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STudy of Alteplase for Respiratory failure in SARS-Cov2 COVID-19 (STARS): A Vanguard Multicenter, Rapidly Adaptive, Pragmatic, Randomized, Controlled Trial

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STudy of Alteplase for Respiratory failure in SARS-Cov2 COVID-19 (STARS): A Vanguard Multicenter, Rapidly Adaptive, Pragmatic, Randomized, Controlled Trial

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Abstract

Background: Pulmonary vascular microthrombi are a proposed mechanism of COVID19 respiratory failure. We hypothesized that early administration of tissue-plasminogen activator(tPA) followed by therapeutic heparin would improve pulmonary function in these patients.

Research Question: Does tPA improve pulmonary function in severe COVID-19 respiratory failure, and is it safe?

Study Design and Methods: Adults with COVID-19-induced respiratory failure were randomized May14,2020-March 3,2021 in two phases: Phase-1(n=36): control (standard-of-care) vs *tPA-Bolus*(50mg tPA IV-bolus followed by 7 days of heparin (goal aPTT=60-80s); Phase-2(n=14): control vs *tPA-Drip*(50 mg of tPA IV-bolus, followed by tPA drip 2mg/hr plus heparin 500U/hour over 24 hours, then heparin to maintain aPTT 60-80s/7 days). Patients were excluded from enrollment if they did not have a neurologic exam or cross-sectional brain imaging within the previous 4.5 hours to rule out stroke and potential for hemorrhagic conversion. The primary outcome was PaO2/FiO2 improvement from baseline at 48 hours post-randomization. Secondary outcomes included: PaO2/FiO2 improvement>50% or PaO2/FiO2>=200 at 48hrs(COMPOSITE), ventilator-free days(VFD) and mortality.

Results: Fifty patients were randomized: Phase 1: 17 control, 19 *tPA-Bolus*; Phase 2: 8 control, 6 *tPA-Drip*. There were no severe bleeding events. In *tPA-Bolus* patients, the PaO2/FiO2 ratio was significantly($p<0.017$) higher than baseline at 6 through 168 hours post-randomization; controls experienced no significant improvements. When compared to controls, *tPA-Bolus* patients' PaO2/FiO2 ratio at 48hours[16.9%(-8.3–36.8) vs 29.8%(4.5–88.7),p=0.11], COMPOSITE outcome (11.8% vs 47.4%,p=0.03), VFD[0.0(0.0–9.0) vs 12.0(0.0–19.0),p=0.11] and in-hospital mortality(41.2% vs 21.1%,p=0.19) did not reach statistically significant differences. tPA-Drip patients did not experience benefit.

Interpretation: The combination *tPA-Bolus*+heparin is safe in severe COVID-19 respiratory failure. A Phase 3 study is warranted given improvements in oxygenation and promising observations in VFD and mortality.

Trial Registration: Registered on ClinicalTrials.gov on April 22, 2020 (NCT04357730).

Keywords: COVID-19; Acute Respiratory Distress Syndrome (ARDS); Tissue Plasminogen Activator (tPA); Pulmonary Failure; Fibrinolysis

Pathologic evaluations of SARS-Cov-2 (COVID-19) patients who succumbed to respiratory failure have identified a common pattern of disseminated pulmonary microvascular thrombosis[1-4]. Whole blood coagulation assessment of critically ill COVID-19 patients with viscoelastic testing has universally demonstrated a hypercoagulable state with increased clot strength[5,6] and fibrinolysis resistance[7-10]. Early in the clinical course of COVID-19 respiratory failure most patients have relatively normal lung compliance with markedly elevated dead-space ventilation[11], a hallmark of vascular occlusive etiologies of respiratory failure that is consistent with the previously described autopsy findings [1-4].

In the large multicenter ACTIV-4a/ATTACC/REMAP-CAP trial, therapeutic anticoagulation improved survival to discharge and clinical outcomes in patients with emerging respiratory failure who were not yet dependent on mechanical ventilation (the "moderate group") compared to the prophylactic anticoagulation group[12]. In contrast, no benefit was observed when therapeutic anticoagulation was initiated after the onset of severe respiratory failure, suggesting therapeutic anticoagulation is only effective if started prior to the accumulation of significant clot burden within the lung vasculature. It is in this cohort of patients with severe COVID-19 respiratory failure and high-risk for death that our group hypothesized a potential role for fibrinolytic therapy with tissue plasminogen activator (tPA) to restore pulmonary microvascular patency, reduce dead-space ventilation and improve oxygenation[13-15].

The use of fibrinolytic therapy to treat organ failure was proposed several decades ago[16], and two small phase I clinical trials have demonstrated safety and potential feasibility in ARDS patients[17]. The use of fibrinolytic therapy in COVID-19 respiratory failure was initially proposed by our group at the outset of the pandemic[14], and there have now been several case series and a small retrospective observational study published suggesting a potential benefit from tPA[18-21]. However, the inherent bleeding risks following tPA administration for other clinical indications have limited enthusiasm for this approach[22]. To determine whether tPA is a potentially useful and safe treatment of severe COVID-19 respiratory failure, we conducted a vanguard, multicenter, randomized controlled trial of tPA (alteplase) combined with varying doses of heparin versus standard of care in severe COVID-19 respiratory failure (NCT04357730). Our hypothesis was that the combination of tPA with heparin would improve oxygenation and reduce adverse outcomes compared to standard of care.

Study Design and Methods

Study design: The STARS trial was a vanguard, phase 2a, multicenter, open-label, rapidly adaptive, pragmatic, randomized, controlled clinical trial designed to evaluate whether different dosing regimens of tPA with heparin administered to patients with COVID-19 associated advanced respiratory failure could improve pulmonary function within 48 hours (compared to immediately before its administration) without a significant increase in life-threatening

hemorrhage. The study design was previously described[23]. A multiphase approach with four analyses at short enrollment intervals (three interim and one final) was proposed to allow for rapid safety and efficacy assessment and adaptations at each interim analysis. At the third interim analysis (n=30) it was decided to implement a tPA drip instead of bolus (detailed below) as the intervention, which encountered logistical challenges given the relatively short shelf-life of tPA and was not implemented until enrollment 37. This resulted in two phases: Phase 1: 36 patients were randomized to either intervention (*tPA-Bolus*, described in detail below) or control (standard-of-care per each institution's protocol); Phase 2: 14 patients were randomized to *tPA-Drip* (described below) vs control (standard-of-care).

Setting: Patients were recruited in eight academic tertiary care hospitals across the US.

Inclusion and exclusion criteria: Eligible patients were: 1) age 18-75 years; 2) confirmed diagnosis of COVID-19 and severe respiratory failure requiring mechanical ventilation (MV) with PaO2/FiO2 ratio <150 for >4 hours; 3) less than 11 MV days at time of enrollment; and 3) non-focal neurologic examination or brain imaging with no evidence of stroke (magnetic resonance imaging (MRI) or computed tomography (CT) within the prior 4.5 hours). When arterial blood gas (ABG) data were not available, imputed PaO2/FiO2 ratios were allowed utilizing the imputation table developed as part of the National Heart, Lung and Blood Institute's PETAL (Prevention and Early Treatment of Acute Lung Injury) Network[24]. Exclusion criteria included: active bleeding, acute myocardial infarction or cardiac arrest on current admission, hemodynamic instability requiring noradrenaline >0.2mcg/Kg/min, acute renal failure requiring dialysis, liver failure (bilirubin >3 times baseline), known or suspected cirrhosis, cardiac tamponade, bacterial endocarditis, severe uncontrolled hypertension (systolic blood pressure >185mmHg or diastolic blood pressure >110mmHg), traumatic brain injury within the prior three months, stroke or prior history of intracerebral hemorrhage, seizure during pre-hospital or hospital course for COVID-19, diagnosis of brain tumor or arteriovenous malformation, presently on extracorporeal membrane oxygenation, major surgery or trauma within the previous two weeks, gastrointestinal or genitourinary bleed within the prior three weeks, known bleeding disorder, arterial puncture at a non-compressible site or lumbar puncture within the past seven days, pregnancy, prothrombin time/international normalized ratio (INR) >1.7 (with or without concurrent use of warfarin), platelet count <100 x 10^9/L ,history of heparin-induced thrombocytopenia (HIT), fibrinogen <300mg/dL, P2Y12 receptor inhibitor medication (anti-platelet) within five days of enrollment, known abdominal or thoracic aortic aneurysm, history within past 5 years of CNS malignancy or other malignancy that commonly metastasizes to the brain (lung, breast, melanoma), or prisoner status.

Ethics Approval: The trial was performed according to the Food and Drug Administration (FDA) Investigational New Drug regulations (IND 149634) and registered with ClinicalTrials.gov (NCT04357730). All participating trial sites had study approval and oversight

from their respective Institutional Review Boards (e-Appendix 1). Due to the nature of the study, which enrolled critically ill patients on mechanical ventilation, informed consent for trial participation was obtained from each patient's Legally Authorized Representative. An independent Data Safety Monitoring Board (DSMB) oversaw the safety of the trial with mandatory reviews at each interim analysis and for all suspected serious adverse events. Data were stored in a REDCap instrument sponsored by NIH/NCATS Colorado CTSA Grant Number UL1 TR002535.

