. Patients who meet defined toxicity and/or efficacy criteria within the 7-day period may cease study therapy at that earlier time point. Patients with a partial response to study therapy by day 7 (defined as > 20% decrease in supplemental oxygen) may continue to receive an additional 7 days of therapy (14 days total therapy). Toxicity (including rate of hemorrhage / bleeding) will be assessed. Each patient will be monitored for 14 days, including 7 days of therapy and 7 days of post-therapy observation.

Sample Size**: 12 patients.** An interim assessment for toxicity and early mortality will be performed after the first six patients have enrolled. Accrual may proceed to a maximum of 12 patients, if the stopping rules are not met within the first six patients.

Other**:** The study will target patients with either early or late-phase ARDS, provided patients have not required mechanical ventilation for > 7 consecutive days. The study allows co-enrollment with other SARS-CoV2 therapy trials, provided those trials are not also utilizing other anti-coagulant or fibrinolytic agents.

2.0 HYPOTHESIS

Severe SARS-CoV2 infections are associated with the development of acute respiratory distress syndrome in the setting of hypercoagulability, endothelial dysfunction / thrombotic microangiopathy. Defibrotide therapy for patients with severe SARS-CoV2 ARDS will be safe and associated with improved overall survival, within 28 days of therapy initiation.

Rationale: To date, therapy for SARS-CoV2 lung injury has focused on agents that either inhibit viral replication and/or blunt the cytokine release syndrome associated with viral-mediated immune activation. Only recently have we begun to realize that the lung injury associated with SARS-CoV2 infections are frequently associated with a ventilation-perfusion mismatch, in which patients have preserved lung compliance, but impaired oxygenation. This raises a new paradigm in SARS-CoV2 therapy. Given the abnormal ventilation-perfusion mismatch with preserved compliance, it is thought that patients with SARS-CoV2 infections exhibit a loss of the normal hypoxic vasoconstriction reflex that helps preserve ventilation-perfusion matching (Gattinoni). This observation in conjunction with the frequent concurrence of multi-organ dysfunction (e.g. renal, hepatic) and hypercoagulability with marked elevations in D-dimer and PAI-1 levels in affected patients, support a dysfunction in the endothelium, and thus we hypothesize that this endothelial dysfunction is a prime target for SARS-CoV2 therapy. The current protocol will examine this hypothesis, utilizing an endothelial stabilizing agent (defibrotide). The agent is currently approved by the Food and Drug Administration (FDA) for use in patients with sinusoidal obstruction syndrome (SOS) with pulmonary or renal dysfunction following hematopoietic cell transplantation (HCT). In summary, t**he current trial is a feasibility trial, to assess the safety and tolerability of this regimen in this patient population.**

The significance of this safety study is high. If successful, this trial will form the foundation for a multicenter, phase 2 trial targeting patients with viral induced ARDS.

3.0 OBJECTIVES

3.1 Primary Objective

To assess the safety and tolerability of defibrotide for the management of SARS-CoV2 related ARDS.

3.2 Secondary Objectives

To assess the therapeutic response of defibrotide therapy for patients with SARS-CoV2 related ARDS, including: Day 14, ventilator free survival.

a. The number of ventilator free days within 14 days of study entry.
b. The time to improvement in oxygenation, with improvement in oxygenation defined as an increase in PaO2/FiO2 of 50 (or greater) compared to the nadir of PaO2/FiO2.
c. To use the "WHO COVID-19 Ordinal Scale" to assess the mean change in the WHO scores during study therapy.
d. Overall Survival at 28 days.

3.3 Exploratory Objectives:

To perform preliminary assessment of endothelial biomarkers in patients with SARS-CoV2 ARDS, with correlation to serum cytokines and clinical response.

4.0 BACKGROUND

General (SARS-CoV2): Coronavirus Disease 2019 (COVID-19), caused by a novel coronavirus (SARS-CoV2), is a highly infectious pathogen that spreads primarily through respiratory droplets and/or aerosol particles. Though many affected patients express only mild symptomatology, with fever, cough and myalgias / fatigue, approximately10-20% of patients may develop a more severe phenotype with rapid progression to acute respiratory distress syndrome (ARDS), septic shock, disseminated intravascular coagulation (DIC) and hypercoagulability. Older age and co-morbid conditions (obesity, diabetes, immunocompromised, etc) are known risk factors for a more severe disease phenotype (Chen N 2020)(Huang C 2020).

Pulmonary Complications associated with SARS-CoV2: Emerging data from centers caring for patients affected with SARS-CoV2, suggest a significant component of endothelial dysfunction and dysregulation of thrombotic/fibrinolytic pathway. Mechanical ventilation in patients with SARS-CoV2 lung injury demonstrate good compliance but severe hypoxemia unlike patients with traditional ARDS, which is typically characterized by significant stiffness and reduction in lung compliance. The severe hypoxemia is attributed to high shunt (>50%), hypoxic vasoconstriction, and microvascular thrombosis in the pulmonary circulation, ultimately leading to ventilatory dead space (Wu 2020)(Xu 2020). The acute precipitous decline in respiratory status of these patients is also linked to inflammatory mediators and "cytokine storm" with a recent retrospective, multicenter study of 150 confirmed COVID-19 cases in Wuhan, China, demonstrating decreased survival with elevated ferritin (mean 1297·6 ng/ml in non-survivors *vs* 614·0 ng/ml in survivors; $p<0·001$) and IL-6 ($p<0·0001$).

Coagulopathy in SARS-CoV2 ARDS: Incidence. The incidence of SARS-CoV2 related coagulopathy is only now being understood, with thrombotic complications reported in 30-35% of affected patients. A current Dutch publication (April 6th, 2020) examined 184 ICU patients with proven SARS-CoV2 ARDS, with a cumulative incidence of thrombotic complications of 31% (95%CI: 20-41), including both arterial thrombotic and pulmonary emboli (Kluk FA 2020). All patients developed thrombotic complications despite standard heparin thrombo-prophylaxis.

Even heparinization in this patient population may NOT prevent thrombotic complications. A study of 26 patients with SARS-Cov2 ARDS in two European intensive care units (ICU) was reported in April 2020, with patients receiving either prophylactic (31%) or therapeutic anticoagulation (69%) for venous thromboembolism prevention (Llitjos 2020). Patients received either low molecular weight or unfractionated heparin, with anti-Xa levels monitored. The overall incidence of thromboembolic complications was 69%, significantly higher in patients treated with prophylactic vs therapeutic heparin (100% vs. 56%, respectively, p=0.03).

Coagulopathy in SARS-CoV2 ARDS: Pathophysiology. Emerging data from centers caring for patients infected with SARS-CoV2 suggests a significant component of endothelial dysfunction and dysregulation of thrombotic/fibrinolytic pathway (Hunt2020)(Thachil 2020)(Nascimento I 2020)(Huang 2020)(Tang 2020). Severe SARS-CoV2 infections, in particular, have been associated with markedly elevated D-dimers levels, with increasing mortality as the D-dimer rises. Day 28 mortality rates of 52.4% have been reported with D-dimer values > 6-fold upper limit of normal (ULN), with mortality approaching 60% with D-dimers > 8 fold ULN (Yin 2020). Of note, hemorrhagic symptomatology are not common with SARS-CoV2 infections, with infrequent reports of pulmonary or gastro-intestinal bleeding (Thachil 2020)(Hunt 2020) in affected patients. Interestingly enough, thrombocytopenia is rarely noted, with < 5% of severely affected patients exhibiting a platelet count < 100,000/uL on admission (Thachil 2020), with hyperfibrinogenemia (not hyofibrinogenemia) commonly described. Thrombo-prophylaxis with anticoagulants (heparin and/or low molecular weight heparin, LMW Heparin) have been shown to decrease mortality in patients with severe SARS-CoV2 infections (Yin 2020). This is especially true in patients with sepsis induced coagulopathy (SIC) scores ≥4 (40.0% vs 64.2%, P=0.029) and patients with markedly elevated D-dimer (> 3.0 mcg/ml, or > 6-fold the upper limit of normal).

