UCL

Full title of trial	A pilot, open label, phase II clinical trial of nebulised recombinant tissue-Plasminogen Activator (rtPA) in patients with COVID-19 ARDS: The Plasminogen Activator COVID-19 ARDS (PACA) trial
Short title	Nebulised rtPA for ARDS due to COVID-19 – The PACA trial
Sponsor	University College London (UCL)
Sponsor protocol number	132151
EudraCT No	2020-001640-26
ISRCTN / Clinicaltrials.gov no:	NCT04356833
Document name:	**PACA protocol version 10.0 (2Feb2021) redacted**
Document date:	**02 Feb 2021**

A pilot, open label, phase II clinical trial of nebulised recombinant tissue-Plasminogen Activator (rtPA) in patients with COVID-19 ARDS: The Plasminogen Activator COVID-19 ARDS (PACA) trial

Full title of trial	A pilot, open label, phase II clinical trial of nebulised recombinant tissue-Plasminogen Activator (rtPA) in patients with COVID-19 ARDS: The Plasminogen Activator COVID-19 ARDS (PACA) trial
Short title	Nebulised rtPA for ARDS due to COVID-19 – The PACA trial
Version and date of protocol	Version 10.0, 2 Feb 2021
Sponsor:	University College London (UCL)
Sponsor protocol number	132151
Funder (s):	Royal Free Charity
EudraCT No	2020-001640-26
ISRCTN / Clinicaltrials.gov no:	NCT04356833
ACTIVE IMP(s):	Nebulised recombinant tissue plasminogen activator (rtPA) alteplase
Phase of trial	Phase II
Sites(s)	Multi Site
Chief investigator: Pratima Chowdary The Katharine Dormandy Haemophilia and Thrombosis Centre Royal Free Hampstead NHS Trust Royal Free Hospital, Pond Street Hampstead, London NW3 2QG	Sponsor Representative: Joint Research Office, UCL, Office address: UCLH/UCL Joint Research Office, part of the Research Directorate 4th Floor, West 250 Euston Road

	London NW1 2PG

Protocol Version History

Version Number	Date	Protocol Update Finalised By (insert name of person):	Reasons for Update
2.0	14 April 2020	Alison Evans	Update to inclusion criteria 4b (removal of 7 day timeline for intubation in IMV patients)and removal of exclusion criteria 'Patients receiving anticoagulation with therapeutic doses'
3.0	17 Apr 2020	Alison Evans	Updates to section 2.5 (assessment and management of risk), 7.10 (stopping criteria), 10.5 (Randomisation methods) and 12.2 (IDMC) in response to GNA from MHRA and comments from MHRA EAG. *In addition:* Updated the inclusion criteria to remove the upper age limit Updated inclusion criteria no 5 to remove HFO definition (and reference to HFO throughout protocol Updated the schedule of collection for research blood samples to baseline, Day 2, 4, 7 and then every week until the end of the trial.
4.0	29 Apr 2020		Amendments made to inclusion/exclusion criteria for consistency. Updated exclusion criteria no 7 to 'Patients considered inappropriate for critical care (~~prior decision re ceiling of care established~~ **e.g. being considered for palliative care**). Certain sections have been corrected regarding fibrinogen monitoring and the action to be taken. In particular, it was unclear whether replacement therapy should be given when fibrinogen levels are <1.5 g/L or <1.0 g/L. This has been clarified. A drop in fibrinogen <1.5 g/L is not a stopping criteria for the trial and accordingly this has not been listed in the Stopping Criteria section (7.10) as the intention has always been that this was a reason for treatment discontinuation in an individual patient. The wording in the protocol has been clarified around this. Updated stopping criteria to remove: • Any major bleeding as reported by ISTH criteria and replace with: • Any fatal bleeding • Symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, pericardial,

			pulmonary or intramuscular with compartment syndrome. therefore not including the following as a stopping criteria: 'Bleeding causing a fall in haemoglobin level of 20 g/L or more, or leading to transfusion of two or more units of whole blood or red cells.' We have also added the caveat that the bleeding events specified in the stopping criteria should occur 'within start of treatment and 30 h after the last dose'. Clinicaltrials.gov number added
5.0	20 May 2020		To update the rtPA dosing schedule and relevant endpoints
6.0	18 Sep 2020		Use of historical matched controls instead of standard of care arm at a ratio of two controls to every one treatment arm. Allowance for telephone consent for matched historical controls. Use of deceased patients as historical controls with no consent sought. Renaming of non-invasive ventilation group to non-invasive oxygen support arm (throughout protocol) and amended to include standard oxygen therapy. Other small changes to the inclusion/exclusion criteria. Extension of pre-treatment baseline period to 3 days. Minor changes to primary and secondary (objectives and) endpoints. Addition of University of Surrey laboratory for exploratory endpoint analyses.
7.0	11 Nov 2020		To document that we will not be collecting consent for historical controls, following discussion with CAG. Updates to data collected for historical control patients to allow for a more complete comparison between treatment patients and control patients (section 7.5.1). Also updates to the criteria to be used for matching of control patients to allow for better matching (section 10.4.2). To make minor clarifications/corrections.
8.0	8 Jan 2021		To include an additional cohort of up to 30 patients to allow further accrual of safety and efficacy data.

9.0	14 Jan 2021		To correct error in the wording in last row of dosing table for NIV patients (section 7.3.2) to clarify that dosing will continue every 12 h (+/- 2 h) on Days 6-14. To make updates to schedule of assessments table in Appendix 1 (section 20) to further clarify assessments to be done in Cohort 2 (in line with updates made to section 7.5 in protocol version 8.0).
10.0	2 Feb 2021		To document that should a patient's condition change such that their ventilation type is changed whilst receiving rtPA treatment (that is, from IMV to NIV, or vice versa) they will switch to the treatment schedule that is applicable to the new ventilation type (section 2.5 and 7.4). To increase the window of flexibility in timing of the dose from 2 hours to 4 hours when dosing is twice a day (section 7.3.2). Section 5.1 – to add in that the IMP may be supplied as 20 mg vials, in addition to 10 mg vials. Section 10.6 Have updated patient numbering of individual groups in line with the patient registration SOP and clarified the definition of the groups.Section 10.6 Clarified that additional descriptions of the results may be performed taking into account changes in ventilation type.

Signatures

The Chief Investigator and the JRO have discussed this protocol. The investigator agrees to perform the investigations and to abide by this protocol

The investigator agrees to conduct the trial in compliance with the approved protocol, EU GCP and UK Regulations for CTIMPs (SI 2004/1031; as amended), the UK Data Protection Act (1998), the Trust Information Governance Policy (or other local equivalent), the current UK Policy Framework for Health and Social Care Research, the Sponsor's SOPs, and other regulatory requirements as amended.

Chief investigator		
Dr Pratima Chowdary		
UCL	Signature	Date
Sponsor		
Dr Rajinder Sidhu		
UCL	Signature	Date

Table of contents

List of abbreviations

ACDP	Advisory Committee of Dangerous Pathogens
ACE	Angiotensin-converting-enzyme
AE	Adverse Event
AESI	Adverse Event of Special Interest
ALI	Acute Lung Injury
APR	Annual Progress Report
APTT	Activated Partial Thromboplastin Time
AR	Adverse Reaction
ARDS	Acute Respiratory Distress Syndrome
AT	Antithrombin
BNP	Brain Natriuretic Peptide
CA	Competent Authority
CI	Chief Investigator
COVID-19	Coronavirus Disease 2019
CPAP	Positive Airway Pressure
CRF	Case Report Form
CRO	Contract Research Organisation
CRP	C-Reactive Protein
CTA	Clinical Trial Authorisation
CTIMP	Clinical Trial of Investigational Medicinal Product
DH	Department of Health
DI	Designated Individual
DIBD	Developmental International Birth Date
DMC	Data Monitoring Committee
DNA	DeoxyriboNucleic Acid
DP	Driving Pressure
DSUR	Development Safety Update Report
EC	European Commission
ECMO	Extracorporeal Membrane Oxygenation
ED	Emergency Department
EMA	European Medicines Agency

EU	European Union
EUCTD	European Clinical Trials Directive
EudraCT	European Clinical Trials Database
EudraVigilance	European database for Pharmacovigilance
FDF	Fibrin Degradation Product
FiO_2	Fraction of inspired Oxygen
GAfREC	Governance Arrangements for NHS Research Ethics
GCP	Good Clinical Practice
GMO	Genetically Modified Organisms
GMP	Good Manufacturing Practice
GP	General Practitioner
HFO	High Flow Oxygen
HSL	Health Services Laboratories
HTA	Human Tissue Authority
IB	Investigator Brochure
ICF	Informed Consent Form
ICU	Intensive Care Unit
IDMC	Independent Data Monitoring Committee
IDW	Ideal Body Weight
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
IMV	Invasive mechanical ventilation
ISF	Investigator Site File
ISRCTN	International Standard Randomised Controlled Trial Number
ISTH	International Society on Thrombosis and Haemostasis
ITT	Intention to treat
IU	International Units
IV	Intravenous
JRO	Joint Research Office
KD	Katharine Dormandy
LHD	Lactate Dehydrogenase
LMWH	Low Molecular Weight Heparin

LVEF	Left Ventricular Ejection Fraction
MA	Marketing Authorisation
MHRA	Medicines and Healthcare products Regulatory Agency
MRC	Medical Research Council
MS	Member State
NEWS	National Early Warning Score
NHS	National Health Service
NHS R&D	National Health Service Research & Development
NIMP	Non Investigational Medicinal Product
NIV	Non-invasive ventilation
PAA	Plasminogen Activator Activity
PACA	Plasminogen Activator COVID-19 ARDS Trial
PAI-1	Plasminogen Activator Inhibitor-1
PaO_2	Partial Pressure of Oxygen
PEEP	Positive and Expiry Pressure
PI	Principal Investigator
PIS	Participant Information Sheet
PL	Product License
PT	Prothrombin Time
QA	Quality Assurance
QC	Quality Control
QP	Qualified Person (for release of trial drug)
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
RSI	Reference Safety Information
rtPA	recombinant tissue plasminogen activator, alteplase
RVEF	Right Ventricular Ejection Fraction
SAE	Serious Adverse Event
SaO_2	Oxygen Saturation measured by arterial blood gas
SAR	Serious Adverse Reaction
SARS	Severe Acute Respiratory Syndrome
SDV	Source Document Verification

SI	Statutory Instrument
SOC	Standard of care
SOFA	Sequential Organ Failure Assessment
SOP	Standard Operating Procedure
SmPC	Summary of Product Characteristics
SpO_2	Oxygen saturation measured by pulse oximeter
SPON	Sponsor
SSA	Site Specific Assessment
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMG	Trial Management Group
tPA	tissue Plasminogen Activator
TSC	Trial Steering Committee
TV	Tidal Volume
UCL	University College of London
UK	United Kingdom
uPA	Urokinase Plasminogen Activator
USA	United States of America
WBC	White Blood Cells
WHO	World Health Organization

Trial personnel

See protocol cover page for Chief Investigator and Sponsor contact details.

Statistician	Cohort 1: To be supported by the UCL/UCLH Biostatistics Group [redacted] Cohort 2: To be supported by WStats Limited (Marie Watissée)
Central laboratories	Ms Anne Riddell Head of specialist coagulation lab KD Haemophilia and Thrombosis Centre Royal Free Hospital, Pond St London NW3 2QG Email: anne.riddell@nhs.net tel: 0207 472 6835 fax: 0207 472 6759

1 Summary

Objectives:	The overall objective of the pilot study is to investigate the potential for clinical efficacy and safety of nebulised recombinant tissue plasminogen activator (rtPA) in patients hospitalised with Severe COVID-19 complicated by mild to severe ARDS as assessed by an improvement in oxygen saturation and incidence of major bleeding events.
Type of trial:	Phase II, open label, single centre, pilot interventional study of repeated doses of nebulised rtPA in patients with COVID-19 ARDS.
Trial design and methods:	Investigation of safety and efficacy includes dose finding and safety of the final selected dose. The initial first cohort will enable identification of a dose to be further studied in a second cohort. This will allow further accrual of safety and efficacy data at the final selected dose. The first cohort will include a treatment group (rtPA + standard of care (SOC)) and a group of matched historical controls that received standard of care alone. The first group of 12 eligible patients will receive nebulised rtPA for 14 days in addition to SOC. Each group will comprise six patients receiving invasive mechanical ventilation (IMV) for moderate to severe ARDS, and six patients with mild ARDS requiring non-invasive ventilation (NIV) or standard oxygen therapy. NIV includes continuous positive airway pressure (CPAP) OR high flow oxygen The primary efficacy assessment will be the change in arterial oxygen saturation levels, specifically arterial hypoxemia using the arterial oxygen to inhaled oxygen ratio (PaO_2/FiO_2 or SaO_2/FiO_2) daily during treatment, at the end of treatment and at 5 days post end of treatment. Primary safety assessments will include monitoring for bleeding events and plasma fibrinogen levels. Patients will be followed up until day 28, discharge from hospital or death - whichever occurs first. These 12 patients will be compared against 24 matched historical controls who received SOC alone. A DMC will be set-up to review emergent safety data during the trial and review and advise on the final study results progression from a pilot study to a statistically powered randomised control trial (RCT) based on the safety and efficacy data that is collected. The second cohort will include a treatment group only. 30 patients will be recruited, which will include a minimum of 10 patients receiving IMV and 10 patients receiving NIV or standard oxygen therapy.

Trial duration per participant:	Informed consent to day 28, discharge or death whichever occurs first.
Estimated total trial duration:	12 months
Planned trial sites:	Multi site: Royal Free Hospital (Royal Free London NHS Foundation Trust) Barnet Hospital (Royal Free London NHS Foundation Trust)
Total number of participants planned:	Cohort 1 - 12 participants receiving rtPA + standard of care (SOC), and 24 matched historical controls receiving SOC only Cohort 2 – 30 participants receiving rtPA + SOC or participating in other trials of investigational medicinal products
Main inclusion/exclusion criteria:	Cohort 1 Inclusion Criteria: 1. Patients with COVID-19 (confirmed by PCR or radiologically) 2. ≥16 years 3. Willing and able to provide written informed consent or where patient doesn't have capacity, consent obtained from a legal representative 4. Patients on IMV must meet both the following criteria: 1. PaO_2/FiO_2 of ≤ 300 2. Intubated > 6 hrs 5. Patients not intubated must meet all the following criteria: 1. PaO_2/FiO_2 ≤ 300 or equivalent imputed by non-linear calculation from SpO_2/FiO_2 (see look-up table in appendices) 2. In-patient >6hours and being actively treated 3. On support with non-invasive ventilation OR continuous positive airway pressure (CPAP) OR high flow OR standard oxygen therapy Exclusion criteria 6. Females who are pregnant 7. Concurrent involvement in another experimental investigational medicinal product 8. Known allergies to the IMP or excipients of IMP 9. A pre-existing bleeding disorder with no definitive treatment (e.g. severe haemophilia) 10. Pre-existing severe cardiopulmonary disease (e.g. incurable lung cancer, severe chronic obstructive lung disease, cardiomyopathy, heart failure or impaired contractility <estimated 40% LVEF or RVEF)

	11. Fibrinogen < 2.0 g/L at time of screening 12. Patients considered inappropriate for active treatment (e.g. being considered for palliative care) 13. Patients with active bleeding in the preceding 7 days 14. Patients who in the opinion of the investigator are not suitable Cohort 2 Inclusion criteria are the same as for cohort 1 Exclusion criteria 1. Known allergies to the IMP or excipients of IMP 2. Fibrinogen < 1.5 g/L at time of screening 3. Patients considered inappropriate for active treatment (e.g. being considered for palliative care) 4. Patients who in the opinion of the investigator are not suitable 5. Females who are pregnant
Statistical methodology and analysis:	Cohort 1 Data will be presented primarily using standard descriptive statistics. In the treatment group (rtPA + SOC) outcomes will be summarised separately for the six treated patients receiving invasive mechanical ventilation (IMV), and for the other six treated patients requiring support with non-invasive ventilation (NIV) Or continuous positive airway pressure (CPAP) OR high flow oxygen or standard oxygen therapy. Similar descriptive statistics will also be presented for the 24 historical controls. If appropriate, outcomes will be compared between the historical SOC group and intervention group using appropriate regression methods, making an adjustment for baseline measurements. Cohort 2 Data will be presented primarily using standard descriptive statistics. Outcomes will be summarised separately for the treated patients receiving invasive mechanical ventilation (IMV), and for the treated patients requiring support with non-invasive ventilation (NIV) Or continuous positive airway pressure (CPAP) OR high flow oxygen or standard oxygen therapy. Given the study is not designed or powered to definitively show efficacy, all analyses should be considered as hypothesis generating rather than providing firm conclusions.
Primary objectives	1. Efficacy: Investigate the potential for efficacy of nebulised rtPA in patients presenting with severe COVID-19 requiring IMV or Non-invasive support with NIV OR continuous positive airway pressure (CPAP) OR high flow oxygen Or standard oxygen therapy 2. Safety: Evaluate the safety of nebulised rtPA treatment.

