Study Protocol

v. 11/30/2020

Regulatory Sponsor: Ernest E. Moore

Ernest E Moore Shock Trauma Center at Denver Health Medical Center

777 Bannock Street, MC 0206

Denver, CO 80204

Phone: 303-602-1820

Email: Ernest.Moore@dhha.org

Funding Sponsor: Genentech, Inc.

1 DNA Way

South San Francisco, CA 94080-4990 U.S.A.

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Protocol title: Fibrinolytic Therapy to treat ARDS in the Setting of COVID-19 Infection: A Phase 2a Clinical Trial (former name "STudy of Alteplase for Respiratory failure in SARS-Cov2 (COVID-19): A Phase 2a Clinical Trial

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COLORADO MULTIPLE INSTITUTIONAL REVIEW BOARD
CAMPUS BOX F-490 TELEPHONE: 303-724-1055 Fax: 303-724-0990

Protocol #: 20-0880
Project Title: Fibrinolytic Therapy to Treat ARDS in the Setting of COVID-19 Infection: A Phase 2a Clinical Trial
Principal Investigator: Ernest E. Moore MD
Version Date: 11/30/2020

Abstract:

The global pandemic COVID-19 has overwhelmed the medical capacity to accommodate a large surge of patients with acute respiratory distress syndrome (ARDS).[1] In the United States, the number of cases of COVID-19 ARDS is projected to exceed the number of available ventilators [1]. Reports from China and Italy indicate that 22-64% of critically ill COVID-19 patients with ARDS will die [2-4]. ARDS currently has no evidence-based treatments other than low tidal ventilation to limit mechanical stress on the lung [5] and prone positioning [6]. A new therapeutic approach capable of rapidly treating and attenuating ARDS secondary to COVID-19 is urgently needed.

The dominant pathologic feature of viral-induced ARDS is fibrin accumulation in the microvasculature and airspaces. Substantial preclinical work suggests antifibrinolytic therapy attenuates infection provoked ARDS. In 2001, a phase I trial [7] demonstrated the urokinase and streptokinase were effective in patients with terminal ARDS, markedly improving oxygen delivery and reducing an expected mortality in that specific patient cohort from 100% to 70%. A more contemporary approach to thrombolytic therapy is tissue plasminogen activator (tPA) due to its higher efficacy of clot lysis with comparable bleeding risk [8]. We therefore propose a phase IIa clinical trial with two intravenous (IV) tPA treatment arms and a control arm to test the efficacy and safety of IV tPA in improving respiratory function and oxygenation, and consequently, successful extubation, duration of mechanical ventilation and survival.

Rationale:

As the COVID-19 pandemic accelerates, cases have grown exponentially around the world. Other countries' experience suggests that 5-16% of COVID-19 in-patients will undergo prolonged intensive care [4,9,10] with 50-70% needing mechanical ventilation(MV)[3,4,11], threatening to overwhelm hospital capacity[1]. ARDS has no effective treatment besides supportive care, the use of ventilation strategies encompassing low tidal volumes that limit trans-pulmonary pressures, and prone positioning in severe disease [5,6]. Most current trials in clinicaltrials.gov for COVID-19-induced ARDS aim at modulating the inflammatory response or test anti-viral drugs. Sarilumab and tocilizumab that block IL-6 effects are being tested in RCT for patients hospitalized with severe COVID-19 (NCT04317092, NCT04322773, NCT04327388). The World Health Organization international trial

[1] While we recognize that the COVID-19 induced respiratory failure may differ somewhat from the prototypical ARDS, this seems to be the term predominantly used in the current literature.

SOLIDARITY will test remdesivir; chloroquine + hydroxychloroquine; lopinavir + ritonavir; and lopinavir + ritonavir and interferon-beta (NCT04321616). Yet studies targeting the coagulation system, which is intrinsically intertwined with the inflammatory response are lacking [12-16]

A consistent finding in ARDS is the deposition of fibrin in the airspaces and lung parenchyma, along with fibrin-platelet microthrombi in the pulmonary vasculature, which contribute to the development of progressive respiratory dysfunction and right heart failure [17-19]. Similar to pathologic findings of ARDS, microthrombi have now been observed in lung specimens from patients infected with COVID-19 [11,20]. Examples of pulmonary microthrombi from an autopsy specimen of a patient with severe ARDS are shown below.

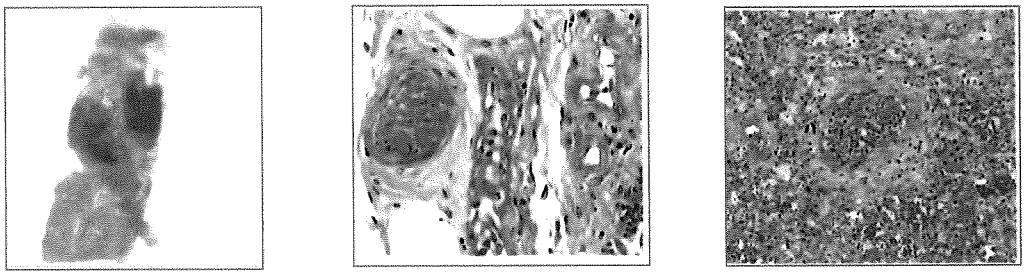


Figure 1. Pulmonary pathology in an autopsy specimen from a COVID-19 ARDS patient. Left panel: Low power view showing extensive pulmonary hemorrhagic changes and infarction. Middle and right panels: microthrombi occluding the pulmonary vasculature.