There is ample evidence to suggest that fibrin deposition is a key feature of SARS-CoV2 related ARDS, with markedly elevated levels of plasminogen activator inhibitor-1 (PAI-1), a critical regulator of fibrinolysis, noted (Giannis 2020). A role for platelet and megakaryocyte activation in viral mediated illnesses has been previously shown, with evidence that coronaviruses directly infect megakaryocytes, and activate platelet function (Yang 2005). A recent lung autopsy series of patients infected in the United States with SARS-CoV2 noted an abundance of megakaryocytes, platelet-rich thrombi, pulmonary vascular microthrombi, and downstream foci of hemorrhage. A notable finding was the lack of significant secondary infection in these cases, suggesting that secondary infections were not the cause of mortality (Fox 2020). The authors concluded that effective therapy for patients with SARS-CoV2 ARDS should not only target the viral pathogen, but also the thrombotic and microangiopathic effects of the virus (Fox 2020).

Defibrotide: Rationale and Mechanism of action. Defibrotide (DF) is a polydeoxyribonucleotide that was FDA approved in March 2016 for the treatment of

sinusoidal obstruction syndrome (SOS) with pulmonary and renal dysfunction following hematopoietic cell transplant (HCT), with significant improvement in day 100 post-HCT survival noted in treated subjects (Richardson 2016). Defibrotide displays significant anticoagulant, anti-inflammatory, and endothelial cell protective properties (Kornblum 2006)(Larocca 2008)(Richardson 2009)(Bianchi 1993).

Though the mechanism of action of DF is not yet completely understood, it is clearly related to its polyanionic nature, interaction with plasma and/or matrix proteins, binding to adenosine receptors (ARs), and protection of the vascular endothelium. A number of in vitro studies have demonstrated that DF blocks tissue factor and plasminogen activator inhibitor-1 (PAI-1) expression induced by lipopolysaccharide (LPS) in endothelial cells (Cella 2001). Dendritic cells, a key mediator in the coagulation-inflammatory pathway serve as a novel target for DF, with DF shown to actively inhibit Toll-like receptors on macrophages and dendritic cells (Franchischetti 2012). Following therapy with DF, marked decreases in PAI-1 levels have been reported in therapy responders, with PAI-1 levels typically unchanged in non-responders (Kaleelrahman 2003). Common toxicities related to DF have included hemorrhagic and/or hypotensive events, though the incidence of pulmonary hemorrhage [11.8% and 15.6%] and gastrointestinal bleeding [7.8% and 9.4%] were similar between DF and control groups in the licensing trial (Richardson 2016). We are now proposing an open label, trial of defibrotide for patients with severe SARS-CoV2 with acute respiratory distress syndrome.

Defibrotide (DF) vs heparin for thrombo-prophylaxis: Several studies have shown that DF is at least as effective, or potentially more effective than heparin (Rizzi 1987) (Strouse 2016). A large, open-label, multicenter study in post-op patients (n=4810), in which defibrotide (n = 2810) vs heparin prophylaxis (n=2000) was administered noted that the incidence of postsurgical DVT and pulmonary embolism (PE) were significantly lower in the defibrotide arm [1.17 vs 2.35% (DVT), p = .002; 0.53 vs 1.15%, p = .025 (PE), respectively] (Gerosa 1989).

Defibrotide dosing: Current FDA approved dosing schedules (25 mg/kg/day in 4 divided doses) were derived from pharmacokinetic (Pk) studies in healthy controls, subjects with hepatic SOS and those with hepato-renal dysfunction (Tocchetti 2016)(Umemura 2016)(Palmer 1993). Peak levels and peak pharmacodynamics effects are noted following 2-hours of infusion, with rapid clearance and short half-life (<1.0 hours)(Umemura 2016). Similar pharmacokinetic profiles are seen in subjects with end-stage renal disease and/or patients undergoing hemodialysis (Tocchetti 2016). Thus, renal impairment does not impact clearance or dosing schedule, with 25 mg/kg/day still administered in 4 divided doses. The current protocol will utilize this same dosing schedule. The frequent association of renal dysfunction with SARS-CoV2 ARDS will not lead to any dosing adjustments.

In summary, SARS-CoV2 induced changes in the fibrinolytic pathway are global in nature, impacting the coagulation-inflammatory pathway. Defibrotide may serve as a potent anti-coagulant, pro-fibrinolytic and endothelial stabilizing agent, potentially playing a key role in lessening thrombo-embolic disease and multi-organ dysfunction associated with this disorder. We hypothesize that through its role in preventing pulmonary microthrombi, decreasing pulmonary endothelial production of inflammatory cytokines (IL-1, IL-6, TNFR1, IL-8), promoting vaso-dilation (increased production of nitric oxide, prostanoids), ability to restore normal endothelium induced vascular responses, inhibition of platelet activation

(reduction in VWF), and regulation of the fibrinolytic pathway (reduction in PAI-1), defibrotide will lead to improvement in oxygenation and promote the resolution of ARDS. The high incidence of renal and cardiac dysfunction in SARS-CoV2 affected patients may be similarly impacted.

5.0 ELIGIBILITY CRITERIA

5.1 Inclusion Criteria

5.1.1. Age ≥ 18 years of age.

5.1.2. Presence of SARS-CoV2 infection, confirmed by real-time reverse transcription polymerase chain reaction (RT-PCR) assay from a nasopharyngeal swab specimen or other diagnostic test for SARS-CoV2.

5.1.3. Serum D-Dimer ≥ 2X ULN.

5.1.4. Patients with Acute Respiratory Distress Syndrome (ARDS) as determined by the following criteria (Berlin criteria adaptation):

a. Radiographic evidence of bilateral lung disease (opacities or ground glass opacification) on chest radiograph (CXR) or computed tomography (CT), and the opacities not fully explained by pleural effusions, cardiac failure or fluid overload.

b. Impairment of oxygenation, defined by either a) the ratio of arterial oxygen tension to fraction of inspired oxygen (PaO_2/FiO_2) ≤ 300 mmHg, or b) the use of supplemental oxygen to support an SpO_2 ≥ 90%.

5.1.5. Patients must provide voluntary written informed consent to be eligible for study. For patients who are medically unable to provide consent, their designated proxy or legal guardian will provide informed consent. The consenting process is described in Appendix II.

5.1.6. Patients actively participating in another clinical trial for the management of SARS-CoV2 are eligible provided those trials do not directly involve an anti-platelet, anti-coagulant or anti-fibrinolytic agent. (Patients enrolled on investigational trials utilizing anti-viral specific agents, cytokine inhibitors, tyrosine kinase inhibitors, or other anti-inflammatory agents are still eligible).

5.2 Exclusion Criteria

5.2.1. Thrombolytic treatment and/or anticoagulant treatment at <u>therapeutic</u> doses within 12 hours of study entry. [*Note: the use of non-therapeutic doses of heparin (≤7,500 units Q8Hours) or low molecular weight heparin (≤ 1 mg/kg/day) is allowed}. In addition, the administration of heparin flushes for centrally placed catheters, fibrinolytic instillation for central venous line occlusion, or in the in-flow circuit for patients on continuous veno-venous hemodialysis is allowed].*

5.2.2. Clinically significant acute bleeding, including (but not limited to one of the following): pulmonary hemorrhage (diffuse alveolar hemorrhage), intracranial bleed, gastro-intestinal hemorrhage (gross hematemesis or

hematochezia), gross hematuria or uncontrolled epistaxis irrespective of the amount of blood loss, within the prior 3 days.

5.2.3. On mechanical ventilation for > 7 consecutive days (> 168 hours).

5.2.4. Serum platelet count < 50,000/uL. Transfusion of platelets to achieve a level > 50,000/uL is not allowed for eligibility.

5.2.5. Serum fibrinogen < 150 mg/dl. Transfusion of fresh frozen plasma or cryoprecipitate to achieve a level > 150 mg/dl is not allowed for eligibility.

5.2.6. The presence of an uncontrolled systemic infection (other than COVID-19).

5.2.7. Hemodynamic instability as defined by a requirement for 2 or more vasopressors (not including renal-doses of dopamine).

5.2.8. Concurrent use of Extracorporeal membrane oxygenation (ECMO).

5.2.9. Patients with a previously known hypersensitivity reaction to defibrotide, or any of its excipients.

5.2.10. Females who are pregnant or breastfeeding.

5.2.11. History of cerebrovascular accident (i.e. thrombotic or hemorrhagic stroke) within 3 months prior to study entry.

Other considerations: There is no exclusion for hepatic or renal failure at the time of study entry, including the use of hemodialysis or peritoneal dialysis.