Primary endpoints	Efficacy 1. Change in PaO_2/FiO_2 ratio from baseline(as defined in sections 7.1.2 and 7.1.3, daily during treatment, end of treatment and 3 and 5 days post treatment in the groups receiving rtPA Safety 1. Incidence and severity of major bleeding events directly attributable to the study drug 2. Decrease in fibrinogen levels to < 1.0 gm/L during treatment period and 48 hrs after the last dose of treatment 3. Number and nature of serious adverse events causally related to the treatment
Secondary objectives	1. Investigate the impact on patient's clinical status over time using the WHO ordinal scale of clinical improvement. 2. Investigate the effect of nebulised rtPA on other respiratory markers (such as lung compliance) and organ dysfunction 3. Investigate the impact on in hospital mortality and resource utilisation 4.
Secondary endpoints	1. Changes in lung compliance (defined as tidal volume / (peak inspiratory pressure – PEEP)) from baseline (as defined in sections 7.1.2 and 7.1.3) and absolute values at day 5, day 7, end of treatment, 3 days post end of treatment and 5 days post end of treatment 2. Clinical status as assessed by a 7-point WHO ordinal scale at baseline (as defined in sections 7.1.2 and 7.1.3), daily up to 5 days post end of treatment and at day 28, discharge or death (whichever comes first) 3. Mean daily Sequential Organ Failure Assessment (SOFA) score at baseline (as defined in sections 7.1.2 and 7.1.3) and daily up to 5 days post end of treatment. 4. In follow up period, number of oxygenation free days, ventilator free days, intensive care stay, up to 28 days or death or discharge, whichever occurs first. 5. Incidence and number of days of new oxygen use, non-invasive ventilation or high flow oxygen devices in the first 28 days. 6. Incidence and number of days of new mechanical ventilation use during in the first 28 days 7. In hospital mortality
Exploratory objectives	Changes to coagulation and inflammatory markers concerning intervention and response to treatment.

Exploratory endpoints	Change in levels of plasma biomarkers of pro- and anti-inflammatory markers (e.g. interleukin-6, interleukin-8, interleukin-10 and interleukin-1Ra), endothelial injury (plasma von Willebrand factor) coagulation (procoagulants, anticoagulants, and fibrinolytic pathway) measure of tissue damage

2 Background and Rationale

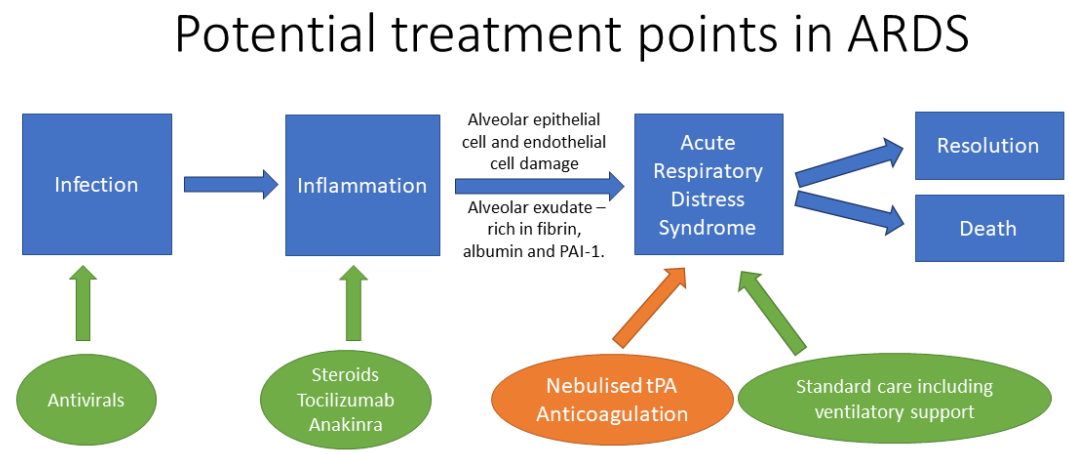
2.1 Background

A novel coronavirus, designated SARS-CoV-2 results in a respiratory illness termed COVID-19 pneumonia and is responsible for the current pandemic. The infection is in the majority of people a self-limiting illness, but in a significant proportion of the population, particularly the elderly, results in acute respiratory distress syndrome (ARDS), with severe progressive pneumonia, multiorgan failure, and death (Yang et al. 2020; Guan et al. 2020). In one study from Wuhan, China 31% of adult hospitalized patients with COVID-19 pneumonia developed ARDS.

Several therapeutic interventions for ARDS caused by SARS-CoV-2 are in clinical trials, including anti-viral (e.g. Remesdivir, EudraCTs: 2020-000936-23, 2020-000842-32 and 2020-000841-15), and anti-inflammatory agents (e.g.Tocilizumab, clinicaltrials.gov: NCT04317092) which have completed recruitment and licensed for use.

The current standard of care for ARDS includes conservative fluid strategies for patients, early empirical antibiotics for suspected bacterial co-infection, lung-protective ventilation and prone positioning. The mortality rate is 25 - 60% with the current standard of care (Aranda-Valderrama and Kaynar 2018).

Currently, the only intervention that has a positive recommendation for routine use is corticosteroids (Siemieniuk et al. 2020) with remdesvir and tocilizumab being used in a selected patient population. We propose that therapeutic interventions targeting different components of the pathophysiological process in patients hospitalised for severe COVID-19 is essential for rapid control of the disease. In addition to antivirals and anti-inflammatory strategies, interventions aimed at salvaging patients with severe disease or with decreased reserved is essential if the overall mortality and morbidity are to be reduced.



Tocilizumab - humanized monoclonal antibody against the interleukin-6 receptor
Anakinara - recombinant interleukin-1 receptor antagonist
tPA – tissue Plasminogen Activator

2.2 Pathophysiological basis for the use of inhaled rtPA by nebulisation

The exact mechanism for this rapid acute lung injury in these patients is not fully understood, but diffuse alveolar damage typically marks the onset of ARDS. Viral replication in alveolar epithelium associated with an intense dysregulated local inflammatory response at the alveolar-capillary level, mediated by various cytokines, chemokines and other inflammatory mediators appears to play a crucial role. This early phase of epithelial and endothelial injury is characterised by loss of integrity of the barrier with plasma rich in albumin and fibrinogen and cellular debris exuding into the alveolar spaces which probably accentuates the inflammatory process (Bhargava and Wendt 2012).

This procoagulant state stems from increased tissue factor expression and suppression of fibrinolytic activity due to a rise in plasminogen activator inhibitor -1 (PAI-1)(Idell, James, and Coalson 1992). This leads to the formation of microthrombi in the lungs, as demonstrated at autopsy (Xu et al. 2020), further compromising gas exchange. Emerging evidence indicates that activation of coagulation as demonstrated by elevated D-dimer and fibrin degradation product (FDP) levels are associated with poorer outcomes in novel coronavirus (Tang et al. 2020). Furthermore, patients treated with heparin show improved outcomes (Tang et al.). The use of concurrent anticoagulation or antiplatelet agents is not forbidden in the trial.

Additionally, fibrin formation within the lungs starts a vicious cycle in which leukocytes localised to the clot through integrin binding sites within the fibrinogen molecule (Cooper, Lo, and Malik 1988) exacerbate inflammation and fibrinolysis (Barrett et al. 2017). The sequestration of neutrophils to fibrin in the lung has been observed in both pre-clinical (Cooper, Lo, and Malik 1988) and clinical contexts and have been shown to induce inflammation and tissue injury in the context of both viral (Ichikawa et al. 2013; Narasaraju and Harshini 2016), and non-viral (Ichikawa et al. 2013; Conhaim et al. 2014) lung injury. The interplay of neutrophils with fibrin in the clot may further exacerbate clotting through the generation of neutrophil extracellular traps (NETs) which slow down fibrinolysis (Varjú et al. 2015) and further increases the risk of thrombosis.

2.3 Rationale for fibrinolytic therapy delivered by inhalation

There is accumulating data in animal, and clinical models that both intravenous and inhalation thrombolytic drugs are associated with increased oxygenation and improved rates of mortality, as a result of clearance of fibrin from the alveolar space (Hofstra et al. 2013). Following an initial case report demonstrating the success of nebulised tissue plasminogen activator (rtPA) (Gram et al. 1999), a recent randomised controlled trial demonstrated that streptokinase improved survival compared to standard of care with the restoration of oxygenation to normal levels within three days (Abdelaal Ahmed Mahmoud et al. 2020).

A recent meta-analysis of pre-clinical data demonstrated an apparent reduction in inflammation (alveolar neutrophils) when rtPA was directly administered to the lung as a nebulised therapy (Liu et al. 2018; Foley 2017). Although rtPA is widely used in clinical practice as a therapy for clot dissolution in thrombotic cardiovascular and cerebrovascular events but hasn't been widely investigated for this indication.

We propose to undertake an interventional study using nebulised rtPA, which has a proven favourable safety profile. Importantly, this molecule targets the pathway at a point with no proposed interventional studies. The molecule can be administered either intravenously or nebulised.

Intravenous administration of rtPA can result in diffusion across the disrupted barrier to facilitate the resolution of the accumulated debris. This diffusion is not predictable, and importantly the intravenous route has an increased risk of bleeding and requires intensive monitoring. In contrast, nebulised rtPA targets the area where it can overcome the increased local PAI-1 activity. Besides, diffusion into the vasculature results in improvement of the pulmonary vascular thrombosis. Anticoagulation can address the issue of pulmonary vessel thrombosis but does not address the primary issue of the fibrin membranes on the alveoli.

We propose to undertake an interventional pilot study using inhaled rtPA by nebulisation. We hypothesise that this route of administration will result in more rapid resolution with the potential to avoid secondary inflammation and cytokine storm triggered by the non-resolving fibrin membranes.

This intervention study will examine whether there is an indication that targeted clearing of fibrin and the resulting leukocytes from the alveolar space early in the progression of COVID-19, would have a protective effect on inflammation, facilitating the restoration of alveolar-capillary barrier and survival. The trial, through accelerated clearance, has the potential to decrease the need for ventilatory support, decreased length of ICU stay and decreased mortality.

2.4 Rationale for rtPA dose – Cohort 1

Rehberg et al. USA (Rehberg et al. 2014) in a sheep model of lung injury post burn and smoke inhalation injury randomly assigned sheep to intravenous saline plus saline nebulization (control), intravenous recombinant human antithrombin (AT, 6 IU/kg/h) started 1 hour after injury plus saline nebulization or intravenous AT combined with nebulized heparin (10,000 IU every 4 hours, started 2 hours after injury), and nebulized tissue plasminogen activator (2 mg every 4 hours, started 4 hours after injury, a total of 22mg) (triple therapy, n = 6 each) with animals mechanically ventilated and fluid resuscitated according to standard protocols during the 48-hour study period. Triple therapy resulted in improved PaO_2/FiO_2

ratio (p = 0.007), attenuated pulmonary obstruction (p = 0.02) and shunting (p = 0.025), as well as reduced ventilatory pressures versus AT i.v. at 48 hours.

Hofstra et al, Netherlands (Hofstra et al. 2013) in a rat model of bacterial infection administered nebulised rtPA (1.25 mg/kg, prophylactically and at 6 and 12 h). Nebulized rtPA enhanced the bronchoalveolar fibrinolytic system, as reflected by a significant reduction of PAI–1 activity levels in bronchoalveolar lavage fluid, and a consequent increase in plasminogen activator activity (PAA) levels to supranormal levels and both treatments also significantly affected systemic fibrinolysis as reflected by a significant increase in PAA levels in plasma to supranormal levels. Neither nebulized rtPA nor anti–PA1–1 affected pulmonary inflammation as evaluated by the neutrophil and mononuclear cell count.

In a metanalysis of 22 studies (Liu et al. 2018) evaluating the potential effects of fibrinolytics on animal models of ALI, both large and small animals who received rtPA, uPA, and plasmin by various routes including inhalational and intravenous were included. Fibrinolytics significantly increased the fibrinolytic activity both in the plasma and bronchoalveolar lavage fluid.

Stringers group (Lackowski et al. 2010) have demonstrated the feasibility of inhaled tPA as evidenced by the retention of protein stability and activity following nebulization. of pulmonary formulation of mouse tPA. In these studies mice received either a low dose (0.30 mg/kg/d) or a high dose (0.60 mg/kg/d) or saline every 12 h for 28 days. Although male mice had a lower incidence of bleeding at 8%, these events occurred at lower mean doses (± SE) of 1.6 (± 0.02) mg/kg/d compared to females who received doses of 1.48 (± 0.03) mg/kg/d and a bleeding incidence of 16%. Further bleeding was seen about six and 12 days in male and female mice, respectively, after the initiation of dosing suggesting potential accumulation of tPA.

Sulfur mustard (SM) inhalation causes life-threatening plastic bronchitis, characterized by bronchial cast formation, resulting in severe airway obstruction that can lead to respiratory failure and death. Mortality in those requiring intubation is greater than 80%. In a rat model exposed to inhalation of the toxic SM analog, 2-chloroethyl ethyl sulfide (CEES) were rescued by intratracheal tPA (0.15 to 0.7 mg/kg)(Veress et al. 2013). Dosing was delayed until 5.5 hrs as case formation starts around 4 hrs with a reduction in saturation to below 90% at 5 hrs post exposure. A second dose was administered an hour later. A dose-dependent improvement in SpO2 was noted with tPA relative to controls with only the highest tPA (0.7 mg/kg) used resulting in near-baseline oxygen saturations (>90%) at 12 hours. Remarkably, tPA completely eliminated mortality in this model. Deaths in control groups occurred mainly between 8 and 28 hours after CEES exposure, and mortality was about 90%. The same group in another rat model exposed to SM, administered intratracheal t-PA (0.7 mg/kg) or placebo every 4 hrs for 48 hrs when oxygen saturation reached less than 85% (median: 6.5 h). An improvement was seen after the first dose with normalisation at the end of 48 hrs (Veress et al. 2015). Further, at the time of euthanasia, well in excess of 50%–65% of the total luminal areas of central conducting airways were obstructed with fibrin casts in both the nontreated and placebo-treated rats, whereas the degree of airway obstruction was nonsignificant in tPA-treated animals. Further no bleeding was observed unlike that seen with nebulised heparin.

Normal rat airways possess endogenous fibrinolytic activity, and none of three native inhibitors of fibrinolysis, namely PAI-1, apha 2 antiplasmin (α2AP), or thrombin activable fibrinolysis inhibitor (TAFI), was detectable. Following exposure to CEES, the fibrin-degrading and plasminogen-activating capabilities of the airways become impaired and analysis demonstrates increased levels of PAI-1, TAFI, and α2AP. Further, RT-PCR analysis indicates a minimal production of TAFI or α2AP mRNA, an indication

that their presence in airways is due to vascular permeability and leak. PAI-1 mRNA expression was increased suggesting an upregulation (Rancourt et al. 2014).

In fact, in the absence of fibrin, human tPA is generally considered a weak protease(Longstaff et al. 2011) and it is reasonable to expect that the dose of rtPA in the clinical situation of ALI/ARDS or plastic bronchitis may be lower. Of note is the limited amount of plasminogen in the exudate and high levels of PAI- 1 all which can all limit the activity.

In patients presenting with plastic bronchitis, nebulised rtPA has been used extensively. A range of doses have been reported in children, ranging from 5mg, four times a day for seven days (Colaneri et al. 2014) to 2 mg/kg in divided doses (Grutter et al. 2012) without any bleeding complications. In single case report of lupus induced ARDS prompt resolution of ARDS was seen with ultrasonic nebulized rtPA (30 mg) and intravenous rtPA (20 mg) over 2 h followed by continuous treatment with ultrasonic nebulized unfractionated heparin (15,000 IU/day) (Gram et al. 1999). A more recent small randomised controlled study of saline, nebulised heparin and nebulised streptokinase showed that patients receiving streptokinase achieved a PaO2/FIO2 ratio >100 at day 1, PaO_2/FiO_2 >200 at day 5, and PaO_2/FiO_2 >300 at day 7 when compared to standard of care group who did not achieve a PaO_2/FiO_2 ratio >100 after eight days of conservative management (Abdelaal Ahmed Mahmoud et al. 2020). In this study patients received 250,000 IU/4 h by a nebulizer, with a total daily dose of nebulized streptokinase of 1,500,000 IU. A personal communication from the authors reveals that most patients did not require treatment beyond 3 to 4 days. Currently there is clinical trial of Inhaled Tissue Plasminogen Activator for Acute Plastic Bronchitis in the USA. (https://clinicaltrials.gov/ct2/show/NCT02315898).

We communicated with Kathleen A. Stringer, University of Michigan who has undertaken a considerable amount of animal work and she confirmed the safety and efficacy of 5 mg of tPA reconstituted per instructions, nebulised every 6 hrs for 3 days was adequate in their paediatric patients.