Randomization and masking: A randomization table was developed using Research Randomizer[25] and automated via REDCap to either the intervention (tPA-Bolus in Phase 1 or tPA-Drip in Phase 2, described below) or control. This was an open label study as the intervention carries a risk of bleeding that could be mitigated with anti-fibrinolytic therapy, thus there was no masking of the patients, research team or primary treatment team. The trial profile is shown in Figure 1.

Procedures: At randomization, patients assigned to the control group continued their current medical care according to their institution's protocols, with no input from the study team. Patients randomized to the intervention arm, received the following regimens:

1) Phase 1 (patients 1 to 36): patients randomized to *tPA-Bolus* intervention received an intravenous (IV) 50mg bolus of 1mg/mL tPA as a 10mg push followed by the remaining 40mg infused over the next 2 hours. Immediately upon completion of the tPA, a 5000 unit bolus of IV unfractionated heparin (UFH) was administered and continued for the next 7 days (or until extubation) as an infusion to maintain activated partial thromboplastin time (aPTT) of 60-80 seconds. At 24 hours after tPA initiation, patients with a PaO2/FiO2 improvement that was at least 20% but did not meet the primary endpoint of a 50% improvement (i.e., 20-49% improvement) and who did not develop any of the above-mentioned exclusion criteria, received a second 50mg tPA bolus, during when the UFH infusion was halted and resumed at its prior rate as soon as the second tPA administration was complete. The heparin regimen was maintained for seven days or until successful extubation.
2) Phase 2 (patients 37 to 50): patients randomized to the intervention received the *tPA-Drip* intervention consisting of a 50mg IV bolus of 1mg/mL tPA as a 10mg push followed by the remaining 40mg infused over the next 2 hours (not to exceed 0.9mg/kg dose). Immediately following this initial tPA infusion, patients received a drip of 2 mg/hr tPA over the ensuing 24 hours (total 48 mg infusion) accompanied by an infusion of a sub-therapeutic dose of 500U/hour of heparin during the tPA drip. Once the tPA drip terminated, the heparin dose was titrated up (no bolus) to maintain an aPTT 60-80 seconds.

Monitoring: Upon enrollment, randomization and at short intervals thereafter (hours 2, 6, 12, 18, 24 and daily until day 7 post-randomization), we collected data on arterial blood gases, complete blood count with platelet count (CBC), prothrombin time (PT)/international normalized ratio (INR), aPTT, fibrinogen, D-dimer, troponin, C-reactive protein (CRP), liver and renal function tests.

Outcomes: All outcomes were pre-specified prior to the beginning of the trial. The primary outcome was improvement in PaO2/FiO2 ratio at 48 hours after randomization over baseline. Pre-planned secondary outcomes reported here include: 1) COMPOSITE: achievement of PaO2/FiO2 ≥ 200 or 50% increase in PaO2/FiO2; 2) National Early Warning Score 2 (NEWS2); 3) 28-day in-hospital mortality; 4) in-hospital mortality; 5) ventilator-free days (VFD) and ICU-free days (IFD)[26].

Cessation rules to terminate treatment in patients enrolled in the intervention arm of the trial are described in e-Appendix 1. Any serious adverse event was to be immediately reported to the Sponsor, IRB, DSMB, FDA and funding agency for discussion and feedback per regulatory and ethical guidelines. Adverse Events are reported according to the National Institutes of Health Common Terminology Criteria for Adverse Events, where Grade 1 = mild, Grade 2 = moderate, Grade 3 = severe, Grade 4 = life-threatening, and Grade 5 = event that leads to death.

Statistical Analysis: The sample size calculations accounted for pairwise comparisons between study groups and were performed using PASS version 14 (NCSS, LLC, Kayseville, Utah, USA). Sample size assumptions were: power=80%, overall confidence=95%, four sequential tests (three interim and one final) using the Pocock alpha spending function to determine test boundaries, a baseline PaO2/FiO2=149 based on a previous study [17] with an overestimated standard deviation of 100. We also assumed a design effect=1.12 due to the study's multicenter nature (intra-class correlation coefficient=0.03, average cluster=5) and 20% inflation to account for premature deaths. A sample size of 50 (25 in each intervention group and 25 in the control group) eligible patients was able to detect a minimum 91% improvement in PaO2/FiO2 between a simultaneously enrolled intervention and control groups. The complete statistical analysis plan and study protocol is included in e-Appendix 2.

Analyses were conducted on SAS version 9.4 (SAS Institute, Cary, NC). We assessed the randomization effectiveness by comparing demographic and baseline clinical characteristics (Table 1). All analyses were conducted initially as an intent-to-treat; there was no treatment cessation or crossover so there was no need to conduct as-treated analyses. As previously recommended [27], we assessed pairwise differences between groups of simultaneously enrolled patients in Phase 1 and Phase 2, i.e., we report separate analyses for Phase 1 and Phase 2. There

were no adjustments for multiple outcomes, as all study outcomes were pre-specified hypotheses, in order to avoid increased type II errors[28,29]. Linear mixed models were conducted (continuous outcomes) or generalized estimating equations (categorical outcomes) to account for intra-hospital cluster effects and repeated measures. All pre-planned comparisons included within group (improvement over baseline) and between two concurrently enrolled groups. All tests were two-tailed with significance declared at $p<0.017$ at each interim analysis according to the alpha defined by the Pocock alpha spending method for an overall trial $alpha<0.05$.

A pre-planned subgroup analysis stratified by median D-dimer at baseline is presented. In ad hoc analyses, we also examined the aPTT time trends in each of the study groups to assess the role of heparin, and trends in COVID-19 severity of enrolled patients during the trial. The trends were statistically assessed by the Cochran-Armitage trend test or by linear regression.

Role of the funding source: The funder of this investigator-initiated study (Genentech, Inc) had no role in the study design, data collection, analysis, interpretation, or manuscript preparation. Five authors (EEM, HBM, CB, MY, AS) served as the steering committee and had full access to the data.

Results

Figure 1 shows the CONSORT diagram for patient eligibility and distribution, with the enrollment period spanning May 14th, 2020 until March 3rd, 2021 at which time the trial was stopped for reaching the target enrollment (n=50).

Phase 1

Table 1 shows the characteristics of the 36 patients enrolled during Phase 1 (Control n=17 vs tPA-Bolus n=19). There were only minor imbalances between the two groups at baseline, with slightly more tPA-Bolus patients being male and having concurrent infections (other than COVID-19), and were less likely to receive dexamethasone. All other baseline variables showed good balance, importantly including a similar baseline PaO2/FiO2 ratio. Of the 19 patients receiving the tPA-Bolus intervention, 8 (42.1%) required a second tPA dose due to transient PaO2/FiO2 improvement as defined in the Methods. No patients crossed over or withdrew. As shown in Table 2 and Figure 2, tPA-Bolus patients showed a larger and statistically significant increase in PaO2/FiO2 ratios relative to baseline at every time point measured (6, 12, 24, 48, 72, 96, 144 and 168 hours post-randomization) while controls did not, resulting in more tPA-Bolus patients reaching the COMPOSITE outcome, although there were no significant differences in PaO2/FiO2 ratios between groups at 48 hours. There were no differences in NEWS2 scores over time between groups (Table 2). Observations between tPA-Bolus patients and controls with respect to VFD, IFD, and in-hospital mortality did not reach significant differences. Control patients maintained significantly shorter aPTT than tPA-Bolus ($p<0.001$, Table 2).

Stratification of the temporal trends of tPA-Bolus patients by receipt of a second tPA dose at 24 hours resulted in a second peak in the PaO2/FiO2 ratio and sustained higher values up to seven days (Figure 2, Panel B). Further stratification by average aPTT over 7 days post-randomization showed that among patients who maintained a 7-day average aPTT>40s (the approximate median value at 48 hours), tPA-Bolus recipients maintained consistently higher PaO2/FiO2 ratios over time (Figure 3, Panel A) which is in contrast to those patients whose 7-day average aPTT was <=40s (Figure 3, Panel B).

As anticipated, the temporal trends of D-dimer differed significantly between intervention and controls (interaction intervention*time p<0.0001). The peak D-dimer levels (Figure 4, Panel A) occurred immediately after completion of the two-hour bolus, while a second smaller peak (also significantly different than baseline) was seen at 36 hours consistent with a repeat tPA bolus in a large number of patients. Baseline D-dimer levels (>1900 vs <=1900, which was the median upon randomization), however, did not modify the differences between tPA-Bolus and controls at 48 hours or affect the temporal trends of these two study groups.

