




METHODOLOGICAL ARTICLE

Study of alteplase for respiratory failure in severe acute respiratory syndrome coronavirus 2/COVID-19: Study design of the phase IIa STARS trial

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Abstract

Background: The coronavirus disease 2019 (COVID-19) pandemic has caused a large surge of acute respiratory distress syndrome (ARDS). Prior phase I trials (non-COVID-19) demonstrated improvement in pulmonary function in patients ARDS using fibrinolytic therapy. A follow-up trial using the widely available tissue-type plasminogen activator (t-PA) alteplase is now needed to assess optimal dosing and safety in this critically ill patient population.

Objective: To describe the design and rationale of a phase IIa trial to evaluate the safety and efficacy of alteplase treatment for moderate/severe COVID-19-induced ARDS.

Patients/Methods: A rapidly adaptive, pragmatic, open-label, randomized, controlled, phase IIa clinical trial will be conducted with 3 groups: intravenous alteplase 50 mg, intravenous alteplase 100 mg, and control (standard-of-care). Inclusion criteria are known/suspected COVID-19 infection with PaO₂/FiO₂ ratio <150 mm Hg for > 4 hours despite maximal mechanical ventilation management. Alteplase will be delivered through an initial bolus of 50 mg or 100 mg followed by heparin infusion for systemic anticoagulation, with alteplase redosing if there is a >20% PaO₂/FiO₂ improvement not sustained by 24 hours.

Results: The primary outcome is improvement in PaO₂/FiO₂ at 48 hours after randomization. Other outcomes include ventilator- and intensive care unit-free days, successful extubation (no reintubation ≤3 days after initial extubation), and mortality. Fifty eligible patients will be enrolled in a rapidly adaptive, modified stepped-wedge design with 4 looks at the data.

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Conclusion: Findings will provide timely information on the safety, efficacy, and optimal dosing of t-PA to treat moderate/severe COVID-19-induced ARDS, which can be rapidly adapted to a phase III trial (NCT04357730; FDA IND 149634).

KEYWORDS

acute respiratory distress syndrome, clinical trial, coagulopathy, COVID-19, fibrinolysis shutdown, tissue-type plasminogen activator

Essentials

- Fibrinolytics have been used in phase I trials to treat acute respiratory distress syndrome (ARDS) with efficacy and low risk of bleeding complications.
- Coronavirus disease 2019 (COVID-19) is associated with microthrombi of the lungs and ARDS.
- STARS is an adaptive, pragmatic, open-label, phase IIa trial using tissue-type plasminogen activator (t-PA) to treat COVID-19-related ARDS.
- Two different dosing regimens of t-PA will be used to test for efficacy in improving oxygenation at 48 hours from study enrollment.

1 | INTRODUCTION

The worldwide incidence of coronavirus disease 2019 (COVID-19) continues to rise, taxing the health care and economic resources of countries throughout the developed world. Based on the clinical experience in China and Italy, it is estimated that 5% to 27% of hospitalized patients with COVID-19 will require prolonged intensive care,¹⁻⁶ with 50% to 99% requiring mechanical ventilation (MV) for viral-induced pneumonitis progressing to acute hypoxemic respiratory failure and acute respiratory distress syndrome (ARDS).^{2,7,8} In patients requiring MV, the reported mortality exceeds 50%^{7,9} and approached 90% in a recent report from New York City.¹⁰ There is no specific treatment for COVID-19 ARDS other than routine mechanical ventilation, although prone positioning seems to be particularly effective in this population,¹¹ either as a consequence of enhanced alveolar drainage or redistribution of perfusion to better aerated portions of the lungs.

A remarkable feature of the pulmonary pathophysiology in COVID-19 ARDS is the preservation of relatively normal lung compliance and a low incidence of barotrauma¹² suggesting extensive shunting, ventilation-perfusion mismatch, and loss of regulation of alveolar perfusion. Autopsy and surgical specimens in these patients show a range of pathologic findings including diffuse alveolar damage, fibrin accumulation in the alveoli, the

presence of mononuclear cell infiltrates and megakaryocytes, as well as fibrin-platelet microthrombi in the pulmonary vasculature.^{1,3} The concept of accumulation of pulmonary microthrombi leading to death dates back to 1845.¹³ The angiographic appearance of filling defects of the pulmonary vasculature in patients with ARDS has been associated with a high mortality rate for decades.¹⁴⁻¹⁷ Animal models of irreversible shock have demonstrated clots in organs driving organ failure.^{18,19} This can be reversed with preemptive heparin^{20,21} or postshock fibrinolytics.²² Autopsies of critically ill patients also demonstrated clots in the organs of patients in the intensive care unit who died from organ failure.^{19,23} These observations were eventually translated to 2 separate phase I human trials,^{24,25} which were not followed up.

Given these vascular and hematologic findings and the distinct nature of the COVID-19 ARDS, with preserved pulmonary mechanics, we postulate that this advanced ARDS is due to the microthrombosis and resistance to clot lysis in the pulmonary circulation. We believe these factors directly contribute to the high-shunt type of hypoxemic respiratory failure seen in COVID-19 ARDS. We hypothesized that administration of alteplase, a tissue-plasminogen activator (t-PA), followed by systemic anticoagulation will improve the PaO₂/FiO₂ ratio 48 hours after treatment.