Inappropriate activation of the clotting system in ARDS results from enhanced activation and propagation of clot formation as well as suppression of fibrinolysis [21-23]. Our group has shown that low fibrinolysis is associated with ARDS [24-28]. Studies starting decades ago have demonstrated the systemic and local effects of dysfunctional coagulation in ARDS, specifically related to fibrin [13-15,29,30]. This occurs largely because of excessive amounts of tissue factor that are produced by alveolar epithelial cells and activated alveolar macrophages [31], and high levels of plasminogen activator inhibitor-1 (PAI-1) produced and released by endothelial cells [32,33]. Consistent with this, generalized derangements of the hemostatic system with prolongation of the prothrombin time, elevated D-dimer and fibrin degradation products have been reported in severely ill COVID-19 patients, particularly in non-survivors [4,34,35]. These laboratory findings, in combination with the large clot burden seen in the pulmonary microvasculature, mirrors that seen in human sepsis [17,36], experimental endotoxemia [37], and massive tissue trauma [24,27]. Targeting the coagulation and fibrinolytic systems to improve the treatment of ARDS has been proposed for at least the past two decades [33,38,39]. In particular, the use of plasminogen activators to limit ARDS progression and reduce ARDS-induced death has received strong support from animal models [40-42], and a phase 1 human clinical trial [7]. In 2001, Hardaway and colleagues [7,43] showed that administration of either urokinase or streptokinase to patients with terminal ARDS reduced the expected mortality from 100% to 70% with no adverse bleeding events. Importantly, the majority of patients who ultimately succumbed died from renal or hepatic failure, rather than pulmonary failure.

Consideration of therapies that are widely available but not recognized for this indication and traditionally considered "high-risk" such as fibrinolytic agents is warranted in this unprecedented public health emergency, since the risk of adverse events from tPA is far outweighed by the extremely high risk of death in the patient's meeting the eligibility criteria for this trial. While the prior

studies by Hardaway et al evaluating fibrinolytic therapy for treatment of ARDS used urokinase and streptokinase, the more contemporary approach to thrombolytic therapy involves the use of tissue-type plasminogen activator (tPA) due to higher efficacy of clot lysis with comparable bleeding risk to the other fibrinolytic agents.

Hypothesis:

We hypothesize that administration of tPA to patients with COVID-19 associated severe ARDS will improve pulmonary gas exchange and oxygenation via a decrease in pulmonary vascular microthrombi.

Study Design:

This is a Phase IIa clinical trial, open label, with a modified stepped-wedge design, testing systemic administration of fibrinolytic therapy with alteplase (tPA) versus standard of care for patients infected with COVID-19 resulting in severe respiratory failure. The design is a rapidly adaptive, pragmatic clinical trial, with 3 interim analyses and 1 final look at the data. Pre-planned adaptations described below will be contemplated at each interim analysis or earlier if recommended by the Data Safety Monitoring Board (DSMB).

Inclusion Criteria: We will include adult patients ages 18-75 years old with known or suspected COVID-19 infection with a PaO2/FiO2 ratio < 150 or inferred PaO2/FiO2 ratio from SpO2 if ABG is unavailable (Table) persisting for > 4 hours despite optimal mechanical ventilation management according to each institution's ventilation protocols, and a neurological exam without focal signs or new deficits at time of enrollment (if patient is on paralytics, patient has been aroused sufficiently to allow a neurological examination to exclude new focal deficits or has MRI/CT scan in the last 4.5 hours with no evidence of stroke. Finally, patients must be on the ventilator for <=10 days to be eligible. Based on experience with critically ill patients, longer ventilation time may be associated with increased risk of bleeding. Patients will be enrolled based on clinical features, without consideration of language (using hospital interpreters and translated consent), race/ethnicity, or gender. A neurological exam or CT/MRI scan to demonstrate no evidence of an acute stroke is needed due to a recent case-report of large-vessel stroke as a presenting feature of COVID-19 in young individuals.[44]

SPO2	FIO2														
	0.3	0.35	0.4	0.45	0.5	0.55	0.6	0.65	0.7	0.75	0.8	0.85	0.9	0.95	1
80%	148	127	111	98	89	81	74	68	63	59	55	52	49	47	44
81%	151	129	113	101	91	82	76	70	65	60	57	53	50	48	45
82%	155	132	116	103	93	84	77	71	66	62	58	55	52	49	46
83%	158	136	119	106	95	86	79	73	68	63	59	56	53	50	47
84%	162	139	122	108	97	89	81	75	70	65	61	57	54	51	49
85%	167	143	125	111	100	91	83	77	71	67	63	59	56	53	50
86%	171	147	129	114	103	94	86	79	73	69	64	61	57	54	51
87%	177	151	132	118	106	96	88	81	76	71	66	62	59	56	53
88%	182	156	137	121	109	99	91	84	78	73	68	64	61	58	55
89%	189	162	141	126	113	103	94	87	81	75	71	67	63	60	57
90%	196	168	147	130	117	107	98	90	84	78	73	69	65	62	59
91%	203	174	153	136	122	111	102	94	87	81	76	72	68	64	61
92%	213	182	159	142	128	116	106	98	91	85	80	75	71	67	64
93%	223	191	168	149	134	122	112	103	96	89	84	79	74	71	67
94%	236	202	177	157	142	129	118	109	101	94	89	83	79	75	71
95%	252	216	189	168	151	138	126	116	108	101	95	89	84	80	76
96%	273	234	205	182	164	149	136	126	117	109	102	96	91	86	82

Patients are eligible to participate even if they are concurrently enrolled in other COVID-19 therapeutic trials, as long as the other trials allow co-enrollment as well.