Phase 2

Table 3 shows the characteristics of the 14 patients enrolled during Phase 2 (Control n=8 vs tPA-Drip n=6). No major imbalances in baseline characteristics were noted. The tPA-Drip group did not show benefit compared to simultaneously enrolled controls (Table 4). Of note, aPTT was short (<40s) in both study groups (tPA-Drip group and the controls), further suggesting the pivotal role of therapeutic heparin in the intervention. Similar to the tPA-Bolus group, the tPA-Drip group demonstrated a large and significant spike in D-dimer levels immediately after initiation of tPA compared to controls (Figure 4, Panel B). There were no severe, life-threatening, or fatal bleeding events in the tPA-Drip group (Tables 4 and 5). Subgroup analyses in this phase were not informative due to the small sample size.

Adverse events

There were no severe, life-threatening, or fatal bleeding events in the tPA-Bolus group (Tables 2 and 5), nor in the tPA-Drip group (Tables 4 and 5).

Evolution of COVID-19 disease severity over the duration of the trial

Over the duration of the trial, we documented a change in the COVID-19 severity of the eligible patients. Figure 5, Panel A shows the monthly mortality by study group over the duration of the trial, which significantly increased over time (Cochran-Armitage trend test p=0.02). The PaO2/FiO2 upon enrollment did not show a significant change over time (Figure 5, Panel B; R-square 0.05, p=0.13), nor the NEWS2 score (Figure 5, Panel C; R-square 0.06, p=0.08).

Discussion

The results of this trial show that the use of tPA (alteplase) as a bolus with immediate therapeutic anticoagulation after its administration for severe COVID-19 respiratory failure is safe and appears to improve oxygenation over baseline in a sustained fashion (from 6 through 168 hours post-randomization), while the control group did not show any significant improvement at any time. The trial was markedly underpowered to detect significant differences in clinically important parameters like VFD and in-hospital mortality, so not surprisingly there were no significant differences found in these outcomes. The trial's power to detect differences was further reduced by a late-stage adaptation. While not significantly different, the observation that the tPA-Bolus group had 12 VFD on median while the control group had 0 VFD on median was a large effect-size signal that persisted at every interim analysis during the trial. The same is true of in-hospital mortality, where the trial was unable to detect significant differences between the tPA-Bolus group and controls, but the low in-hospital mortality observed for the tPA-Bolus group, at just 21%, was a promisingly low rate for patients with such severe COVID respiratory failure.

These findings are of elevated clinical significance during a global pandemic of a disease with high morbidity and mortality. Of particular importance is safety, as there were no major bleeding events, including intracranial hemorrhage, associated with tPA and heparin, answering an important question for future investigations and clinical use. The safe outcomes were likely aided by careful selection, as a non-focal neurologic examination or cross-sectional brain imaging was required within the preceding 4.5 hours prior to enrollment to rule out a stroke prior to use of tPA, in addition to ensuring no laboratory or medical findings that posed unacceptable increased risk for bleeding prior to enrollment.

In COVID-19 respiratory failure there seems to be a substantial contribution of microvascular thrombosis and occlusion leading to dead-space ventilation[1-4]. This would explain the findings reported by Gattinoni et al[11] and others observing relatively preserved lung compliance despite profound respiratory failure early in the course of disease, and would also explain the results of the ACTIV-4a/ATTACC/REMAP-CAP trial that therapeutic heparin therapy is only beneficial if initiated early before decompensation requiring mechanical ventilation (i.e. before too much of the pulmonary microvasculature clots off) [12]. These observations support our findings of improved oxygenation with tPA-Bolus plus therapeutic heparin, as therapeutic heparinization alone cannot re-establish vascular patency and reduce dead-space ventilation once the microvasculature is clotted off; thrombolysis is required to accomplish this. It is also noteworthy that there was significant spike in D-dimer levels in the tPA-Bolus group that temporally aligned with dosing of tPA (and repeat dosing of tPA), verifying that we accomplished lysis of mature, cross-linked clot just as hypothesized.

In contrast to the tPA-Bolus group, the tPA-Drip group had no benefit. The major limitation of these findings are that they are based on six patients at a different time of the pandemic, when

mortality rates in the control group were also increasing. Moreover, an important protocol difference in the tPA Drip group was the low-dose/subtherapeutic heparin infusion during the 24-hour tPA drip that resulted in consistently short aPTT values. In Phase 1 of our trial, the highest benefit of the tPA-Bolus was observed among patients for whom the heparin regimen resulted in longer aPTT values. This raises the possibility that, in the tPA-Drip group, any revascularization effect of the initial tPA bolus before the drip ensued was lost by the lack of therapeutic anticoagulation during the low-dose tPA infusion over those subsequent 24 hours. This is supported by the coagulation data, where the aPTT at 24 hours was essentially within normal limits in the tPA-Drip group, whereas the UFH infusion and aPTT values in the tPA-Bolus group were fully therapeutic at that time. Thus, re-thrombosis of the microvasculature due to subtherapeutic heparin could explain failure to improve in the tPA-Drip group.

There are a number of important limitations of this study, in large part due to funding restrictions. First, it was underpowered to detect significant differences between the majority of the clinically important outcomes. Second, the "standard of care" of COVID-19 patients changed over the duration of the trial, although the randomization process would be expected to have controlled for these changes. Additionally, three of the eight enrolling centers made up over half of the enrollments and could have impacted the outcomes despite a relatively even randomization. Finally, this was an open label study, thus it is possible that the primary treatment teams changed portions of the tPA patients' care that could have impacted the trial outcomes.

Interpretation

In summary, bolus dosing of tPA with immediate therapeutic heparin anticoagulation in well-selected patients with severe COVID-19 respiratory failure is safe and improves oxygenation. The trial was underpowered to detect differences in clinical outcomes like VFD and in-hospital mortality, although the observations in these outcomes were promising. A Phase 3 study is warranted.

Take-Home Points

Study Question: Does fibrinolytic therapy with tPA improve pulmonary function in severe COVID-19 respiratory failure, and is it safe (specifically with respect to bleeding) in this setting?

Results: The tPA bolus group had improved oxygenation with no intracranial hemorrhages, and additional promising observations were noted in VFD and mortality.

Interpretation: The STARS trial provides the first known prospective evidence of potential benefit of fibrinolytic therapy in COVID-19 respiratory failure with an acceptable risk profile when patients are carefully selected.

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Author Contributions: CDB, HBM, EEM, AS and MBY had access to all data and contributed to all components of the study and manuscript generation. All other STARS trial authors meet at least the minimum three criteria for *CHEST* authorship.

Financial/Nonfinancial Disclosures: CDB, HBM, EEM, and MBY have patents pending related to both coagulation/fibrinolysis diagnostics and therapeutic fibrinolytics, and are passive co-founders and holds 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 (tPA) from Genentech, and owns stock options as a co-founder of Merrimack Pharmaceuticals. CDB, HBM, EEM, JW, NH, DST, AS, and MBY have received research grant funding from Genentech. JW receives consulting fees from Camurus A. B.. All other authors have nothing to disclose.

Role of the Sponsors: The funder of this investigator-initiated study (Genentech, Inc) had no role in the study design, data collection, analysis, interpretation, or manuscript preparation. Five authors (EEM, HBM, CB, MY, AS) served as the steering committee and had full access to the data.

Trial Registration and Protocol: The STARS trial was registered on ClinicalTrials.gov on April 22, 2020 (NCT04357730), where the trial protocol is also available.

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Figure Legends

Figure 1. CONSORT diagram

Figure 2. A: PaO2/FiO2 over time in Phase 1 estimates with 95% confidence bands based on the linear mixed model (interaction time*intervention p=0.14) for the tPA-Bolus vs Control groups. Asterisks indicate significant (p<0.017) differences **compared to baseline**; only the tPA-Bolus group showed significant improvements in PaO2/FiO2 compared to baseline; there were no significant improvements in PaO2/FiO2 in the control group.

B: PaO2/FiO2: same as A but further stratifying by requirement of a second tPA bolus at 24 hours

Figure 3. Role of average aPTT in PaO2/FiO2 temporal trends in Phase 1: A: 7-day average aPTT≤40s (n=15); B: 7-day average aPTT>40s (n=21)

Figure 4. D-Dimer temporal trends by study group in:

A: Phase 1 (tPA-Bolus vs Control): the intervention significantly changed the temporal trends of the study groups (interaction intervention*time p<0.0001). Asterisks indicate significant (p<0.003, adjusted for multiple comparisons by false-discovery rate) differences compared to baseline; only the tPA-Bolus group showed significant changes in D-Dimer levels compared to baseline; there were no significant changes in D-Dimer levels in the control group.

B: Phase 2 (tPA-Drip vs Control): the intervention changed (albeit not significantly at the p<0.017) the temporal trends of the study groups (interaction intervention*time p<0.013). Asterisks indicate significant (p<0.003, adjusted for multiple comparisons by false-discovery rate) differences compared to baseline; only the tPA-Drip group showed significant changes in D-Dimer levels compared to baseline; there were no significant changes in D-Dimer levels in the control group.

Figure 5. Trends in disease severity during the trial: A: mortality; B: PaO2/FiO2 ratio at eligibility; C: NEWS2 score at eligibility.