1.1 | Objective

We aimed to describe the design and rationale of a phase IIa trial (NCT04357730) that will evaluate the safety and efficacy of tPA (alteplase) treatment for moderate to severe ARDS in the setting of COVID-19 infection.

2 | METHODS

This is a phase IIa, open-label clinical trial with a modified stepped-wedge design, testing systemic administration of fibrinolytic therapy with alteplase (using Activase manufactured by Genentech, Inc) versus standard of care for patients infected with COVID-19 resulting in severe ARDS. The study is registered at clinicaltrials.gov (NCT04357730), has received approval to proceed by the US Food and Drug Administration (FDA; IND 149634), and by all institutions' institutional review boards (IRBs). The design is a rapidly adaptive, pragmatic clinical trial, with 3 interim analyses and 1 final look at the data. Preplanned adaptations described below will be contemplated at each interim analysis or earlier if recommended by the Data Safety Monitoring Board (DSMB).

2.1 | Inclusion criteria

We will include patients ages 18-75 years, with known or suspected COVID-19 infection, with a normal neurological exam at time of enrollment (if patient is on paralytics, the patient has been awakened and showed no new neurological deficits in a complete neurological exam or had a magnetic resonance imaging (MRI)/computed tomography (CT) scan in the past 4.5 hours with no evidence of stroke), with a PaO₂/FiO₂ ratio <150 mm Hg (at sea level or adjusted for altitude) persisting for >4 hours despite maximal MV management according to each institution's ventilation protocols (FiO₂ ≥ 60% and positive end-expiratory pressure [PEEP] ≥ 10 cm H₂O). If obtaining arterial blood gases is not possible due to a surge-related shortage of blood gas syringes, as we have experienced previously, we will infer the PaO₂/FiO₂ ratio from percent saturation of hemoglobin with oxygen as measured by pulse oximetry (SpO₂), using the nonlinear imputation developed by the National Heart, Lung, and Blood Institute's PETAL (Prevention and Early Treatment of Acute Lung Injury) Network Collaborators.²⁶ A normal neurological exam or CT/MRI scan to demonstrate no evidence of an acute stroke is needed due to recent reports of large-vessel stroke as a presenting feature of COVID-19 in young individuals.²⁷

Patients will be enrolled based on clinical characteristics, without consideration of language (using hospital interpreters and translated consent), race/ethnicity, or sex/gender. Patients are eligible to participate even if they are concurrently enrolled in other COVID-19 therapeutic trials. Exclusion criteria are listed in Table 1.

There are 3 treatment arms:

TABLE 1 Inclusion and exclusion criteria for the STARS trial

Inclusion criteria: age 18-75 y old with known or suspected COVID-19 infection with a PaO₂/FiO₂ ratio <150 (at sea level) or (inferred PaO₂/FiO₂ ratio from SpO₂ if an arterial blood gas is unavailable) persisting for >4 h despite maximal mechanical ventilation management according to each institution's ventilation protocols.

Absolute exclusion criteria (documented at the time of enrollment):

Stroke or inability to demonstrate a normal neurological exam unless a CT scan within 4.5 h of enrollment excludes a cerebral vascular event

Active bleeding

Acute myocardial infarction or history of myocardial infarction within the past 3 wk or cardiac arrest during hospitalization

Hemodynamic instability with noradrenaline >0.2 µg/kg/min

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 >185 mm Hg or DBP >110 mm Hg

History of severe head injury within prior 3 mo, or prior history of intracranial hemorrhage

Seizure during prehospital course or during hospitalization for COVID-19

Diagnosis of brain tumor, arteriovenous malformation (AVM), or ruptured aneurysm

Currently on ECMO

Major surgery or major trauma within the past 2 wk

GI or GU bleed within the past 3 wk

Known bleeding disorder

Arterial puncture at a noncompressible site within the past 7 d

Lumbar puncture within past 7 d

Pregnancy

INR > 1.7 (with or without concurrent use of warfarin)

Platelet count <100 × 10⁹/L or history of HIT

Fibrinogen <300 mg/dL

Known abdominal or thoracic aneurysm

History of CNS malignancy or CNS metastasis within past 5 y

History of non-CNS malignancy within the past 5 y that commonly metastasizes to the brain (lung, breast, melanoma)

Prisoner status

1. Group tPA50 (n = 20) will receive 50 mg of alteplase intravenous bolus administration over 2 hours, given as a 10-mg push followed by the remaining 40 mg over a total time of 2 hours. Immediately following the alteplase infusion, 5000 U of unfractionated heparin (UFH) will be delivered; the heparin drip will be continued to maintain the activated partial thromboplastin time (aPTT) at 60 to 80 seconds (2.0-2.5 times the upper limit of normal). This t-PA protocol is a modification of the GUSTO (Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries) I to III trials.^{28,29}

2. Group tPA100 ($n = 20$) will receive 100 mg of t-PA intravenous bolus administration over 2 hours, given as a 10-mg push followed by the remaining 90 mg over a total time of 2 hours. Immediately following the t-PA infusion, 5000 U of UFH will be delivered, and the heparin drip will be continued to maintain the aPTT at 60 to 80 seconds (2.0-2.5 times the upper limit of normal). This t-PA protocol is similar to that used by Konstantinides et al.³⁰
3. Control: institution's standard-of-care protocol for ARDS.

