Statistical Analysis Plan

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.

Study Product: Alteplase (Activase®)

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)

NCT#: 04357730

IND#: 149634

Statistical Analysis Plan (SAP) for STARS ("STudy of Alteplase for Respiratory failure in SARS-Cov2 (COVID-19)": A Phase 2a Clinical Trial

This SAP follows the recently published Guidelines for the Content of Statistical Analysis Plans in Clinical Trials. [1]

Section 1: Administrative Information

1a Title: STARS ("STudy of Alteplase for Respiratory failure in SARS-Cov2 (COVID-19)"

1b Trial registration number: NCT

2. SAP version 4, 11/30/2020

Section 2: Introduction

2.1. Synopsis of trial background and rationale

Similar to pathologic findings of ARDS, microthrombi have now been observed in lung specimens from patients infected with COVID-19 [2,3]. Generalized derangements of the hemostatic system with prolongation of the prothrombin time, reduced prothrombin time activity, and elevated D-dimer and fibrin degradation products have been reported in severely ill COVID-19 patients, particularly in non-survivors [4-6]. These laboratory findings, in combination with the large clot burden seen in the pulmonary microvasculature, mirrors that seen in human sepsis [7,8], experimental endotoxemia [9], and massive tissue trauma [10,11]. Targeting the coagulation and fibrinolytic systems to improve the treatment of ARDS has been proposed for at least the past two decades [12-14]. In particular, the use of plasminogen activators to limit ARDS progression and reduce ARDS-induced death has received strong support from animal models [15-17], and a phase 1 human clinical trial [18]. In 2001, Hardaway and colleagues [18,19] 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.

Tissue plasminogen activator (alteplase) is a more modern approach to fibrinolysis, for which there is extensive experience in myocardial infarction, stroke and major pulmonary embolism [20-22]. Most current trials included in clinicaltrials.gov for the COVID-19-induced SARS 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 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. [23-27]

Alteplase is already being used in several centers off label for salvage therapy in patients with severe COVID-19 induced SARS (SARS-Cov2) with promising results These investigators recognize that in the context of a pandemic with no known treatment, it is important to have a

rigorous yet efficient study design with a control group [28]. Thus, we propose a Phase 2a, open label, rapidly adaptive, randomized trial to test the use of two doses of alteplase followed by heparin compared to controls receiving standard of care.

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.

2.2 Outcomes

Primary Outcome: PaO2/FiO2 improvement from pre-to-post intervention at 48 hours post randomization. The 48 hours PaO2/FiO2 should ideally be obtained in the same position and the same dose of paralytic agents as in baseline, however, given the pragmatic nature of the trial, this decision must be made by the care team. The 48 hours post-randomization outcome PaO2/FiO2 will be measured under the optimal ventilation strategy within 42 to 54 hours (48 hours +/- 6 hours) post randomization.

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

- Per amendment after first interim analysis Nov 5, 2020, we added two secondary outcomes:
- 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

Section 3: Study Methods

3.1. Trial design Multicenter, pragmatic, randomized, open label, rapidly adaptive, controlled, clinical trial

The trial has a novel design with modified stepped-wedge and adaptive features in order to rapidly identify the intervention group with the highest chance of benefit. This unconventional design is appropriate for the current circumstances of the pandemic caused by a new, lethal and morbid virus infection (COVID-19) for which no known treatment exists. The below figure illustrates the design. In brief, the patients will be sequentially assigned to one of three following groups:

- 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. 44,45

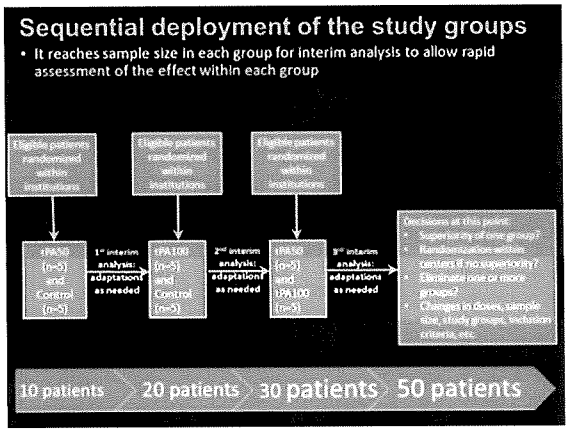
- Group tPA50 plus drip will receive 50 mg of tPA intravenous bolus administration over 2 hours, given as a 10 mg push followed by the remaining 40 mgs over a total time of 2 hrs (not to exceed 0.9mg/kg dose). Immediately following this initial tPA infusion, we will initiate a drip of 2 mg/hr tPA over the ensuing 24 hours (total 48 mg infusion) accompanied by an infusion of 500 U/hr 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.