Table 1: Baseline characteristics in Phase 1 (categorical variables are expressed in N (%) and numerical variables in median (interquartile range))

PHASE 1	**Total**	**control**	**tPA-Bolus + Heparin**
N	36	17	19
AGE (years)	60.0 (52.0–64.0)	60.0 (57.0–62.0)	59.0 (47.0–65.0)
SEX=Male	25 (69.4)	10 (58.8)	15 (78.9)
BMI (kg/m2)	36.8 (30.7–42.0)	36.8 (29.6–42.0)	37.1 (32.1–43.7)
DAYS FROM ADMISSION TO RANDOMIZATION	2.0 (1.0–4.0)	2.0 (1.0–4.0)	2.0 (1.0–4.0)
PULMONARY HYPERTENSION	5 (14.3)	3 (18.8)	2 (10.5)
DIABETES	12 (34.3)	6 (37.5)	6 (31.6)
CARDIAC DISEASE	32 (91.4)	14 (87.5)	18 (94.7)
HYPERTENSION	10 (27.8)	6 (35.3)	4 (21.1)
CHRONIC OBSTRUCTIVE PULMONARY DISEASE	28 (80.0)	13 (81.3)	15 (78.9)
IMMUNOSUPPRESSION	33 (94.3)	14 (87.5)	19 (100)
HYPERLIPIDEMIA	11 (31.4)	6 (37.5)	5 (26.3)
OTHER COMORBIDITY	12 (36.4)	5 (35.7)	7 (36.8)
CONCURRENT INFECTIONS	23 (63.9)	10 (58.8)	13 (68.4)
DEXAMETHASONE	20 (55.6)	11 (64.7)	9 (47.4)
REMDESIVIR	17 (47.2)	8 (47.1)	9 (47.4)
RECEIVED SECOND TPA DOSE	14 (63.6)	3 (100)	11 (57.9)
POSITION			
Prone	14 (38.9)	7 (41.2)	7 (36.8)
Supine	16 (44.4)	9 (52.9)	7 (36.8)
Side	4 (11.1)	1 (5.9)	3 (15.8)

PAO2:FiO2	112.3 (87.0–134.5)	107.1 (85.0–128.9)	113.3 (89.0–135.0)
NEWS2	6.0 (5.0–9.0)	6.0 (5.0–9.0)	6.0 (5.0–9.0)
aPTT	30.5 (27.5–33.7)	30.0 (28.5–33.1)	32.3 (26.3–34.9)
INR	1.2 (1.1–1.3)	1.3 (1.1–1.3)	1.1 (1.1–1.2)
FIBRINOGEN (mg/dl)	685.0 (597.0–827.0)	668.5 (599.5–843.0)	685.0 (527.0–819.0)
D-DIMER (ng/mL)	1900.0 (1089.0–4800.0)	1900.0 (910.0–6137.0)	2105.0 (1169.5–4294.0)

NEWS2: National Early Warning Score 2; aPTT: activated Partial Thromboplastin Time; INR: international normalized ratio for prothrombin time

Table 2: Outcomes for Phase 1 (categorical variables are expressed in N (%) and numerical variables in median (interquartile range))

Variables	Total	control	tPA-Bolus + Heparin	P-value
N	36	17	19	
PAO2:FiO2 at 24HRS	145.0 (110.5–193.5)	146.7 (98.8–174.0)	144.0 (122.9–217.1)	0.5471
PAO2:FiO2 % IMPROVEMENT OVER BASELINE AT 24HRS	39.7 (-4.9–72.1)	37.0 (-6.4–64.5)	44.4 (-3.4–78.0)	0.6573
PAO2:FiO2 at 48HRS	138.2 (105.0–181.0)	125.0 (87.5–147.5)	157.1 (130.0–188.0)	0.0458
PAO2:FiO2 % IMPROVEMENT OVER BASELINE AT 48HRS	24.6 (-1.5–59.8)	16.9 (-8.3–36.8)	29.8 (4.5–88.7)	0.1131
COMPOSITE OUTCOME: PAO2:FiO2 % IMPROVEMENT AT 48 HRS>50% OR PAO2:FiO2>=200	11 (30.6)	2 (11.8)	9 (47.4)	0.0312
PARALYTICS AT 48HRS	18 (50.0)	10 (58.8)	8 (42.1)	0.5051
POSITION AT 48HRS				0.8166
Prone	14 (38.9)	6 (35.3)	8 (42.1)	
Supine	16 (44.4)	9 (52.9)	7 (36.8)	
Left Side	2 (5.6)	1 (5.9)	1 (5.3)	
Right Side	4 (11.1)	1 (5.9)	3 (15.8)	
NEWS2 % INCREASE OVER BASELINE AT 48HRS	0.0 (-22.2–30.0)	0.0 (-22.2–40.0)	0.0 (-22.2–20.0)	0.9241
PTT AT 24HRS (s)	38.9 (32.7–58.6)	32.9 (28.0–36.1)	51.7 (36.9–65.6)	0.0004
PTT AT 48HRS (s)	41.2 (30.0–65.6)	30.0 (26.9–36.2)	64.3 (55.7–73.6)	<.0001
INR AT 24HRS	1.2 (1.1–1.3)	1.3 (1.2–1.3)	1.2 (1.1–1.2)	0.1521
INR AT 48HRS	1.2 (1.1–1.3)	1.2 (1.1–1.3)	1.2 (1.1–1.3)	0.5685

FIBRINOGEN AT 24HRS (mg/dl)	613.0 (562.0–814.0)	595.0 (521.0–828.0)	627.0 (567.0–800.0)	0.7513
FIBRINOGEN AT 48HRS (mg/dl)	586.0 (498.0–798.0)	612.0 (450.0–817.0)	567.0 (520.0–786.0)	0.9725
D-DIMER AT 24HRS (ng/ml)	1895.0 (969.0–4422.0)	1426.0 (730.0–3970.0)	2296.0 (1330.0–9700.0)	0.0615
D-DIMER AT 48HRS (ng/ml)	1641.0 (900.0–3450.0)	1326.0 (870.0–2970.0)	1975.0 (1010.0–3650.0)	0.4282
ADVERSE EVENT INCIDENCE	26 (72.2)	13 (76.5)	13 (68.4)	59%
BLEEDING EVENT INCIDENCE	5 (13.9)	2 (11.8)	3 (15.8)	73%
VENTILATION DAYS	14.0 (9.0–28.0)	18.0 (9.0–28.0)	13.0 (8.0–25.0)	0.2088
VFD	0.0 (0.0–17.0)	0.0 (0.0–9.0)	12.0 (0.0–19.0)	0.1064
ICU DAYS	16.5 (11.5–28.0)	18.0 (12.0–28.0)	16.0 (11.0–28.0)	0.8990
IFD	0.0 (0.0–14.0)	0.0 (0.0–10.0)	6.0 (0.0–15.0)	0.4200
IN-HOSPITAL MORTALITY	11 (30.6)	7 (41.2)	4 (21.1)	0.1907
28 DAY MORTALITY	9 (25.0)	5 (29.4)	4 (21.1)	0.5631

NEWS2: National Early Warning Score 2; aPTT: activated Partial Thromboplastin Time; INR: international normalized ratio for prothrombin time; VFD: ventilation-free-days; ICU: Intensive care unit; IFD: Intensive care unit-free-days

Table 3: Baseline characteristics of Phase 2(categorical variables are expressed in N (%) and numerical variables in median (interquartile range))

Phase 2	**Total**	**Control**	**tPA-Drip + Heparin**
N	14	8	6
AGE (years)	63.5 (56.0–66.0)	60.5 (51.0–65.0)	64.5 (62.0–68.0)
SEX=Male	12 (85.7)	6 (75.0)	6 (100)
BMI (kg/m2)	30.3 (27.7–35.4)	30.9 (26.1–35.4)	29.5 (27.7–36.1)
DAYS FROM ADMISSION TO RANDOMIZATION	1.5 (1.0–4.0)	1.0 (0.5–1.5)	5.0 (2.0–8.0)
DIABETES	5 (35.7)	3 (37.5)	2 (33.3)
CARDIAC DISEASE	1 (7.1)	1 (12.5)	
HYPERTENSION	8 (57.1)	4 (50.0)	4 (66.7)
CHRONIC OBSTRUCTIVE PULMONARY DISEASE	3 (21.4)	0	3 (50.0)
HYPERLIPIDEMIA	2 (14.3)	1 (12.5)	1 (16.7)
OTHER COMORBIDITY	9 (64.3)	5 (62.5)	4 (66.7)
INFECTIONS	9 (64.3)	5 (62.5)	4 (66.7)
DEXAMETHASONE	6 (46.2)	3 (42.9)	3 (50.0)
REMDESIVIR	3 (23.1)	2 (28.6)	1 (16.7)
POSITION			
Prone	8 (57.1)	6 (75.0)	2 (33.3)
Supine	3 (21.4)	1 (12.5)	2 (33.3)
Side	3 (21.4)	1 (12.5)	2 (33.3)
PAO2:FiO2	99.5 (77.0–128.3)	99.5 (75.5–123.9)	109.7 (77.0–132.9)
NEWS2	7.5 (5.0–10.0)	10.0 (7.5–11.0)	6.0 (5.0–7.0)

aPTT (sec)	31.1 (27.0–33.7)	31.1 (27.0–33.7)	31.0 (26.7–41.3)
INR	1.2 (1.0–1.3)	1.3 (1.1–1.3)	1.1 (1.0–1.3)
FIBRINOGEN (mg/dl)	695.0 (560.0–870.0)	560.0 (507.0–992.0)	695.5 (692.0–717.0)
D-DIMER (ng/mL)	3940.0 (1364.0–5510.0)	4180.0 (1434.0–9170.0)	2652.0 (963.0–5510.0)