Rebolusing of tPA is permitted in the first two intervention groups, particularly in those patients who show an initial transient response, but is not sustained (<50% PaO₂/FiO₂ improvement by 24 hours). All exclusion criteria (Table 1) also apply to the second t-PA (alteplase) bolus.

Other modifications of the alteplase dosing are as follows:

1. Fibrinogen monitoring: For all t-PA administration groups, fibrinogen levels will be measured before and after t-PA intravenous 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 t-PA (alteplase) will not be given.
2. Heparin dosing: An infusion of unfractionated heparin will be continued for up to 7 days or until the patient is extubated and has an O₂ requirement of ≤ 4 L/min by nasal cannula, and titrated to maintain the activated partial thromboplastin time to 60 to 80 seconds (2.0-2.5 times the upper limit of normal). 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 t-PA effects causing hypercoagulability. If necessary, an infusion of antithrombin concentrate will be administered in heparin-resistant patients.

2.2 | Diverse positioning and/or paralytic agents for ventilation

If the position or use of paralytics must be changed before the 24- and 48-hours post-randomization, the PaO₂/FiO₂ measured immediately before these changes (within <6 hours of the 48-hour postrandomization end point) will be used as primary outcome.

2.3 | Outcomes

The primary outcome of interest is change in PaO₂/FiO₂ at 48 hours from randomization. Secondary outcomes are listed in Table 2.

2.4 | Rapidly adaptive design (Figure 1)

The design is a rapidly adaptive, pragmatic clinical trial, with 3 interim analyses and 1 final look at the data, with test boundaries

determined by the Pocock method to maintain overall experiment error at <0.05. Preplanned adaptations described below will be contemplated at each interim analysis or earlier if recommended by the DSMB. 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 fashion³¹, with preplanned adaptations(below) at each interim analysis Figure 1.

2.5 | Data collection and storage

Study data will be collected and managed using REDCap (Research Electronic Data Capture) electronic data capture tools hosted by the University of Colorado Anschutz Medical Campus.³²

2.6 | Randomization

All randomizations will be conducted intrahospital (ie, no cluster randomization) to avoid the confounding effect of practice variation, in blocks of 10 to allow better distribution between groups at each interim analysis. It will be done by the Data Coordinating Center and automated in a REDCap instrument. Upon confirmed eligibility and consent, the REDCap instrument will reveal the assignment (Group tPA50, Group tPA100, Control) to the pharmacy of the enrolling institution, which will then release the drug if the patient was assigned to one of the intervention groups. Time 0 is assigned as the time of randomization. We anticipate that each of the 5 centers will enroll 5 to 10 patients.

2.7 | Sample size rationale

The sample size was fixed at $n = 50$ (with 20 patients in each intervention group and 10 patients in the control group) due to budgetary and feasibility constraints. The minimum detectable difference was then calculated using PASS version 14.0 (NCSS LLC, Kaysville, UT, USA), focusing on the primary outcome (PaO₂/FiO₂ improvement) and assuming (i) power = 80%, confidence = 95%, and four sequential tests (3 interim + 1 final), using the Pocock method to determine test boundaries; (ii) potential improvement assumptions based on a previous study²⁵ as well as a more favorable scenario with mean baseline PaO₂/FiO₂ = 149 with an overestimated standard deviation of 100, (iii) design effect = 1.12 due to the study's multicenter nature (intraclass correlation coefficient = 0.03,^{33,34} average cluster = 5); and (iv) 20% inflation to account for premature death or withdrawal for any reasons.

A sample size of 50 (20 in each intervention group and 10 in the control group) patients would detect a $\geq 68\%$ improvement in PaO₂/FiO₂ between the 2 intervention groups and $\geq 73\%$ improvement between intervention groups and controls. While balanced group sizes will maximize a study's statistical power, unequal randomization ratios will only significantly reduce the power of a study if the ratio is

TABLE 2 Primary and secondary outcomes for the STARS trial

Outcome	Timing
Primary outcome: PaO ₂ /FiO ₂ improvement from pre-to-post intervention	48 h after randomization.
Secondary outcomes	
Achievement of PaO ₂ /FiO ₂ ≥200 or 50% increase in PaO ₂ /FiO ₂ (whichever is lower)	48 h after randomization
National Early Warning Score (NEWS) ^a	48 h after randomization
NIAID ordinal scale ^b	14 d
Reduction of FiO ₂ <80% (if started on higher concentration)	
Return to supine or lateral position (if started in prone position)	
Reduction of positive end expiratory pressure	
Reduction of inhaled prostanoids (if started before t-PA therapy)	
Reduction of inhaled nitric oxide (if started before t-PA therapy)	
Reduction of paralytic (if on before t-PA therapy)	
Receiver operating characteristic curve of coagulation variables associated with achievement a PaO ₂ /FiO ₂ >50%	
Receiver operating characteristic curve of coagulation variables associated with bleeding complications	
48 h in-hospital mortality	
14 d in-hospital mortality	14 d
28 d in-hospital mortality	28 d
ICU-free days (up to 28 d)	28 d
In-hospital coagulation-related event-free (arterial and venous) days (up to 28 d)	28 d
Ventilator-free days (up to 28 d)	28 d
Successful extubation (no reintubation <3 d after initial extubation)	28 d
Survival to discharge	Discharge