In the context of ARDS higher PAI-I activity associated with ARDS may require a higher dose, further compared to children adults also require higher doses. Therefore, a dose of 10 mg every 6 hrs for 72 hrs was initially chosen (now updated – see below*), although this dose is not equivalent to the dose used in the study by Ahmed Abdelaal Ahmed Mahmoud M. Alkhatip et al. This dose was finalised in consultation with Professor Kathleen A. Stringer, PharmD, University of Michigan.

***Please note protocol version 5.0 has updated this dosing schedule – please see rationale below**.*

Further, dosing for the first two patients will be staggered to allow adequate safety monitoring (see section 4.1), before enrolling the rest of the cohort.

An autopsy study on COVID-19 patients has shown that COVID-19 ARDS is characterised in the early stages by a lymphocytic infiltrate, but subsequently an increasingly extensive intra-alveolar fibrin deposition accumulation (or fibrin “balls”)(Copin et al. 2020)). However, it is not possible to evaluate the fibrin burden in an individual patient, so we believe that treatment should be extended to a total of 14 days unless they meet a stopping criterion. The treatment duration is based on another trial of nebulised heparin which was conducted for 14 days with no evidence of excessive bleeding (Dixon et al. 2010). Similarly, pre-clinical data in rats show intratracheal rtPA administered over 28 days was associated with bleeding if the dose exceeded 1mg/kg/day(Lackowski et al. 2010).

In this trial, one patient on non-invasive ventilation responded to the treatment given for 72 hrs and was discharged from ICU to ward on nasal oxygen (2 litres per minute) at 96hrs. 36 hrs after the last dose his

oxygenation has decreased again and the trial management group was of the opinion that the patient required additional treatment in view of the previous response.

This led to the implementation of an urgent safety measure in this one patient (patient PACA-201) to allow re-treatment. Retreatment consisted of a second 3 day block of treatment with rtPA, in line with the treatment schedule as already described in the protocol. During retreatment, the patient's PaO_2/FiO_2 ratio increased and showed periods of normalisation. After treatment was discontinued for a second time, the patient's oxygenation has decreased but not as dramatically as it had when treatment was discontinued for the first time.

The protocol (version 5.0) has now been updated with a new dosing schedule to allow treatment for maximum of 14 days treatment (see section 7.3 for details of schedule). This updated dosing schedule has been discussed and agreed with the TMG and IDMC.

2.5 Dosing schedule of rtPA for cohort 2 – rationale

During the first surge 9 patients were recruited to the pilot study, with 6 patients receiving IMV and 3 patients receiving non-invasive oxygen support. When the first surge died out, recruitment was not feasible and following discussion with DMC and TMG cohort 1 was closed for further recruitment and historical controls were recruited for evaluation of efficacy to enable a sample size calculation for a larger phase 3 study.

None of the bleeding events were considered related to the tPA. See preliminary safety data for cohort 1 in section 2.7. Final analysis of data is awaited; preliminary review of data is described here.

Preliminary data from the 9 patients from cohort 1 indicated a potential increase in arterial oxygen saturation levels, and importantly no evidence of consumption of fibrinogen nor a decrease in plasminogen levels was observed unlike the changes associated with intravenous administration.

Protocol v8.0 has been prepared at the start of the second UK surge. 30 patients will be recruited to this second cohort, with a minimum recruitment of 10 IMV patients and minimum of 10 patients receiving non-invasive ventilation. Early initiation of treatment appears to more effective, and this may be related either to lower fibrin burden or higher amount of plasminogen from the plasma exudate the substrate for rtPA. In the NIV and standard oxygen therapy group, a loading dose of 20 mg three times a day for 2 days followed by 20 mg twice a day is reasonable as intermittent fibrinolysis is effective. The loading dose potentially can help overcome the accumulation of PAI-1 early in the disease process, and is strong inhibitor of fibrinolysis.

The effect on arterial oxygen saturation levels appears to be less in IMV patients and drug losses can be expected because of the dead space created by the ventilator circuit. To account for this, we propose 20 mg every 8 hrs in patients receiving IMV.

Although the pilot study has used nebulisation every 6hrs, it is very reasonable to decrease the frequency to either twice a day for the non-invasive oxygen support group, or three times a day for the IMV group. The less frequent administration should make it easier for delivery teams.

The maximum duration of treatment will continue to be 14 days with a provision for early termination as described in section 7.3.3.

Should a patient's condition change such that their ventilation type is changed whilst receiving rtPA treatment (that is, from IMV to NIV, or vice versa) they will switch to the treatment schedule that is applicable to the new ventilation type.

2.6 Assessment and management of risk

Deposition and removal of fibrin are critical processes in reducing blood loss, wound healing and repair. Thrombin is the final enzyme of the coagulation cascade and converts soluble fibrinogen into a fibrin meshwork that traps red cells and other cellular debris. The removal of the fibrin meshwork occurs via fibrinolysis that requires plasmin generated from zymogen plasminogen that normally circulates in plasma and is incorporated into the clot at the time of formation. Plasminogen is activated by two proteases: urokinase-type plasminogen activator (uPA) or tissue plasminogen activator (tPA), both of which are primarily synthesized by the endothelium (Weisel and Litvinov 2014). Fibrinolysis is tightly regulated under normal physiological conditions. The catalytic activities of tPA and uPA are curtailed through the inhibitory proteins plasminogen activator inhibitor-1 (PAI-1) and α_2-antiplasmin which inhibits free tPA but not that bound to a fibrin clot. Finally, circulating tPA has a very short half-life of 5–8 min (Sheehan and Tsirka 2005).

Recombinant tPA (rtPA, alteplase) is licensed for the thrombolytic treatment of acute myocardial infarction, massive pulmonary embolism with haemodynamic instability and acute ischaemic stroke. The dosages used in these conditions are typically around 0.9 to 1.2 mg/Kg administered over 1 to 2 hrs (Aleplase June 2019). rtPA administered by the normal intravenous route carries a risk of bleeding and the extent of this risk is related to the drug dose (Lee et al. 2015). The highest number of bleeding events were in seen in trials involving acute ischemic stroke where the reported complications were symptomatic intracranial haemorrhage (6%), other major haemorrhage (2%), and angioedema (5%) (Miller, Simpson, and Silver 2011). Angioedema is related to the rapid generation of bradykinin from high-molecular-weight kininogen by plasmin (Fugate, Kalimullah, and Wijdicks 2012). The risk factors for symptomatic intracranial haemorrhage include age, male gender, obesity, increased stroke severity, diabetes, hyperglycemia, uncontrolled hypertension, combination antiplatelet use, large areas of early ischemic change, atrial fibrillation, congestive heart failure, and leukoaraiosis. A risk factor for angioedema is the use of angiotensin-converting enzyme inhibitor.

Similarly in patients with acute myocardial infarction, the risk of intracranial haemorrhage is <1%, and in multivariable models the main risk factors were older age, female sex, black ethnicity, systolic blood pressure of 140 mm Hg or more, diastolic blood pressure of 100 mm Hg or more, history of stroke, tPA dose more than 1.5 mg/kg, and lower body weight(Gurwitz et al. 1998). In another study where the dose of r-tPA was investigated major or minor haemorrhagic events were associated with the extent of fibrinogen breakdown, peak rtPA levels, thrombocytopenia, prolongation of the activated partial thromboplastin time (APTT) to more than 90 seconds, weight of 70 kg or less, female gender, and physical signs of cardiac decompensation (Bovill et al. 1991). The outcomes in patients with stroke and acute myocardial infarction, is related to both the indication for thrombolysis and potentially fatal brain haemorrhages.

The other common site of bleeding is gastrointestinal bleeding, which is often not fatal and can be managed as per standard protocols. There has been an increasing interest in catheter directed thrombolysis over extended duration in venous thrombosis to decrease the risk of bleeding with

potential similar efficacy. In a randomised trial of pharmacomechanical-thrombolysis, in the treatment group major bleeding was seen in 1.7% of patients (6 of 336 treated patients) which consisted of two patients with gastrointestinal bleeding, two patients with retroperitoneal bleeding and two patients with bleeding at the site of the procedure. All patients were receiving concomitant anticoagulation (Vedantham et al. 2017). In another retrospective registry of around 500 patients, one intracranial haemorrhage was seen with the majority of major bleeding limited to the site of procedure.

The potential for pulmonary haemorrhage has been carefully considered during the study design. This risk is probably related to the generation of plasmin in the alveoli, responsible for the fibrinolysis. To date no bleeding has been seen in anecdotal reports using 1 to 2mg/Kg of nebulised rtPA per day, over 3 - 14 days in patients with plastic bronchitis. Plasmin generation, in addition to the amount of rtPA inhaled, is also related to the availability of plasminogen in the alveoli, which is limited by the amount that can cross the alveolar capillary barrier. Indeed this limited availability has the potential to result in therapeutic failure. The other factor that may contribute to the bleeding is the use of therapeutic anticoagulation. Concurrent therapeutic anticoagulation is common with the use of rtPA as it prevents recurrence of thrombosis. There is emerging evidence that these patients have pulmonary thrombin at the segmental and sub segmental , and importantly they appear to a very strong prothrombotic tendency. It is important to note that the bleeding risk in the literature typically includes the concurrent use of anticoagulants. A strong prothrombotic tendency can result in relative resistance to the action of anticoagulants. Indeed patients presenting with elevated troponin in our organisation have been noted to have atypical coronary thrombosis with regards extent and clot burden .

We hypothesise that this risk will be much reduced if administered by inhalation. This is because relatively little of the drug is expected to cross the alveolar-capillary interface and enter the bloodstream. The doses being used in this study are lower than that used in studies for myocardial infarction and stroke and similar to that used in venous thrombosis. Additionally, as the absorption rate is slow, the rapid inactivation of rtPA in the circulation will prevent accumulation to the level normally associated with bleeding. The background risk of pulmonary bleeding in COVID-19 pneumonia is low, with this symptom reported in only 5% of cases (Huang et al. 2020). The bleeding risk is accurately predicted from the plasma fibrinogen level which provides a measure of the rtPA absorbed. This will be monitored and if plasma fibrinogen levels fall to <1.0 g/L, the further dosing of rtPA in that patient will be stopped. Replacement therapy, either in the form of fibrinogen concentrate or cryoprecipitate will be administered to return fibrinogen to >1.0 g/L.

The table below summarises the risks, frequencies and mitigations of the IMP(s) and NIMP(s):

Name of IMP(s)	Potential risk	Risk Frequency	Risk Management
rtPA (alteplase)	Pulmonary haemorrhage	Uncommon when administered intravenously, unknown when administered by ventilation	Stop rtPA nebulisation for this patient, and administer fibrinogen concentrate or cryoprecipitate if fibrinogen is < 1.0 g/L, and other supportive treatment including IV tranexamic acid. May trigger stopping rule for trial if considered a

			major bleed by ISTH criteria (see section 7.10)
rtPA (alteplase)	Minor bleeding particularly traumatic – suction through endotracheal tube or at site of venepuncture	Common when administered intravenously, unknown when administered by ventilation	Supportive treatment, and use caution during suction
rtPA (alteplase)	hypersensitivity reactions (e.g. rash, urticaria, bronchospasm, angio-oedema, hypotension, shock)*	Rare when administered intravenously, unknown when administered by ventilation	Anti- allergic medications
rtPA (alteplase)	serious anaphylaxis	very rare when administered intravenously, unknown when administered by ventilation	Treat per local protocols and stop further administration of rtPA for this patient.
rtPA (alteplase)	Major Bleeding including intracerebral haemorrhage	Common when administered intravenously, unknown when administered by ventilation.	Rapid absorption is required to achieve high enough levels to result in bleeding. the plasma half-life is 5 minutes. Stop rtPA nebulisation for that patient, and administer fibrinogen concentrate or cryoprecipitate if fibrinogen is < 1.0 g/L. May trigger stopping rule for trial (see section 7.10)

The table below summarise the risks and mitigations of all test above standard care that are being performed in a table:

Intervention	Potential risk	Risk Management
Additional blood samples	Related to venepuncture, which is done as standard of care.	Press on the site of blood tests

2.7 Safety data from cohort 1

Preliminary data from the 9 treatment arm patients is summarised below (this data is preliminary data and is not the final analysis):

All bleed events were identified as adverse events of special interest and evaluated for severity (mild, moderate and severe as described in section 8.3.1) and causality. In addition, the ISTH (International Society of Haemostasis and Thrombosis) classification for bleed severity as used for anticoagulation studies was implemented.

In total, 7 bleeding events were identified. A bleed was considered not related to alteplase if the event happened 30 hrs after the last dose of treatment, alteplase exposure time period. Six of the seven bleeds were beyond alteplase exposure time period, and one was within the time period.

One bleed each met the criteria for ISTH major and minor bleed. Five bleeds did not meet the ISTH criteria for minor or major bleeds. The severity of bleeds as assessed for adverse event reporting resulted in five bleeds being categorised as mild and two events as moderate.

The severity assessment as used for AE reporting was used to further describe the bleeds.

2.7.1 Mild bleeds

These bleeds did not require any intervention, and neither did they meet the criteria for minor or major ISTH bleeding events. All patients were on therapeutic anticoagulation, and no intervention was required.

- Two mild bleeds were due to collection of blood around the site of insertion of the jugular line under mepore transparent dressing. Both were precipitated by proning of patients. One of the bleed around the insertion site was within alteplase exposure time window and was considered unrelated to use of alteplase.
- One mild bleed in from the oral cavity followed an injury from a tracheal tube bitten by the patient and presented as blood-streaked saliva.
- One mild bleed presented as blood-streaked thick secretions on one occasion.
- One mild event was due to bleeding from the tracheostomy site along with a small clot in the tube.

2.7.2 Moderate bleeds

- Two moderate bleeds were noted during the trial, both of which required interruption of anticoagulation and additional interventions. Both were outside the alteplase exposure time period.
- One moderate bleed was gastrointestinal bleed secondary to ulceration and met the criteria for major bleed as per ISTH classification. In addition to the interruption of anticoagulation, the patient required red cell transfusion and endoscopy.
- One moderate bleed for two days was intermittent bleeding around the tracheostomy site, that increased over time. Interruption of anticoagulation was initiated on the second day of the bleed, and the patient also required intravenous tranexamic acid and local measures.

2.7.3 Lab safety data

Trial stopping criteria included a decrease in fibrinogen to < 1.5gm/L at any time, and more than 50% decrease over 24hrs in the first couple of patients. No patient had <1.5gm/L during cohort 1, and notably, no reductions of fibrinogen as described above were seen during the treatment period and 48hrs after the last dose.

2.7.4 Exploratory biomarkers for fibrinolytic pathway

The plasma fibrinolytic pathway was evaluated through measurement of plasminogen, the precursor of plasmin and the alpha-2 antiplasmin its major inhibitor, and these were within normal limits. Preliminary analysis of the tPA data show no changes were seen during tPA administration. Similarly, no changes in concentration of tPA antigen, PAI-1 antigen and tPA-PAI-1 complexes were seen. The abnormalities were consistent with COVID -19 with no evidence of any significant absorption of tPA or activation of the fibrinolytic pathway.

	baseline		**day 3**		**day 5**		**day 13**
	Median	10% - 90%	Median	10% - 90%	Median	10% - 90%	
No. of patients	9		9		8		2
Plasminogen activity IU/dL	108	63.5 - 124.7	111	36.2 - 127.8	97.5	76.5 - 130	70 - 112
Alpha 2 antiplasmin IU/dL	112.5	103.6 - 129 .4	116	104.6 - 128	119	106.1 - 139	112 - 116
PAI -1 antigen ng/mL	41	31.6 - 58.4	39	28 - 54.2	38	26.8 - 92.2	25 - 35
tPA antigen ng/mL	16.5	7.8 - 37.2	14	9 - 23	14	11.1 - 40	26 - 10

In accordance with the MRC/DH/MHRA Joint Project Risk-adapted Approaches to the Management of Clinical Trials of Investigational Medicinal Products, this trial is categorised as:

- Type B = Somewhat higher than the risk of standard medical care

3 Objectives

3.1 Primary Objectives

1. Efficacy: Investigate the potential for efficacy of nebulised rtPA in patients presenting with severe COVID-19 requiring IMV or non invasive support with NIV OR continuous positive airway pressure (CPAP) OR high flow oxygen OR standard oxygen therapy
2. Safety: Evaluate the safety of nebulised rtPA treatment.

3.2 Secondary Objectives

1. Investigate the impact on patient's clinical status over time using the WHO ordinal scale of clinical improvement.

2. Investigate the effect of nebulised rtPA on other respiratory markers (such as lung compliance) and organ dysfunction
3. Investigate the impact on in hospital mortality and resource utilisation

3.3 Exploratory Objectives

1. Changes to coagulation and inflammatory markers concerning intervention and response to treatment.

4 Trial design

4.1 Overall design

This is a phase II, open label, single centre, uncontrolled, repeated dose, pilot trial of nebulised rtPA in patients with COVID-19 ARDS.

The study will recruit patients requiring either IMV or non-invasive oxygen support. Eligible patients (or if patients lack capacity, their legal representative) will be provided with an information sheet and informed consent will be sought (see section 6.3). Eligibility will be assessed via routine clinical assessments, which may have been done prior to consent. The only exceptions are a pregnancy test (blood or urine), and possibly any assessments listed in section 7.1 that were not done as per routine care. These must be done following consent, and all screening assessments must have been done during the 24-hour period before dosing with rtPA.