- Control: institution's protocol for ARDS

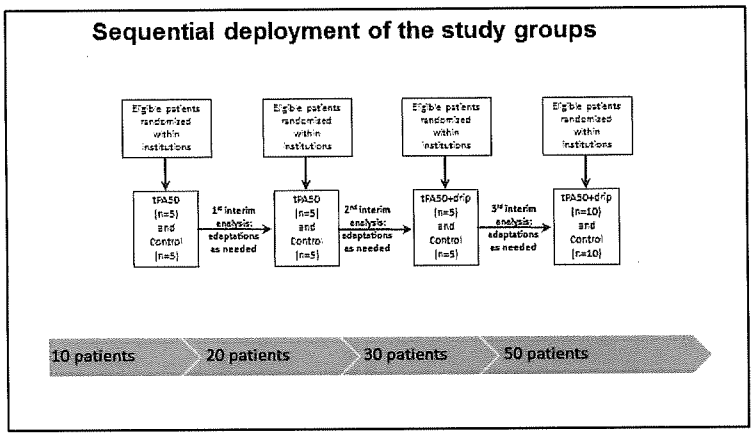
In order to rapidly accrue patients in one intervention group with a concurrent control, the first 10 patients will be randomly assigned to Group tPA50 or Control (Standard-of-care) when the first interim analysis occurs. If no stopping criteria are met, the next 10 patients are randomly

assigned to Group tPA50 or Control when the second interim analysis is done. If no criteria for stopping or dropping an arm are met, the next 10 patients are randomly assigned to Group tPA50 plus drip or Control when the third interim analysis is done. Finally, if no criteria for stopping or dropping an arm are met, the trial progresses with patients being randomized to Group tPA50 plus drip or Control up to the final analysis at n=50.

INITIAL DESIGN ANALYSIS



NEW DESIGN AFTER 1st INTERIM



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/hr 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.

3.2. Randomization details

Randomization is performed by the leading institution (Ernest E Moore Shock Trauma Center at Denver Health, DH) using Research Randomizer [1] and automated via REDCap. All randomizations are conducted intra-hospital (i.e., no cluster randomization), to avoid the confounding effect of practice variation. Randomization is conducted in blocks of 10 to allow better distribution between groups at each interim analysis.

Each center will identify potentially eligible patients, obtain their consent, and enter in/exclusion criteria into the web-based REDCap instrument; if the patient is indeed eligible, the random assignment will be revealed to the institution's pharmacy, which will then release the drug at the appropriate dose (in case the patient is assigned to one of the intervention groups) or no drug will be released if the patient is randomized to the control group. This is defined as TIME ZERO.

3.2. Sample size

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

[1] Urbaniak, G. C., & Plous, S. (2013). Research Randomizer (Version 4.0) [Computer software]. Retrieved on April 10, 2020, at http://www.randomizer.org/

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.

The table below shows the details of the interim analyses with the Pocock spending method:

Look	N of Patients	Lower Bndry	Upper Bndry	Nominal Alpha	Inc Alpha	Total Alpha	Inc Power	Total Power
1	10	-2.43798	2.43798	0.015	0.015	0.015	0.18976	0.190
2	20	-2.42677	2.42677	0.015	0.011	0.026	0.25179	0.442
3	30	-2.41014	2.41014	0.016	0.009	0.035	0.21191	0.653
4	50	-2.39658	2.39658	0.017	0.008	0.043	0.14655	0.800

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 [29], 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.

3.3. Statistical interim analyses and stopping guidance:

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 [30]:

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).

3.4. 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.

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Section 4: Statistical Principles

4.1. Level of statistical significance: see above description of p-value for interim analyses.

4.2. Description and rationale for any adjustment for multiplicity of time points: 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. [31,32]

4.3. Analysis populations: all analyses, including safety analyses, will conducted initially as **intention-to-treat** and then **as-treated**.

Section 5: Trial Population

5.1. Representativeness of trial sample: The patients included in the sample will be compared to current reports of patients with SARS-Cov2 with similar severity.