NEWS2: National Early Warning Score 2; aPTT: activated Partial Thromboplastin Time; INR: international normalized ratio for prothrombin time

Table 4: Outcomes in Phase 2 (categorical variables are expressed in N (%) and numerical variables in median (interquartile range))

PHASE 2	**Total**	**control**	**tPA Drip+Heparin**	**P-value**
N	14	8	6	
PAO2:FiO2 AT 24HRS	114.1 (87.1–124.0)	119.2 (111.9–131.3)	94.5 (71.0–114.5)	0.0814
PAO2:FiO2 % IMPROVEMENT OVER BASELINE AT 24HRS	14.5 (-19.5–45.8)	19.9 (-0.3–80.8)	-16.7 (-37.4–36.5)	0.1376
PAO2:FiO2 AT 48HRS	104.5 (84.3–116.7)	113.7(88.8–160.0)	103.5(78.8–105.0)	0.4014
PAO2:FiO2 % IMPROVEMENT OVER BASELINE AT 48HRS	-19.6 (-22.6–101.9)	-11.9 (-24.3–136.1)	-19.6 (-21.7–2.3)	0.7469
COMPOSITE OUTCOME: PAO2:FiO2 % IMPROVEMENT AT 48 HRS>50% OR PAO2:FiO2>=200	4 (28.6)	3 (37.5)	1 (16.7)	0.5804
PARALYTICS AT 48HRS	10 (71.4)	6 (75.0)	4 (66.7)	1.0000
POSITION AT 48HRS				0.8601
Prone	7 (50.0)	3 (37.5)	4 (66.7)	
Supine	4 (28.6)	3 (37.5)	1 (16.7)	
Left Side	2 (14.3)	1 (12.5)	1 (16.7)	
Right Side	1 (7.1)	1 (12.5)		
NEWS2 % INCREASE OVER BASELINE AT48HRS	0.0 (-25.0–60.0)	-12.5 (-26.8–18.8)	65.7 (0.0–80.0)	0.0794
PTT AT 24HRS (s)	30.5 (26.9–38.8)	35.6 (29.0–51.2)	27.7 (26.8–30.0)	0.1752
PTT AT 48HRS (s)	34.4 (28.5–73.4)	53.1 (28.5–95.9)	33.0 (28.5–57.4)	0.5181
INR AT 24HRS	1.1 (1.0–1.2)	1.1 (1.0–1.3)	1.1 (1.0–1.2)	0.7453
INR AT 48HRS	1.2 (1.1–1.4)	1.2 (1.1–1.3)	1.2 (1.1–1.4)	0.9477

FIBRINOGEN AT 24HRS (mg/dl)	606.0 (474.0–822.0)	588.5 (441.0–768.0)	612.0 (542.0–822.0)	0.8465
FIBRINOGEN AT 48HRS (mg/dl)	564.5 (420.0–706.0)	480.5 (395.5–638.5)	698.5 (542.0–821.0)	0.1748
D-DIMER AT 24HRS (ng/ml)	5420.0 (3320.0–11510)	3855.0 (1996.0–8500.0)	8477.0 (5540.0–11510)	0.1066
D-DIMER AT 48HRS (ng/ml)	4060.5 (3460.0–5890.0)	3480.5 (2713.5–4750.0)	4957.5 (4261.0–7650.0)	0.0612
ADVERSE EVENT INCIDENCE	7 (50.0)	5 (62.5)	2 (33.3)	0.2801
BLEEDING EVENT INCIDENCE	1 (7.1)	1 (12.5)		0.3688
VENTILATION DAYS	20.5 (16.0–26.0)	24.5 (12.0–27.0)	17.5 (16.0–25.0)	0.6979
VFD	0.0 (0.0–0.0)	0.0 (0.0–1.0)	0.0 (0.0–0.0)	0.9284
ICU DAYS	22.5 (17.0–29.0)	27.0 (12.0–30.5)	19.0 (17.0–25.0)	0.6982
IFD	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.3123
IN-HOSPITAL MORTALITY	9 (64.3)	4 (50.0)	5 (83.3)	0.1977
28 DAY MORTALITY	8 (57.1)	4 (50.0)	4 (66.7)	0.5329

NEWS2: National Early Warning Score 2; aPTT: activated Partial Thromboplastin Time; INR: international normalized ratio for prothrombin time; VFD: ventilation-free-days; ICU: Intensive care unit; IFD: Intensive care unit-free-days

Table 5: Adverse events by study group and severity grade (1: mild to 5: life threatening/fatal; includes death only as a consequence of an adverse event)

	Study groups			
	Control	**tPA-Bolus**	**tPA-Drip**	**Total**
	N=25	**N=19**	**N=6**	
Severity grade 5				
Arrest	2	0	0	2
Liver failure	0	0	1	1
Renal failure	0	0	1	1
Worsening of lung function	4	3	1	8
Total number of events	6	3	3	12
Number of subjects	6	3	1	10
% of subjects	24.0%	12.0%	4.0%	40.0%
Severity grade 4				
Cardiac arrhythmia	2	0	0	2
Failed extubation	0	1	0	1
Hyperkalemia	1	0	0	1
Hypotension	2	1	0	3
Liver failure	1	0	0	1
Multiple organ failure	0	0	1	1
Peritonitis	1	0	0	1
Pneumonia	3	0	0	3
Septic shock	1	0	0	1
Worsening of lung function	1	0	1	2
Total number of events	12	2	2	16
Number of subjects	5	2	1	8

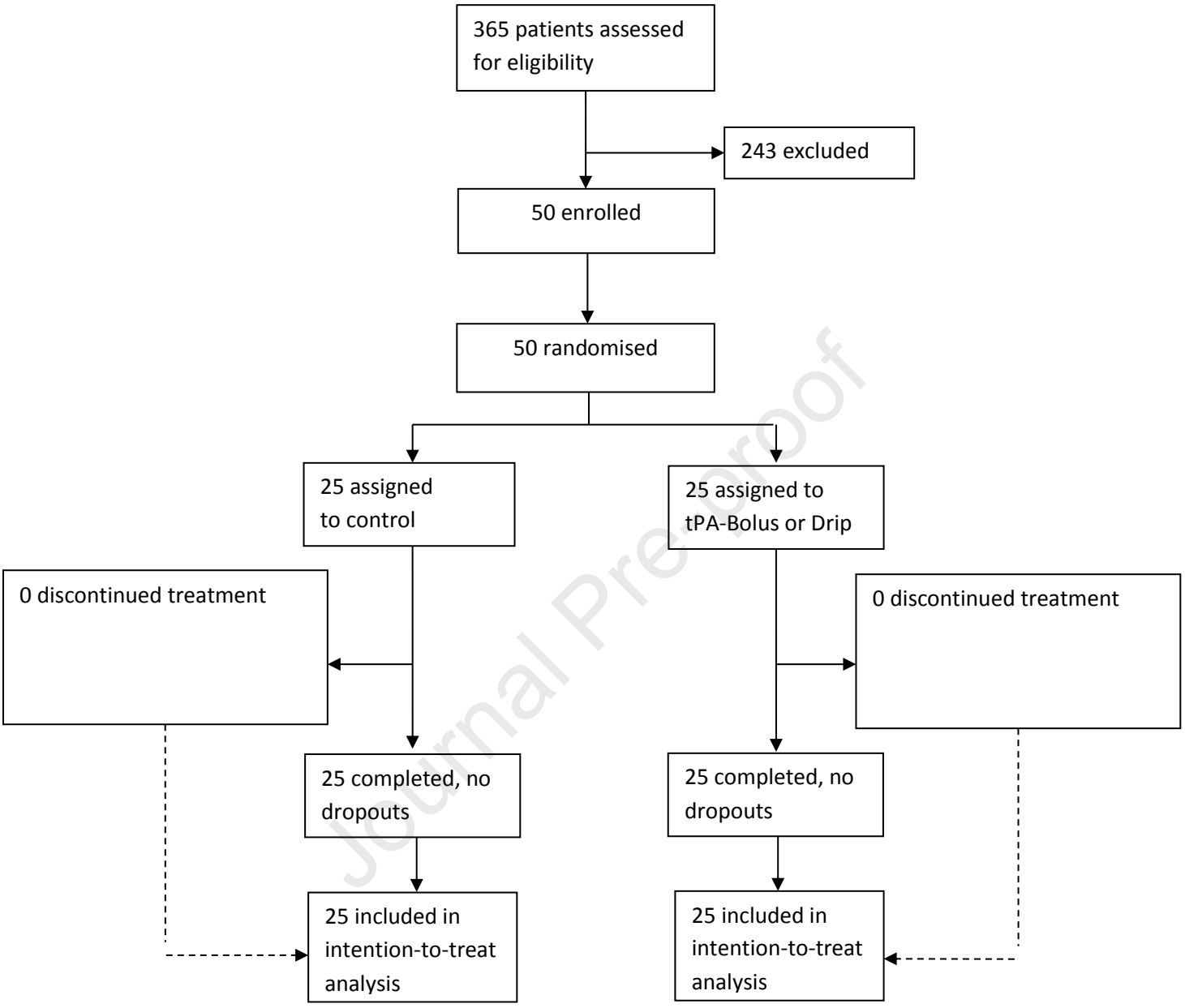
	Study groups			
	Control	**tPA-Bolus**	**tPA-Drip**	**Total**
	N=25	**N=19**	**N=6**	
% of subjects	20.0%	8.0%	4.0%	32.0%
Severity grade 3				
Candidiasis	0	1		1
Cardiac arrhythmia	1	0		1
Delirium	0	1		1
Deep venous thrombosis	1	0		1
Hypervolemia	0	1		1
Hypotension	0	1		1
Ileus	0	1		1
Pulmonary embolism	1	1		2
Pneumonia	1	2		3
Renal failure	4	0		4
Septic shock	1	0		1
Urinary tract infection	1	1		2
Worsening of lung function	2	0		2
Total number of events	12	9		21
Number of subjects	8	6		14
% of subjects	32.0%	24.0%		56.0%
Severity grade 2				
Acidosis (respiratory)	1	0	0	1
Alkalosis metabolic/respiratory	1	0	0	1
Aspiration	1	1	0	2
Bacteremia	2	0	1	3