The study will run two cohorts sequentially. In cohort 1, 9 consented patients received nebulised rtPA in addition to SOC. 6 patients were receiving IMV and 3 were receiving non invasive support with NIV or CPAP or high flow oxygen or standard oxygen therapy. As an observational arm, matched historical controls who received standard of care were also recruited at a ratio of 2 controls to every 1 treatment arm patient, resulting in 18 historical controls. Originally, the study aimed to recruit 12 patients with 6 on each ventilation type (IMV and non-invasive oxygen support). This would have resulted in 24 historical controls. After the first wave of COVID-19 cases decreased in August 2020 in the UK it became difficult to continue recruitment, so recruitment closed for cohort 1.

With a second surge underway in early 2021, cohort 2 will aim to recruit more patients during this period to provide more data on the safety of rtPA. Based on the analysis of cohort 1, fewer timepoints will be collected (see sections 7.1.2 and 7.5.2), which will allow for more rapid recruitment while at the same time not compromising safety monitoring. A more flexible dosing regimen for rtPA will be utilised which is described in section 7.3.2. 30 patients will be recruited in total, with an aim to recruit a minimum of 10 IMV patients and 10 patients on non-invasive oxygen support.

A statistically powered randomised controlled trial (RCT) would be ideal to define the magnitude of benefit and impact on overall survival and is the typical design in patients with ARDS. Although there is clinical data on the safety of nebulised rtPA, but there is no clinical data in this clinical condition to facilitate a sample size calculation. There is data from a small RCT that has used another fibrinolytic agent, but the doses are not comparable(Abdelaal Ahmed Mahmoud et al. 2020). When a patient is randomised to receive no treatment, this precludes participation in other interventional studies which might decrease the risk of mortality. The most recent figures suggest a 50% mortality in patients receiving IMV there is 50% mortality. (https://www.icnarc.org/About/Latest-News/2020/04/04/Report-

On-2249-Patients-Critically-Ill-With-Covid-19) Currently there is a concerted effort to introduce multiple therapies based on our understanding of the pathophysiologic basis of disorder. Further, if this pilot study shows significant effect in a subset of patients, there would be justification to progress to a statistically powered RCT. We would progress to a RCT only after submission of a substantial amendment to this trial protocol or submission of a new clinical trial application. In the event of major adverse drug reactions or minor improvement in the oxygenation as assessed by PaO_2/FiO_2, there are minimal gains to be had with a larger randomised control study.

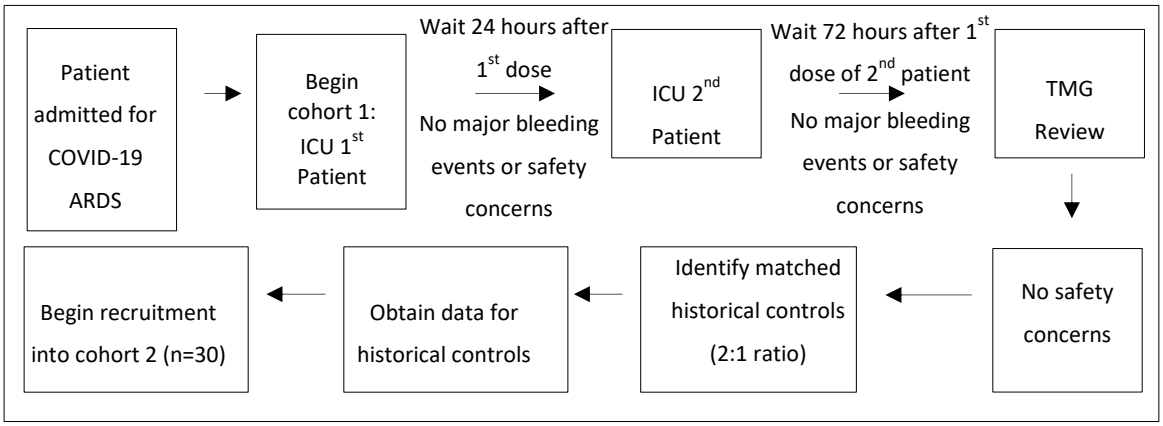
For patients in the rtPA group, 10 mg of rtPA dissolved in 5 ml of diluent will be given every 6 hrs for a maximum of 14 treatment days. Efficacy will be assessed by the monitoring of arterial oxygen saturation.

Safety monitoring will be performed by assessment of the incidence and severity of bleeding events, and by the monitoring of plasma fibrinogen levels and routine coagulation parameters. Additional samples will be taken for exploratory assessment of potential biomarkers, including (but not restricted to) PAI- 1, alpha 2 antiplasmin and a range of inflammatory cytokines and coagulation proteins. All other monitoring will be done as per SOC. From the end of the treatment phase both groups will be followed up in accordance with SOC.

Although there is extensive experience with the use of nebulised rtPA in the context of the underlying inflammation, safety measures been included. A gap of 24hrs will be maintained between first and second patient. At 24hrs if patient 1 has no evidence of major pulmonary bleeding suggesting exaggerated alveolar fibrinolysis and no evidence of fibrinogen reduction of more than 50%, suggestive of systemic absorption a second patient will be dosed. Both patients will be evaluated for 72 hrs. If no major bleeding is noticed and fibrinogen levels have not decreased more than 50% in either patient (in line with stopping criteria – section 7.10), and no other stopping criteria have been met, then the rest of the cohort can be recruited after the review of the safety data by the trial management group comprised of the investigators. If there are any concerns with the data this will be referred to the DMC for review. If the safety profile is acceptable, dosing of the third and subsequent patients in the rtPA group will resume with no required interval between patients.

Efficacy will be described as a change in PaO_2/FiO_2 (or SaO_2/FiO_2) ratio assessed daily, end of treatment and 5 days post treatment in relation to baseline. A Data Monitoring Committee (DMC) will be set-up to review safety data within the trial along with the final study results and advise on progressing from a pilot study to a randomised control trial based on the safety and efficacy data that is collected. The DMC will receive weekly reports on bleeding complications, both major and minor along with fibrinogen levels. If at any time a patient has major pulmonary bleeding, further dosing of patients will be stopped and an adhoc DMC review will be arranged before resuming dosing (see section 12.2 Data Monitoring Committee).

4.2 Schematic diagram(s) of overall trial design



5 Investigational Medicinal Products and Non-Investigational Medicinal Products

5.1 Name and description of IMP(s) - rtPA, alteplase (Actilyse®)

Actilyse 10 mg powder and solvent for solution for injection and infusion or Actilyse 20 mg powder and solvent for solution for injection and infusion.

1 vial with powder contains:

- 10 mg rtPA (alteplase) (corresponding to 5,800,000 IU) or
- 20 mg rtPA (alteplase) (corresponding to 11,600,000 IU)

Alteplase is produced by recombinant DNA technique using a Chinese hamster ovary cell-line.

The powder is presented as a colourless to pale yellow lyophilizate cake. The reconstituted preparation is a clear and colourless to pale yellow solution.

The reconstitution solvent is water for injections.

Actilyse is authorised as an intravenous formulation:

- Thrombolytic treatment in acute myocardial infarction
- Thrombolytic treatment in acute massive pulmonary embolism with haemodynamic instability
- Fibrinolytic treatment of acute ischaemic stroke

Actilyse is not authorised for administration via a nebulised route.

5.2 Source of IMP, Manufacture, Distribution and Storage

Actilyse will be supplied from existing hospital stock and replenished as required free-of-charge directly to the pharmacy by the manufacturer (Boehringer Ingelheim Ltd).

In this study Actilyse will be administered by nebulisation rather than intravenous injection. The rationale for selecting this route of administration is described in section 3.3.

A proof-of-concept nebulisation study (personal communication from H. Wachtel, Boehringer Ingelheim) indicated that at the planned concentration of 2 mg/mL nebulisation produces a fine particle fraction of approximately 60%. This means that, in principle, 60% of the dose is able to reach the deep lungs.

The potential irritant effect of nebulised rtPA (with excipients: arginine, phosphoric acid for pH-adjustment, polysorbate 80) on the lungs is not known, but experience within the critical care setting indicates that risk will be low and manageable.

5.3 Storage and handling of IMP(s) at site

All IMP aspects of the trial at participating sites are the responsibility of the PI, who may delegate this duty to the local pharmacist or other appropriately trained personnel. The delegation of duties must be recorded on the Staff Signature and Delegation of Tasks.
Storage must be in the original package in order to protect from light. Do not store above 25 °C.
Actilyse will be reconstituted with water for injection in accordance with the instructions in the Summary of Drug Arrangements (SoDa) for the trial.

The reconstituted solution has been demonstrated to be stable for 24 hours at 2 °C – 8 °C and for 8 hours at 25 °C. From a microbiological perspective, the product should be used immediately after reconstitution. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and would normally not be longer than 24 hours at 2 to 8°C.

For cohort 1, 10 mg of Actilyse reconstituted in 5ml of diluent provided will be used in an ultrasonic nebuliser available in the hospital. For cohort 2, 20mg will be reconstituted as directed in the SoDa, and will be used in an ultrasonic nebuliser available in the hospital.

Detailed instructions are contained in the summary of drug arrangements.

5.4 Accountability of IMP(s)

IMP will be supplied by Boehringer Ingelheim Ltd to the hospital site. Details of initial supply and resupply will be detailed in the summary of drug arrangements.

The IMP Drug Accountability Log must be completed to record each dose of IMP dispensed for each trial participant. This log must be retained in the relevant section of the Pharmacy Site File, and a copy must be submitted to the sponsor upon request. It is the responsibility of the Pharmacy Lead to maintain drug accountability records.

All used/unused IMPs dispensed will be destroyed and not returned to pharmacy.

Detailed instructions are contained in the summary of drug arrangements.

5.5 Concomitant medication

Concomitant medications will be recorded in the Participant's medical records/CRF.

No formal interaction studies with Actilyse and medicinal products commonly administered in patients with acute myocardial infarction have been performed.

Drugs affecting coagulation/platelet function

The risk of haemorrhage is increased if coumarine derivatives, oral anticoagulants, platelet aggregation inhibitors, unfractionated heparin or LMWH or active substances which interfere with coagulation are

administered (before, during or within the first 24 hours after treatment with Actilyse). This is particularly the case with intravenous administration; however, bleeding risk associated with nebulised administration, where absorption and systemic exposure is expected to be low, is not known.

There are now reports that standard prophylactic doses of anticoagulation have a high failure rate in COVID-19 pneumonia. As a result higher than standard doses are being used (including therapeutic anticoagulation) in these patients(Klok et al. 2020; Bikdeli et al. 2020) As the plasma concentration of rtPA is expected to be relatively low after nebulised administration, we do not expect concomitant administration of higher than standard doses of thromboprophylaxis to result in higher bleeding rates during this study. Use of anticoagulation at higher than standard doses is therefore not an exclusion criteria.

ACE inhibitors

Concomitant treatment with ACE inhibitors may enhance the risk of suffering a hypersensitivity reaction (see section 4.4).

Concomitant use of GPIIb/IIIa antagonists increases the risk of bleeding.

Where bronchoscopy is undertaken, diagnostic or therapeutic, it is reasonable to use topical tPA at a dose not exceeding 10 mg for the session.

5.6 Post-trial IMP arrangements

There will be no arrangements to provide IMP to patients after trial participation as patients would have either recovered or succumbed to their disease.

6 Selection of Participants

6.1 Eligibility of trial participants

6.1.1 Trial participant inclusion criteria – Cohort 1 and Cohort 2

All patients must meet the following criteria (ward or ICU based):

1. Patients with COVID-19 (confirmed by PCR or radiologically)
2. ≥16 years
3. Willing and able to provide written informed consent or where patient doesn't have capacity, consent obtained from a legal representative if available
4. Patients on mechanical ventilation must meet the following criteria:
    a. PaO_2/FiO_2 of ≤ 300
    b. Intubated > 6 hrs
5. Patients not intubated must meet the following criteria:
    a. PaO_2/FiO_2 ≤ 300 or equivalent imputed by non-linear calculation from SpO_2/FiO_2 (see look-up table in appendices)
    b. In-patient >6 hours and being actively treated
    c. On support with non-invasive ventilation OR continuous positive airway pressure (CPAP) OR high flow OR standard oxygen therapy

6.1.2 Trial participant exclusion criteria – Cohort 1

None of the following criteria must apply:

1. Females who are pregnant
2. Concurrent involvement in another experimental investigational medicinal product
3. Known allergies to the IMP or excipients of IMP
4. A pre-existing bleeding disorder (e.g. severe haemophilia) with no definitive treatment
5. Pre-existing severe cardiopulmonary disease (e.g. incurable lung cancer, severe chronic obstructive lung disease, cardiomyopathy, heart failure or impaired contractility <estimated 40% LVEF or RVEF)
6. Fibrinogen < 2.0 g/L at time of screening
7. Patients considered inappropriate for active treatment (e.g. being considered for palliative care)
8. Patients with active bleeding in the preceding 7 days
9. Patients who in the opinion of the investigator are not suitable

6.1.3 Eligibility of matched historical controls

Historical controls with COVID-19 (confirmed either via PCR or radiologically) will be selected on the basis of the matching criteria stated in section 10.4.2 only. Historical controls must be aged 16 years or over.

6.1.4 Trial participant exclusion criteria – Cohort 2

1. Females who are pregnant
2. Known allergies to the IMP or excipients of IMP
3. Fibrinogen < 1.5 g/L at time of screening
4. Patients considered inappropriate for active treatment (e.g. being considered for palliative care)
5. Patients who in the opinion of the investigator are not suitable

6.2 Recruitment

Patients will be identified by the clinical team involved in the patient's routine care in either wards or ICU. As described in section 6.3 patients will either go through the consenting process themselves or, if they do not have the capacity, consent will be obtained from their legal representative.

All trial related activities will ideally be carried out by site staff delegated to the trial. As nebulisation will need to be regularly undertaken over 14 days, trained nurses who are used to nebulisation will be permitted to undertake this procedure, with two nurses double checking the dilution and amount of rtPA being reconstituted.

The only procedures that may be performed in advance of written informed consent being taken are those that would have been performed on all participants in the same situation as routine clinical practice.

Participant recruitment at a site will only commence when the trial has been issued with the 'Open to Recruitment' letter by the sponsor.

6.3 Informed consent procedure

It is the responsibility of the Investigator, or a person delegated by the Investigator to obtain written informed consent from each participant prior to participation in the trial, following adequate explanation of the aims, methods, anticipated benefits and potential hazards of the trial.

The person taking consent will be GCP-trained, suitably qualified and experienced, and will have been delegated this duty by the CI/ PI on the Staff Signature and Delegation of Tasks.

"Adequate time" must be given for consideration by the participant/legal representative before taking part. Consent will ideally be sought at least 24 hours after being given the study documentation. However, given the need to urgently begin treatment in many of these patients, it may not be justifiable to seek consent at least 24 hours after providing the study documentation, as the patient's condition may deteriorate over this time. In these instances, consent may be sought less than 24 hours after providing the study documentation. It must be recorded in the medical notes when the participant information sheet (PIS) has been given to the participant/legal representative.

The Investigator or designee will explain that participants are under no obligation to enter the trial and that they can withdraw at any time during the trial, without having to give a reason.

However, if the patient lacks capacity to give consent due to the severity of their medical condition (e.g. acute respiratory failure or need for immediate ventilation), then consent may be obtained from a relative acting as the patient's personal legal representative. If there is no personal legal representative available (which due to restrictions of visitors to the hospital is likely) a professional legal representative (independent clinical responsible for the patients' medical care or other person appointed by the hospital) will be asked to provide consent on behalf of the patient. Further consent will then be sought from a personal legal representative if one becomes available or from the patient if they recover sufficiently. If the patient refuses consent after recovery further assessments will be stopped.

In the event that a patient loses capacity as a result of treatment for their primary medical condition while on the study, an appropriate consultee will be sought as described above. No clinical trial procedures will be conducted prior to the participant/legal representative giving consent by signing the Consent form. Consent will not denote enrolment into trial.

A copy of the signed informed consent form will be given to the participant. The original signed form will be retained in the trial file at site and a copy placed in the medical notes.

The PIS and consent form will be reviewed and updated if necessary throughout the trial (e.g. where new safety information becomes available) and participants will be re-consented as appropriate.

6.4 Consent for historical standard of care controls (cohort 1 only)

Patients will be identified as historical controls by reviewing site admissions for cases that match treatment arm patients according to the characteristics listed in section 10.4.2. Historical controls can be identified from either site (i.e. Royal Free Hospital treatment arm patients can be matched with historical controls from Barnet Hospital). There is no limit to how far back in time the search for historical controls will go.

Initial collection of data will be done without patient consent under The Health Service (Control of Patient Information) Regulations 2002 and the notices issued by the secretary of state to healthcare

organisations, GP's, local authorities and arm's length bodies to allow sharing of information to support efforts against coronavirus (COVID-19).