5.2. Eligibility:

Inclusion Criteria: We will include patients ages 18 to 75 years, with known or suspected COVID-19 infection, with a neurological examination without focal signs or new deficits at time of enrollment (if patient is on paralytics, the 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), with a PaO2/FiO2 ratio < 150 (at sea level or adjusted for altitude) persisting for longer than 4 hours despite maximal mechanical ventilation management according to each institution's ventilation protocols (FiO2 of 60% or more and PEEP greater than or equal of 10cmH2O). If obtaining arterial blood gas is not possible, 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 (NHLBI) PETAL (Prevention and Early Treatment of Acute Lung Injury) Network Collaborators [33] A 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.[34]

Exclusion Criteria (At the Time of Enrollment)

1. Active bleeding
2. Acute myocardial infarction or history of myocardial infarction within the past 3 weeks or cardiac arrest during hospitalization
3. Hemodynamic instability with Noradrenaline >0.2mcg/Kg/min

4. Acute renal failure requiring dialysis
5. Liver failure (escalating liver failure with ALT> 3 times baseline)
6. 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
7. Cardiac tamponade
8. Bacterial endocarditis
9. Severe uncontrolled hypertension defined as SBP>185mmHg or DBP>110mmHg
10. CVA (stroke), history of severe head injury within prior 3 months, or prior history of intracranial hemorrhage
11. Seizure during pre-hospital course or during hospitalization for COVID-19
12. Diagnosis of brain tumor, AVM or ruptured aneurysm
13. Is currently on ECMO
14. Major surgery or major trauma within the past 2 weeks
15. GI or GU bleed within the past 3 weeks
16. Known bleeding disorder
17. P2Y12 receptor inhibitor medication (anti-platelet) within 5 days of enrollment
18. Arterial puncture at a non-compressible site within the past 7 days
19. Lumbar puncture with in past 7 days
20. Pregnancy
21. INR > 1.7 (with or without concurrent use of warfarin)
22. Platelet count < 100 x 10^9/L or history of HITT
23. Fibrinogen < 300mg/dL
24. Known abdominal or thoracic aneurysm
25. History of CNS malignancy or CNS metastasis within past 5 years
26. History of non-CNS malignancy within the past 5 years that commonly metastasizes to the brain (lung, breast, melanoma)

5.3. Reasons and details of withdrawal: the data will be presented and compared between the study groups.

5.4. Baseline patient characteristics: patients in the study groups will be compared regarding demographic characteristics, comorbidities, previous and current medications, presence of infectious and non-infectious complications, mechanical ventilation variables, and function of the central nervous, pulmonary, renal, liver, cardiovascular, inflammatory, coagulation and hematological systems.

5.5. Multicenter description: we will report the enrollment, withdrawal and outcomes of each participating hospital.

Section 6: Analysis of outcomes

6.1. Primary and Secondary Outcomes: see above

6.2. Any calculation or transformations used to derive the outcomes: all outcomes 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 (from literature). All outcomes will also be analyzed as change from baseline.

6.3. Effectiveness of the randomization: we will compare the study groups regarding above variables to determine baseline comparability of the groups. Per the CONSORT statement, p-values should not be used, as any differences are, by definition, by chance. We will use the absolute standardized mean difference (SMD)$\leq$0.20 to 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.

6.4. Outcomes comparison:
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. The primary outcome will be assessed within group and between groups Differences in the primary outcome will be assessed using linear mixed models, with appropriate transformations if normality departure of residuals is detected. Linear mixed models allows us to adjust for potential confounders detected in the comparison of the groups at baseline using inverse probability weighting by a propensity score, the covariance structure, the repeated measures and the intra-hospital 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 intra-hospital correlation. 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, as premature death is, unfortunately, expected. Survival analysis with inverse probability weighted Cox proportional hazards model and robust sandwich variance estimate with clustering for hospitals will be used for mortality and for survivor-bias outcomes censoring for death (e.g., thrombotic complication). As all outcomes are in-hospital, loss to follow-up is not likely. The pre-planned comparisons include within group (improvement over baseline) and between groups, all two-tailed with significance declared as defined by the Pocock spending method.

6.5. Pre-planned 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.

- Baseline PaO2/FiO2 <100 and <50
- Hemodynamic instability with vasopressors
- Age <35, <50, <65 years
- D-Dimers median levels
- Fibrinolysis shutdown (by TEG or ROTEM)
- Fibrinogen median levels
- Prone/supine positioning
- Requirement of re-bolusing of alteplase
- Received dose of alteplase as premature death or adverse event or other reasons may preclude the administration of complete treatment
- 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 SAP with the IRBs, DSMB, funder and FDA.