;

6.0 STUDY THERAPY

6.1 General

The study is designed as a single-center, open label trial investigating the use of defibrotide for the treatment of SARS-CoV2 related ARDS. The investigational agent is supplied by Jazz Pharmaceuticals.

6.2 Defibrotide dosing and administration

All patients will receive 25 mg/kg/day of defibrotide, given in 4 divided doses (approximately Q6H), each dose infused intravenously (IV) over an approximate 2-hour period. No dose shall be infused within 3 hours of a prior dose. The defibrotide will be administered in 0.9% Sodium Chloride or 5% dextrose in water IV and mixed to a concentration of 4 - 20 mg/mL.

The dose of Defibrotide (6.25 mg/kg Q6H) will be based on the patient's recorded weight at the time of study entry. Each of the 4 divided doses per day will be rounded to the nearest 10 mg for adults (>35 kg) and the nearest 5 mg for children (<35 kg) in order to facilitate efficient drug administration. Total daily dose for all patients will be 25 mg/kg. No premedication is required with dosing. Vitals (Blood Pressure, Heart Rate, Respiratory Rate) should be obtained at the start and completion of the first infusion, and with subsequent infusions where clinically possible. There is no dosing adjustment for hepatic or renal dysfunction, including hemodialysis.

Administer the diluted defibrotide solution using an infusion set equipped with a 0.2 micron inline filter. Flush the intravenous administration line (peripheral or central) with 5% Dextrose Injection, USP or 0.9% Sodium Chloride Injection, USP immediately before and after administration. Do not co-administer defibrotide and other intravenous drugs concurrently within the same intravenous line.

6.3 Therapy duration

The planned duration of study therapy is 7 days (168 hours) from study entry, with the following qualifications:

a. Patients who respond to study therapy prior to the 7-day period, as evidenced by a decline in the WHO Ordinal Score by ≥ 2 points (APPENDIX 1) for > 48 consecutive hours, may discontinue study therapy at that earlier time point.

b. Patients who have not responded to study therapy by day 7 of therapy, evidenced by <20% reduction (or a worsening) of the amount of supplemental oxygen they are receiving, will discontinue study therapy at that time point (day 7).

c. Patients who have evidence of a partial pulmonary response by day 7 (≥20% reduction in supplemental O2 requirement, but still require supplemental oxygen) may elect to continue to receive study drug through an additional 7 days of study (total 14-day therapy course).

6.4 Criteria to Hold Study Therapy

Study drug will be held in the following settings:

Patients who develop signs of “major bleeding” as defined by International Society on Thrombosis and Haemostasis (ISTH) criteria (Appendix 3), will have defibrotide held at that time.

Per ISTH criteria:

- The term “major bleeding” is defined as symptomatic bleeding in a critical area or organ, including intracranial, intra-spinal, intraocular, retroperitoneal, intra-articular, pericardial, or intramuscular with compartment syndrome.
- Any bleeding associated with a decline in hemoglobin level of ≥ 2.0 g/dl, leading to transfusion of two or more units of whole blood or red cells will constitute a major bleeding episode, and defibrotide will be held.

Additional criteria:

- In addition, defibrotide therapy will be held if symptomatic alveolar hemorrhage, macroscopic hematuria, uncontrolled menorrhagia (i.e. requiring therapy), uncontrolled epistaxis (> 10 minutes duration) requiring cauterization and/or nasal packing, or uncontrolled bleeding from a wound site (i.e. bleeding requiring therapeutic intervention) occur.
- Blood tinged endotracheal secretions, microscopic hematuria, mild menorrhagia, and/or epistaxis < 10 minutes duration do not require withholding of drug therapy unless the treating medical team deems that the event requires direct medical intervention.

Therapy may resume once the bleeding diathesis has been resolved for at least 24 hours, and may be restarted at the same dose and dosing schedule. If any further signs of bleeding develop upon re-initiation of therapy, defibrotide would be discontinued and not re-started. Patients who develop signs of CNS hemorrhage or diffuse alveolar hemorrhage

should discontinue study therapy immediately, and will not resume therapy at any time point.

Defibrotide will be held for surgical procedures or to accommodate other urgent intervention (central line placement) without necessitating dose modification. For surgical procedures it is recommended that defibrotide administration be completed > 2 hours prior to the procedure. The DF may be resumed 24 hours after completion of the procedure (or later) based upon the clinical discretion of the treating physician.

In situations in which defibrotide therapy was temporarily held and then resumed, no additional doses of defibrotide will be given to account for any doses that were missed while the drug was being held.

Defibrotide will be discontinued permanently for the development of a "severe or life-threatening (anaphylaxis) hypersensitivity reaction.

Defibrotide will be discontinued permanently if a patient initiates extra-corporeal membrane oxygenation (ECMO) therapy for any reason.

Defibrotide dosing for acute hypersensitivity reactions is discussed in section 6.5 below.

6.5 Management of Acute Hypersensitivity Reactions.

If an infusion-related hypersensitivity reaction occurs, the infusion will be emergently held as outlined below. One of the principal investigators, Gregory Yanik (pager 3390), Vibha Lama (pager 12599), or David Frame (pager 1803) MUST be contacted immediately (prior to the next dose) so that the study team can determine if further dosing should be held, and/or discontinued altogether. One of the principal investigators will be available 24/7 to discuss potential adverse events or infusion related hypersensitivity reactions that develop in patients receiving study therapy.

Acute Intravenous Infusion Reactions: Emergency equipment and medication for the treatment of infusion reactions must be available for immediate use. All infusion reactions must be reported as AEs (as defined in section 10) and graded using the grading scales as instructed in Section 10.

Interruption of the Intravenous Infusion: The infusion should be interrupted if any of the following AEs are observed:

- Hypotension (blood pressure > 2 standard deviations below baseline)
- Rigors/chills
- Rash, pruritus
- Urticaria (hives, welts, wheals)
- Diaphoresis
- Worsening dyspnea or ventilator status
- Vomiting
- Flushing

The reaction(s) should be treated symptomatically, and the infusion may be restarted at 50% of the original rate, after approval by the study investigators. If study investigators feel there is a medical need for treatment or discontinuation of the infusion other than described above, they should use clinical judgment to provide the appropriate response according to typical clinical practice.

Termination of the Intravenous Infusion: The infusion should be terminated and NOT restarted if any of the following adverse events occur:

- anaphylaxis*
- laryngeal/pharyngeal edema
- severe bronchospasm
- chest pain
- seizure
- severe hypotension
- other neurological symptoms (confusion, loss of consciousness, paresthesia, paralysis, etc.) any other symptom or sign that, in the opinion of the investigator, warrants termination of the IV infusion

Consider anaphylaxis if the following is observed (Sampson, 2006): acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (eg, generalized hives, pruritus or flushing, swollen lips-tongue-uvula) AND AT LEAST ONE OF THE FOLLOWING:

- Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced peak expiratory flow, hypoxemia)
- Reduced blood pressure or associated symptoms of end-organ dysfunction (eg, hypotonia [collapse], syncope, incontinence).

As noted above, one of the principal investigators [Gregory Yanik (pager 3390), Vibha Lama (pager 12599), or David Frame (pager 1803)] MUST be contacted immediately if any potential hypersensitivity reaction occurs, so that the study team can determine if further dosing should be immediately held, and/or discontinued.

6.6 Concurrent Medications

Patients may NOT be treated concurrently with therapeutic dosing of antifibrinolytics, or anticoagulants, including (but not limited to) therapeutic dosing with heparin, systemic t-PA, or warfarin. The use of non-therapeutic doses of heparin (≤7,500 units Q8Hours) or low molecular weight heparin (≤ 1 mg/kg/day) is allowed. Patients may also receive heparin or other anticoagulants for routine central venous line management, intermittent dialysis or ultrafiltration. Fibrinolytic instillation for central venous line occlusion is also permitted.

6.7 Supportive care

Hematologic: Platelet transfusions should be given to maintain a platelet count ≥ 50,000/mm3 at all times, and fresh frozen plasma or cryoprecipitate given to maintain a serum fibrinogen level > 150 mg/dl during the duration of study therapy.

Infectious disease: Antimicrobial therapy for prophylaxis or treatment of acute infections should continue as clinically indicated, per investigator discretion.

Hepato-renal: There is no change in defibrotide dosing with hepatic and/or renal dysfunction. Patients receiving hemodialysis, peritoneal dialysis or ultrafiltration may continue study therapy without dosing modification.