These notices are currently due to end on 31 March 2021. Consent will not be sought from historical controls because the use of their personal identifiable information is covered by this notice.

The Royal Free trial team will retain a subject ID log that links the patient's trial identification number with their full name and hospital number. The log will indicate whether a patient is alive or dead at the time of enrolment. Because the both hospitals are part of the same Trust (Royal Free London NHS Trust), as per routine practice, the study team at the Royal Free Hospital also have access to the medical records of Barnet Hospital patients. The patient's identifiable information will not be recorded on the study database or case report forms, they will only be referred to by their unique trial ID number.

7 Trial procedures

7.1 Pre-treatment Assessments

7.1.1 Screening – Cohorts 1 and 2

The results from the following routine procedures may be used to assess the participant's eligibility. Where routine results are not available at the time of consent, the procedure(s) will be carried out after consent. All must have been done within 24 hours prior undertaken as part of their standard care before their first dose of rtPA. These will also constitute the baseline assessments for cohort 2.

Patients receiving rtPA and SOC will undertake all assessments:

- Informed consent
- Demographics
- Review of medical history (including inpatient history and COVID-19 diagnosis – see section 7.7).
- Eligibility review
- Pregnancy test (blood or urine) for female participants of childbearing potential
- Concomitant medications
- Directed physical examination including vital signs (heart rate, temperature, blood pressure, and body weight)
- Documentation of respiratory status: Respiratory status of patients is monitored hourly and data points will be extracted for every four hour where available, but one data point is required as a minimum. The time of measurement should be documented. The variables include:
    - Details of oxygen and respiratory support including the type of device and Fi02 Respiratory rate
    - PaO_2
    - For IMV patients, details of ventilation including but not exclusively the below
        - Tidal volume (in mL)
        - Full sedation or not – not for cohort 2
        - Paralysis or not – not for cohort 2
        - Patient position – not for cohort 2
        - Peak inspiratory pressure (for computing compliance)
        - PEEP (for computing compliance)

- For non-invasive support patients:
  - SpO_2
  - Oxygen flow rate
  - National Early Warning Score (NEWS 2) – not for cohort 2
- For CPAP patients
  - Positive end expiry pressure (PEEP)

- Local blood testing as per section 7.6.2
- Research blood sampling as per section 7.6.2
- Clinical status as assessed by 7-point ordinal scale (see section 7.7)
- Sequential Organ Failure Assessment (SOFA) score (see section 7.7)
- Glasgow coma scale (see section 7.7)

7.1.2 Baseline assessments (for duration of 3 days prior to treatment) – Cohort 1 only

Screening assessments will be combined with daily routine assessments taken from the 3 days prior to dosing to establish a baseline:

- Documentation of respiratory status: Respiratory status of patients is monitored hourly and data points will be extracted for every four hours where available, but one data point is required as a minimum. The time of measurement should be documented. The variables include:
  - Mode of Ventilation
  - Oxygenation index
  - Respiratory rate
  - PaO_2
  - SpO_2
  - FiO_2
  - For IMV patients, details of ventilation including but not exclusively the below
    - Tidal volume (in mL)
    - Full sedation or not
    - Paralysis or not.
    - Patient position
    - Peak inspiratory pressure (collected for computing compliance)
    - PEEP (collected for computing compliance)
  - For non-invasive oxygen support patients:
    - National Early Warning Score (NEWS 2)
  - For CPAP patients
    - Positive end expiry pressure (PEEP)
- Clinical status as assessed by 7-point clinical ordinal scale (see section 7.7)
- Sequential Organ Failure Assessment (SOFA) score (see section 7.7)
- Glasgow coma scale (see section 7.7)

7.2 Registration Procedures

Participant registration will be undertaken by the coordinating trial team at site on a trial specific registration form.

Participants are considered to be enrolled into the trial following: consent, pre-treatment assessments (see section 7.1), confirmation of eligibility, completion of the registration process, allocation of the participant trial number.

7.3 Treatment Schedule for nebulisation

Patients in the rtPA and SOC group will receive the first dose as soon as possible after enrolment, and in accordance with the dosing schedule below. The time of initiation of the first dose will be recorded and the timing of the initiation of subsequent doses will be relative to this time. 10mg rtPA will be administered by nebulisation every 6 hours. Treatment with rtPA will be administered at a dose of 10 mg every 6hrs for a minimum of 5 days initially and a maximum of 14 treatment days overall.

7.3.1 Cohort 1

rtPA treatment schedule

Study Day	Time (hours)	Dose No.	Dose (mg)	Daily Dose (mg)
1	0 (± 1hr)	1	10	40
	6 (± 1hr)	2	10	
	12 (± 1hr)	3	10	
	18 (± 1hr)	4	10	
2	24 (± 1hr)	5	10	40
	30 (± 1hr)	6	10	
	36 (± 1hr)	7	10	
	42 (± 1hr)	8	10	
3	48 (± 1hr)	9	10	40
	54 (± 1hr)	10	10	
	60 (± 1hr)	11	10	
	66 (± 1hr)	12	10	

4	72 (± 1hr)	13	10	40
	78 (± 1hr)	14	10	
	84 (± 1hr)	15	10	
	90 (± 1hr)	16	10	
5	96 (± 1hr)	17	10	40
	102 (± 1hr)	18	10	
	108 (± 1hr)	19	10	
	114 (± 1hr)	20	10	
6 to 14	*Patients will continue to be dosed every 6 hours (± 1hr) from the time of the first dose, resulting in 56 doses in total being administered.*			

The duration of therapy can be extended from five days to a maximum of 14 treatment days in total. This decision can only be made in the absence of any dose-limiting toxicity. Treatment cannot be given for more than 56 doses.

7.3.2 Cohort 2

The treatment schedule will differ depending on the ventilation type

rtPA treatment schedule for IMV patients

Study Day	Time (hours)	Dose No.	Dose (mg)	Daily Dose (mg)
1	0	1	20	60
	8 (± 2hrs)	2	20	
	16 (± 2hrs)	3	20	
2	24 (± 2hrs)	4	20	60
	32 (± 2hrs)	5	20	

	40 (± 2hrs)	6	20	
3	48 (± 2hrs)	7	20	60
	56 (± 2hrs)	8	20	
	64 (± 2hrs)	9	20	
4	72 (± 2hrs)	10	20	60
	80 (± 2hrs)	11	20	
	88 (± 2hrs)	12	20	
5 to 14	*Patients will continue to be dosed every 8 hours (± 2hrs) from the time of the first dose, resulting in 42 doses in total being administered.*			

rtPA treatment schedule for patients on non-invasive oxygen support

This dosing schedule will consist of a higher loading dose for the first 2 days.

Study Day	Time (hours)	Dose No.	Dose (mg)	Daily Dose (mg)
1	0	1	20	60
	8 (± 2hrs)	2	20	
	16 (± 2hrs)	3	20	
2	24 (± 2hrs)	4	20	60
	32 (± 2hrs)	5	20	
	40 (± 2hrs)	6	20	
3	48 (± 4hrs)	7	20	40
	60 (± 4hrs)	8	20	

4	72 (± 4hrs)	9	20	40
	84 (± 4hr)	10	20	
5	96 (± 4hr)	11	20	40
	108 (± 4hr)	12	20	
6 to 14	*Patients will continue to be dosed every 12 hours (± 4hrs) from the time of the first dose, resulting in 30 doses in total being administered.*			

7.3.3 Treatment discontinuation

Treatment may be stopped at any point in a patient if any of the following rules are met:

- If plasma fibrinogen levels fall to <1.0 g/L, the further dosing of rtPA in the patient will be stopped (see section 7.4).
- Patient maintains saturation on room air that is either normal or normal for them for 48 hours as assessed by the investigator
- A trial stopping rule has been met (see section 7.10)

7.3.4 Re-treatment with rtPA after treatment discontinuation due to improvement in patient saturation (as described above)

If there is a recurrence of COVID-19 ARDS related symptoms or a worsening of PaO_2/FiO_2 ratio, which the investigator considers could be related to treatment discontinuation, treatment can be restarted. The decision to re-treat will be made within 5 days (120 hrs) from the last dose of treatment. Treatment may not be restarted more than once.

The rationale for restarting of treatment must be documented in the notes, along with the absence of toxicity. The patients monitoring will done according to main treatment phase.

7.3.5 Re-treatment of patients previously consented and treated on the trial – Cohort 1

For patients that have already been treated under the previous 3 day dosing schedule, if they remain eligible for the trial, they will be offered the option to continue treatment as above – the maximum number of days of extra treatment for these patients will be 11 days. Patients (or their legal representatives) must give consent to this re-treatment.

The total duration of trial participation for these patients may exceed 28 days (to approximately 5-6 weeks) to allow us to extend treatment to the maximum 14 days and to allow us to collect 5 days of post treatment data. We will inform the patient or their legal representative of this possibility and this will be documented in the patient information sheet.

7.4 Dose Modifications

Modifying the dose of rtPA will not be permitted. However, should a patient's condition change such that their ventilation type is changed whilst receiving rtPA treatment (that is, from IMV to NIV, or vice versa) they will switch to the treatment schedule that is applicable to the new ventilation type.

If plasma fibrinogen levels fall to <1.0 g/L, the further dosing of rtPA in the patient will be stopped. Replacement therapy, either in the form of fibrinogen concentrate or cryoprecipitate will be administered to return fibrinogen to >1.0 g/L.

7.5 Visit schedule and assessments post initiation of treatment

7.5.1 Cohort 1 and 2

Any assessments that are done as per routine care do not need to be repeated.

- Documentation of respiratory status: Respiratory status of patients is monitored hourly and data points will be extracted for every four hours where available until 5 days after the last dose for cohort 1, but one data point per day is required as a minimum. For cohort 2 data will be extracted once a day coinciding with the worst PF ratio. As PF ratio is the primary endpoint, in addition to the worst PF ratio over the preceding 24 hrs up to a maximum of 6 data points spread across 24 hrs that represents patients clinical status will be extracted. Similar to cohort 1 respiratory data will be collected until 5 days after the last dose. After this period, respiratory status is to be documented weekly (+/- 1 day) until discharge, death, or 28 days whichever occurs first. The time of measurement should be documented.
- The variables include:
    - Details of oxygen and respiratory support including the type of device, FiO2 Respiratory rate
    - PaO_2
    - For IMV patients, details of ventilation including but not exclusively the below
        - Tidal volume (in mL)
        - Full sedation or not – not for cohort 2
        - Paralysis or not - – not for cohort 2.
        - Patient position - – not for cohort 2
        - Peak inspiratory pressure (collected for computing compliance)
        - PEEP (collected for computing compliance)
    - For non-invasive oxygen support patients:
        - National Early Warning Score (NEWS 2) – not for cohort 2
        - Oxygen flow rate
        - SpO_2
    - For CPAP patients
        - Positive end expiry pressure (PEEP)

The following tests are to be done at a minimum once a day until 5 days after the last dose (day 7 for SOC patients) in cohort 1, a minimum every other day in cohort 2, and then as required until day 28, discharge or death whichever occurs first.

- Local blood testing as per section 7.6.2

The following tests will be done once at 48 h (+/-24 h) and 96 h (+/-24h) after start of treatment and then twice weekly while on treatment until 5 days after stopping treatment. After this period, testing should be weekly (+/- 1 day) until day 28, discharge or death whichever occurs first. Twice weekly testing should be evenly spaced across weekdays if possible.

- Research blood sampling as per section 7.6.2

The following tests are to be done daily until 5 days after the last dose (day 7 for SOC patients), and then weekly (+/- 1 day) until day 28, discharge or death whichever occurs first:

- Directed physical examination at baseline, and thereafter weekly only in cohort 2
- vital signs (heart rate, temperature, blood pressure)
- Concomitant medications
- Review of Adverse events and Adverse drug reactions including bleeding episodes

The following assessments are to be done daily until 5 days after the last dose (day 7 for SOC patients) after the baseline assessments were done for these tests:

- Clinical status as assessed by a 7-point ordinal scale
- SOFA score
- Glasgow coma scale

The following end of study data will also be collected at day 28, discharge or death whichever occurs first:

- Documentation of any Oxygenation free days in the first 28 days
- Documentation of any Ventilator-free days in the first 28 days
- Incidence and duration of new oxygen use, non-invasive ventilation or high flow oxygen devices in the first 28 days.
- Documentation of incidence and duration of new mechanical ventilation use during the trial
    - Documentation of intensive care unit (ICU) free days in the first 28 days Death and cause of death (if applicable)
    - Documentation of any Oxygenation free days
    - Documentation of any Ventilator free days
- At 28 days on trial (or death or discharge, whichever comes first), a single measurement will be taken on the 7-point ordinal scale (see section 7.74)

7.5.2 Cohort 1 -Data collection for matched historical controls

The following data will be collected for matched historical controls:

- Method of COVID-19 confirmation (PCR or radiological)
- Matching criteria (type of oxygen support, age, severity of disease according to PaO_2/FiO_2 ratio, ethnicity, gender
- COVID-19 admission history – to include PCR and CXR findings, date of admission to hospital, date of ICU admission, date of oxygen support and the progression of oxygen support to death or discharge from the study, presence or absence of pulmonary embolism and deep vein thrombosis. Presence of haemophagocytic syndrome will also be recorded, as this impacts on fibrinogen levels.
- Date of admission to hospital and duration of stay
- PaO_2

- FiO_2
- Patient position
- Ongoing 7-point ordinal scale
- Medical history – medical history to be reviewed and relevant history as assessed by the clinician to be recorded in the CRF, including an ISARIC style categorisation for all the major organ systems
- Concomitant medications
- SOFA score
- Glasgow coma scale
- Local blood results as per section 7.6.2
- Results from other standard of care assessments may also be collected if they explain the rate of change for the PaO_2/FiO_2 ratio, such as secondary infections, medication changes.
  This data will be collected where available for 28 days from the date of admission to hospital.

7.6 Laboratory Assessments and Procedures

7.6.1 Sample Collection

The Advisory Committee of Dangerous pathogens (ACDP) in conjunction with the Health and Safety Executive and Public Health England have classified COVID-19 as Hazard Group 3. Work with specimens that may contain the virus has been given dispensation to have diagnostic work carried out at less than full containment level 3 conditions subject to local risk assessment. The view of ACDP is that blood samples from confirmed COVID-19 patients can be handled in CL2 after local risk assessment of the possibility of aerosol generation for each kind of test/analyser.

7.6.2 Summary of laboratory tests

The following tests will be carried out at Local Laboratories as part of clinical care.

1. Confirmatory tests for coronavirus
2. Blood

Haematology	Full blood count including: haemoglobin, WBC, platelet count,
Serum chemistry	Routine clinical biochemistry including: renal, liver, bone, CRP, ferritin, LDH, BNP, troponin T (the last two baseline only)
Conventional coagulation tests	PT, APTT, Fibrinogen and D-dimers

3. Research blood tests

Procoagulants, anticoagulants, fibrinolytic proteins, fibrinolytic inhibitors, chemokines and cytokines

Research blood samples will be analysed in one of two laboratories:

1. Haemophilia research lab, Royal Free Hospital.
2. Department of Biochemical Sciences, University of Surrey, Guildford

Testing frequency will be presented in the schedule of assessments.

7.6.3 Storage of Sample Aliquots

Serum and plasma samples will be stored at in the Haemophilia and Thrombosis Laboratory Containment level 2 -80°C freezer prior to analysis and any additional analysis, the left-over aliquots will be stored in the Haemophilia and Thrombosis Centre Plasma Bank.

Samples for testing by University of Surrey will be shipped in batches. At the end of the trial, and on completion of all analyses, any remaining blood samples and the derivatives at University of Surrey thereof will be returned to CI for storage in Biobank/further analysis in ethics approved study.

Samples will be stored up to 5 years after the end of study for use in other ethically approved research projects.

7.7 Clinical Procedures and Data Collection

7.7.1 Directed physical examination:

A review of relevant body systems will be conducted by a study clinician. Vital signs will also be undertaken; these can be done by a clinician or study nurse. Vital signs include heart rate, temperature, blood pressure, and (at baseline only) body weight.

7.7.2 Review of medical history (including inpatient history and COVID-19 diagnosis) and document concomitant medications

A review of the patient's relevant medical history must be undertaken and documented in the medical notes and CRF. The patient's COVID-19 diagnosis (clinical or laboratory - confirmed by PCR or radiologically) must also be documented.

Inpatient history must include:

- Hospital admission time
- Hospital admission source (Community vs transfer from other institution)
- ICU admission time
- ICU admission source (ward/ED/OR/Transfer from other institution)
- Planned ICU admission (yes/No)

All over-the-counter or prescription medication, vitamins, and/or herbal supplements will be recorded.