^a National Early Warning Score (NEWS2): based on 7 clinical parameters (respiration rate, oxygen saturation, any supplemental oxygen, temperature, systolic blood pressure, heart rate, level of consciousness); ^bNIAID ordinal scale: The ordinal scale is an assessment of the clinical status as follows: (i) death; (ii) hospitalized, on invasive mechanical ventilation or extracorporeal membrane oxygenation; (iii) hospitalized, on noninvasive ventilation or high-flow oxygen devices; (iv) hospitalized, requiring supplemental oxygen; (v) hospitalized, not requiring supplemental oxygen—requiring ongoing medical care (COVID-19 related or otherwise); (vi) hospitalized, not requiring supplemental oxygen—no longer requires ongoing medical care; (vii) not hospitalized, limitation on activities and/or requiring home oxygen; (viii) not hospitalized, no limitations on activities (we will combine 7 and 8 as discharge from hospital to home, as the trial is limited to in-hospital morbidity/mortality).

3:1 or more. Reasons for the unequal randomization include (i) more safety information, an essential component of a phase IIa study; (ii) experience with dosing of t-PA; and (iii) to allow 3 equal sequential phases that would inform the remainder of the trial.³⁵ The initial 2 phases (t-PA 50 mg vs control; t-PA 100 mg vs control) will provide a signal that allows the termination of the control arm.

Recruitment will assume at least a 30% increase to account for refusal or inability to consent. We anticipate enrolling enough individuals to result in a sample of 50 eligible patients, to be reevaluated during each of the interim analyses. A legally authorized representative, as defined by each state and each institution's legislation and policies, will be able to consent.

2.8 | Criteria for stopping the clinical trial early for efficacy or harm

Criteria include reaching adjusted *P* value for the primary outcome and at least 1 of the secondary outcomes at all follow-up time points, or DSMB deemed the harm profile unacceptable.

2.9 | Criteria for stopping for futility

We will follow the guidelines established by Jitlal et al³⁶. These criteria are (i) low conditional power (<15%), calculated using bootstrapping simulations, based on the target minimum differences for all primary and secondary outcomes; (ii) observed difference size in the primary or secondary outcomes favor the control group (<5%); (iii) the DSMB and trial team agree that enough patients and events have been observed so far to produce a reliable effect; (iv) only 1 center interested in continuing enrollment; and (v) no evidence of an effect in any prespecified subgroups. If the DSMB deemed the adverse events profile acceptable, we may wish to continue to ensure that a modest effect is not missed.