7.0 REQUIRED OBSERVATIONS

For sections 7.2 through 7.4, if a laboratory parameter has been obtained within 48 hours prior to study enrollment, it does not need to be repeated for study purposes and will be considered the *baseline* value. Following the last day of study drug therapy, no further "study related" blood tests will be obtained.

Guidelines for monitoring hematologic and coagulation parameters were derived from Thachil, J., et al., *ISTH interim guidance on recognition and management of coagulopathy in COVID-19.* J Thromb Haemost, 2020. **18**(5): p. 1023-1026.

7.1 Pulmonary Indices: Pulmonary parameters will be recorded daily for subjects from the time of study entry to cessation of study drug therapy. Pulmonary assessment will include a subject's current level of supplemental oxygen support (%FiO2), current modality of support (mechanical ventilation, BiPaP, CPAP, nasal cannula, or none), and the results of any chest radiographs (if performed) that day. For patients that remain hospitalized on day 14 of study, pulmonary indices will also be made at that time point.

Blood gas values will be obtained as clinically indicated. Scoring from the WHO COVID Ordinal Scale (Appendix 1) will be determined daily by study investigators, till cessation of study drug therapy.

7.2 Hematologic parameters:

A complete blood count including platelets (CBCP) should be obtained at baseline (study entry), and daily till cessation of study therapy. Additional blood counts may be obtained as clinically indicated.

7.3 Chemistries:

Serum chemistries (Electrolytes, BUN, creatinine, liver panel) should be obtained at baseline (study entry), and upon cessation of study therapy. Additional chemistries should be obtained as clinically indicated.

7.4 Coagulation Labs:

D-dimer, Prothrombin time (PT), and serum fibrinogen should be obtained at baseline (study entry) and daily till cessation of study therapy. Additional D-dimer, PT, and serum fibrinogen testing may be obtained as clinically indicated.

7.5 Serologic Cytokine Profiles:

Approximately 3-5 ml of plasma will be drawn at baseline (±1 day), day 4 (±2 days), day 7 (±2 days), and upon completion of study therapy (±2 days) for cytokine analysis. For subjects completing study therapy prior to day 7, samples will be acquired upon completion of study therapy (±2 days), without a day 7 sample acquired. For subjects completing study therapy after day 7, an additional sample will be acquired upon completion of study therapy (±2 days). The inability to obtain these ancillary tests on the specified days will not be considered a protocol violation.

7.6 **Biomarkers of hemostasis and endothelial dysfunction:**
Biomarkers of hemostasis and endothelial dysfunction will be obtained from the 3-5 ml of plasma as described in section 7.5. Serologic biomarkers of hemostasis and endothelial dysfunction may include (but are not limited to): PAI-1, Protein C, tPA, VWF. The inability to obtain these ancillary tests on the specified days will not be considered a protocol violation.

For subjects completing study therapy prior to day 7, samples will be acquired upon completion of study therapy (±2 days), without a day 7 sample acquired. For subjects completing study therapy on day 7, samples will be acquired at baseline, day 4 (±2 days) and day 7 (±2 days) of therapy. For subjects completing study therapy after day 7, samples will be acquired at baseline, day 4 (±2 days), day 7 (±2 days), and upon completion of study therapy (±2 days).

7.7 Study Schedule of Events

	Baseline	Day 1	Day 2[f]	Day 3[f]	Day 4[f]	Day 5[f]	Day 6[f]	Day 7[f]	30 days post[h]
Eligibility screen	X								
Informed consent	X								
Medical history	X[a]	X	X	X	X	X	X	X	
Physical exam	X[a]	X	X	X	X	X	X	X	
CBC (platelets)	X[a]	X	X	X	X	X	X	X	
Chemistries	X[a]							X	
D-dimer	X[a]	X	X	X	X	X	X	X	
PT	X[a]	X	X	X	X	X	X	X	
Fibrinogen	X[a]	X	X	X	X	X	X	X	
ABG	X[a]								
CXR	X[b]								
Biomarkers[c]	X				X			X	
Urine or serum pregnancy test[d]	X								
Pulmonary Indices[e]	X	X	X	X	X	X	X	X	
Defibrotide		X	X	X	X	X	X	X	
Vital signs[g]		X	X	X	X	X	X	X	
Adverse Event and Follow-up[h]		X	X	X	X	X	X	X	X

Key: CBC-complete blood count; CXR-chest X ray; ABG-arterial blood gas; BP-blood pressure; HR-heart rate; RR-respiratory rate, Tx-treatment.

Notes:

a, Laboratory tests performed within 48 hours prior to study entry do not need to be repeated at baseline. The D-Dimer, serum fibrinogen, PT and CBCP should be performed daily (till cessation of study drug therapy), and should be viewed as standard medical care. Arterial blood gas (ABG) measurements, and chest radiographs should be performed per routine medical care. Patients who continue therapy beyond day 7, should continue to have laboratory tests performed (as indicated above) till cessation of study therapy.

b, Chest XRay (CXR): The CXR does not need to be repeated at baseline, if a CXR was done on admission and revealed bilateral infiltrates.

c. Biomarkers. Five ml of plasma will be drawn at baseline (±1 day), day 4 (±1 day), day 7 (±1 day), and upon completion of study therapy (±1 day) for cytokine analysis. Patients discontinuing drug prior to day 7 are not required to have a day 7 biomarker assessment drawn."

d. Pregnancy test: Perform in subjects of child-bearing potential, if not already performed this admission for clinical care reasons.

e. Pulmonary indices will be recorded daily till cessation of drug therapy, and again at day 14 (if a patient remains hospitalized at that time). Patients who are discharged prior to day 14 do not need to have pulmonary indices assessed on day 14.

f. Should be done **"**till "cessation of study therapy", if the defibrotide is given for a duration other than 7 days.

g. Vital signs: heart rate, respiratory rate, blood pressure prior to and upon completion of the first infusion, and if clinically possible with subsequent infusions.

h. Adverse Event Reporting and Follow-up: Patients will be assessed for adverse events daily during receipt of study therapy, and for 7 days following completion of study drug therapy, provided they remain hospitalized during this 7-day post-therapy period. In addition, patients will be contacted 30 days (±2 days) after receiving the last dose of study therapy, to assess their clinical status and any active adverse events at that time. This final assessment can be done virtually, by telephone or electronically (email) if the patient cannot be contacted by phone. No in-person visit is required.

8.0 STATISTICAL ANALYSIS:

We are proposing a single arm, single center open label trial to assess the safety and tolerability of defibrotide for patients with SARS-CoV2 induced ARDS.

8.1 Primary study endpoint

The primary safety endpoint will be the occurrence of major toxicity, specifically hemorrhagic complications within 14 days of initiation of treatment.

8.2 Sample size justification

This study is intended to be a feasibility study of potential hemorrhagic complications associated with defibrotide in our study population. Thus, this study will enroll a maximum of **twelve patients**, which is the number of patients that we can expect to accrue in the current setting of the SARS-CoV2 pandemic.

8.3 Primary stopping rule

An incidence of > 20% hemorrhagic complications would be unacceptable in this setting. Thus, we will continually monitor the observed proportion of patients with major bleeding complications. We start with prior Beta(2,8) distribution, which has mean 0.2. If ever we have more than 80% posterior probability that the true proportion is greater than 0.20,

accrual to the protocol will be suspended to allow the DSMB to review the incidence of major bleeding.

Specifically, if we see at least **three** patients with major bleeding among the first six patients, or at least **four** patients with major bleeding among the first eleven patients, the study will be suspended. If the true proportion of major bleeding events is 0.20, the study has a probability of 0.19 of being suspended. If the true proportion of major bleeding events is 0.30 or 0.40, the probability of trial suspension is 0.47 and 0.73, respectively. Note that if the trial is suspended to accrual, the accrual is expected to be seven patients.

8.4 Secondary endpoints

There are two secondary endpoints: (i) Day 14 overall survival and (ii) Day 14 ventilator-free survival, both of which are binary endpoints. Day 14 overall survival will be summarized by the proportion of the twelve patients who are alive at Day 14 after starting treatment, and Day 14 ventilator-free survival will be summarized by the proportion of the twelve patients who are both alive and not using a ventilator at Day 14 after starting treatment. Both proportions will be accompanied by an exact 95% confidence interval.