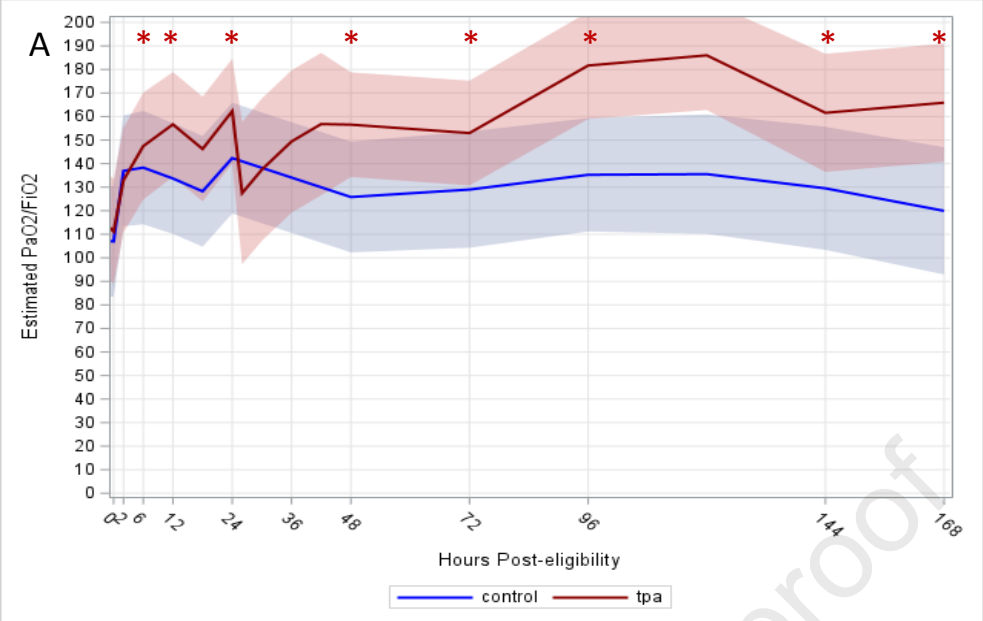
	Study groups			
	Control	**tPA-Bolus**	**tPA-Drip**	**Total**
	N=25	**N=19**	**N=6**	
Bleeding abdominal	1	0	0	1
Bleeding hemoptysis	0	1	0	1
Bleeding rectal tear	1	0	0	1
Bleeding urinary	0	1	0	1
Candidiasis	1	0	0	1
Cardiac arrhythmia	1	2	0	3
Delirium	2	1	0	3
Diarrhea	1	0	0	1
Deep venous thrombosis	3	1	0	4
EBV	1	0	0	1
Failure to wean off ventilation	0	1	0	1
Fracture	1	0	0	1
HSV	1	0	0	1
Hyperfibrinogenemia	1	0	0	1
Hyperglycemia	0	0	1	1
Hyperkalemia	0	1	0	1
Hypervolemia	1	0	1	2
Hypotension	2	1	0	3
Myopathy	2	1	0	3
Pleural effusion	0	1	1	2
Pneumonia	4	2	0	6
Pneumothorax	1	0	0	1
Renal failure	0	2	1	3

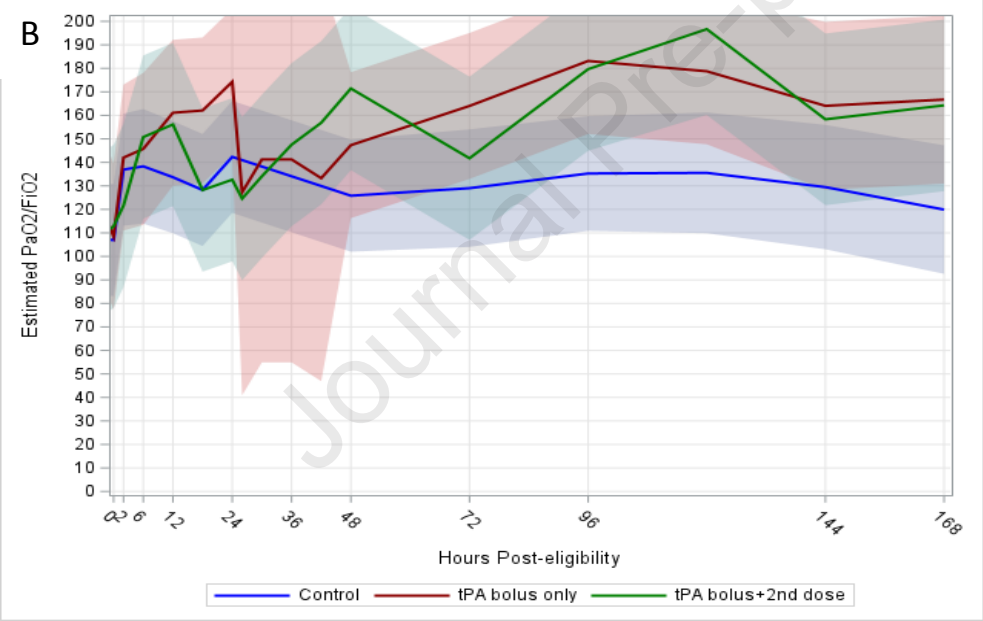
	Study groups			
	Control	**tPA-Bolus**	**tPA-Drip**	**Total**
	N=25	**N=19**	**N=6**	
Sepsis	0	1	0	1
Thrombosis arterial	1	0	0	1
Tongue edema	0	0	1	1
Urinary retention	1	0	0	1
Urinary tract infection	1	0	0	1
Worsening of lung function	2	0	0	2
Total number of events	34	17	6	57
Number of subjects	14	6	3	23
% of subjects	56.0%	24.0%	12.0%	92.0%
Severity grade 1				
Agitation	0	1	0	1
Alkalosis metabolic	0	2	0	2
Anemia	5	4	0	9
Aspiration	2	1	0	3
Bacteremia	1	0	0	1
Benzodiazepine/opiate withdrawal	0	1	0	1
Biliary dilation	0	1	0	1
Bleeding nasal	1	0	0	1
Bleeding oral	2	2	0	4
Bleeding vaginal	0	1	0	1
Bronchial obstruction	2	0	0	2
Cardiac arrhythmia	2	3	0	5