Absolute Exclusion Criteria (documented at the time of enrollment):

- Active bleeding
- Acute myocardial infarction or history of myocardial infarction within the past 3 weeks or cardiac arrest during hospitalization
- Hemodynamic instability with Noradrenaline >0.2mcg/Kg/min
- Acute renal failure requiring dialysis
- Liver failure (escalating liver failure with total Bilirubin > 3 mg/dL)
- Suspicion of cirrhosis due to history of cirrhosis diagnosis, hepatic encephalopathy, documentation of portal hypertension, bleeding from esophageal varices, ascites, imaging or operative finding suggestive of liver cirrhosis, or constellation of abnormal laboratory test results suggestive of depressed hepatic function
- Cardiac tamponade
- Bacterial endocarditis
- Severe uncontrolled hypertension defined as SBP>185mmHg or DBP>110mmHg
- CVA (stroke), history of severe head injury within prior 3 months, or prior history of intracranial hemorrhage
- Seizure during pre-hospital course or during hospitalization for COVID-19
- Diagnosis of brain tumor, arterio-venous malformation (AVM) or ruptured aneurysm
- Currently on ECMO
- Major surgery or major trauma within the past 2 weeks
- GI or GU bleed within the past 3 weeks
- Known bleeding disorder

- P2Y12 receptor inhibitor medication (anti-platelet) within 5 days of enrollment
- Arterial puncture at a non-compressible site within the past 7 days
- Lumbar puncture within past 7 days
- Pregnancy
- INR > 1.7 (with or without concurrent use of warfarin)
- Platelet count < 100 x 10^9/L or history of HITT
- Fibrinogen < 300mg/dL
- Known abdominal or thoracic aneurysm
- History of CNS malignancy or CNS metastasis within past 5 years
- History of non-CNS malignancy within the past 5 years that commonly metastasizes to the brain (lung, breast, melanoma)
- Prisoner status

Patients will be sequentially assigned to one the following three groups as follows:

1. **Group tPA50** will receive 50 mg of tPA intravenous bolus administration over 2 hours, given as a 10 mg push followed by the remaining 40 mgs over a total time of 2 hrs (not to exceed 0.9mg/kg dose). Immediately following the tPA infusion, 5000 U of UFH will be delivered and the heparin drip will be continued to maintain the activated partial thromboplastin time at 60-80sec (2.0 to 2.5 times the upper limit of normal). This tPA protocol is a modification of the GUSTO I to III trials. [45,46]
2. **Group tPA50 plus drip** will receive 50 mg of tPA intravenous bolus administration over 2 hours, given as a 10 mg push followed by the remaining 40 mgs over a total time of 2 hrs (not to exceed 0.9mg/kg dose). Immediately following this initial tPA infusion, we will initiate a drip of 2 mg/hr tPA over the ensuing 24 hours (total 48 mg infusion) accompanied by an infusion of 500U/hour heparin during the tPA drip. After this, heparin dose will be increased slowly to maintain a PTT between 60 and 80 sec, titrated per attending's discretion. [47]
3. **Control:** standard of care according to the institution's protocol for ARDS

Amendment Nov 4, 2020: please see below description for more detailed information on adaptation. The rationale for adjusting the tPA administration to a 50 mg bolus followed by a 2mg/hr infusion over the ensuing 24 hr is based on the facts that:

1) a convincing improvement in P/F was observed at 24 hr, but was lost over the ensuing 24 hr,
2) there was no discernable response to the second tPA bolus at the 24 hr time point,
3) we believe the initial 50 mg bolus has provoked fibrinolysis shutdown ;ie, PAI-1 generation in response to the tPA bolus blunting a response to a second bolus at 24 hr,
4) an infusion of low dose tPA will consume the PAI-1 generated in response to the initial tPA bolus and provide an additional fibrinolytic intervention over the ensuing 24 hr,
5) the bolus/infusion regimen was employed in the seminal 2001, Hardaway study [7] demonstrating an improvement in oxygenation in patients with advanced ARDS,

6) the 2mg/ml tPA and 500 U heparin is the standard combination employed by interventional radiology for directed fibrinolysis (personal communication Q Meissner MD, Director of IR, UCSD), and
7) we are reticent to increase the tPA dose to 100 mg considering overall risk: benefit.
The above modification was predicted in the original COMIRB submission, in which we anticipated both dose and mode of administration changes is our adaptive plan.
The rationale and change to a bolus/infusion regimen was discussed with the DSMB, and they concurred without reservation (see letter from DSMB, in which they concur with proposed plan).