We will also continually monitor the study for excessive Day 14 mortality. **In parallel with our decision rule related to excessive severe hemorrhagic events, we will suspend accrual if we see if we see at least five deaths by Day 14 among the first six patients, or at least seven deaths by Day 14 among the first eleven patients.** If the true Day 14 mortality rate is 70% (which is the historical rate for patients with respiratory comprise), the study has a probability of 0.80 of being suspended. Conversely, if the Day 14 mortality rate is 50%, the study has a much lower probability of 0.30 of being suspended.

8.5 Definition of evaluable subjects

Subjects who receive at least one dose of the study drug (defibrotide) would be considered evaluable for the primary and secondary study endpoints. Subjects who die following consent, but prior to initiation of the study therapy (defibrotide), are not evaluable for safety, and will be replaced on study.

9.0 OFF STUDY CRITERIA

Defibrotide will be discontinued for any of the following reasons:

- Withdrawal of consent / patient choice.
- The patient experiences an adverse event which, in the opinion of the Investigator, contraindicates continuation in the study.
- Protocol violation, including disallowed concomitant therapy.
- The Investigator considers it not in the patient's best interest to continue.
- Defibrotide-related toxicity (including hemorrhage as defined in section 6.4)
- Subject death.

10.0 ADVERSE EVENT REPORTING REQUIREMENTS

10.1 Purpose

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents.

10.2 Adverse Event Reporting Requirements

The descriptions and grading scales used in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. The CTCAE v5.0 mapping document can be downloaded at the CTEP website: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf

Reporting requirements may include the following considerations: 1) the characteristics of the adverse event including the *grade* (severity); 2) the *relationship to the study therapy* (attribution); and 3) the *prior experience* (expectedness) of the adverse event.

Adverse events (AE) refer to any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)). By way of example and without limitation, an AE can be any unfavorable and unintended sign (for example, an abnormal laboratory finding), symptom, or disease temporally associated with the use of the Study Drug.

Protocol-defined and serious AE will collected for 30 days following receipt of the last DF dose, or death, whichever comes first. In addition, patients with drug related AE will be monitored until symptom resolution, even if the symptoms extend beyond the 30-day period.

AEs may include:

- Exacerbation (i.e., an increase in the frequency or severity) of a pre-existing condition. Illness present before study entry should be recorded in the medical history section of the CRF and only be reported as an AE if there is an increase in frequency or severity of the condition during the study.
- Intercurrent illnesses with an onset after administration of defibrotide begins.

AEs DO NOT include:

- Any medical condition or laboratory abnormality with an onset date before initial study treatment is considered to be pre-existing in nature. Any known pre-existing conditions that are ongoing at time of study entry should be considered medical history.
- Medical or surgical procedures (the condition that leads to the procedure is the AE)
- Situations where an untoward medical occurrence has not taken place. For example:
- Planned hospitalizations due to pre-existing conditions, which have not worsened.
- Hospitalizations that occur for procedures not due to an AE.
- Hospitalization for the diagnostic procedure where the hospital stay is less than 24 hours in duration or for normal management procedures (i.e. chemotherapy).

Laboratory findings DO NOT need to be reported as AEs in the following cases:

- Laboratory parameters already beyond the reference range at screening.
- Abnormal laboratory parameters caused by mechanical or physical influences on the blood sample (eg, in vitro hemolysis) and flagged as such by the laboratory in the laboratory report.
- An abnormal laboratory value that cannot be confirmed after repeat analysis, preferably in the same laboratory.

10.3 Definition of Serious Adverse Events (SAE):

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- **Death** – If death results from (progression of) the disease, the disease should be reported as event (SAE) itself.
- **A life-threatening adverse event** – The term “life threatening” refers to an event in which the subject was at risk for death at the time of the event; it does not refer to an event that hypothetically might have caused death if it had been more severe.
- **Requires inpatient hospitalization or prolongation of existing hospitalization for at least 24 hours** - Hospital admission for planned surgery or for normal disease management procedures are not considered as defining criteria for SAEs.
- **Results in persistent or significant disability or incapacity or substantial disruption of the ability to conduct normal life functions**
- **Is a congenital anomaly/birth defect**
- **Is medically significant** - Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

10.4 Causality

The investigator will assess the relationship of an adverse event to the treatment intervention. If possible, the investigator should distinguish the relationship between the event and (a) the clinical course of ARDS and (b) the investigational intervention.

Causality will be defined as follows:

Definite – The AE is clearly related to the study treatment.
Probable – The AE is likely related to the study treatment.
Possible – The AE may be related to the study treatment.
Unlikely – The AE is doubtfully related to the study treatment.
Unrelated – The AE is clearly NOT related to the study treatment/intervention.

10.5 Expectedness

An adverse event (AE) is considered “expected” if those adverse events are described in the approved Package Insert (Label), the clinical protocol or informed consent document.

Unexpected serious adverse events are defined as any experience that has not been described in the package insert, protocol, or the informed consent document. Expedited reporting is required for serious adverse events that are unexpected.

10.6 Additional Protocol-Defined Serious Adverse Events

- Bleeding / Hemorrhage: The development of a bleeding diathesis that in the opinion of the treating physicians may be potentially life-threatening, including (but not limited to) pulmonary hemorrhage, CNS bleed / hemorrhage, gastro-intestinal hemorrhage (frank hematochezia or hematemesis), genito-urinary bleeding (gross

hematuria), or uncontrolled epistaxis / oral mucosal bleeding as defined in section 6.4.

- *Acute Renal Dysfunction:* Abnormal kidney function requiring dialysis (including hemofiltration) in patients who did not require this procedure prior to study entry, or a rise in serum creatinine of greater than 3 times baseline for over 48 hours.
- *Deterioration of Respiratory Status:* Impairment of respiratory function requiring tracheostomy or the institution of high frequency oscillatory ventilation (HFOV) or ECMO, if not present at study entry. This excludes intubation for re-operation or temporary intubation for diagnostic or therapeutic procedures.
- *Multi-System Organ Failure:* The development of potentially reversible physiological derangement involving 2 or more organ systems not involved in the ARDS, provided this organ dysfunction was not present at the time of study entry.

10.7 Reporting of Unanticipated Problems:

All serious adverse events (SAEs) regardless of causality to study drug, will be reported to the Principal Investigator within 24 hours of first awareness of the event. Follow-up information must also be reported within 24 hours of receipt of the information by the investigator.

All SAEs will be reported to the IRB per current institutional standards. In this trial, serious, unexpected adverse events believed to be definitely, probably or possibly related to the study treatment will be reported to the Food and Drug Administration via the MedWatch 3500A. The Michigan IND/IDE Assistance Program (MIAP) will assist the Sponsor in submitting SAEs to the FDA that meet the reporting requirements in 21 CFR 312.32.

In addition, all SAEs as defined by the Protocol and discontinuations of subject treatment as a result of safety related events during the study will also be reported immediately by email (AEreporting@jazzpharma.com) to Jazz Pharmaceuticals, and in any event no later than 1 business day following Institution's or Investigator's receipt of notice of such occurrence, in full compliance with Applicable Law governing protection of personal data. The Institution and/or study investigators shall provide a copy of all Data Safety Monitoring Board (DSMB) reports to Jazz Pharmaceuticals by email (ClinicalSafety@jazzpharma.com) within five (5) business days of a DSMB meeting. As between the parties, the Institution is responsible for complying with all the relevant Governmental Authority's reporting requirements for the Study that are applicable to the Sponsor of a clinical trial. The obligations set forth in this paragraph will continue until the later of (a) the expiration of this Agreement; or (b) 180 days after the last administration of the product in the study.

10.8 Reporting of Unanticipated Problems

There are types of incidents, experiences and outcomes that occur during the conduct of human subjects research that represent unanticipated problems but are not considered adverse events. For example, some unanticipated problems involve social economic harm instead of the physical or psychological harm associated with adverse events. In other cases, unanticipated problems place subjects of others at increased risk of harm, but not harm occurs.

Upon becoming aware of any incident, experience, or outcome (not relaed to an adverse event) that may represent an unanticipated problem, the investigator should assess whether the incident, experience, or outcome represents an unanticipated problem. The

incident, experience or outcomes is considered unanticipated if it meets all of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency);
2. Related or possibly related to participation in the research; and
3. Suggests that the research places subjects or others at a greater risk of harm than was previously known or recognized.