2.10 | Preplanned adaptations at each interim analysis

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

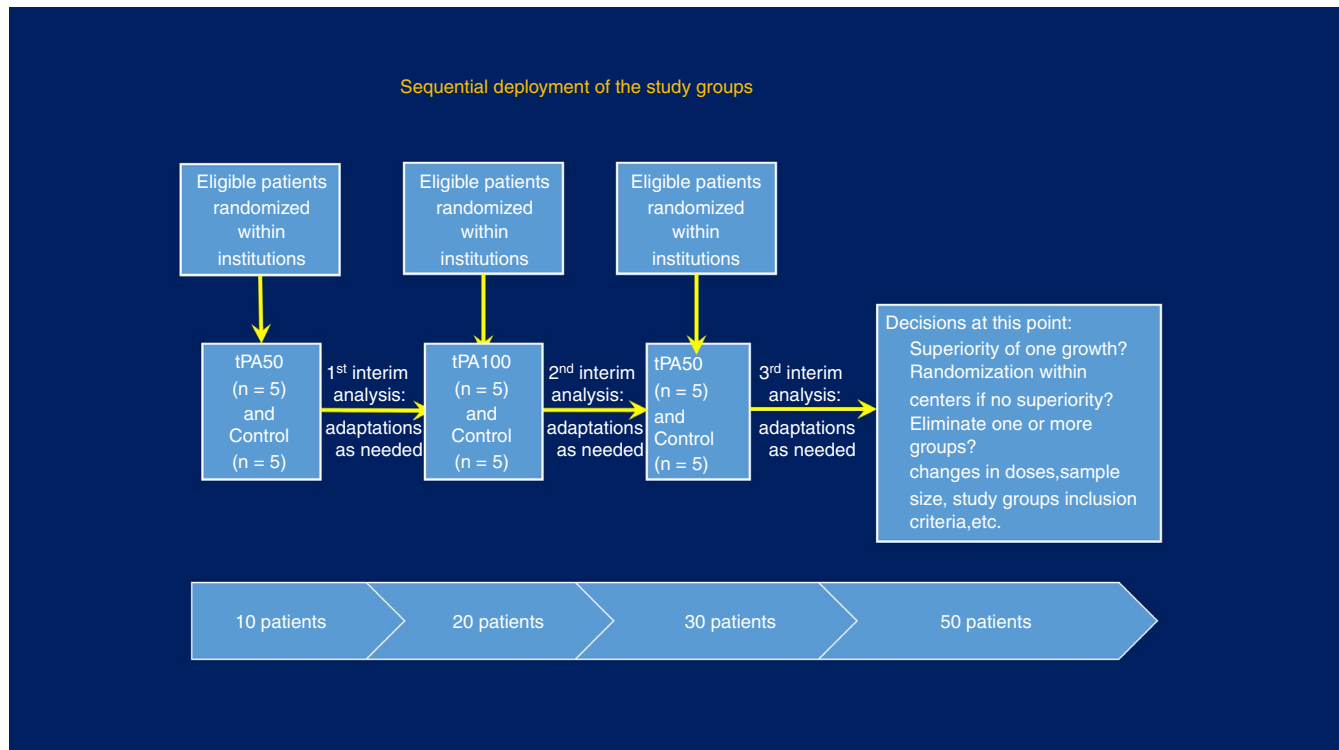


FIGURE 1 Study design of the STARS trial

- 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 that 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, or mode of administration of the study drugs.
- Inclusion criteria: Although currently the trial entry criteria are based on age and $\text{PaO}_2/\text{FiO}_2$, we recognize the potential role of coagulation assays (eg, 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 the stratified analysis on initial $\text{PaO}_2/\text{FiO}_2$ shows benefit or harm in low and moderate $\text{PaO}_2/\text{FiO}_2$, the $\text{PaO}_2/\text{FiO}_2$ 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 constraints 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: Modifications on enrollment and consent procedure may be proposed to remedy low enrollment and high refusal rate. One potential alternative is the addition of an observational arm as done by Pieracci et al.³⁷
- Crossover: If 1 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 “cross over” 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 2 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 (eg, criteria for entry may be modified to $\text{PaO}_2/\text{FiO}_2 < 150$ in prone position).
- Doses/duration/administration mode of t-PA and heparin: As more is learned during this trial as well as other clinical trials about the administration of t-PA 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 (eg, bolus vs continuous drip), it may be beneficial for study subjects to modify the study arms.

2.11 | Laboratory measurements

Laboratory tests obtained on all patients will include:

1. Arterial blood gases, fibrinogen and D-dimer levels, prothrombin time/International Normalized Ratio, aPTT, C-reactive protein, and complete blood count with platelet count: pre and post alteplase intravenous bolus (only baseline for controls), then every 6 hours for the first 24 hours, and once a day for 7 days (or earlier if patient is extubated) following treatment intervention in all the groups.
2. Thrombelastography (TEG) or rotational thromboelastometry (ROTEM) (where available): pre and post alteplase intravenous bolus (baseline for controls), then every 6 hours for the first 24 hours, and once a day for 7 days (or earlier if patient is extubated) following treatment intervention in all the groups.
3. Troponin, creatinine, bilirubin, alanine aminotransferase, and creatine kinase pre-alteplase (baseline for controls) and at 24 and 72 hours after infusion.

2.12 | Safety assessment

Safety considerations and monitoring with plan during the intervention administration are listed in Table 3. The estimated risk of the most feared complication, intracranial hemorrhage, is estimated to be 0.72%²⁸ if total t-PA dose is <1.4 mg/kg over total infusion. Estimated risk of severe or life-threatening bleeding other than intracranial is 0.4%³⁸ if total t-PA dose is <1.4 mg/kg over total infusion. Given the emergence of data on the increased rate of thrombotic strokes in young patients with COVID-19,²⁷ exclusion of stroke prior to fibrinolytic therapy is essential. The inability to demonstrate a normal neurological exam unless a CT scan within 4.5 hours³⁹ of enrollment excludes a cerebral vascular event represents an absolute exclusion to study enrollment. Safety assessments will consist of monitoring and reporting adverse events and serious adverse events per protocol. This includes any adverse event, defined as any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of alteplase or other protocol-imposed intervention (eg, heparin, blood sampling). Given alteplase and heparin relative short half-life (<72 minutes and <90 minutes, respectively), any adverse event 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. Methods and timing of safety check assessments are listed in Table 3.

2.13 | Follow-up

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

2.14 | Statistical analysis

The statistical analysis plan followed the recently published guidelines⁴⁰ and is available in the Supplemental Material. All outcome variables will be examined for distribution. If very skewed, we will attempt log and Box-Cox power transformations to approximate normality. If those are unsuccessful, the outcomes will be categorized using the median or previously defined cutoff. All outcomes will also be analyzed as relative change from baseline. Effectiveness of the randomization to determine baseline comparability of the groups will be done using the absolute standardized mean difference (SMD <0.20 defined as acceptable balance). Any differences deemed clinically relevant or with absolute SMD >0.2 will be adjusted for using inverse probability weighting methods as described below.

All outcome comparison 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. The primary outcome will be assessed within groups and between groups. Differences in the primary outcome will be evaluated using linear mixed models, with appropriate transformations if normality departure of residuals is detected. Linear mixed models allow (i) adjustment for potential confounders detected in the comparison of the groups at baseline using inverse probability weighting by a propensity score; and (ii) change in the covariance structure to account for repeated measures and the intrahospital correlation (as this is a multicenter study). In addition, it tolerates missing observations. We will also compare percent change over baseline, using *t* tests with the appropriate adjustment for heteroscedasticity if needed. Categorical outcomes will be compared using generalized estimating equations to account for confounders (as above), covariance structure and intrahospital correlation. In addition, we will compare the “dose” of the intervention (ie, how much of the treatment the patient received) as an effect of interest, as premature death and withdrawals are expected. Survival analysis with inverse probability weighted Cox proportional hazards model and robust sandwich variance estimate to account for clustering for hospitals will be used for mortality as well as for survivor-bias subject outcomes (eg, pulmonary embolism) censoring for death. As all outcomes are in-hospital, loss to follow-up is not likely. The preplanned comparisons include within group (improvement over baseline) and between groups, all 2-tailed with significance declared as defined by the Pocock spending method.