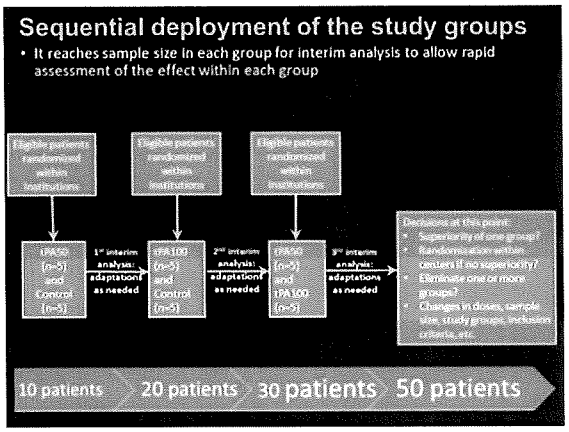
Design:

This is a multicenter (Ernest E Moore Shock Trauma Center at Denver Health Medical Center (DHMC), University of Colorado Hospital (UCH), National Jewish Health / St. Joseph Hospital (NJH/SJH), Beth Israel Deaconess Medical Center (BIDMC), Long Island Jewish Hospital / North Shore University Hospital (LIJH/NSUH), Scripps Health (SH), St. Mary's Medical Center (SM), University of Miami (UM), Ben-Taub General Hospital (BT) and Dallas Methodist (DM)) Phase IIa clinical trial. The design is a pragmatic, open label, randomized, controlled, rapidly adaptive clinical trial, with 3 interim analyses and 1 final look at the data. For rapid efficacy assessment to isolate the arm(s) with the highest likelihood of success and lowest bleeding risk, we will deploy each intervention arm sequentially up to each interim analysis, in a modified stepped-wedge fashion, with pre-planned adaptations (below) at each interim analysis. Pre-planned adaptations described below will be contemplated at each interim analysis or earlier if recommended by the Data Safety Monitoring Board (DSMB).

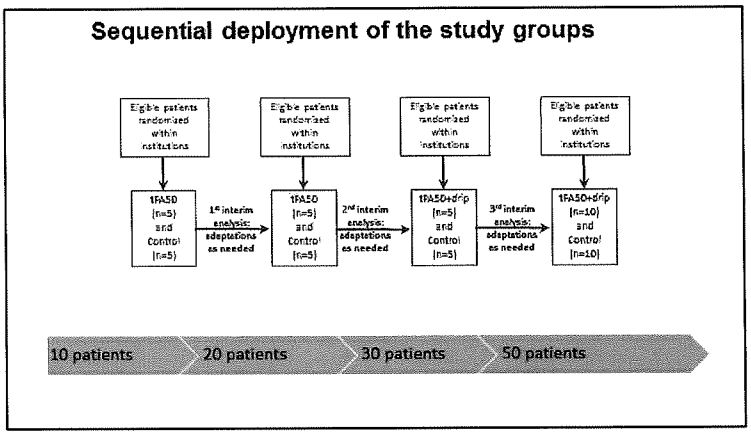
All randomizations are conducted intra-hospital (i.e., no cluster randomization), to avoid the confounding effect of practice variation. Randomization will be conducted in blocks of 10 to allow better distribution between groups at each interim analysis. It will be done by the Data Coordinating Center (DCC) and automated in an web-based REDCap data collection instrument. Upon confirmed eligibility and consent, the REDCap will reveal the group assignment to institution's pharmacy, which will then release the drug if the patient is assigned to the intervention group (Group tPA50, Group tPA50+drip). Time zero is assigned as the time of randomization. We anticipate that each of the five centers will enroll 8-12 patients.

The figure below illustrates the initial planned sequential, stepped-wedge design and the proposed new design after the adaptations driven by the first interim analysis. Study will continue during the interim analyses, unless a safety issue occurs.

INITIAL DESIGN



NEW DESIGN AFTER 1st INTERIM ANALYSIS



Re-bolusing of tPA, at the same dose, is permitted in the tPA50 intervention group in those patients who show an initial transient response (>20% improvement of PaO2/FiO2 over pre-infusion of alteplase at any of the measurements at 2, 6, 12 or 18 hours, but <50% improvement of PaO2/FiO2 at 24 hours after randomization); the repeat dose will be given between 24 and 36 hours after the initial tPA administration.

The same EXCLUSION criteria apply to the second tPA (alteplase) bolus. The heparin drip should be stopped two hours before the second tPA (alteplase) and re-started after the second alteplase infusion ends, at the same dose as before the second alteplase.

Other modifications of the dosing are as follows:

1. **Fibrinogen monitoring:** For all tPA administration groups, fibrinogen levels will be measured before and after tPA IV bolus, 6 hours after the start of the infusion, then every 6 hours for first 24 hours, and once a day for 6 days following treatment intervention in all the groups (see detailed lab testing schedule below). **If fibrinogen levels fall below 300 mg/dL, the second bolus of tPA (alteplase) is NOT indicated.**
2. **Heparin dosing for the tPA50 group and for after the 48mg tPA drip in the tPA50 drip group:** An infusion of unfractionated heparin infusion will be continued for up to 7 days or until the patient is extubated and has an O2 requirement of ≤ 4L/min by nasal cannula, and titrated to maintain the activated partial thromboplastin time to 60-80sec (2.0 to 2.5 times the upper limit of normal) per attending's discretion. In case an additional tPA (alteplase) infusion is needed, the heparin drip should be stopped two hours before and re-started after the alteplase infusion ends, at the same dose as before the alteplase. The goal of this treatment is to prevent recurrent microvascular thrombotic hypoxemia or macrovascular complications (stroke, myocardial infarction or venous thromboembolism) due to possible rebound tPA effects causing hypercoagulability. Intravenous heparin is currently a standard of care (SOC) for COVID-19 patients in the ICU due to their extreme hypercoagulability.