7.7.3 Bleeding Episodes:

Bleeding episodes will be documented in the medical notes and CRF, and will include:

- Number
- Site, date and time of onset, Aetiology (spontaneous, traumatic, etc)
- Control of Anticoagulation Subcommittee of the International Society on Thrombosis and Haemostasis (Schulman and Kearon 2005) recommendation for major and minor bleeding will be implemented.
- Major bleeding in non-surgical patients is said to be present if the bleeding is
    - Fatal bleeding, and/or
    - Symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intra-articular or pericardial, pulmonary or intramuscular with compartment syndrome, and/or

- Minor bleed, if any sign or symptom of haemorrhage that does not fit ISTH definition of major bleed and one of (requiring medical intervention by a healthcare professional) leading to hospitalization or increased level of care prompting a face to face (i.e., not just a telephone or electronic communication) evaluation
- Any coagulation treatment required

7.7.4 7-point clinical status ordinal scale:

The ordinal scale is an assessment of the clinical status at a given study day:

1. Limitation of activities
2. Hospitalized, no oxygen therapy
3. Oxygen by mask or nasal prongs
4. Non-invasive ventilation or high-flow oxygen
5. Intubation and mechanical ventilation
6. Ventilation+ additional organ support (vasopressor, RRT, ECMO)
7. Death

7.7.5 Sequential Organ Failure Assessment (SOFA) score:

The Sequential Organ Failure Assessment (SOFA) is a morbidity severity score and mortality estimation tool developed from a large sample of ICU patients throughout the world. The SOFA was designed to focus on organ dysfunction and morbidity, with less of an emphasis on mortality prediction. It requires the following (worst value within past 24 hours):

- FiO_2
- PaO_2
- Mechanical ventilation y/n
- Platelets
- Bilirubin
- Glasgow coma scale
- Mean arterial pressure
- Vasopressors y/n
- Creatinine
- Urine output

This can be calculated by entering the results above into an online tool:
https://clincalc.com/IcuMortality/SOFA.aspx

7.7.6 Glasgow Coma Scale:

The Glasgow coma scale is used to assess patients in a coma. The initial score correlates with the severity of brain injury and prognosis. The Glasgow Coma Scale provides a score in the range 3-15; patients with scores of 3-8 are usually said to be in a coma.

Scoring is done according to the table below:

	1	2	3	4	5	6
Eye	Does not open eyes	Opens eyes in response to pain	Opens eyes in response to voice	Opens eyes spontaneously	N/A	N/A
Verbal	Makes no sounds	Makes sounds	Words	Confused, disoriented	Oriented, converses normally	N/A
Motor	Makes no movements	Extension to painful stimuli (decerebrate response)	Abnormal flexion to painful stimuli (decorticate response)	Flexion / Withdrawal to painful stimuli	Localizes to painful stimuli	Obeys commands

Maximum score is 15 which has the best prognosis

Minimum score is 3 which has the worst prognosis

Scores of 8 or above have a good chance for recovery

Scores of 3-5 are potentially fatal, especially if accompanied by fixed pupils or absent oculovestibular responses.

7.7.7 National Early Warning Score (NEWS - 2)

The NEWS, like many existing Early Warning Score systems, is based on a simple scoring system in which a number is allocated to physiological measurements (Vital Signs) already routinely measured in hospital and recorded on the patient clinical chart. The six simple physiological parameters form the basis of the scoring system:

1. Respiratory rate
2. Oxygen saturations
3. Temperature
4. Systolic blood pressure
5. Heart rate
6. Level of consciousness.

Values are added to each variable using the table below:

Physiological Parameters	3	2	1	0	1	2	3
Respiration Rate (BPM)	≤8		9-11	12-20		21-24	≥25
Oxygen Saturations (%)	≤91	92-93	94-95	≥96			
Any Supplemental Oxygen		Yes		No			
Temperature (°C)	≤35		35.1-36.0	36.1-38.0	38.1-39.0	≥39.1	
Systolic Blood Pressure (mmHg)	≤90	19-100	101-110	111-219			≥220
Heart Rate (BPM)	≤40		41-50	51-90	91-110	111-130	≥131
Level of Consciousness				A			V, P or U

Clinical risk is assigned based on the total score:

- Aggregate score 1-4: Low
- Aggregate score 5-6: Medium
- Aggregate score 7 or more: High

Refer to https://www.activ8rlives.com/support/data-collected/cardiovascular-and-respiratory/national-early-warning-score-news/

7.8 Discontinuation/withdrawal of participants

In consenting to participate in the trial, participants or legal representatives on their behalf are consenting to trial treatment, assessments, follow-up and data collection.

7.8.1 Discontinuation of Trial Treatment for clinical reasons

A participant may be withdrawn from trial treatment whenever continued participation is no longer in the participant's best interests, but the reasons for doing so must be recorded.

The decision to withdraw a participant from treatment must be recorded in the CRF and medical notes, and the sponsor when required should be notified in writing.

7.8.2 Participant withdrawal from trial treatment and/or follow-up

If a participant expresses their wish to withdraw from trial treatment or trial follow-up, sites should explain the importance of remaining on trial follow-up and seek permission to allow use of routine follow-up data to be used for trial purposes. The importance of safety follow-up should be emphasised to the participant in the Participant Information Sheet.

The decision of the participant to withdraw from treatment or follow-up must be recorded in the CRF and medical notes.

The participant may withhold their reason for withdrawal however, if the participant gives a reason for their withdrawal, this should be recorded.

7.8.3 Withdrawal of Consent to Data Collection

If a participant explicitly states, they do not wish to contribute further data to the trial their decision must be respected and recorded in the CRF and medical notes.

7.9 Replacements

Replacement of withdrawn patients may occur at the discretion of the Investigator.

7.10 Stopping rules

The trial may be stopped before completion for the following reasons:

- On the recommendation of the sponsor and CI
- A major pulmonary bleeding that is symptomatic or fatal that occurs between start of treatment and 30 hours after the last dose.
- Cohort 1 - A 50% or greater reduction of fibrinogen at 72 hrs (from baseline value at screening) in either of the first 2 patients after dosing. If we see a 50% or greater drop in fibrinogen in a patient, it would only be a particular safety concern in that patient if the fibrinogen were to go below 1.5 g/L (hence why this level is stated as a reason to stop dosing in an individual patient). However, if we do see a 50% drop in fibrinogen from baseline values in either of the first 2 patients it might suggest that the dose is too high overall with secondary systemic absorption. As such stopping the trial will provide us the opportunity to discuss within the TMG and IDMC to make a decision on the dose going forward. A substantial amendment would be made to the MHRA and REC.
- Any fatal bleeding that occurs between start of treatment and 30 hours after the last dose.
- Symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, pericardial, pulmonary or intramuscular with compartment syndrome, that occurs between start of treatment and 30 hours after the last dose.

Further enrolment into the study will be put on hold in the event of any of the stopping rules and a substantial amendment will be submitted to temporarily halt the trial. Whenever the study is stopped in addition to a review of the data by the trial management group consisting of the investigators, the DMC will also be convened for review of the data to assess the risk benefit of progressing the pilot study. If, following an internal safety review by the DMC, the Sponsor deems it appropriate to restart the trial; this can only be done following approval by the MHRA and REC and along with any local approvals required.

7.11 Definition of End of Trial

The end of trial is the date of discharge, death, or day 28 of the last patient whichever is sooner.

8 Recording and reporting of adverse events and reactions

Collection, recording and reporting of adverse events (including serious and non-serious events and reactions) to the sponsor will be completed according to the sponsor's SOP (INV/S05).

8.1 Definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a patient or clinical trial participant administered a medicinal product and which does not necessarily have a causal relationship with this treatment. *Therefore an AE can be any unfavourable or unintended change in the structure (signs), function (symptoms) or chemistry (laboratory data) in a participant to whom an IMP or procedural intervention has been administered, including occurrences which are not necessarily caused by or related to that product.*
Adverse Reaction (AR)	Any untoward and unintended response in a participant to an investigational medicinal product which **is related** to any dose administered to that participant. This includes medication errors, uses outside of protocol (including misuse and abuse of product)
Serious Adverse Event (SAE), Serious Adverse Reaction (SAR) or Unexpected Serious Adverse Reaction	Any adverse event, adverse reaction or unexpected adverse reaction, respectively, that: • results in death, • is life-threatening*, • requires hospitalisation or prolongation of existing hospitalisation**, • results in persistent or significant disability or incapacity, or • consists of a congenital anomaly or birth defect *A life- threatening event, this refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe. ** Hospitalisation is defined as an in-patient admission, regardless of length of stay. Hospitalisation for pre-existing conditions, including elective procedures do not constitute an SAE. Some medical events may jeopardise the subject or may require an intervention to prevent one of the above characteristics/consequences. Such **important medical events** should also be considered as serious. The term "**severe**" is often used to describe the intensity of an event or reaction (e.g. mild, moderate or severe) and should not be confused or interchanged with the term "**serious**".

Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature, severity or outcome of which is not consistent with the Reference Safety Information.
Reference Safety Information (RSI)	A list of medical events that defines which reactions are expected for the IMP being administered to clinical trial subjects, and so do not require expedited reporting to the Competent Authority. It is contained in a specific section in the Summary of product characteristics (SmPC) or the Investigator Brochure (IB).

8.2 Recording adverse events

All adverse events will be recorded in the medical records in the first instance. Non-serious AEs will not be collected in the CRFs for this trial, apart from the adverse events of special interest (AESI) listed below:

- Bleeding events

In addition, any Serious Adverse Events (SAEs) assessed as likely to be related to IMP administration will be collected. See section 8.4 for further details on recording and reporting of Serious Adverse Reactions (SARs).These AESIs and Serious Adverse Reactions (SARs) will be recorded with clinical symptoms and accompanied with a simple, brief description of the event, including dates as appropriate, until the participant completes the trial.

8.3 Assessments of Adverse Events

Each adverse event will be assessed for severity, causality, seriousness and expectedness as described below.

8.3.1 Severity

Category	Definition
Mild	The adverse event does not interfere with the participant's daily routine, and does not require intervention; it causes slight discomfort
Moderate	The adverse event interferes with some aspects of the participant's routine, or requires intervention, but is not damaging to health; it causes moderate discomfort
Severe	The adverse event results in alteration, discomfort or disability which is clearly damaging to health

8.3.2 Causality

The assessment of relationship of adverse events to the administration of IMP is a clinical decision based on all available information at the time of the completion of the case report form.

The following categories will be used to define the causality of the adverse event:

Category	Definition

Related	*A causal relationship between an IMP/investigational treatment and an adverse event is at least a reasonable possibility, i.e., the relationship cannot be ruled out.*
Not related	*There is no reasonable possibility of a causal relationship between an IMP/investigational treatment and an adverse event.*

8.3.3 Expectedness

Category	Definition
Expected	An adverse event which is consistent with the information about the IMP listed in the current approved Reference Safety Information (RSI) for the trial.
Unexpected	An adverse event which is not consistent with the information about the IMP listed in the current approved Reference Safety Information (RSI) for the trial.

The RSI for this trial is the Investigator Brochure for Recombinant tissue plasminogen activator (rtPA) – alteplase. As the IMP rtPA (Actilyse) is being used outside its authorised indication and route of administration all SARs will be assessed as unexpected and classified as SUSARs, and will be subject to expedited reporting to the MHRA and REC.

8.3.4 Seriousness

All events are assessed for seriousness as defined for an SAE in section 8.1.

All SAEs and reported in line with the procedures set-out in section 8.4 apart from regulatory reporting.

8.4 Procedures for recording and reporting Serious Adverse Reactions

All SAEs will be recorded in the medical records in the first instance. Only serious adverse events assessed as likely to be related to IMP administration, Serious Adverse Reactions (SARs), will be recorded in the CRF, and the sponsor's SAE log. The SAE log will be reported to the sponsor at the end of the study.

SARs will be recorded from the start of treatment until the end of the study.

All SARs must be recorded on a SAE Reporting Form. The CI/PI or designated individual will complete the sponsor's SAE form and email to the Sponsor at SAE@ucl.ac.uk immediately and no later than 24 hours of his / her becoming aware of the event. The Chief or Principal Investigator will respond to any SAE queries raised by the sponsor as soon as possible.

Completed SAE forms must be sent within 24 hours of becoming aware of the event to the Sponsor Email forms to SAE@ucl.ac.uk

8.5 Notification of deaths

Only deaths assessed as likely related to IMP administration will be reported to Sponsor, see section 9.4 above.

8.6 Reporting of SUSARS

The sponsor will notify the main REC and MHRA of all SUSARs. SUSARs that are fatal or life-threatening must be notified to the MHRA and REC within 7 days after the sponsor has learned of them. Other SUSARs must be reported to the REC and MHRA within 15 days after the sponsor has learned of them.

8.7 Development Safety Update Reports

The sponsor will provide the main REC and the MHRA with Development Safety Update Reports (DSUR) which will be written in conjunction with the trial team and the Sponsor's office. The report will be submitted within 60 days of the Developmental International Birth Date (DIBD) of the trial each year until the trial is declared ended.

8.8 Pregnancy

Not applicable. All female patients of child bearing potential must have a confirmed negative pregnancy test prior to enrolment (within 24hrs) and will remain in-patients in hospital during the course of the study.

8.9 Reporting Urgent Safety Measures and other safety events

If any urgent safety measures are taken the CI/ PI shall immediately and in any event no later than 3 days from the date the measures are taken, give written notice to the MHRA, the relevant REC and Sponsor of the measures taken and the circumstances giving rise to those measures.

8.10 Notification of Serious Breaches to GCP and/or the protocol (SPON/S15)

A "serious breach" is a breach which is likely to effect to a significant degree –

(a) the safety or physical or mental integrity of the participants of the trial; or

(b) the scientific value of the trial.

The sponsor of a clinical trial shall notify the licensing authority in writing of any serious breach of –

(a) the conditions and principles of GCP in connection with that trial; or (b) the protocol relating to that trial, as amended from time to time, within 7 days of becoming aware of that breach.

The sponsor will be notified immediately of any case where the above definition applies during the trial conduct phase. The sponsor's SOP on the 'Notification of violations, urgent safety measures and serious breaches' will be followed.

9 Data management and quality assurance

9.1 Confidentiality

All data will be handled in accordance with the UK Data Protection Act 2018.

The Case Report Forms (CRFs) will not bear the participant's name or other personal identifiable data. The participant's initials, date of birth and trial identification number, will be used for identification and this will be clearly explained to the patient in the Patient information sheet. Patient consent for this will be sought.

9.2 Data collection tools and source document identification

Data will be collected on Trial specific case report forms (CRFs).

Source data are contained in source documents (medical notes and laboratory reports) and must be accurately transcribed on to the CRF.

It is the responsibility of the investigator to ensure the accuracy of all data entered in the CRFs. The delegation log will identify all those personnel with responsibilities for data collection and handling, including those who have access to the trial database.

9.3 Completing Case Report Forms

All CRFs must be completed and signed by staff that are listed on the site staff delegation log and authorised by the CI/ PI to perform this duty. The CI/ PI is responsible for the accuracy of all data reported in the CRF. Once completed the original will be kept with the study team at site.

9.4 Data handling and analysis

A trial specific data management SOP will be in place for the trial. This will contain details of the software to be used for the database, the process of database design, data entry, data quality checks, data queries, data security, database lock.

Where data are transferred electronically this will be in accordance with the UK Data Protection Act 2018 as well as UCL Information Security Policy and Trust Information Governance Policy. There will be a documented record of data transfer and measures in place for the recovery of original information after transfer.

10 Statistical Considerations

10.1 Primary endpoints

10.1.1 Efficacy

1. Change in PaO_2/FiO_2 ratio from baseline (as defined in sections 7.1.2 and 7.1.3), daily during treatment, end of treatment, 3 days post end of treatment and 5 days post end of treatment.

10.1.2 Safety

1. Incidence and severity of major bleeding events directly attributable to the study drug
2. Decrease in fibrinogen levels to < 1.0 gm/L during treatment period and 48 hrs after the last dose of treatment
3. Number and nature of serious adverse events causally related to the treatment

10.2 Secondary endpoints

1. Changes in lung compliance (defined as tidal volume / (peak inspiratory pressure – PEEP)) from baseline and absolute values at day 5 (96 hrs ± 2 hrs), day 7 (144 hrs ± 4hrs), end of treatment, 3 and 5 days post end of treatment

2. Clinical status as assessed by a 7-point WHO ordinal scale at baseline and daily up to 5 days post end of treatment and at day 28, discharge or death (whichever comes first).
3. Mean daily Sequential Organ Failure Assessment (SOFA) score at baseline through to 5 days post end of treatment
4. In follow up period, number of oxygenation free days, ventilator free days, intensive care stay, up to 28 days or death or discharge, whichever occurs first.
5. Incidence and number of days of new oxygen use, non-invasive ventilation or high flow oxygen devices in the first 28 days.
6. Incidence and number of days of new mechanical ventilation use during in the first 28 days
7. In hospital mortality

10.3 Exploratory endpoints

Change in levels of plasma biomarkers of pro- and anti-inflammatory markers (e.g. interleukin-6, interleukin-8, interleukin-10 and interleukin-1Ra), endothelial injury (plasma von Willebrand factor) coagulation (procogulants, anticoagulants, and fibrinolytic pathway) measure of tissue damage. Historical controls will not provide data for exploratory endpoints.