There will be no adjustment for multiple outcomes, as all were preplanned. Adjustments for multiple comparisons in preplanned hypotheses leads to more type II errors.^{41,42}

TABLE 3 Safety check assessments: methods and timing

Serious adverse events	Method for safety check	Safety check frequency	Cessation rule ^a
Death	NA	NA	NA
Cardiopulmonary arrest	NA	NA	Any cardiopulmonary arrest
Allergic reactions including angioedema	Clinical exam	Clinical exam before, during, and immediately after alteplase infusion; every 6 h after alteplase infusion up to 24 h; at least every 24 h 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 applicable per care provider's decision. Most patients will use GCS without verbal component	Clinical exam before, during, and immediately after alteplase infusion; every 6 h post alteplase infusion up to 24 h; at least every 24 h after alteplase infusion during heparin infusion or more frequently if any abnormality detected Imaging per attending's discretion	GCS decrease of >2 points or focal deficit within 24 h of study drug infusion or new hemorrhage on CT scan or MRI
Worsening of pulmonary function	Arterial blood gas and ventilation indices	Every 6 h in the first 24 h and every 12 until 48 h; if second alteplase dose, every 6 h until 48 h	>30% PaO ₂ /FiO ₂ baseline reduction
External bleeding	Clinical exam	Clinical exam before, during, and immediately after alteplase infusion; every 6 h post alteplase infusion up to 24 h; at least every 24 h after alteplase infusion during heparin infusion or more frequently if any abnormality detected	Unresponsive to compression
Gastrointestinal bleeding	Clinical exam and hemoglobin	Clinical exam before, during, and immediately after alteplase infusion; every 6 h post alteplase infusion up to 24 h; at least every 24 h after alteplase infusion during heparin infusion or more frequently if any abnormality detected. Endoscopic exam per attending's discretion.	Hemoglobin reduction >3 g/dL within 24 hours of study drug intervention or requiring RBC transfusion with suspected gastrointestinal bleeding
Hemoptysis	Clinical exam	Clinical exam before, during, and immediately after alteplase infusion; every 6 h after alteplase infusion up to 24 h; at least every 24 h after alteplase infusion during heparin infusion or more frequently if any abnormality detected. Endoscopic exam per attending's discretion.	Persistent hemoptysis for ≥4 h or compromising airway
Hematuria	Clinical exam	Clinical exam before, during, and immediately after alteplase infusion; every 6 h post alteplase infusion up to 24 h; at least every 24 h after alteplase infusion during heparin infusion or more frequently if any abnormality detected. Endoscopic exam per attending's discretion.	Persistent hematuria for ≥4 h or urinary obstruction
Retroperitoneal bleeding		Clinical exam pre, during and immediately post alteplase infusion; every 6 h post alteplase infusion up to 24 h; at least every 24 h after alteplase infusion during heparin infusion or more frequently if any abnormality detected. Endoscopic exam per attending's discretion.	Hemoglobin reduction >3 g/dL within 24 h of infusion of study drug infusion or requiring RBC transfusion
Tube thoracotomy	Clinical exam and Hgb	Clinical exam before, during, and immediately after alteplase infusion; every 6 h post alteplase infusion up to 24 h; at least every 24 h after alteplase infusion during heparin infusion or more frequently if any abnormality detected	Hemoglobin reduction >3 g/dL within 24 h of infusion of study drug infusion or requiring RBC transfusion
Any of the below listed criteria developing during or up to 3 h after alteplase or heparin infusion ^b	Clinical exam and laboratory		Any of listed exclusion criteria developing during or up to 3 h after alteplase or heparin infusion, except for fibrinogen, for which we will set cessation cutoff at 100 mg/dL

^aCriteria or attending's decision.

^bCriteria: Acute myocardial infarction; acute renal failure (escalating renal failure with creatinine >3 times baseline); liver failure (escalating liver failure with ALT >3 times baseline); cardiac tamponade; bacterial endocarditis; severe uncontrolled hypertension defined as SBP >185 mm Hg or DBP >110 mm Hg; seizure; placement on ECMO; major surgery required; requirement of lumbar puncture; INR >1.7; platelet count <100 × 10⁹/L or history of HIT; fibrinogen <100 mg/dL.

2.15 | Preplanned subgroup analyses

We anticipate the following subgroup analyses, which will assist in determining whether there is a subgroup of patients for whom the intervention is more beneficial/harmful: (i) baseline PaO₂/FiO₂ < 100 and < 50; (ii) hemodynamic instability with vasopressors; (iii) age <35, 36-50, 51-65, 66-75 years; (iv) D-dimers median levels; (v) fibrinolysis shutdown (by TEG or ROTEM); (vi) fibrinogen median levels; (vii) prone/supine positioning; (viii) requirement of rebolusing of Iteplase; (ix) received dose of alteplase as premature death or adverse event or other reasons may preclude the administration of complete treatment; and (x) elimination of centers contributing <2 cases.

Additional subgroup analyses may be defined at an interim analysis and will be added for the subsequent interim analyses. This will be documented by filing another version of this statistical analysis plan with the IRBs, DSMB, funder, and FDA.