If the investigator determines that the incident, experience, or outcome represents an unanticipated problem, the investigator must report to the IRB according to the local IRB policies.

11.0 DATA SAFETY MONITORING:

Protocol Monitoring Committee: A protocol monitoring committee consisting of the principal investigators, one or more co-investigators, study coordinator, and data manager will meet monthly during the conduct of the study to discuss expected and unexpected adverse events, safety concerns, adherence to protocol therapy, study enrollment, and any potential protocol amendments that may be required. Recommendations for study continuation, amendments, suspension and/or study termination will be made at each of the monthly protocol monitor meetings and formally submitted to an independent Data Safety Monitoring Board.

Data Safety Monitoring Board (DSMB): Membership shall consist of two external (non-Michigan Medicine) and two internal (Michigan Medicine) physicians. The DSMB will be an independent body, with none of the DSMB members serving as study co-investigators. The DSMB will meet quarterly to review the conduct of the study, including review of all monthly reports submitted from the protocol monitoring committee. More frequent DSMB meetings may be required, pending any issues raised in the monthly reports from the protocol monitoring committee. In addition, the DSMB will review the study after the first 6 patients have completed study drug therapy, to determine if the study stopping rules for early termination have been met, and if the study enrollment should be terminated or allowed to continue. Enrollment will be held once the 6th patient has completed study drug therapy, to allow the DSMB to make this determination. Reports from the DSMB meetings will be furnished to IRBMED in accordance with Michigan Medicine policies.

12.0 ADDITIONAL CLINICAL MONITORING PROCEDURES

To further assure adequate protection of the rights of human subjects, this study will also be monitored by the University of Michigan Institute of Clinical and Health Research (MICHR). Routine monitoring will be scheduled at appropriate intervals, with more frequent visits occurring at the beginning of the study. A site initiation visit will take place, followed by routine monitoring visits. Additional visits can be scheduled at the request of the Sponsor-Investigator.

The established monitoring plan will ensure the quality and integrity of the data throughout the study conduct to verify adherence to the protocol, completeness and accuracy of study data and samples collected, dispensing and inventory of the drug, and compliance with regulations. During the time of COVID-19 pandemic, monitoring will be conducted remotely.

13.0 DRUG INFORMATION (Defibrotide [Defitelio®])

Jazz Pharmaceuticals will supply the commercial Defitelio product labeled for investigational use. All patients will receive 6.25 mg/kg Q6h (25 mg/kg/day) of defibrotide. Defibrotide will be administered in 0.9% sodium chloride or 5% dextrose in water IV (given in 4 divided doses approximately Q6H) mixed to a concentration of between 4 - 20 mg/mL, each infused over approximately 2 hours. No dose shall be infused within 3 hours of a prior dose. (See SECTION 6.0 for additional details).

Initiation: The patient should receive their first dose of defibrotide within 24 hours of consent to participate in the trial.

Dose: The daily dose of defibrotide (6.25 mg/kg Q6H) will be based on the patient's recorded weight at the time of study entry. Each of the 4 divided doses per day will be rounded to the nearest 10 mg in order to facilitate efficient drug administration. Total daily dose for all patients will be 25 mg/kg. Dosing modification for an individual patient is not allowed. However, study doses may be held per guidelines in section 6.0. There are no dose adjustments required for renal dysfunction and/or use of hemodialysis. Management of acute reactions is noted in SECTION 6.0.

Drug Formulation and Procurement

Drug formulation: Defibrotide is a clear light yellow to brown solution supplied as 200 mg/2.5 mL (concentration of 80 mg/mL) in single-patient-use clear, glass vials.
The drug should be stored at controlled room temperature. Do not store above 30°C (86°F). Do not freeze. See the Defibrotide Drug Ordering and Handling Guide for the handling of storage condition deviations.

Procurement: Defibrotide will be supplied by Jazz Pharmaceuticals, INC, Palo Alto, CA 94304. Supply must be coordinated through Jazz Pharmaceuticals and its designee.

See the Defibrotide Drug Ordering and Handling Guide for complete details on drug ordering and on handling of the study drug.

Storage/Preparation/Handling

All defibrotide shipped to investigators should be stored in a locked, secure location in the pharmacy at controlled room temperature not above 30°C (86°F). Do not freeze.

Dilute defibrotide in 5% Dextrose Injection, USP or 0.9% Sodium Chloride Injection, USP to a concentration of 4 mg/mL to 20 mg/mL. The vials contain no antimicrobial preservatives and are intended for a single-patient-use only. Partially used vials should be discarded. Use the diluted solution within 4 hours if stored at room temperature or within 24 hours if stored under refrigeration. Up to four doses of defibrotide solution may be prepared at one time, if refrigerated.

Study Drug Return

The site is responsible for destruction of unused or expired defibrotide vials per local procedures.

Drug Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of the investigational drug defibrotide. The drug accountability records will capture drug receipt, drug dispensing, drug return and final disposition.

Adverse Reactions (Defitelio [defibrotide] package insert. Palo Alto, CA: Jazz Pharmaceuticals, inc.; 2016 Mar.)

The safety of defibrotide was determined in 176 adult and pediatric patients with hepatic veno-occlusive disease (VOD) with pulmonary and/or renal dysfunction following hematopoietic stem-cell transplantation (HSCT) who were treated with defibrotide 6.25 mg/kg every 6 hours

Information about adverse reactions of any grade was available for all 176 patients. The most common adverse reactions (incidence ≥10% and independent of causality) were hypotension, diarrhea, vomiting, nausea, and epistaxis. The most common serious adverse reactions (incidence ≥5% and independent of causality) were hypotension (11%) and pulmonary alveolar hemorrhage (7%). Hemorrhage events of any type and any grade were reported for 104 (59%) of the patients, and the events were grade 4-5 in 35 (20%).

The Table below presents adverse reactions[a] independent of causality ≥10% any grade or Grade 4/5 ≥2% reported in patients treated with defibrotide.

Adverse Reaction [a]	**DEFITELIO (n=176)**	
	Any grade	**Grade 4-5[b]**
Hypotension	65 (37%)	12 (7%)
Diarrhea	43 (24%)	0
Vomiting	31 (18%)	0
Nausea	28 (16%)	0
Epistaxis	24 (14%)	0
Pulmonary alveolar hemorrhage	15 (9%)	12 (7%)
Gastrointestinal hemorrhage	15 (9%)	5 (3%)
Sepsis	12 (7%)	9 (5%)
Graft versus host disease	11 (6%)	7 (4%)
Lung infiltration	10 (6%)	5 (3%)
Pneumonia	9 (5%)	5 (3%)
Pulmonary hemorrhage	7 (4%)	4 (2%)
Infection	6 (3%)	4 (2%)
Hemorrhage intracranial	5 (3%)	4 (2%)
Hyperuricemia	4 (2%)	4 (2%)
Cerebral hemorrhage[c]	3 (2%)	3 (2%)

a Excludes events considered to be due to the underlying disease: multi-organ failure, veno-occlusive disease, respiratory failure, renal failure, and hypoxia
b Adverse reactions considered life-threatening or fatal
c Cerebral hemorrhage has been included in the table due to clinical relevance

14.0 REFERENCES:

Bianchi G, Barone D, Lanzarotti E, Tettamanti R, Porta R, Moltrasio D, Cedro A, Salvetti L, Mantovani M, Prino G. Defibrotide, a single-stranded polydeoxyribonucleotide acting as an adenosine receptor agonist. Eur J Pharmacol**.** 1993; *238*: 327– 334.

Cella G, Sbarai A, Mazzaro G, Motta G, Carraro P, Andreozzi GM, Hoppensteadt DA, Fareed J. Tissue factor pathway inhibitor release induced by defibrotide and heparins. Clin Appl Thromb Hemost**.** 2001; *7*: 225– 228.

Chen N, Zhou M, Dong X et al (2020) Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 395(10223):507–513 2.

Francischetti MB et al. Defibrotide interferes with several steps of the coagulation-inflammation cycle and exhibits therapeutic potential to treat severe malariaArteriosclerosis, Thrombosis, and Vascular Biology. 2012;32:786–798.

Fox SE, Akmatbekov A, Harbert JL, Li G, Brown JQ, Vander Heide RS. Pulmonary and Cardiac Pathology in Covid-19: The First Autopsy Series from New Orleans. https://doi.org/10.1101/2020.04.06.20050575.