10.4 Sample size and recruitment

10.4.1 Sample size calculation

The sample size for this pilot study is not based on a statistical consideration. The recruitment target of 12 patients treated with rtPA and SOC is based on feasibility and the planned recruitment rate.

Similarly, for cohort 2 the sample size of 30 patients is not based on statistical consideration, but has been selected on the grounds of what is reasonable amid a second surge of COVID-19 admissions in early 2021 and the number of patients we might reasonably accept to recruit across the 2 sites over the next 2 to 6 weeks.

10.4.2 Historical control recruitment – Cohort 1 only

Historical controls will be recruited at a ratio of 2 controls to every 1 rtPA + SOC arm patient, and will be matched according to the following characteristics:

1. Ventilation and oxygen type (IMV and non-invasive oxygen support)
2. Severity as determined by PaO_2/FiO_2 ratio
3. Gender
4. Age (+/- 2 years, up to a maximum of 10 years)
5. Ethnicity

Matching will be done in the order that the characteristics are presented above.

Deceased patients can be considered for matching as controls. However, as a certain number of observations are required for developing a P/F ratio of the curve, patients who died within 48 hrs of admission are excluded.

Baseline data during admission will be used for matching.

An age range of +/- two years was used in the first instance, and this was widened until two eligible patients were identified that matched the treatment patient's ethnicity, up to a maximum of +/- 10 years.

Where more than two controls are identified, two controls will be drawn at random without replacement among all considered historical patients that match according to the characteristics above.

10.5 Randomisation methods

No randomisation will be employed in this pilot study due to practical and ethical considerations.

A statistically powered RCT would be ideal to define the magnitude of benefit and impact on overall survival and is the typical design in patients with ARDS Although there is clinical data on the safety of nebulised rtPA, but there are no clinical data on this condition to facilitate a sample size calculation. There are data from a small RCT that has used another fibrinolytic agent, but the doses are not comparable (Abdelaal Ahmed Mahmoud et al. 2020). If a patient is randomised to receive no treatment, this precludes participation in other interventional studies which might increase the risk of mortality. The most recent figures suggest a 50% mortality in patients receiving IMV.(https://www.icnarc.org/About/Latest-News/2020/04/04/Report-On-2249-Patients-Critically-Ill-With-Covid-19) Currently there is a concerted effort to introduce multiple therapies based on our understanding of the pathophysiologic basis of disorder. Ethical concerns were raised about the potential for denying patients new treatment strategies when randomised to standard of care arm. The management strategies are evolving on an unprecedented scale with repurposing of existing medications, many of which are proof of concept of studies. Further, if this pilot study shows significant effect in a subset of patients, there would be justification to progress to a statistically powered RCT. We would progress to a RCT only after submission of a substantial amendment to this trial protocol or submission of a new clinical trial application In the event of major adverse drug reactions or minor improvement in the oxygenation as assessed by PaO_2/FiO_2 , there are minimal gains to be had with a larger randomised control study.

A Data Monitoring Committee (DMC) will be set-up to review safety data within the trial along with the final study results and advise on progressing from a pilot study to a statistically powered RCT based on the safety and efficacy data that is collected.

10.6 Statistical analysis plan

All analyses will be descriptive in nature and should be considered as hypothesis generating rather than providing firm conclusions.

Analysis of cohorts 1 and 2 will be undertaken separately.

All study data will be listed and all relevant data will be tabulated and summarised separately by cohort and by group (rtPA treated, historical controls), separately for the patients receiving IMV, and for other patients with mild ARDS requiring non-invasive oxygen support at enrolment, and overall only where appropriate, as follows:

Planned recruitment for Cohort 1:

Group 1: Patients with ARDS receiving IMV at enrolment treated with rtPA (n=6)

Group 2: Patients with ARDS requiring non-invasive support at enrolment treated with rtPA (n=6)

Group 3: Patients with ARDS receiving IMV - historical controls (n=12)

Group 4: Patients with ARDS requiring non-invasive support - historical controls (n=12)

Planned recruitment for Cohort 2:

A total of 30 patients will be recruited, with a minimum of 10 from each ventilation type.

Group 5: Patients with ARDS receiving IMV at enrolment treated with rtPA

Group 6: Patients with ARDS requiring non-invasive support at enrolment treated with rtPA

Data will be presented using standard descriptive statistics. Continuous variables will be summarised using number of observations, mean, and standard deviation, median, inter-quartile range, minimum, and maximum values. Categorical values will be summarised using number of observations and percentages.

A statistical analysis plan will be written and finalised prior to database lock. This plan will give a detailed description of all summaries and results that will be presented. Cohort 1 data will be locked separately from the cohort 2 data and a separate SAP will be produced for the analysis of the cohort 2 data.

10.6.1 Summary of baseline data and flow of participants

All pre-treatment assessments data in the protocol will be listed and all relevant data will be tabulated for each group separately.

For patients treated with rtPA + SOC, the last available value prior to the first administration of IMP will be considered the baseline value.

For patients receiving SOC only (historical controls), the last available value prior to enrolment, as defined in section 8.2, will be considered the baseline value.

All data necessary to produce a consort flow diagram (http://www.consort-statement.org/) will be summarised: the number of patients in each group that were enrolled, assessed for eligibility, excluded (with reasons listed), assigned to a group, treated with rtPA + SOC, followed up, discontinued (with reasons such as lost to follow-up, withdrawn, or death), and number of patients analysed in each group, and where applicable excluded from analyses (with reasons).

10.6.2 Primary outcome analysis

The PaO2/FiO2 ratio measured over time will be described for each group. The change from baseline in the PaO2/FiO2 ratio will be summarised at each post baseline timepoint. Plots will be used, where appropriate, to show the PaO2/FiO2 summarised for patients over time.

PaO2/FiO2 will be a calculated field on the trial database derived from the fields for PaO2 and FiO2. For cohort 2 data will be extracted once a day coinciding with the worst PF ratio. As PF ratio is the primary endpoint, in addition to the worst PF ratio, up to a maximum of 6 data points spread across 24 hrs that represents patients clinical status will be extracted. Details of further data derivation and analyses will be described in the SAP.

All patient data will be used, regardless of any intercurrent events that may have occurred after enrolment:

- Data from patients recruited into the rtPA + SOC group will be summarised whether or not patients have received all planned doses of the IMP.
- Any change in requirement for oxygenation and ventilation support will be summarised and described
- Changes in PaO2/FiO2 ratio over time will be described and interpreted taking into consideration that a patient's condition may have changed and led to their ventilation type changed post enrolment. If data allows, additional descriptions of the PaO2/FiO2 ratio over time may be performed in each subgroup of patients experiencing such changes (e.g. IMV to NIV, or vice versa).

The incidence and severity of bleeding events will be summarised in each group, and the change in fibrinogen levels will also be summarised at each post-baseline timepoint.

For cohort 1, subject to the final sample size and data quality, outcomes will also be compared between the SOC only group and intervention (rtPA + SOC) group using appropriate regression methods to make adjustment for baseline characteristics. Appropriate data transformations required for model assumptions will be considered if necessary.

10.6.3 Secondary outcome analysis

All secondary outcomes measured overtime will be summarised at each post-baseline timepoint, for each group, and overall where appropriate:

- Clinical status as assessed by a 7-point ordinal scale and change in clinical status
- Changes in lung compliance (computed in the database using tidal volume / (peak inspiratory pressure – PEEP)) from baseline and absolute values at day 5 (96 hrs ± 2 hrs), day 7 (144 hrs ± 4hrs), end of treatment, 3 and 5 days post end of treatment.
- The Mean daily Sequential Organ Failure Assessment (SOFA) score and change from baseline

The following secondary outcomes will also be summarised for each group, and overall, where appropriate:

- The number of oxygenation free days in the first 28 days
- Number of ventilator-free days in the first 28 days
- The incidence and number of days of new oxygen use, non-invasive ventilation or high flow oxygen devices in the first 28 days.
- Incidence and number of days of new mechanical ventilation use during the trial
- Intensive care unit (ICU) free days in the first 28 days
- In hospital mortality
- Length of ICU stay post treatment
- Length of ventilation
- Use of vasopressors

10.6.4 Sensitivity and other planned analyses

A description of plans for sensitivity and other analyses will be provided in the SAPs.

11 Record keeping and archiving

At the end of the trial, all essential documentation will be archived securely by the CI and trial sites for a minimum of 25 years from the declaration of end of trial.

Essential documents are those which enable both the conduct of the trial and the quality of the data produced to be evaluated and show whether the site complied with the principles of Good Clinical Practice and all applicable regulatory requirements.

The sponsor will notify sites when trial documentation can be archived. All archived documents must continue to be available for inspection by appropriate authorities upon request.

12 Oversight Committees

12.1 Trial Management Group (TMG)

The TMG will include the Chief Investigator, co-investigators and trial staff. The TMG will assess the safety of the trial specifically at 24 hrs after the first dose (to confirm progression to treatment of the second patient) and 72 hrs after the second patient's first dose (to confirm dosing can continue). If there are any safety issues identified the TMG will refer to the DMC for their input. The TMG will be responsible for overseeing the trial. The group will meet regularly during the estimated 3-week recruitment / treatment period to review efficacy and safety.

The TMG will review recruitment figures, SAEs and substantial amendments to the protocol prior to submission to the REC and/or MHRA.

During cohort 2, the TMG will convene after every 10 patients have been recruited.

12.2 Data Monitoring Committee (DMC)

The role of the DMC is to provide advice on data and safety aspects of the trial and recommend if the trial should progress to a statistically powered RCT. We would progress to a RCT only after submission of a substantial amendment to this trial protocol or submission of a new clinical trial application.

Cohort 1:

If at the 24 hr post dose review of patient one and 72 hr post first dose review of patient two there are any safety issues noted, the TMG will refer to the DMC for their input.

The DMC will receive weekly reports on bleeding complications, both major and minor along with fibrinogen levels. If at any time a patient meets one of the stopping criteria, further dosing of patients will be stopped and an adhoc DMC review will be arranged before resuming dosing.

Cohort 2:

During cohort 2, the DMC will convene after every 10 patients have been recruited. If at any time a patient meets one of the stopping criteria (as per section 7.10), further dosing of patients will be stopped and an adhoc DMC review will be arranged before resuming dosing.

The DMC will review the final study results and advise on progressing from a pilot study to a statistically powered randomised control trial based on the safety and efficacy data that is collected.

13 Direct Access to Source Data/Documents

The investigator/ institution will permit trial-related monitoring, audits, REC review, and regulatory inspection(s), providing direct access to source data/documents. Trial participants are informed of this during the informed consent discussion. Participants will consent to provide access to their medical notes.

14 Ethics and regulatory requirements

The sponsor will ensure that the trial protocol, participant information sheet, consent form, GP letter and submitted supporting documents have been approved by the appropriate regulatory body (MHRA in UK) and an appropriate research ethics committee and the HRA, prior to any participant recruitment. The protocol, all other supporting documents including and agreed amendments, will be documented and submitted for ethical and regulatory approval as required. Amendments will not be implemented prior to receipt of the required approval(s).

Before the site may be opened to recruit participants, the Chief Investigator or designee must receive confirmation of capability and capacity in writing from the Trust Research & Development (R&D). It is the responsibility of the CI to ensure that all subsequent amendments gain the necessary approvals, including NHS Permission (where required) at the site. This does not affect the individual clinician's responsibility to take immediate action if thought necessary to protect the health and interest of individual participants (see section 8.9 for reporting urgent safety measures).

An annual progress report (APR) will be submitted to the REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the trial is declared ended. The chief investigator will prepare the APR.

Within 90 days after the end of the trial, the CI/Sponsor will ensure that the main REC and the MHRA are notified that the trial has finished. If the trial is terminated prematurely, those reports will be made within 15 days after the end of the trial.

The CI will supply the Sponsor with a report of the clinical trial which complies with the format as defined by the EMA. This will then be uploaded to EudraCT for availability to the MHRA and a copy of the report will be submitted to the main REC, within 1 year after the end of the trial.

15 Monitoring requirement for the trial

In the context of this pandemic, visits to hospital sites are generally not appropriate as they could increase the risks of spreading infection. Therefore, no prospective monitoring will be planned for this trial. If necessary, for cause telephone monitoring calls will be undertaken with site and documented.

16 Finance

This trial will be funded by the Royal Free Hospital Charity.

17 Insurance

University College London holds insurance against claims from participants for injury caused by their participation in the clinical trial. Participants may be able to claim compensation if they can prove that UCL has been negligent. However, as this clinical trial is being carried out in a hospital, the hospital continues to have a duty of care to the participant of the clinical trial. University College London does not accept liability for any breach in the hospital's duty of care, or any negligence on the part of hospital employees. This applies whether the hospital is an NHS Trust or otherwise.

Participants may also be able to claim compensation for injury caused by participation in this clinical trial without the need to prove negligence on the part of University College London or another party. Participants who sustain injury and wish to make a claim for compensation should do so in writing in the first instance to the Chief Investigator, who will pass the claim to the Sponsor's Insurers, via the Sponsor's office.

Hospitals selected to participate in this clinical trial shall provide clinical negligence insurance cover for harm caused by their employees and a copy of the relevant insurance policy or summary shall be provided to University College London, upon request.

18 Publication policy

The sponsor intends to publish the results of this study in accordance with external guidelines regardless of whether the outcomes are perceived as positive, neutral, or negative.

All publications relating to the sponsor's projects must undergo appropriate review, and require sponsor agreement to publish prior to release of information.

No publication that incorporates the sponsor's confidential information shall be submitted for publication without the sponsor's prior written agreement to publish, and shall be given to the sponsor for review at least 30 days prior to submission for publication.

19 References

Abdelaal Ahmed Mahmoud, Ahmed, Hatem Elmoutaz Mahmoud, Mohamed Ali Mahran, and Marwa Khaled. 2020. 'Streptokinase Versus Unfractionated Heparin Nebulization in Patients With Severe Acute Respiratory Distress Syndrome (ARDS): A Randomized Controlled Trial With Observational Controls', *Journal of Cardiothoracic and Vascular Anesthesia*, 34: 436-43.

Aleplase, electronic Medicines Compendium (eMC) -. June 2019. 'factor VIII inhibitor bypassing fraction - Summary of Product Characteristics', Accessed Jan 2021. https://www.medicines.org.uk/emc/product/898/smpc.

Aranda-Valderrama, P., and A. M. Kaynar. 2018. 'The Basic Science and Molecular Mechanisms of Lung Injury and Acute Respiratory Distress Syndrome', *Int Anesthesiol Clin*, 56: 1-25.

Barrett, C. D., H. B. Moore, A. Banerjee, C. C. Silliman, E. E. Moore, and M. B. Yaffe. 2017. 'Human neutrophil elastase mediates fibrinolysis shutdown through competitive degradation of plasminogen and generation of angiostatin', *J Trauma Acute Care Surg*, 83: 1053-61.

Bhargava, Maneesh, and Chris H. Wendt. 2012. 'Biomarkers in acute lung injury', *Transl Res*, 159: 205-17.

Bikdeli, B., M. V. Madhavan, D. Jimenez, T. Chuich, I. Dreyfus, E. Driggin, C. Nigoghossian, W. Ageno, M. Madjid, Y. Guo, L. V. Tang, Y. Hu, J. Giri, M. Cushman, I. Quéré, E. P. Dimakakos, C. M. Gibson, G. Lippi, E. J. Favaloro, J. Fareed, J. A. Caprini, A. J. Tafur, J. R. Burton, D. P. Francese, E. Y. Wang, A. Falanga, C. McLintock, B. J. Hunt, A. C. Spyropoulos, G. D. Barnes, J. W. Eikelboom, I. Weinberg, S. Schulman, M. Carrier, G. Piazza, J. A. Beckman, P. G. Steg, G. W. Stone, S. Rosenkranz, S. Z. Goldhaber, S. A. Parikh, M. Monreal, H. M. Krumholz, S. V. Konstantinides, J. I. Weitz, and G. Y.

H. Lip. 2020. 'COVID-19 and Thrombotic or Thromboembolic Disease: Implications for Prevention, Antithrombotic Therapy, and Follow-up', *J Am Coll Cardiol*.

Bovill, Edwin G., Michael L. Terrin, David C. Stump, Andrew D. Berke, Margaret Frederick, Désiré Collen, Frederick Feit, Joel M. Gore, L. David Hillis, Costas T. Lambrew, Roy Leiboff, Kenneth G. Mann, John E. Markis, Craig M. Pratt, Scott W. Sharkey, George Sopko, Russell P. Tracy, and James H. Chesebro. 1991. 'Hemorrhagic Events during Therapy with Recombinant Tissue-Type Plasminogen Activator, Heparin, and Aspirin for Acute Myocardial Infarction: Results of the Thrombolysis in Myocardial Infarction (TIMI), Phase II Trial', *Ann Intern Med*, 115: 256-65.

Colaneri, M., A. Quarti, M. Pozzi, S. Gasparini, I. Carloni, and F. M. de Benedictis. 2014. 'Management of plastic bronchitis with nebulized tissue plasminogen activator: another brick in the wall', *Ital J Pediatr*, 40: 18.