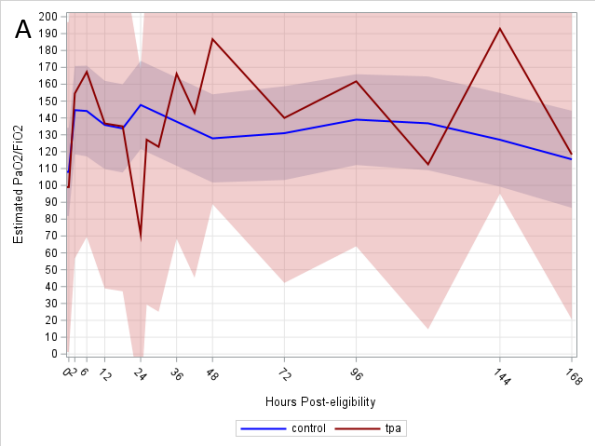
	Study groups			
	Control	**tPA-Bolus**	**tPA-Drip**	**Total**
	N=25	**N=19**	**N=6**	
Constipation	2	4	0	6
Dehydration	0	1	0	1
Delirium	4	2	0	6
Diarrhea	1	2	0	3
Deep venous thrombosis	3	0	0	3
Dysphagia	0	2	0	2
Dysphonia	2	0	0	2
Encephalopathy	2	0	0	2
Eosinophilia	1	1	0	2
Facial edema	0	0	1	1
Fall	0	1	0	1
Fever	7	2	1	10
Hyperglycemia	3	1	0	4
Hyperkalemia	1	0	0	1
Hypernatremia	2	3	0	5
Hypertension	2	2	0	4
Hypervolemia	1	0	0	1
Hypoglycemia	1	0	0	1
Hypokalemia	0	1	0	1
Hyponatremia	0	1	0	1
Hypotension	5	1	0	6
Hypovolemia	1	0	0	1
Ileus	1	3	0	4

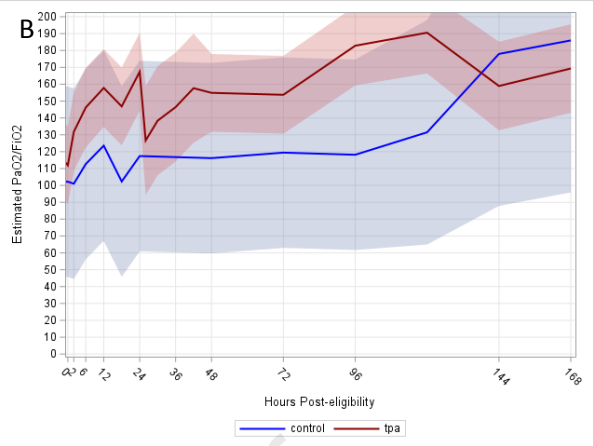
	Study groups			
	Control	**tPA-Bolus**	**tPA-Drip**	**Total**
	N=25	**N=19**	**N=6**	
Leukocytosis	2	3	0	5
Myopathy	4	4	0	8
Paraphimosis	1	0	0	1
Pleural effusion	0	1	0	1
Pneumatocele	0	1	0	1
Pneumonia	2	3	1	6
Pressure ulcer	1	2	0	3
Pulmonary hypertension	2	0	0	2
Rash	0	1	0	1
Renal failure	2	2	0	4
Renal tubular acidosis	1	0	0	1
Sinusitis	0	1	0	1
Thrombocytosis	0	1	0	1
Thrombocytosis	0	1	0	1
Transaminitis	1	1	0	2
Urinary retention	0	2	0	2
Urinary tract infection	0	2	0	2
Vomit	0	2	0	2
Worsening of lung function	3	0	0	3
Total number of events	73	70	3	146
Number of subjects	11	11	2	24
% Of subjects	44.0%	44.0%	8.0%	96.0%