Gerosa C, Calvani AB, Cornelli U, et al. A multicenter study of defibrotide in the prevention of deep venous thrombosis. Minerva Chir. 1989;44(10):1507-1516.

Giannis D, Ziogas IA, Gianni P, Coagulation disorders in coronavirus infected patients: COVID-19, SARS-CoV-1, MERS-CoV and lessons from the past, *Journal of Clinical Virology* (2020).

Huang C, Wang Y, Li X et al (2020) Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395(10223):497–506.

Hunt B, Retter A, McClintock C. Practical guidance for the prevention of thrombosis and management of coagulopathy and disseminated intravascular coagulation of patients infected with COVID-19. Thrombosis and Hemostasis March 25th, 2020.

Nascimento I, Cacic N, Abdulazeem HM et al. Novel Coronavirus Infection (COVID-19) in Humans: A Scoping Review and Meta-Analysis. J Clin Med. 2020 Mar 30;9(4).

Kaleelrahman M, Eaton JD, Leeming D et al. Role of plasminogen activator inhibitor-1 (PAI-1) levels in the diagnosis of BMT-associated hepatic veno-occlusive disease and monitoring of subsequent therapy with defibrotide (DF). Hematology. 2003 Apr;8(2):91-5.

Klok FA, Kruip MJ, van der Meer JM et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. Thrombosis Res (2020) in print.

Kornblum N, Ayyanar K, Benimetskaya L, Richardson P, Iacobelli M, Stein CA. Defibrotide, a polydisperse mixture of single-stranded phosphodiester oligonucleotides with lifesaving activity in severe hepatic veno-occlusive disease: clinical outcomes and potential mechanisms of action. Oligonucleotides**.** 2006; *16*: 105– 114.

Larocca A, Cavallo F, Magarotto V, Rossi D, Patriarca F, Boccadoro M, Palumbo A. Defibrotide: a review on clinical use and future development. Expert Opin Biol Ther**.** 2008; *8*: 1201– 1212.

Llitjos JF, Leclerc M, Chochois C et al. High incidence of venous thromboembolic events in anticoagulated severe COVID-19 patients. J Thrombosis and Hemostas Pre-printed ahead of publication. April 2020.

Palmer KJ, Goa KL. Defibrotide. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in vascular disorders. Drugs. 1993 Feb;45(2):259-94. PMID 7681375.

Richardson P, Linden E, Revta C, Ho V. Use of defibrotide in the treatment and prevention of veno-occlusive disease. Expert Rev Hematol**.** 2009; *2*: 365– 376.
Richardson PG, Riches ML, Kernan NA et al. Phase 3 trial of defibrotide for the treatment of severe veno-occlusive disease and multi-organ failure. Blood. 2016 Mar 31;127(13):1656-65.

Rizzi A, Radaelli F, Pisano M et al. Prevention of deep venous thrombosis with defibrotide in chest surgery. Controlled multicenter study versus heparin. Minerva Med. 1987 Jun 15;78(11):745-50.

Schultz MJ, Haitsma JJ, Zhang H, Slutsky AS (2006) Pulmonary coagulopathy as a new target in therapeutic studies of acute lung injury or pneumonia—a review. Crit Care Med 34(3):871–877 6. National Health Commission of China (2020) The diagnosis and treatment plan for the novel coronavirus disease, 7th edn. National Health Commission of China, Bejing.

Strouse C, Richardson P, Prentice G et al. Defibrotide for Treatment of Severe Veno-Occlusive Disease in Pediatrics and Adults: An Exploratory Analysis Using Data from the CIBMTR. Biol Blood Marrow Transplant. 2016 Jul; 22(7): 1306–1312.

Tang N, Li D, Wang X, Sun Z (2020) Abnormal Coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost. 2020 Mar 27.

Thachil J (2020). The versatile heparin in COVID-19. J Thromb Haemost. 2020 Apr 2

Thachil J, Tang N, Gando S et al. ISTH interim guidance on recognition and management of coagulopathy in COVID-19*.* J Thromb Haemost, 2020;**18**(5):1023-1026.

Tocchetti P, Tudone E, Marier JF, Marbury TC, Zomorodi K, Eller M. Pharmacokinetic profile of defibrotide in patients with renal impairment. Drug Des Devel Ther. 2016 Aug 16;10:2631-41. PMID: 27574402.

Umemura K, Iwaki T, Kimura T et al. Pharmacokinetics and Safety of Defibrotide in Healthy Japanese Subjects. Clin Pharmacol Drug Dev. 2016 Nov;5(6):548-551.

Vincent JL, A deMa, Cantraine F et al. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Crit Care Med 1998;26:1793–800.

Wu C, Chen X, Cai Y, et al. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. JAMA Intern Med. 2020;e200994.

Xu Z, Shi L, Wang Y, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome 2020 Feb 18] [published correction appears in Lancet Respir Med. 2020 Feb 25;:]. Lancet Respir Med. 2020;S2213-2600(20)30076-X.

Yang M, Ng MHL, Li CK. Thrombocytopenia in patients with severe acute respiratory syndrome (review). Hematology 2005;10(2):101–5.

Yang M, Li CK, Li K, Hon KL, Ng MH, Chan PK, Fok TF. Hematological findings in SARS patients and possible mechanisms (review). Int J Mol Med. 2004 Aug;14(2):311-5.

Yin S, Huang M, Li D, Tang N et al. Difference of coagulation features between severe pneumonia induced by SARS-CoV2 and non-SARS-CoV2. J Thromb Thrombolysis. 2020 Apr 3.

15.0 APPENDIX 1: WHO Ordinal scale to assess clinical improvement

Patient State	Descriptor	Score
Ambulatory	No limitation of activities	1
	Limitations of activities	2
Hospitalized (mild disease)	No oxygen therapy	3
	Oxygen by mask or nasal prongs	4
Hospitalized (severe disease)	Non-invasive ventilation or high-flow oxygen	5
	Intubation and mechanical ventilation	6
	Mechanical ventilation + additional organ support – pressors, RRT, ECMO	7
Dead	Death	8

Ordinal Score:

16.0 APPENDIX 2: INFORMED CONSENT PROCESS

Consenting SARS-CoV2 positive patients may present special challenges. In order to reduce possibilities for viral transmission, preserve personal protective equipment (PPE), and comply with the FDA's 2020 guidance for obtaining consent during the SARS-CoV2 pandemic, the following IRBMED guidance (**v.4.17.20**) will be used to obtain informed consent: https://research.medicine.umich.edu/announcements/novel-coronavirus-covid-19-irbmed-news-and-updates .

Per IRBMED recommended guidelines, one of four options are to be utilized, depending upon the patient's clinical status:

Option #1: Patient Electronic Signature
Patient is able to sign informed consent electronically via SignNow (web App).

Option #2: Wet Ink (Paper Signature):
Patient is able to physically sign a paper informed consent, but are unable to sign electronically.

Option #3. Use of Patient / Witness proxy
Patient is able to give informed consent, but is unable to sign the consent form (either electronic or paper).

Option #4: Use of Legal authorized representative (LAR).
Patient is unable to give informed consent (i.e. patient sedated on mechanical ventilation),

Study teams must work with clinical care teams on the COVID unit to assess the following options and select the method most appropriate option for each individual patient. These methods may also be applicable in outpatient settings that include COVID-19 patients.

Option 1: Patient Electronic Signature. This option only applies to patients who are medically competent for decision making and can sign an electronic consent. The option will make use of an electronic signature via SignNow (web App), with the following criteria:

a) The patient must have access to email via a smartphone or computer to view the informed consent documents and apply their electronic signature. Due to current infection control procedures, the patient's device must be used, as one will not be provided by Michigan Medicine.

b) A member of the patient's clinical care team will first provide the patient with a paper copy of the unsigned consent document and any accompanying explanatory coversheet prior to the call from the study team. The clinical team is NOT expected to review the paper copy of the informed consent with the patient. **It is the duty of the study team to review the informed consent with the patient.** The clinical care team is ONLY expected to provide the patient with the paper copy. The paper copy will be disposed of at an appropriate time, according to infection control standards.

c) A member of the study team will call and instruct the patient to download the SignNow E-signature application onto their smartphone, using the site www.signnow.com . SignNow is a cloud-based provider of electronic signature technology. The company's Software-as-a-Service platform enables individuals to sign, and manage documents from any computer or smart-phone device. The e-signature product is available for free on iPhone, iPad, and Android devices, and allows individuals to upload documents from their smartphone's e-mail and tap to insert their signature.

d) A member of the study team will subsequently conduct the discussion with the patient by calling the patient's smartphone or hospital-room telephone. A 3rd-party, impartial witness must be available on the call. This witness should not be a study investigator or study team member. A 3-way phone call with thus be established for the consent process, including a study investigator, witness and the patient.

d) After the patient's questions have been answered by the study team and they agree to participate, the patient will utilize SignNow on their electronic device to complete the signature process. The witness should be present on the call to attest that the consent has been reviewed, that the patient's questions were answered and that an electronic signature was present.

e) The electronic copy of the signed document will be placed into the electronic health record (MiChart) and the study's research records. The study team will assure that the patient receives a signed electronic copy via SignNow.