Laboratory Measurements:

1. Arterial blood gases, fibrinogen and D-Dimer levels, PT/INR, aPTT, and CBC. Interval for measurement: before and after tPA IV bolus, 6 hours after the start of the infusion, then every 6 hours for first 24 hours, and once a day for 6 days following treatment intervention in all the groups.
2. Thrombelastography or ROTEM (where available) Interval for measurement: Before and after tPA bolus, 6 hours after the start of the infusion, and every 6 hours for the first 24 hours, then daily thereafter for 6 days or until discharge
3. Troponin and CRP pre-treatment and at 24 hours post-infusion
4. Total Bilirubin test pre-treatment (for baseline liver function)

Laboratory tests will follow the schedule below:

	ABG	CBC with platelet count	PT/ INR	PTT	Fibrinogen	D-dimer	TEG or ROTEM	Troponin	CRP	Total Bilirubin
Pre-Treatment	X	X	X	X	X	X	X	X	X	X
After Treatment	X	X	X	X	X	X	X			
Hour 6	X	X	X	X	X	X	X			
Hour 12	X	X	X	X	X	X	X			
Hour 18	X	X	X	X	X	X	X			
Hour 24	X	X	X	X	X	X	X	X	X	
Hour 48	X	X	X	X	X	X	X			
Hour 72	X	X	X	X	X	X	X			
Hour 96	X	X	X	X	X	X	X			
Hour 120	X	X	X	X	X	X	X			
Hour 144	X	X	X	X	X	X	X			
Hour 168	X	X	X	X	X	X	X			

All patients enrolled in the trial, regardless of randomization group, will follow the above lab testing schedule. Total amount of blood to be collected during 7 days of study is 200mL. We are also planning on using the blood and other tissues leftover after the safety labs (discarded samples) for research purposes.

Additional two tubes (total volume up to 8.0mL) of blood will be drawn at each timepoint to be processed in the research lab.

Thrombo Therapeutics, Inc. is not involved in this study. TPA-challenged TEG assay is used for research purposes only. The data from this test is not accessible for the clinical care providers and

does not affect the patient care. We are using this assay to study the clot sensitivity and as a marker for hypercoagulability. The results of tPA-challenged TEG assay will not be used for any clinical decision or research subject assignment. At the moment there is no plan to submit tPA-challenged TEG data to the FDA.

Follow-up:

Patients will be followed to death or discharge up to 28 days. Laboratory measurements related to the research study, however, will end at day 7 after randomization.

Outcomes:

Primary Outcome: PaO2/FiO2 improvement from pre-to-post intervention at 48 hours post randomization. Ideally, the PaO2/FiO2 will be measured with the patient in the same prone/supine position as in baseline, as change in positions may artificially reduce the improvement attributable to the study drug. However, given the pragmatic nature of the trial, the prone/supine position will be determined by the attending physician, in which case, we will use as an outcome the PaO2/FiO2 closest to the 48 hours obtained prior to the change in position as the outcome.

Secondary outcomes (assessed 48 hours after randomization or as indicated):

- PaO2/FiO2 improvement from pre-to-post intervention at **24 hours** post randomization.
- Amendment Nov 5, 2020: add the outcome "Decrease in ventilatory dead space as estimated by the ventilatory ratio at **48 hours."** Based on a recent study, (Orfanos S, El Husseini I, Nahass T, et al. Observational study of the use of recombinant tissue-type plasminogen activator in COVID-19 shows a decrease in physiological dead space. ERJ Open Res 2020; 6: 00455-2020), this outcome seems to appropriate to assess the efficacy of the intervention. As most of the subjects do not have capnography data, we will be using the ventilatory ratio, which has been shown to correlate well with dead space.
- Achievement of PaO2/FiO2 ≥ 200 or 50% increase in PaO2/FiO2 (whatever is lower)
- National Early Warning Score (NEWS2): based on 7 clinical variables (respiration rate, oxygen saturation, any supplemental oxygen, temperature, systolic blood pressure, heart rate, level of consciousness).
- NIAID ordinal scale: The ordinal scale is an assessment of the clinical status as follows: 1) Death; 2) Hospitalized, on invasive mechanical ventilation or extracorporeal membrane oxygenation (ECMO); 3) Hospitalized, on non-invasive ventilation or high flow oxygen devices; 4) Hospitalized, requiring supplemental oxygen; 5) Hospitalized, not requiring supplemental oxygen - requiring ongoing medical care (COVID-19 related or otherwise); 6) Hospitalized, not requiring supplemental oxygen - no longer requires ongoing medical care; 7) Not hospitalized, limitation on activities and/or requiring home oxygen; 8) Not hospitalized, no limitations on activities. (combined items 7 and 8 as our study is limited to hospital)

