Full title of trial	A single-site, randomised, controlled, parallel design, open-label investigation of an approved nebulised recombinant human DNase enzyme (dornase alfa) to reduce hyperinflammation in hospitalised participants with COVID-19 (The COVASE trial)
Short title	Dornase alfa in COVID-19
Version and date of protocol	V2.0, 25 April 2020
Sponsor:	University College London (UCL)
Sponsor protocol number	132333 NCT 04359654
Funder (s):	
EudraCT no	2020-001937-11
ISRCTN / Clinicaltrials.gov no:	[Insert ISRCTN or Clinicaltrials.gov reference no]
Active treatment	Dornase alfa (Pulmozyme: recombinant human DNAse I)
PLACEBO IMP(s):	None
Phase of trial	Phase IIa
Sites(s)	UCLH

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Protocol Version History

Version Number	Date	Protocol Update Finalised By (insert name of person):	Reasons for Update
2.0	25 April 2020		Response to REC/MHRA

1 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

Professor Joanna Porter

UCL Respiratory

Signature Date

Sponsor

Dr Nick McNally
UCL

Signature Date

1.1 Contents

1.2 List of abbreviations

AE	Adverse Event
AR	Adverse Reaction
ARDS	Acute respiratory distress syndrome
BAC	Best Available Care
BID	Twice per day
CA	Competent Authority
CF	Cystic Fibrosis
cfDNA	Cell-free DNA
CI	Chief Investigator
Conmeds	Concomitant medications
CoV	Coronavirus
CRF	Case Report Form
CRO	Contract Research Organisation
CRP	C-reactive protein
CT	Computer tomography
CTA	Clinical Trial Authorisation
CTIMP	Clinical Trial of Investigational Medicinal Product
DI	Designated Individual
DMC	Data Monitoring Committee
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
DSUR	Development Safety Update Report
EC	European Commission
EMA	European Medicines Agency
EU	European Union
EUCTD	European Clinical Trials Directive
EudraCT	European Clinical Trials Database
EudraVigilance	European database for Pharmacovigilance
FDA	Federal drug administration
FVC	Forced vital capacity
GAfREC	Governance Arrangements for NHS Research Ethics

GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
H3	Histone 3
HTA	Human Tissue Authority
ICF	Informed Consent Form
ICU	Intensive care unit
IL-1 β	Interleukin -1 beta
IL-6	Interleukin-6
IL-8	Interleukin-8
ISARIC	International Severe Acute Respiratory and Emerging Infection Consortium
ISF	Investigator Site File
ISRCTN	International Standard Randomised
MA	Marketing Authorisation
MHRA	Medicines and Healthcare products Regulatory Agency
MPO	Myeloperoxidase
MS	Member State
MV	Mechanical Ventilation
NIHR	National Institute for health research
NHS R&D	National Health Service Research & Development
NETs	Neutrophil extracellular traps
NOCRI	NIHR Office for Clinical Research Infrastructure
PCR	Polymerase chain reaction
PCT	ProCalcitonin
PD	Pharmacodynamics
PI	Principal Investigator
PO	Purchase order
PIS	Participant Information Sheet
PL	Product License
QD	Once per day
QA	Quality Assurance
QC	Quality Control

RECOVERY	Randomised evaluation of COVID-19 therapy
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
RSI	Reference Safety Information
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SAE	Serious Adverse Event
SDV	Source Document Verification
SOFA	Sepsis-related Organ Failure Assessment
SOP	Standard Operating Procedure
SMPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TNFα	Tumour necrosis factor alpha
TRC	Translational Research Collaboration
UCL	University College London
UCLH	University College London hospital
WHO	World health organisation

1.3 Trial personnel

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

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2 Summary

The clinical spectrum of SARS-CoV-2 infection (COVID-19) appears to be wide, encompassing asymptomatic infection, mild upper respiratory tract illness (majority of cases) and severe viral pneumonia with respiratory failure and even death in the minority of subjects. In severe viral pneumonia excessive and inappropriate activation of neutrophils can result in the formation of neutrophil extracellular traps (NETs) which exacerbate the clinical course of the pneumonia. These NETs consist of DNA, histones and other components of neutrophils (e.g. myeloperoxidase (MPO)). These NETs are found in the lungs and in the circulation and contribute to organ damage. A treatment that reduces NET formation is likely to reduce the exuberant inflammatory response and thereby improve the clinical course of viral pneumonia and save lives.