Option #2: Wet Ink (Paper Signature): This options will apply to patients without an electronic device, or for **p**atients who are unable to sign electronically. It is anticipated that not all patients will possess an electronic device such as a smartphone or computer to complete the electronic SignNow process. Some patients possessing a device may not feel well enough to perform necessary electronic functions to complete the tasks. These patients may still be able to sign a paper copy of the informed consent document.

Wet Ink Signature on Paper Process:
a) The clinical care team will provide an *unsigned* consent form (with any applicable explanatory coversheet) and pen to the patient. In order to meet infection control standards, the paper documents and pen must not leave the patient's room.

b) A member of the study team (study investigator) calls the patient's mobile phone or hospital-room telephone and arranges a three-way call or video conference with the patient, an impartial witness, and if desired and feasible, additional family or friends as requested by the patient.

The impartial witness cannot be the same person conducting the informed consent discussion. An individual who also happens to be a legal authorized representative (LAR) may be considered the impartial witness in this process, but in their role as a witness they are only witnessing the patient's agreement to participate in the research study and are not signing on behalf of the patient.

c) At the start of the teleconference, all participants on the call or video conference should identify themselves. The study team member (investigator) reviews the informed consent document with the patient and responds to any questions from the patient.

e) The witness confirms that the patient's questions have been answered.

f) The study team member confirms that the patient is willing to: 1) participate in the trial and 2) sign the informed consent document while the witness is either present (if the LAR) or listening on the phone.
g) The patient provides verbal confirmation that they: 1) would like to participate in the trial and 2) that they have *signed and dated the informed consent document that is in their possession*.

h) If the signed informed consent document cannot be collected from the patient's location and included in the study records, FDA considers the following options as acceptable documentation that the patient signed the informed consent document:

• Attestations by the witness who participated in the process, and by the investigator that the patient confirmed that they agreed to participate in the study and signed the informed consent. The Study Team and Witness Attestation Form via SignNow should be completed. Another acceptable option is to take a photograph of the informed consent document with attestation by the person entering the photograph into the study record that states how the photograph was obtained and that it is a photograph of the informed consent document signed by the patient.

i) The following materials should be entered into MiChart and the research study's source documentation:

- A copy of the full informed consent document signed by the study team and witness and a photograph of the patient signature (if any) OR
- A copy of the full informed consent and the Study Team and Witness Attestation Form prepared via SignNow, AND
- A notation by the investigator of how the consent was obtained (e.g., telephone) and how it was confirmed that the patient signed the consent form (i.e., either by attestation of the witness and investigator or the photograph of the signed consent). The note should include a statement of why the informed consent document signed by the patient was not retained (e.g., due to contamination of the document by infectious material).

j) A copy of the informed consent document is available to the patient (now research participant) via a medical record request from MiChart.

Option 3. Use of Patient / Witness proxy. Patients who are able to give informed consent, but are physically unable to sign the informed consent document (paper or electronic).

a) The clinical care team will provide an *unsigned* consent form (with any applicable explanatory coversheet) to the patient. The clinical team is NOT expected to review the paper copy of the informed consent with the patient. In order to meet infection control standards, the paper document must not leave the patient's room. An electronic copy of the document may also be sent to the patient (e.g., by email).

b) A study team member will contact the patient by calling the patient's smartphone or hospital-room telephone and arrange a three-way call or video conference with the patient, an impartial witness (see option #1), and if desired and feasible, additional family or friends as requested by the patient.

c) The impartial witness cannot be the same person conducting the informed consent discussion. An individual who also happens to be an LAR may be considered the impartial witness in this process, but in their role as a witness they are only witnessing the patient's agreement to participate in the research study and are not signing on behalf of the patient.]

d) Before proceeding, all participants on the call or video conference should identify themselves. The study team member will review the informed consent document with the patient and respond to any questions.

e) The impartial witness will confirm that the patient's questions have been answered.

f) The patient provides verbal confirmation to the study team and the impartial witness that they would like to participate in the study.

The study team and witness provide attestation to document that the patient confirmed their decision to participate in the study, either by completing the consent document and signature for the witness (the witness should not sign as an LAR).

g) The following materials should be placed into MiChart and the research source documentation, including a copy of the full informed consent document with investigator and witness signature, either on the consent or via SignNow, plus a notation by the investigator how the consent was attained, how questions were answered and that an impartial witness was present.

h) A copy of the informed consent document must be made available to the patient via a medical record request from MiChart.

Option 4. Use of Legal Authorized Representative (LAR) for patients who are unable to give informed consent. This situation involves patients who are incapable of decision making, including (for example) patients who are sedated, on mechanical ventilation. In such situations, their LAR may assume this responsibility.

If the LAR possesses an electronic device capable of receiving email, then the same process as outlined in option 1 will be followed, in which the study investigator and LAR present, and SignNow.com technology is used.

If the LAR does not possess a suitable electronic device, then either a paper informed consent document will be given to the LAR (in-person), or mailed to the LAR. The methodology followed in option 2 will apply, with the investigator and LAR speaking (in-person) or via telephone or similar audio device.

The LAR must return the signed informed consent document to the study team. Options to return the consent document to the study team include:

- Taking a picture of the signature page via a smartphone or camera, and sending the picture back to the study team via email or text message
- Scanning the signature page of the informed consent document and electronically returning to the study team via email
- Faxing the signature page back to the study team

The following materials will be placed in MiChart and the research study's source documentation:

- A copy of the full informed consent document and the image of the signature page signed by the LAR and a signature page signed by the person obtaining consent. The individual obtaining consent may sign/date on the same image as the LAR or a print a clean version of the signature page. The study team signature could also be applied via the SignNow process. AND
- A notation by the investigator of how the consent was obtained (e.g., telephone).

A copy of the informed consent document will be made available to the patient (now research participant)/LAR via a medical record request from MiChart.

17.0 APPENDIX 3. International Society on Thrombosis and Haemostasis Bleeding Scale

ISTH Definitions of Bleeding: Major bleeding has been used as a primary endpoint for the evaluation of safety in clinical trials. The ISTH definitions of major bleeding in non-surgical and surgical patients are as follows:

Major Bleeding in Non-Surgical Patient:

1. Fatal Bleeding, and/or

2. Symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intra-articular, pericardial, or intramuscular with compartment syndrome, and/or

3. Bleeding associated with a decline in hemoglobin level of $\geq$ 2.0 g/dl, leading to transfusion of two or more units of whole blood or red cells.

Minor Bleeding:

All non-major bleeds will be considered minor bleeds. Minor bleeds will be further divided into those that are clinically relevant and those that are not.

Clinically Relevant Minor Bleed

A clinically relevant minor bleed is an acute or subacute clinically overt bleed that does not meet the criteria for a major bleed but prompts a clinical response, in that it leads to at least one of the following:

- A hospital admission for bleeding, or
- A physician guided medical or surgical treatment for bleeding, or
- A change in antithrombotic therapy (including interruption or discontinuation of study drug).

References:

1. Schulman, S.; Kearon, C. (2005). "Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients". *J Thromb Haemost*. **3** (4): 692–4.

2. Rodeghiero, F.; Tosetto, A.; Abshire, T.; Arnold, DM.; Coller, B.; James, P.; Neunert, C.; Lillicrap, D. (2010). "ISTH/SSC bleeding assessment tool: a standardized questionnaire and a proposal for a new bleeding score for inherited bleeding disorders". *J Thromb Haemost*. **8** (9): 2063–5.