- 48 hour in-hospital mortality
- 14 days in-hospital mortality
- 28 days in-hospital mortality
- ICU-free days (up to 28 days)
- In-hospital coagulation-related event-free (arterial and venous) days (up to 28 days)
- Ventilator-free days (up to 28 days)
- Successful extubation (no re-intubation for >3 days after initial extubation)
- Successful weaning from paralysis for > 3 days after initial termination
- Survival to discharge

Sample size and Power Analysis: (Pass, 14.0, NCSS, LLC, Kaysville, Utah, USA)

We anticipate that each of the five centers will enroll 8-12 patients. Sample size and power Analysis was conducted using Pass, vs 14.0 (NCSS, LLC, Kaysville, Utah, USA) and focused on the primary outcome as defined above. Sample size assumptions:

- power=80%, confidence=95%, and 4 sequential tests(3 interim+1 final), using the Pocock spending function to determine test boundaries,
- potential improvement assumptions based on a previous study [40] with appropriate(e.g., Denver) altitude correction as well as mean baseline PaO2/FiO2=149 with an overestimated standard deviation of 100,
- design effect=1.12 due to the study's multicenter nature (intra-class correlation coefficient=0.03, average cluster=5);
- ~20% inflation to account for premature death

A sample size of 50 (25 in each intervention group and 25 in the control group) patients detects a >70% improvement in PaO2/FiO2 between intervention groups (both tPA50 and tPA 50 plus drip) and controls, and >91% improvement in PaO2/FiO2 between intervention tPA 50 plus drip) and controls. Recruitment will assume at least 30% increase to account for refusal or inability to consent.

Consent: We anticipate consenting enough patients to result in 50 eligible patients, thus we plan on a max of 60, to be re-evaluated during each of the interim analyses. LAR, as defined by the state and institutions legislation/policies, will be able to consent

It should be noted that using the traditional (yet arbitrary) confidence level of 95% (alpha=0.05) is not adequate in the current circumstances. The rigid cutoff around what we believe to be a 95% level of certainty, as eloquently put it by Nuzzo in one of the most cited Nature articles [48], is inappropriate. Thus, for all comparisons, we will present the effect size with appropriate confidence intervals depicting the uncertainty surrounding our estimation. The clinical experience of the investigators working together with the independent DSMB will produce the appropriate interpretation of the results, which can then inform current medical decisions and the next Phase III trial.

Criteria for stopping the clinical trial early for efficacy or harm:

Stopping the clinical trial early for efficacy or harm
Reaching adjusted p-value for the primary outcome and at least one of the secondary outcomes at all follow-up time points
DSMB deemed harm profile unacceptable

Criteria for stopping for futility: we will follow the guidelines established by Jitlal et al [49]:

Stopping the clinical trial early for futility
•Low conditional power (<15%), calculated using PASS 14 with bootstrapping simulations, based on the target minimum differences for all primary and secondary outcomes at all follow-up time points.
•Observed difference size in the primary or secondary outcomes favor the control group (<5%) at all follow-up time points.
•The DSMB and trial team agree that enough patients and events have been observed so far to produce a reliable effect
•There are less than 2 centers interested in continuing enrollment
•There is no evidence of an effect in any pre-specified subgroups.
•The DSMB deemed the adverse events profile acceptable (if there are no safety concerns, we may wish to continue to ensure that a modest effect is not missed).

Adaptive design:

The study interim analyses will be used to propose pre-planned modifications based on observed effects, recruitment, eligibility and other aspects of the study as determined below.

- Drop/add study arms: deploying study arms sequentially (vs in-parallel) allows sufficient sample sizes in each arm to assess outcomes and adverse events. Study arms that show significant

improvement may ethically preclude the deployment of other arms. Similarly, study arms which show adverse events (as listed) attributable to the intervention (per trial team with DSMB /IRB determination) or minimal/no improvement may be eliminated. Study arms may be added if concurrent trials demonstrate significant evidence of benefit of a different route, dose, mode of administration of the study drugs.

- Inclusion criteria: although currently the trial entry criteria are based on age and PaO2/FiO2 we recognize the potential role of coagulation assays (for example D-Dimer, fibrinogen, fibrinolysis) in better defining the group most likely to benefit from the fibrinolytic intervention. Thus, such assays may be added as entry criteria if identified as predictors of good results during interim analyses or in other clinical trials. In addition, if stratified analysis on initial PaO2/FiO2 shows benefit or harm in low and moderate PaO2/FiO2, the PaO2/FiO2 level for entry in the study may be modified to increase the probability of benefit.
- Sample size: the current sample size is defined by budget and feasibility constrains, and may prove insufficient if the effect detected is substantial but there is low power to detect it. A larger sample size may be recommended by the trial team and the DSMB, in which case we will pursue additional resources to increase enrollment.
- Cessation rules: based on interim analyses, coagulation and oxygenation variables may become important determinants of benefit/risk for the subjects as explained above, thus these variables may be proposed as further determinants for cessation rules.
- Enrollment/refusal rates
- Crossover: if one treatment arm shows a signal of benefit (as defined in our proposed outcomes), we are under the ethical mandate to offer it to patients who were enrolled in the other arms but did not show improvement. These patients "crossover" to the alternative arm. The analysis will be conducted as an intent-to- treat approach (patients are analyzed according to their initial assigned group) and subsequently in a separate as-treated analysis considering the combination of the two treatments.
- Comparison of prone/supine position: additional arms or change in entry criteria may have to added if the prone position for ventilation is demonstrated to have a major benefit (e.g., criteria for entry may be modified to PaO2/FiO2 <150 in prone position).
- Doses/duration/administration mode of tPA and heparin: as more is learned during this trial as well as other clinical trials about the administration of tPA in relation to other ventilation techniques (prone position, PEEP, pulmonary vasodilators, etc.) and the risk/benefit associated with the doses, duration and model of administration (e.g., bolus versus continuous drip), it may be beneficial for study subjects to modify the study arms.