Conhaim, R. L., K. E. Watson, W. F. Dovi, and M. L. Bates. 2014. 'Inhaled thrombolytics reduce lung microclot and leukocyte infiltration after acute blood loss', *Shock*, 41: 528-36.

Cooper, J A, S K Lo, and A B Malik. 1988. 'Fibrin is a determinant of neutrophil sequestration in the lung', *Circ Res*, 63: 735-41.

Copin, M. C., E. Parmentier, T. Duburcq, J. Poissy, and D. Mathieu. 2020. 'Time to consider histologic pattern of lung injury to treat critically ill patients with COVID-19 infection', *Intensive Care Med*: 1-3.

Dixon, B., M. J. Schultz, R. Smith, J. B. Fink, J. D. Santamaria, and D. J. Campbell. 2010. 'Nebulized heparin is associated with fewer days of mechanical ventilation in critically ill patients: a randomized controlled trial', *Crit Care*, 14: R180.

Foley, J. H. 2017. 'Plasmin(ogen) at the Nexus of Fibrinolysis, Inflammation, and Complement', *Semin Thromb Hemost*, 43: 135-42.

Fugate, J. E., E. A. Kalimullah, and E. F. Wijdicks. 2012. 'Angioedema after tPA: what neurointensivists should know', *Neurocrit Care*, 16: 440-3.

Gram, J., A. M. Münster, B. Dilling-Hansen, H. Al Lavassani, A. X. Lahoz, and J. Jespersen. 1999. 'Inhalation/intravenous recombinant tissue plasminogen activator and inhaled heparin in a patient with acute respiratory distress syndrome', *Fibrinolysis and Proteolysis*, 13: 209-12.

Grutter, G., D. Di Carlo, F. Gandolfo, R. Adorisio, S. Alfieri, G. Michielon, A. Carotti, and G. Pongiglione. 2012. 'Plastic bronchitis after extracardiac Fontan operation', *Ann Thorac Surg*, 94: 860-4.

Guan, W. J., Z. Y. Ni, Y. Hu, W. H. Liang, C. Q. Ou, J. X. He, L. Liu, H. Shan, C. L. Lei, D. S. C. Hui, B. Du, L. J. Li, G. Zeng, K. Y. Yuen, R. C. Chen, C. L. Tang, T. Wang, P. Y. Chen, J. Xiang, S. Y. Li, J. L. Wang, Z. J. Liang, Y. X. Peng, L. Wei, Y. Liu, Y. H. Hu, P. Peng, J. M. Wang, J. Y. Liu, Z. Chen, G. Li, Z. J. Zheng, S. Q. Qiu, J. Luo, C. J. Ye, S. Y. Zhu, and N. S. Zhong. 2020. 'Clinical Characteristics of Coronavirus Disease 2019 in China', *N Engl J Med*.

Gurwitz, J. H., J. M. Gore, R. J. Goldberg, H. V. Barron, T. Breen, A. C. Rundle, M. A. Sloan, W. French, and W. J. Rogers. 1998. 'Risk for intracranial hemorrhage after tissue plasminogen activator treatment for acute myocardial infarction. Participants in the National Registry of Myocardial Infarction 2', *Ann Intern Med*, 129: 597-604.

Hofstra, J. J., A. D. Cornet, P. J. Declerck, B. Dixon, H. Aslami, A. P. Vlaar, J. J. Roelofs, T. van der Poll, M. Levi, and M. J. Schultz. 2013. 'Nebulized fibrinolytic agents improve pulmonary fibrinolysis but not inflammation in rat models of direct and indirect acute lung injury', *PLoS One*, 8: e55262.

Huang, C., Y. Wang, X. Li, L. Ren, J. Zhao, Y. Hu, L. Zhang, G. Fan, J. Xu, X. Gu, Z. Cheng, T. Yu, J. Xia, Y. Wei, W. Wu, X. Xie, W. Yin, H. Li, M. Liu, Y. Xiao, H. Gao, L. Guo, J. Xie, G. Wang, R. Jiang, Z. Gao, Q. Jin, J. Wang, and B. Cao. 2020. 'Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China', *Lancet*, 395: 497-506.

Ichikawa, A., K. Kuba, M. Morita, S. Chida, H. Tezuka, H. Hara, T. Sasaki, T. Ohteki, V. M. Ranieri, C. C. dos Santos, Y. Kawaoka, S. Akira, A. D. Luster, B. Lu, J. M. Penninger, S. Uhlig, A. S. Slutsky, and Y. Imai. 2013. 'CXCL10-CXCR3 enhances the development of neutrophil-mediated fulminant lung injury of viral and nonviral origin', *Am J Respir Crit Care Med*, 187: 65-77.

Idell, S., K. K. James, and J. J. Coalson. 1992. 'Fibrinolytic activity in bronchoalveolar lavage of baboons with diffuse alveolar damage: trends in two forms of lung injury', *Crit Care Med*, 20: 1431-40.

Klok, F. A., M. J. H. A. Kruip, N. J. M. van der Meer, M. S. Arbous, D. A. M. P. J. Gommers, K. M. Kant, F. H. J. Kaptein, J. van Paassen, M. A. M. Stals, M. V. Huisman, and H. Endeman. 2020. 'Incidence of thrombotic complications in critically ill ICU patients with COVID-19', *Thromb Res*.

Lackowski, N. P., J. E. Pitzer, M. Tobias, Z. Van Rheen, R. Nayar, M. Mosharaff, and K. A. Stringer. 2010. 'Safety of prolonged, repeated administration of a pulmonary formulation of tissue plasminogen activator in mice', *Pulm Pharmacol Ther*, 23: 107-14.

Lee, K., A. Istl, L. Dubois, G. DeRose, T. L. Forbes, D. Wiseman, A. Mujoomdar, S. Kribs, and A. H. Power. 2015. 'Fibrinogen Level and Bleeding Risk During Catheter-Directed Thrombolysis Using Tissue Plasminogen Activator', *Vasc Endovascular Surg*, 49: 175-9.

Liu, C., Y. Ma, Z. Su, R. Zhao, X. Zhao, H. G. Nie, P. Xu, L. Zhu, M. Zhang, X. Li, X. Zhang, M. A. Matthay, and H. L. Ji. 2018. 'Meta-Analysis of Preclinical Studies of Fibrinolytic Therapy for Acute Lung Injury', *Front Immunol*, 9: 1898.

Longstaff, C., C. Thelwell, S. C. Williams, M. M. Silva, L. Szabo, and K. Kolev. 2011. 'The interplay between tissue plasminogen activator domains and fibrin structures in the regulation of fibrinolysis: kinetic and microscopic studies', *Blood*, 117: 661-8.

Miller, Daniel J., Jennifer R. Simpson, and Brian Silver. 2011. 'Safety of thrombolysis in acute ischemic stroke: a review of complications, risk factors, and newer technologies', *The Neurohospitalist*, 1: 138-47.

Narasaraju, T., and A. Harshini. 2016. 'Neutrophils as Possible Therapeutic Targets in Severe Influenza Pneumonia', *J Infect Pulm Dis*, 2.

Rancourt, Raymond C., Aftab Ahmad, Livia A. Veress, Jacqueline S. Rioux, Rhonda B. Garlick, and Carl W. White. 2014. 'Antifibrinolytic mechanisms in acute airway injury after sulfur mustard analog inhalation', *American journal of respiratory cell and molecular biology*, 51: 559-67.

Rehberg, S., Y. Yamamoto, L. E. Sousse, C. Jonkam, R. A. Cox, D. S. Prough, and P. Enkhbaatar. 2014. 'Advantages and pitfalls of combining intravenous antithrombin with nebulized heparin and tissue plasminogen activator in acute respiratory distress syndrome', *J Trauma Acute Care Surg*, 76: 126-33.

Schulman, S., and C. Kearon. 2005. 'Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients', *J Thromb Haemost*, 3: 692-4.

Sheehan, John J., and Stella E. Tsirka. 2005. 'Fibrin-modifying serine proteases thrombin, tPA, and plasmin in ischemic stroke: A review', *Glia*, 50: 340-50.

Siemieniuk, Reed, Bram Rochwerg, Thomas Agoritsas, François Lamontagne, Yee-Sin Leo, Helen Macdonald, Arnav Agarwal, Linan Zeng, Lyubov Lytvyn, John Adabie Appiah, Wagdy Amin, Yaseen Arabi, Lucille Blumberg, Erlina Burhan, Frederique Jacquerioz Bausch, Carolyn S Calfee, Bin Cao, Maurizio Cecconi, Duncan Chanda, Graham Cooke, Bin Du, Jake Dunning, Heike Geduld, Patrick Gee, Madiha Hashimi, David S Hui, Sushil Kabra, Seema Kanda, Leticia Kawano-Dourado, Yae-Jean Kim, Niranjan Kissoon, Arthur Kwizera, Jon Henrik Laake, Flavia R Machado, Imelda Mahaka, Hela Manai, Greta Mino, Emmanuel Nsutebu, Natalia Pshenichnaya, Nida Qadir, Saniya Sabzwari, Rohit Sarin, Michael Sharland, Yinzhong Shen, Shalini Sri Ranganathan, Joao Souza, Sebastian Ugarte, Sridhar Venkatapuram, Vu Quoc Dat, Dubula Vuyiseka, Ananda Wijewickrama, Brittany Maguire, Dena Zeraatkar, Jessica Bartoszko, Long Ge, Romina Brignardello-Petersen, Andrew Owen, Gordon Guyatt, Janet Diaz, Michael Jacobs, and Per Olav Vandvik. 2020. 'A living WHO guideline on drugs for covid-19', *BMJ*, 370: m3379.

Tang, N., D. Li, X. Wang, and Z. Sun. 2020. 'Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia', *J Thromb Haemost*.

Tang, Ning, Huan Bai, Xing Chen, Jiale Gong, Dengju Li, and Ziyong Sun. 'Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy', *Journal of Thrombosis and Haemostasis*, n/a.

Varjú, I., C. Longstaff, L. Szabó, Z. Farkas Á, V. J. Varga-Szabó, A. Tanka-Salamon, R. Machovich, and K. Kolev. 2015. 'DNA, histones and neutrophil extracellular traps exert anti-fibrinolytic effects in a plasma environment', *Thromb Haemost*, 113: 1289-98.

Vedantham, S., S. Z. Goldhaber, J. A. Julian, S. R. Kahn, M. R. Jaff, D. J. Cohen, E. Magnuson, M. K. Razavi, A. J. Comerota, H. L. Gornik, T. P. Murphy, L. Lewis, J. R. Duncan, P. Nieters, M. C. Derfler, M. Filion, C. S. Gu, S. Kee, J. Schneider, N. Saad, M. Blinder, S. Moll, D. Sacks, J. Lin, J. Rundback, M. Garcia, R. Razdan, E. VanderWoude, V. Marques, and C. Kearon. 2017. 'Pharmacomechanical Catheter-Directed Thrombolysis for Deep-Vein Thrombosis', *N Engl J Med*, 377: 2240-52.

Veress, L. A., D. R. Anderson, T. B. Hendry-Hofer, P. R. Houin, J. S. Rioux, R. B. Garlick, J. E. Loader, D. C. Paradiso, R. W. Smith, R. C. Rancourt, W. W. Holmes, and C. W. White. 2015. 'Airway tissue plasminogen activator prevents acute mortality due to lethal sulfur mustard inhalation', *Toxicol Sci*, 143: 178-84.

Veress, Livia A., Tara B. Hendry-Hofer, Joan E. Loader, Jacqueline S. Rioux, Rhonda B. Garlick, and Carl W. White. 2013. 'Tissue plasminogen activator prevents mortality from sulfur mustard analog-induced airway obstruction', *American journal of respiratory cell and molecular biology*, 48: 439-47.

Weisel, John, and Rustem Litvinov. 2014. 'Mechanisms of Fibrinolysis and Basic Principles of Management.' in.

Xu, Z., L. Shi, Y. Wang, J. Zhang, L. Huang, C. Zhang, S. Liu, P. Zhao, H. Liu, L. Zhu, Y. Tai, C. Bai, T. Gao, J. Song, P. Xia, J. Dong, J. Zhao, and F. S. Wang. 2020. 'Pathological findings of COVID-19 associated with acute respiratory distress syndrome', *Lancet Respir Med*.

Yang, X., Y. Yu, J. Xu, H. Shu, J. Xia, H. Liu, Y. Wu, L. Zhang, Z. Yu, M. Fang, T. Yu, Y. Wang, S. Pan, X. Zou, S. Yuan, and Y. Shang. 2020. '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*.

20 Appendix 1 - Schedule of assessments

	Screening/ 3 day baseline period[a]	During treatment until 5 days after last dose				From 6 days after last dose until day 28/discharge/death	Day 28/discharge/death
		Every 4 hours	Every 6 hours	Once Daily	24 and 48 hours, then twice weekly	Once daily	
Informed consent	X						
Demographics	X						
Review of medical history (including inpatient history and COVID-19 diagnosis)	X						
Eligibility review	X						

Pregnancy test (blood or urine) for female patients of childbearing potential	X						
Review of concomitant medication	X[f]			X		X - Weekly	X
Review of adverse events/adverse reactions (including bleeding episodes)				X		X - Weekly	X
Directed physical examination including vital signs (heart rate, temperature, blood pressure and body weight)	X			X (cohort 1)	X – weekly only (cohort 2)	X (cohort 1 daily) X (cohort 2 weekly)	X
Documentation of respiratory status[a]	X	X				X - weekly)	X
Local blood testing[b]	X			X (cohort 1 daily)	X (cohort 2) every other day	as required	X
Research blood sampling[c]	X				X	X – weekly	
Administration of rtPA[d]			X				

Clinical status as per 7-point ordinal scale	X			X			X
Sequential Organ Failure Assessment (SOFA) score	X			X			
Glasgow Coma scale	X			X			
End of study data collection [e]							X

[a] Cohort 1 baseline period is 3 days, Cohort 2 baseline is screening assessments only. Respiratory status of patients is monitored routinely hourly, but data points will be extracted for every four hours where available until 5 days after the last dose (day 7 for SOC patients) for cohort 1, but one data point per day is required as a minimum. For cohort 2 data will be extracted once a day coinciding with the worst PF ratio. As PF ratio is the primary endpoint, in addition to the worst PF ratio over the preceding 24 hrs up to a maximum of 6 data points spread across 24 hrs that represents patients clinical status will be extracted. Similar to cohort 1 respiratory data will be collected until 5 days after the last dose. After this period, respiratory status is to be documented weekly (+/- 1 day) until discharge, death, or 28 days whichever occurs first. See section 7.5 for variables required.

[b] Local blood testing as per section 7.6.2 are to be done at a minimum once a day for cohort 1, or every other day for cohort 2, until 5 days after the last dose, and then as required until day 28, discharge or death whichever occurs first.

[c] Research blood sampling will be done once at baseline at 48 hours (+/- 24 hours), 96 hours (+/- 24 hours), and then twice weekly as per section 7.6.2. Twice weekly testing should be evenly spaced across weekdays if possible.

[d] Dosing schedule as per section 7.3

[e] End of study data will be collected at day 28, discharge or death whichever occurs first as per section 7.5.

[f] to be taken where available once daily for 3 days prior to dosing (cohort 1 only). Screening assessments can be used as part of this baseline period.

21 Appendix 2 - Lookup table for imputed PaO2 for a given SpO2 based on non-linear equation (from Brown SM et al. Nonlinear Imputation of PaO2/FIO2 From SpO2/FIO2 Among Mechanically Ventilated Patients in the ICU: A Prospective, Observational Study. Crit Care Med. 2017; Aug;45(8):1317-1324)

Measured SpO_2 (%)	Imputed PaO_2 (mmHg)
100*	167*§
99*	132*
98*	104*
97*	91*
96	82
95	76
94	71
93	67
92	64
91	61
90	59
89	57
88	55
87	53
86	51
85	50

Measured SpO_2 (%)	Imputed PaO_2 (mmHg)
84	49
83	47
82	46
81	45
80	44
79	43
78	42
77	42
76	41
75	40
74	39
73	39
72	38
71	37
70	37

*Generally considered unreliable on the basis of the sigmoidal shape of the haemoglobin-oxygen dissociation curve

§Based on SpO2 99.5%.

Imputed values calculated using the following equation:

$$PO_2 = \left\{ \frac{11{,}700}{\left(1/S - 1\right)} + \left[50^3 + \left(\frac{11{,}700}{1/S - 1} \right)^2 \right]^{1/2} \right\}^{1/3}$$

22 Appendix 3 – Oxygen flow rate calculation (used for calculating FiO2 in patients receive non-invasive oxygen support

Oxygen delivery devices				
Device			**Flow rate**	**% Oxygen delivered (approx..)**
Nasal Cannula			1 L/min	24%
			2 L/min	28%
			4 L/min	36%
Venturi Valve & Mask		**Blue**	2-4 L/min	24%
		White	4-6 L/min	28%
		Yellow	8-10 L/min	35%
		Red	10-12 L/min	40%
		Green	12-15 L/min	60%
Non-rebreather Mask			15 L/min	85%