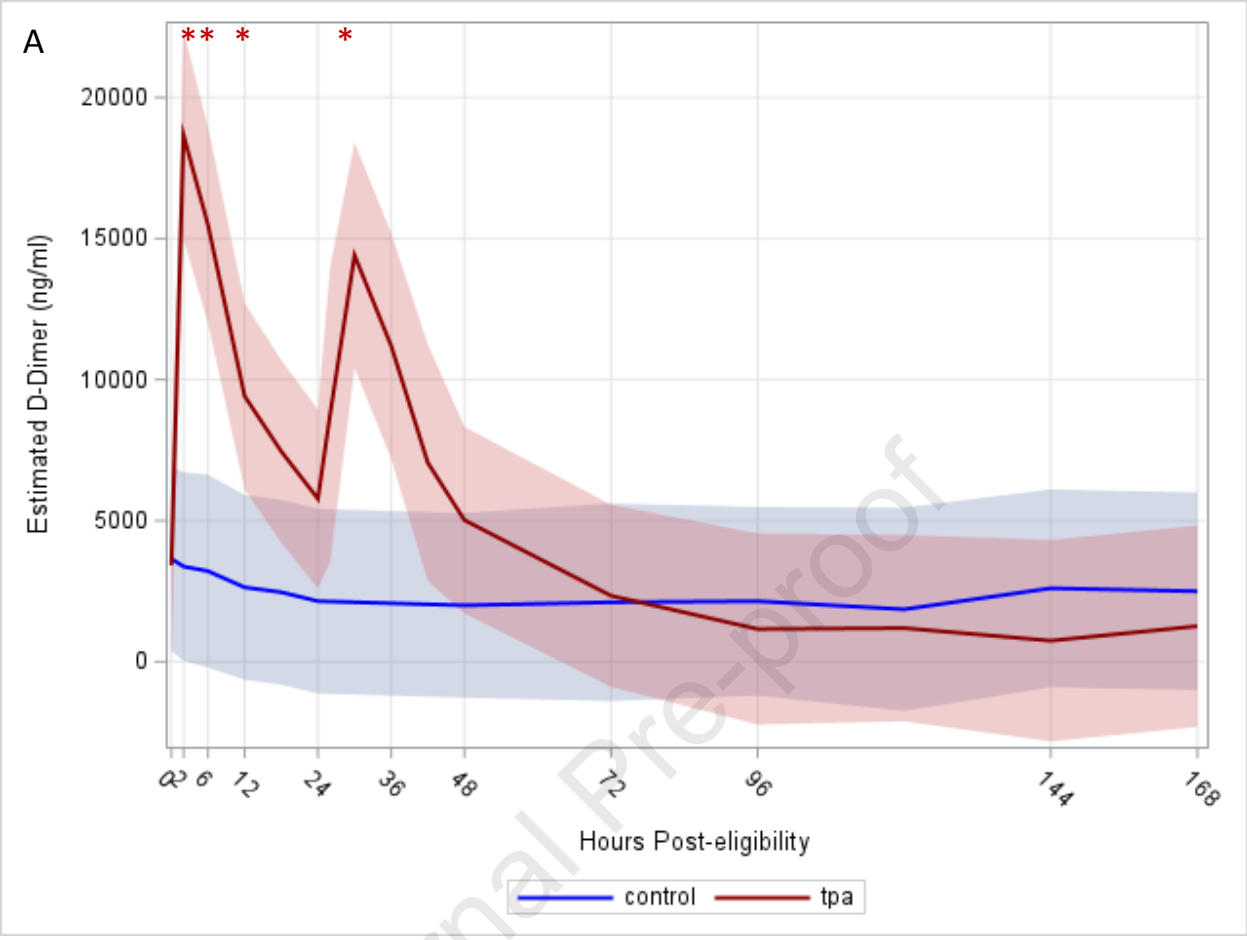


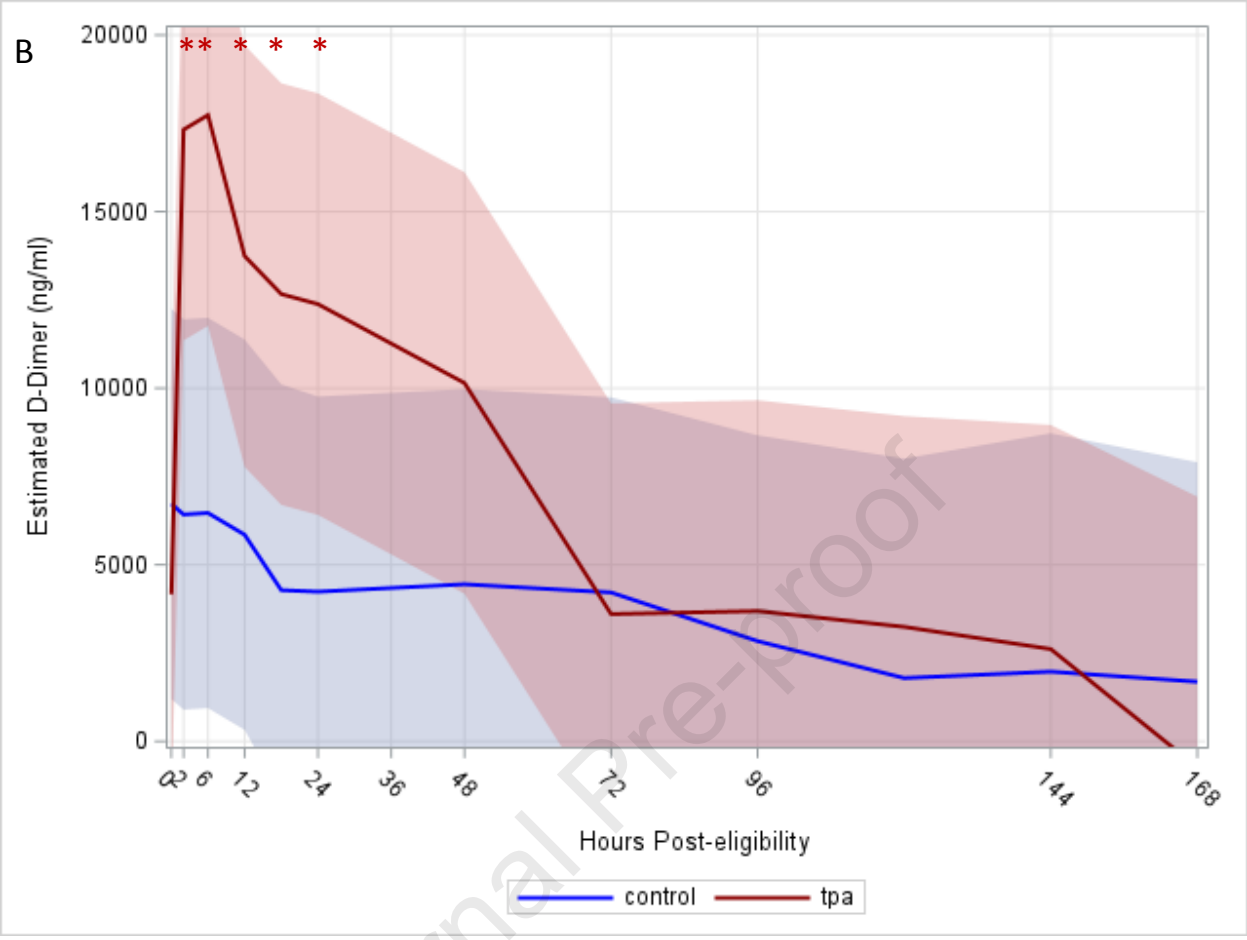


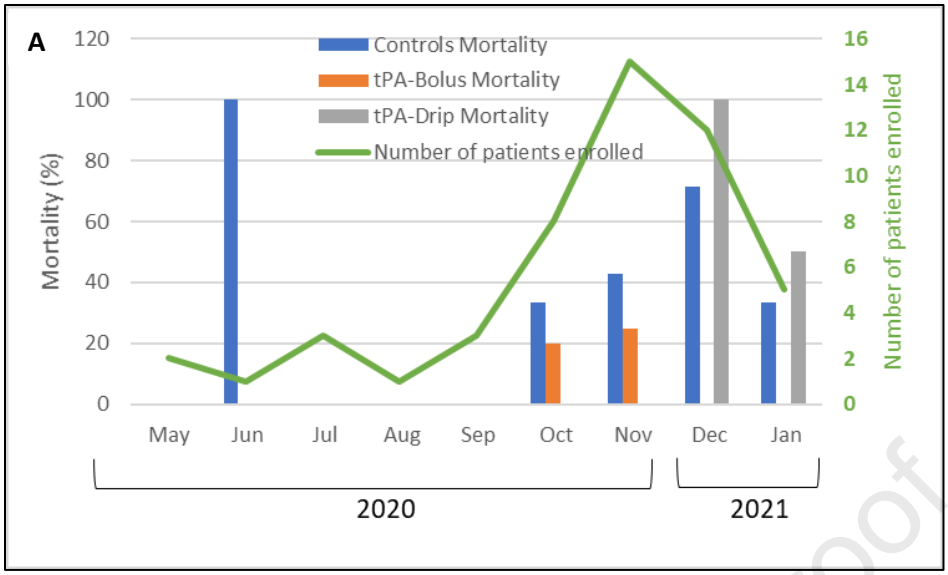


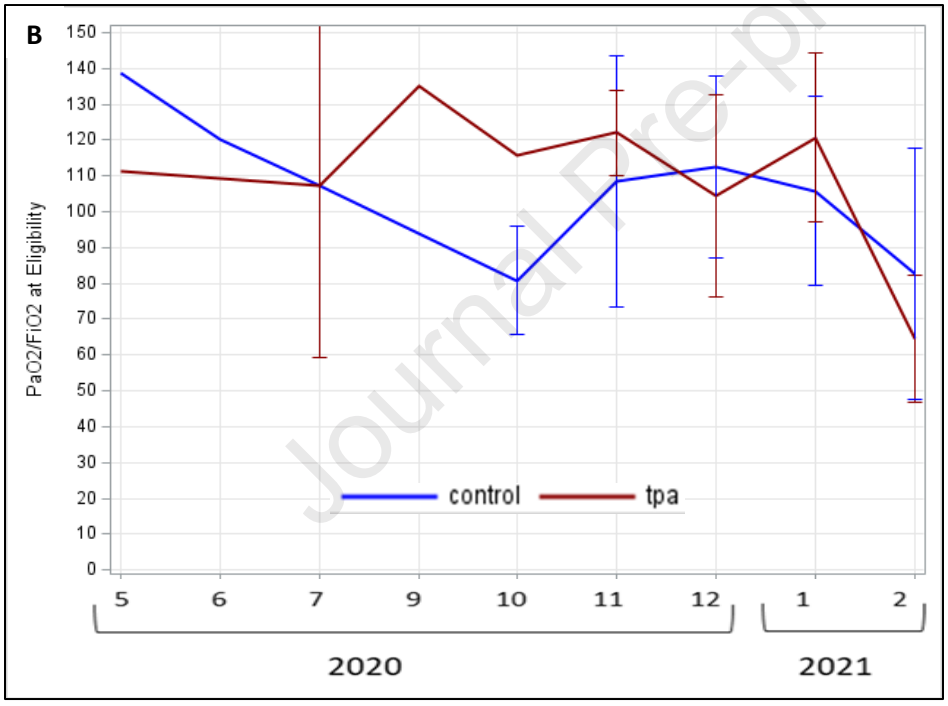


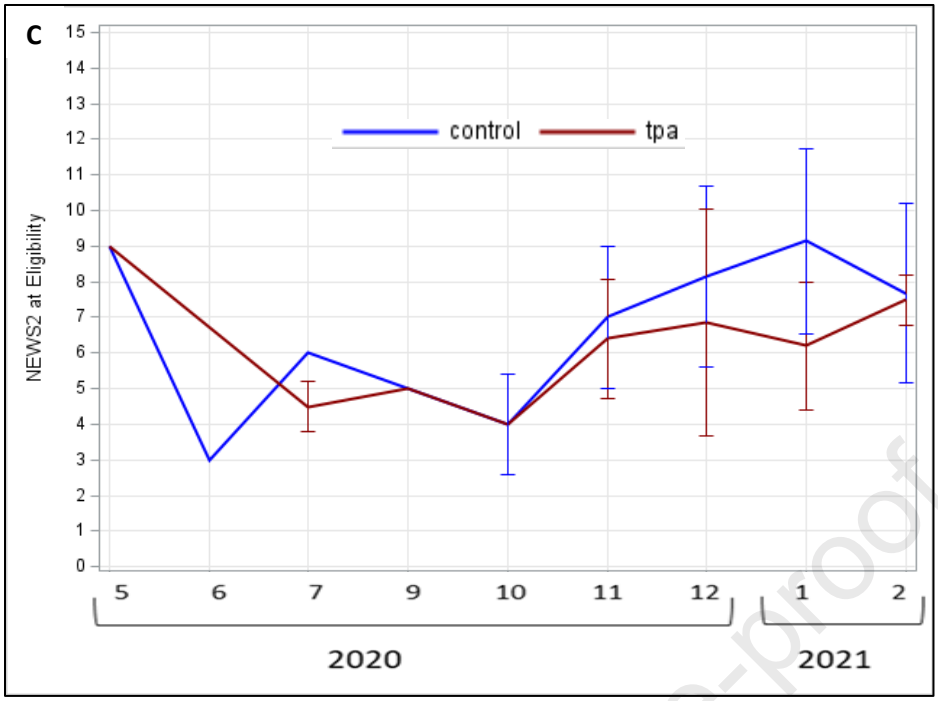












Abbreviations: COVID-19 (SARS-Cov-2, Coronavirus Disease 2019), tissue plasminogen activator (tPA), VFD (ventilator-free days), IFD (ICU-free days), ARDS (acute respiratory distress syndrome), DIC (disseminated intravascular coagulation), ACTIV-4 (Antithrombotics for Adults Hospitalized with COVID-19 trial), NEWS2 (National Early Warning System-2), ICU (intensive care unit), BMI (body mass index), PE (pulmonary embolism), DVT (deep venous thrombosis), ICH (intracranial hemorrhage), ECMO (extracorporeal membrane oxygenation), DSMB (data safety monitoring board), PETAL (Prevention and Early Treatment of Acute Lung Injury network), MV (mechanical ventilation), MRI (magnetic resonance imaging), CT (computed tomography), ABG (arterial blood gas), INR (international normalized ratio), aPTT (activated partial thromboplastin time), U (units), HIT (heparin-induced thrombocytopenia), FDA (United States Food and Drug Administration), NIH (National Institutes of Health), UFH (unfractionated heparin), RBC (red blood cell)