Proposed amendment 11/5/2020: Per the adaptive plan above, changes in dose and mode of administration of tPA were pre-planned. Based on the first interim analysis and feedback from our DSMB, we propose to change the study arm in the second deployment of the intervention to the same dose and mode of administration of the first phase (i.e. 50mg bolus dose, with a second dose if necessary as prescribed above).

Assuming NO stopping criteria (as described in the protocol) are met, we will proceed as follows:

1) If at the end of the second intervention deployment (total of 20 patients randomized), the primary outcome (PaO2/FiO2 ratio at 48 hours) improves by >= 50%, the study group will continue to receive the dose of the first phase (i.e. 50mg bolus dose, with a second dose if necessary as prescribed above) till the end of the study (total of 50 randomized patients). Interim analyses will be conducted as planned above, and submitted expeditiously to the DSMB, IRB and FDA for approval, yet enrollment will continue while these agencies deliberate and their feedback is received.
2) If at the end of the second intervention deployment (total of 20 patients randomized), the primary outcome improves (PaO2/FiO2 ratio at 48 hours) by >= 20%, or the secondary outcomes PaO2/FiO2 ratio at 24 hours improves >=20%, or dead space decreases by 15%, or ICU- or ventilator-free days continue to exhibit a positive response (at least 2 days difference), we will modify the mode of administration of the second dose of tPA as follows: following the 50mg tPA bolus, we will initiate a drip of 2 mg/hr tPA over the ensuing 24 hours (total 48 mg infusion) accompanied by an infusion of 500 U/hour heparin during the tPA drip. After this, heparin dose will be increased slowly to maintain a PTT between 60 and 80 sec, titrated per attending's discretion. Interim analyses will be conducted as planned above, and submitted expeditiously to the DSMB, IRB and FDA for approval, yet enrollment will continue while these agencies deliberate and their feedback is received.

See above for the rationale for the tPA50+drip.

Statistical analysis (see attached Statistical Analysis Plan):

All analyses will be conducted initially as an intent-to-treat (patients are analyzed in the group they were randomized to), followed by an as-treated analysis. We will use linear mixed models (continuous outcomes, appropriate transformations if residuals normality departure is detected) or generalized estimating equations (categorical outcomes) to account for intra-hospital cluster effects and repeated measures, with FINAL (OVERALL) significance at $p<0.05$ (two-tailed tests). If the groups are not comparable, we will compare relative (as opposed to absolute improvement over baseline (with patients as their own controls) to minimize the effect of baseline disparities. In addition, as the sample permits, we will adjust for confounders using a single propensity score adjustor in an inverse probability weighting approach. In addition, we will compare the "dose" of the intervention (i.e., how much of the treatment the patient received) as the effect of interest. The pre-planned final comparisons include within group (improvement over baseline) and between groups, all two-tailed with significance declared according to the alpha defined above by the Pocock spending method.

There will be no adjustment for multiple outcomes, as all were pre-planned. The investigator-statistician in charge (A. Sauaia, MD, PhD) strongly agrees with the argument that adjustment for multiple comparisons in pre-planned hypotheses leads to more type II errors. [50,51]

Data Storage:

Data will be captured in the University of Colorado web-based REDCap instrument. The data collection form is in production in REDCap.

Criteria for cessation of administering drugs and safety assessments:

The estimated risk of intracranial hemorrhage is 0.72% [45] if total tPA dose is < 1.4 mg/kg over total infusion. Estimated risk of severe or life-threatening bleeding other than intracranial is 0.4% [45] if total tPA dose is <1.4mg/kg over total infusion. Per FDA guidelines will deliver bolus over 2 hours.

Specification of Safety Variables

Safety assessments will consist of monitoring and reporting adverse events (AEs) and serious adverse events (SAEs) per protocol. This includes any AE, defined as **any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product (IMP) or other protocol-imposed intervention, regardless of attribution**.

This protocol is limited to infusion of two IMPs, alteplase and heparin and blood sampling; thus given their relative short half-life of infusion drugs alteplase (<72 minutes) and heparin (<90 minutes), any effect listed below manifesting within 3 hours of administration of the intervention drugs would be considered potentially temporally related to the intervention drugs. Adverse events associated with blood sampling are considered temporally related if happening within 30 minutes of the sampling.