6.6. Missing data

Missing data are expected to be minimal. If less than 15% and non-differential 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 two strategies to the complete dataset analyses:

1) Multiple imputation by chained equations (MICE): although MICE is better for missing at random data, we will attempt to use this method.
2) Sensitivity analyses: we will assume worst and best clinical scenarios and compare the results with the complete dataset.

7. Assessment of safety timing and methods and cessation methods

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 applicable per care provider's decision. Most patients will use GCS without verbal component	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. Imaging per attending's discretion.	GCS decrease of >=2 points or focal deficit within 24 hours of study drug infusion or new hemorrhage on CT-Scan or MRI
Worsening of pulmonary function	Arterial blood gas and ventilation indices		$\geq$30% P/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	Hemoglobin reduction >3g/dL within 24 hours of study drug intervention or requiring RBC transfusion with suspected GI

Serious Adverse Events	Method for safety check	Safety check frequency	Cessation rule *
		heparin infusion or more frequently if any abnormality detected. Endoscopic exam per attending's discretion.	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. Endoscopic exam per attending's discretion.	Persistent hemoptysis for >=4 hours or compromising airway
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	Hemoglobin reduction >3g/dL within 24 hours of infusion of study drug infusion or requiring RBC transfusion

Serious Adverse Events	Method for safety check	Safety check frequency	Cessation rule *
		heparin infusion or more frequently if any abnormality detected.	
Any of the below listed exclusion criteria developing during or up to 3 hours after alteplase or heparin infusion, except for fibrinogen, for which we will set cessation cutoff at 300mg/dL **	Clinical exam and laboratory		Any of listed exclusion criteria developing during or up to 3 hours after alteplase or heparin infusion, except for fibrinogen, for which we will set cessation cutoff at 300mg/dL

*criteria or attending's decision

** criteria: 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>185mmHg or DBP>110mmHg; Seizure; Placement on ECMO; Major surgery required; Requirement of lumbar puncture; INR > 1.7; Platelet count < 100 x 109/L or history of HITT; Fibrinogen < 300mg/dL

8. Dataset availability: a de-identified dataset will be made available to other investigators who may submit proposals to the PI for additional analyses or validation.

References

1. Gamble C, Krishan A, Stocken D, et al. Guidelines for the Content of Statistical Analysis Plans in Clinical Trials. *Jama.* 2017;318(23):2337-2343.
2. Guan WJ, Ni ZY, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med.* 2020.
3. Tian S, Hu W, Niu L, Liu H, Xu H, Xiao SY. Pulmonary Pathology of Early-Phase 2019 Novel Coronavirus (COVID-19) Pneumonia in Two Patients With Lung Cancer. *J Thorac Oncol.* 2020.
4. Han H, Yang L, Liu R, et al. Prominent changes in blood coagulation of patients with SARS-CoV-2 infection. *Clin Chem Lab Med.* 2020.
5. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J Thromb Haemost.* 2020.
6. Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med.* 2020.
7. Bone RC, Francis PB, Pierce AK. Intravascular coagulation associated with the adult respiratory distress syndrome. *Am J Med.* 1976;61(5):585-589.
8. Schouten M, Wiersinga WJ, Levi M, van der Poll T. Inflammation, endothelium, and coagulation in sepsis. *J Leukoc Biol.* 2008;83(3):536-545.