2.16 | Missing data

Missing data are expected to be minimal. If <15% and nondifferential between study groups, we will proceed with analyses of complete data. If >15% or differential between groups (possibly missing not at random), we will add 2 strategies to the complete data set analyses: (i) multiple imputation by chained equations (MICE), recognizing that MICE is better for missing at random data; and (ii) sensitivity analyses: we will assume worst and best clinical scenarios and compare the results with the complete data set.

3 | DISCUSSION

The mechanistic rationale for the administration of t-PA (alteplase) is based on (i) autopsy findings of patients who succumbed to COVID-19 ARDS showing presence of microthrombi in the lungs and other organs^{1,3}; (ii) COVID-19 poor outcomes associated with hypercoagulability/hypofibrinolysis⁴³⁻⁴⁵; (iii) the high rate of pulmonary thrombotic complications while on prophylactic heparin dosing⁴⁶; (iv) and previous demonstrations of improved pulmonary function in ARDS with plasminogen activators with no bleeding complications.²⁵ The pathophysiology of COVID can be conceptualized into an early phase, dominated by symptoms directly attributable to the virus, and a later phase manifesting the consequences of the inflammatory response to the invading pathogen. Much of the therapy currently being investigated focuses on reducing the viral load and attenuating cell entry lacking and reducing inflammation.⁴⁷⁻⁵¹ While the conspicuous hypercoagulable state has been widely documented, the role of suppressed fibrinolysis remains largely overlooked.

COVID-19 has a clear association with thrombotic complications, which predominantly occur in the lungs.⁴⁶ Coagulation biomarkers have been associated with poor prognosis.⁵² Functional coagulation measurements have further supported the hypercoagulable state of these patients.⁴³ While the mechanism of thrombosis and hypercoagulability remains unclear, inflammation driving cytokine release is

believed to be the initiator of coagulation changes based on prior work in sepsis.⁵³ Cytokine production is believed to drive tissue factor product, resulting in systemic activation of coagulation.⁵⁴ Tissue factor expression is upregulated on macrophages and endothelial cells in response to elevated tumor necrosis factor- α (TNF- α), interleukin (IL)-6 and IL-1.⁵⁵ At the same time, the cytokine storm damages the endothelium, reducing the antithrombotic capacity of the systemic circulation via suppression of protein C, protein S, antithrombin, and tissue factor pathway inhibitor.⁵⁶ This is compounded by the severe acute respiratory syndrome coronavirus 2 directly infecting the endothelium of the lungs, heart, and small bowel.⁵⁷ COVID also is commonly associated with a high fibrinogen level that correlates with IL-6 levels.⁴⁵ IL-6 has previously been reported to be the main stimulator of fibrinogen synthesis.⁵⁸ With the combination of coagulation activation and hyperfibrinogenemia it is not surprising that this population is prone to thrombosis.

Endotoxin leading to cytokine production has also been demonstrated to activate of the fibrinolytic system 2 hours after infusion, followed by a shutdown of fibrinolysis within the following hour due to elevated plasminogen activator inhibitor -1 (PAI-1) levels.⁵⁹ Fibrinolysis activation with rapid suppression from PAI-1 was appreciated with endotoxin infusion in nonhuman primates, with concurrent increases in thrombin generation.⁶⁰ Pentoxifylline attenuates these fibrinolytic changes in this animal model, whereas IL-6 and TNF- α inhibitors have no effect.⁶¹ These experiments were followed up with the hypothesis that an antifibrinolytic (eg, tranexamic acid) would prevent progression to disseminated intravascular coagulation by blocking plasmin activation; however, tranexamic acid had no impact on the prothrombotic component of endotoxin infusion in healthy subjects and did not alter cytokine production.⁶² Our group's recent work has demonstrated that COVID-19 patients with a thrombelastography Lysis 30 (LY30) of 0% and D-dimer level >2600 ng/mL have a venous thrombosis rate of 50%.⁶³ Due to D-dimers having a half-life that exceeds 12 hours,⁶⁴ this value is reflective of the cumulative amount of polymerized fibrin present over the past day or longer, while low fibrinolytic activity measures the current fibrinolytic systemic state of the patient. Therefore, an elevated D-dimer with low fibrinolytic activity is consistent with prior activation of fibrinolysis with current low fibrinolytic activity, fulfilling the definition of fibrinolysis shutdown, which has been described for the past half-century.^{65,66} This fibrinolytic phenotype has been associated with poor outcomes in trauma.⁶⁷⁻⁷¹ Elevated D-dimer and low fibrinolytic state has previously been mislabeled *overt hyperfibrinolysis*⁷²; however, the overt hyperfibrinolytic phenotype in trauma does not commonly bleed to death and receives significantly fewer transfusions compared to patients with elevated D-dimer and elevated LY30s that represents true hyperfibrinolysis. Hyperfibrinolytic trauma patients have the hallmark signs of excessive fibrinolysis with excessive bleeding and low fibrinogen levels that can be reversed with an antifibrinolytic medication.⁷³ Patients with COVID-19 have elevated fibrinogen but are not bleeding to death and should not be classified as hyperfibrinolytic, which has already been proposed.⁷⁴ The combination of prothrombotic and lack

of fibrinolysis poses a major logistical challenge in treating COVID-19, as both ends of coagulation likely require treatment for effective outcomes. There is a potential that a combination of IL-6 blockage to attenuate hyperfibrinogenemia in combination with t-PA could provide a more durable response, which can be adapted from this phase II trial.