Safety check assessments: methods and timing

Serious adverse events and frequency of safety checks are as follows:

Serious Adverse Events	**Method for safety check**	**Safety check frequency**	**Cessation rule ***
Death	NA	NA	NA
Cardio-pulmonary arrest	NA	NA	Any cardio-pulmonary arrest
Allergic reactions including angioedema	Clinical exam	Clinical exam pre, during and immediately post alteplase infusion; every 6 hours post alteplase infusion up to 24 hours; at least every 24 hours after alteplase infusion during heparin infusion or more frequently if any abnormality detected.	Any allergic reaction
Worsening of neurological function	Clinical neurological exam and imaging if	Clinical exam pre, during and immediately post alteplase infusion; every 6 hours post alteplase infusion up to 24 hours;	GCS decrease of >=2 points or focal deficit within 24 hours of study drug infusion or new

Serious Adverse Events	Method for safety check	Safety check frequency	Cessation rule *
	applicable per care provider's decision. Most patients will use GCS without verbal component	at least every 24 hours after alteplase infusion during heparin infusion or more frequently if any abnormality detected. Imaging per attending's discretion.	hemorrhage on CT-Scan or MRI
Worsening of pulmonary function	Arterial blood gas and ventilation indices		≥30% PaO2/FiO2 baseline reduction
External bleeding	Clinical exam	Clinical exam pre, during and immediately post alteplase infusion; every 6 hours post alteplase infusion up to 24 hours; at least every 24 hours after alteplase infusion during heparin infusion or more frequently if any abnormality detected.	Unresponsive to compression
Gastro-intestinal (GI) bleeding	Clinical exam and hemoglobin	Clinical exam pre, during and immediately post alteplase infusion; every 6 hours post alteplase infusion up to 24 hours; at least every 24 hours after alteplase infusion during heparin infusion or more frequently if any abnormality detected. Endoscopic exam per attending's discretion.	Hemoglobin reduction >3g/dL within 24 hours of study drug intervention or requiring RBC transfusion with suspected GI bleeding
Hemoptysis	Clinical exam	Clinical exam pre, during and immediately post alteplase infusion; every 6 hours post alteplase infusion up to 24 hours; at least every 24 hours after alteplase infusion during heparin infusion or more frequently if any abnormality detected.	Persistent hemoptysis for >=4 hours or compromising airway

Serious Adverse Events	Method for safety check	Safety check frequency	Cessation rule *
		Endoscopic exam per attending's discretion.	
Hematuria	Clinical exam	Clinical exam pre, during and immediately post alteplase infusion; every 6 hours post alteplase infusion up to 24 hours; at least every 24 hours after alteplase infusion during heparin infusion or more frequently if any abnormality detected. Endoscopic exam per attending's discretion.	Persistent hematuria for >=4 hours or urinary obstruction
Retroperitoneal bleeding		Clinical exam pre, during and immediately post alteplase infusion; every 6 hours post alteplase infusion up to 24 hours; at least every 24 hours after alteplase infusion during heparin infusion or more frequently if any abnormality detected. Endoscopic exam per attending's discretion.	Hemoglobin reduction >3g/dL within 24 hours of infusion of study drug infusion or requiring RBC transfusion
Tube thoracotomy	Clinical exam and Hgb	Clinical exam pre, during and immediately post alteplase infusion; every 6 hours post alteplase infusion up to 24 hours; at least every 24 hours after alteplase infusion during heparin infusion or more frequently if any abnormality detected.	Hemoglobin reduction >3g/dL within 24 hours of infusion of study drug infusion or requiring RBC transfusion
Any of the below listed exclusion criteria developing during or up to 3 hours after alteplase or heparin infusion. **	Clinical exam and laboratory		Any of listed exclusion criteria developing during or up to 3 hours after alteplase or heparin infusion.

*criteria or attending's decision

** criteria: Acute myocardial infarction; Acute Renal failure requiring dialysis; Liver failure (escalating liver failure with total Bilirubin > 3 mg/dL); Cardiac tamponade; Bacterial endocarditis; Severe

uncontrolled hypertension defined as SBP>185mmHg or DBP>110mmHg; Seizure; Placement on ECMO; Major surgery required; Requirement of lumbar puncture; INR > 1.7; Platelet count < 100 x 10^9/L or history of HITT.

Reporting of adverse events:

Any adverse event will be immediately and properly reported to IRB and DSMB, FDA and funding agency for discussion and feedback per regulatory guidelines.

Major Milestones, Tasks, Timelines and Deliverables

			Week From Secured Funding									Week 16
	Done	**Essential For Initiation**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**		
PREPARATION												
Establish Core Research Team	X											
Review existing literature	X											
Implement Protocol	X											
Finalize Study Protocol	X											
Finalize Statistical Analysis Plan	X											
Finalize Case Report Form	X											
Enlist Collaborating Institutions	X											
Submit Protocol for Funding	X											
Review Protocol Data	X											
REGULATORY OVERSIGHT												
FDA IND		X										
IRB Approval		X										
ClinicalTrial.gov Registration		X										
FUNDING SECURED												
Secure DSMB			X									
Hire Research Team	X											
INITIATE STUDY PROTOCOL AT CORE INSTITUTIONS												
Patient Enrollment and Group Assignments				X	X	X						
Adaptive Refinement					X	X	X					
EXPAND TO COLLABORATING												

			Week From Secured Funding									Week 16
	Done	**Essential For Initiation**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**		
INSTITUTIONS AS APPROPRIATE												
National								X				
DELIVERABLES												
First Interval Analysis					X	X						
Second Interval Analysis							X	X				
Third Interval Analysis								X	X			
Final Data Analysis										X		
Report to Funding Agency						X		X		X		
SUBMIT STUDY RESULTS TO PUBLICATION												X

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