9. Ostrowski SR, Berg RM, Windelov NA, et al. Discrepant fibrinolytic response in plasma and whole blood during experimental endotoxemia in healthy volunteers. *PLoS One.* 2013;8(3):e59368.
10. Moore HB, Moore EE. Temporal Changes in Fibrinolysis following Injury. *Seminars in thrombosis and hemostasis.* 2020;46(2):189-198.
11. Moore HB, Moore EE, Liras IN, et al. Acute Fibrinolysis Shutdown after Injury Occurs Frequently and Increases Mortality: A Multicenter Evaluation of 2,540 Severely Injured Patients. *J Am Coll Surg.* 2016;222(4):347-355.
12. MacLaren R, Stringer KA. Emerging role of anticoagulants and fibrinolytics in the treatment of acute respiratory distress syndrome. *Pharmacotherapy.* 2007;27(6):860-873.
13. Schultz MJ, Haitsma JJ, Zhang H, Slutsky AS. Pulmonary coagulopathy as a new target in therapeutic studies of acute lung injury or pneumonia--a review. *Crit Care Med.* 2006;34(3):871-877.
14. Ware LB, Camerer E, Welty-Wolf K, Schultz MJ, Matthay MA. Bench to bedside: targeting coagulation and fibrinolysis in acute lung injury. *Am J Physiol Lung Cell Mol Physiol.* 2006;291(3):L307-311.
15. Hardaway RM, Williams CH, Marvasti M, et al. Prevention of adult respiratory distress syndrome with plasminogen activator in pigs. *Crit Care Med.* 1990;18(12):1413-1418.
16. Liu C, Ma Y, Su Z, et al. Meta-Analysis of Preclinical Studies of Fibrinolytic Therapy for Acute Lung Injury. *Front Immunol.* 2018;9:1898.
17. Stringer KA, Hybertson BM, Cho OJ, Cohen Z, Repine JE. Tissue plasminogen activator (tPA) inhibits interleukin-1 induced acute lung leak. *Free Radic Biol Med.* 1998;25(2):184-188.
18. Hardaway RM, Harke H, Tyroch AH, Williams CH, Vazquez Y, Krause GF. Treatment of severe acute respiratory distress syndrome: a final report on a phase I study. *The American surgeon.* 2001;67(4):377-382.
19. Hardaway RM, Harke H, Williams CH. Fibrinolytic agents: a new approach to the treatment of adult respiratory distress syndrome. *Advances in therapy.* 1994;11(2):43-51.
20. Wardlaw JM, Murray V, Berge E, del Zoppo GJ. Thrombolysis for acute ischaemic stroke. *Cochrane Database Syst Rev.* 2014(7):CD000213.
21. investigators G. An international randomized trial comparing four thrombolytic strategies for acute myocardial infarction. *N Engl J Med.* 1993;329(10):673-682.
22. Konstantinides S, Geibel A, Heusel G, et al. Heparin plus alteplase compared with heparin alone in patients with submassive pulmonary embolism. *N Engl J Med.* 2002;347(15):1143-1150.
23. He XL, Liu Z, Xia SY. Vascular endothelial injuries and changes of blood coagulation and fibrinolysis indexes in patients with acute respiratory distress syndrome. *Chinese medical sciences journal = Chung-kuo i hsueh k'o hsueh tsa chih.* 2004;19(4):252-256.
24. Hofstra JJ, Haitsma JJ, Juffermans NP, Levi M, Schultz MJ. The role of bronchoalveolar hemostasis in the pathogenesis of acute lung injury. *Seminars in thrombosis and hemostasis.* 2008;34(5):475-484.
25. Idell S. Coagulation, fibrinolysis, and fibrin deposition in acute lung injury. *Crit Care Med.* 2003;31(4 Suppl):S213-220.
26. Idell S, Koenig KB, Fair DS, Martin TR, McLarty J, Maunder RJ. Serial abnormalities of fibrin turnover in evolving adult respiratory distress syndrome. *The American journal of physiology.* 1991;261(4 Pt 1):L240-248.
27. Olson TM, Driscoll DJ, Edwards WD, Puga FJ, Danielson GK. Pulmonary microthrombi. Caveat for successful modified Fontan operation. *The Journal of thoracic and cardiovascular surgery.* 1993;106(4):739-744.

28. Rome BN, Avorn J. Drug Evaluation during the Covid-19 Pandemic. *New England Journal of Medicine.* 2020.
29. Nuzzo R. Scientific method: statistical errors. *Nature.* 2014;506(7487):150-152.
30. Jitlal M, Khan I, Lee SM, Hackshaw A. Stopping clinical trials early for futility: retrospective analysis of several randomised clinical studies. *Br J Cancer.* 2012;107(6):910-917.
31. Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology (Cambridge, Mass).* 1990;1(1):43-46.
32. Perneger TV. *What's wrong with Bonferroni adjustments.* Vol 3161998.
33. Etchill E, Sperry J, Zuckerbraun B, et al. The confusion continues: results from an American Association for the Surgery of Trauma survey on massive transfusion practices among United States trauma centers. *Transfusion.* 2016.
34. Oxley TJ, Mocco J, Majidi S, et al. Large-Vessel Stroke as a Presenting Feature of Covid-19 in the Young. *N Engl J Med.* 2020.