The 48-hour assessment of PaO₂/FiO₂ in patients after t-PA treatment will also have limitations in quantifying pulmonary dysfunction improvement. There are numerous ventilator adjuncts to improve oxygenation in the setting of severe ARDS, including proning the patient,¹¹ paralytics,⁷⁵ nitric oxide,⁷⁶ and prostanooids.⁷⁷ Prolonged use of these interventions is associated with adverse events.⁷⁸ Therefore, getting the patients off of these medications or prone positioning would be considered a beneficial outcome with t-PA regardless of change in PaO₂/FiO₂ over 48 hours. This also includes reducing toxic levels of oxygen (FiO₂ > 80%) and reducing PEEP. In addition to assessing improvements in each of these individual variables, a composite score of each adjunctive measure will also be conducted to represent a global change in requirement of adjuncts for improving oxygenation at 48 hours.

Due to different treatment practices at the 5 enrolling centers, we anticipate that there will be variability in the techniques used before patient enrollment to optimize oxygenation, an acknowledged limitation. This is in line with the pragmatic nature of the trial. Moreover, the crisis created by the pandemic without available treatments precluded the development of agreed-upon standard operating procedures for ventilation as well as other intensive care procedures.

During the current pandemic, there has been a call for rigorous trials with concurrent control groups.⁷⁹ However, randomized controlled trials bring ethical dilemmas, especially when no current treatment exists. Thus, it is imperative that creativity and the rigorous application of the scientific method are combined to produce an innovative, efficient study design. Our design uses the adaptive framework,^{80,81} which allows preplanned modifications to improve the efficiency of the trial and detect effect or harm more promptly, and a modified stepped-wedge design. More recently, the stepped-wedge randomized trials have gained popularity have been proposed.^{82,83} The modified stepped-wedge pragmatic design is different than the usual parallel randomized controlled trials, in which the intervention and control groups run, as the name implies, in parallel. The traditional stepped-wedge approach involves a sequential rollout of an intervention to participants (individuals or clusters) over a number of time periods, such that at the end of the study, all participants will have received the intervention. The name of the design (stepped-wedge) comes from the schematic illustration of the design. The 1987 Gambia hepatitis intervention study⁸⁴ was the pioneer stepped-wedge study, and tested the effectiveness of a hepatitis B vaccine. We modified the stepped-wedge design to deploy the intervention groups sequentially to more quickly accrue the sample size with one of the intervention groups with a parallel control. In a traditional parallel design, the first interim analyses would require sufficient number in 3 (as opposed to 2) groups, thus increasing the

efficiency of the trial and increasing the likelihood of isolating the more successful arm.

It should be noted that using the traditional (yet arbitrary) confidence level of 95% ($\alpha = 0.05$) is overly stringent for the current circumstances. The rigid cutoff of 95% level of certainty, as eloquently summarized by Nuzzo⁸⁵ in one of the most cited *Nature* articles should not be applied without regard to the conditions in which the study is conducted. Is it appropriate or necessary to be 95% certain when the condition in question has high lethality and morbidity, threatening to exceed the health care system's capacity, and has no known prevention (except for isolation) or treatment? While we do not believe this is the precise time to reignite a discussion about the traditional probability ($P < .05$), we will, in our study, for all comparisons 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 and IRBs will produce the appropriate interpretation of the results, which can then inform current medical decisions and a subsequent phase III trial if appropriate. Peer review and the readers can then assess the results applying their own tolerance for uncertainty.

Two phase I clinical trials support the potential beneficial effect of thrombolytics in severe ARDS with no reported bleeding complications^{24,25} and prompted our group's interest in the potential use of t-PA in COVID-19.⁸⁶ An important observation was that patients can have transient responders to lytic therapy, with autopsy confirmation of subsequent pulmonary rethrombosis.²⁴ Our early off-label use of t-PA in COVID-19-related ARDS demonstrated similar transient or long-lasting improvement on patients using lower doses of this medication.⁸⁷ Redosing of fibrinolytics in the Hardaway et al²⁵ study when indicated demonstrated an overall improvement in oxygenation in 80%. These observations stress the importance of continued heparin anticoagulation following fibrinolytic therapy, as proposed in our trial, together with redosing t-PA as needed. In both prior phase I clinical trials using fibrinolytics in the setting of ARDS,^{24,25} the conclusions made clear that future follow-up studies were required. Our proposal for a phase II study is the natural progression to expand upon those preliminary findings to identify the optimal dosing of a fibrinolytic in the setting of ARDS and ensure safety in regards to treatment strategies using t-PA, which is a commonly used fibrinolytic agent in clinical practice.

RELATIONSHIP DISCLOSURE

HBM, CDB, EEM, and MBY have patents pending related to both coagulation/fibrinolysis diagnostics and therapeutic fibrinolytics, and are passive cofounders and hold stock options in Thrombo Therapeutics, Inc. HBM and EEM have received grant support from Haemonetics and Instrumentation Laboratories. MBY has previously received a gift of alteplase (t-PA) from Genentech and owns stock options as a cofounder of Merrimack Pharmaceuticals. RJ, RCM, PKM, JW, NH, DST, and AS declare nothing to report.

AUTHOR CONTRIBUTIONS

HBM, CDB, EEM, AS, and MBY prepared the manuscript, with critical input and revisions from RJ, RCM, PKM, JW, NH, and DST.

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