NETs have been shown to drive disease in influenza pneumonia as well as in subjects with cystic fibrosis. In addition, their role has been explored in various pre-clinical models of viral infection (mouse and bovine) and shown that reduction of the NETs improves symptoms and increases survival. High neutrophil infiltration is prominent in the lungs of COVID-19 patients and evidence of NET components in the circulation and lung biopsies has been reported in clinical study of COVID-19 patients.

Dornase alfa is a recombinant human DNase I that has been approved since 1994 for the treatment of cystic fibrosis (CF). It is delivered directly to the lungs by nebulisation and has been shown to:

- reduce NETs and inflammation
- reduce the relative risk of developing a respiratory tract infection
- improve pulmonary function in both chronic and acute exacerbation of inflammatory CF

Dornase alfa is safe and well-tolerated in children and adults with CF at doses ranging from 2.5mg QD up to a maximum of 10mg BID.

This study proposes to treat hospitalised subjects with COVID-19 by administration of 2.5mg Dornase alfa BID for 7 days. The effect on NETs, inflammation and clinical course will be closely monitored. We expect to see a reduction in circulating NETS and inflammatory biomarkers that will result in clinical benefit. Historic controls will be derived from an existing database of 120 subjects with COVID-19 that have been admitted to UCL since the beginning of the outbreak.

It is worth noting that dornase alfa can be self-administered at home. Therefore, dornase alfa has the potential to provide benefit in subjects with COVID-19 who have mild disease and are self-isolating and in those discharged from hospital to recuperate at home.

Objectives:	**Primary objective**: to assess the effect of nebulised dornase alfa on C-reactive Protein (CRP) in hospitalised participants with COVID-19. **Secondary objective**: to assess the effect of nebulised dornase alfa on clinical responses in hospitalised participants with COVID-19. **Exploratory objective**: to assess the effect of nebulised dornase alfa on inflammation, biomarkers of NETs, coagulation, complement activation and haemolysis in hospitalised participants with COVID-19.
Type of trial:	A single-site, randomised, controlled, parallel design, open-label trial of an approved nebulised recombinant human DNase enzyme (dornase alfa) to reduce hyperinflammation in hospitalised participants with COVID-19 (The COVASE Trial).
Trial design and methods:	An open-label, randomised, Best-Available-Care (BAC) and historic-controlled trial of nebulised dornase alfa [2.5 mg BID] for 7 days in participants with COVID-19 who are admitted to hospital and are at risk of ventilatory failure (the COVASE study). Controls will include a randomised arm to receive BAC, historic data from UCLH patients with COVID-19 and biobanked samples will be used to demonstrate an effect of dornase alfa. CRP will be measured to assess the effect of dornase alfa on inflammation. Clinical endpoints and biomarkers (e.g. d-dimer) will be used to assess the clinical response. Exploratory endpoints will explore the effects of dornase alfa on features of neutrophil extracellular traps (NETs).
Trial duration per participant:	Six weeks from consent to last trial assessment.
Estimated total trial duration:	Four - five months from when first participant enrolled to last participant follow-up.
Planned trial sites:	Single site
Total number of participants planned:	40 participants will be enrolled. A sample size re-estimation is planned to occur approximately one third of the way through the study (e.g. when 12 participants have been randomised). At this point, the decision may be made to randomise additional participants if the variability of the primary endpoint is higher than anticipated.
Main inclusion/exclusion criteria:	Participants who are hospitalised for COVID-19 will be recruited into the study.

Inclusion criteria:

1. Male and female participants, aged ≥ 18 years.
2. Participants who are hospitalised for suspected Coronavirus (SARS-CoV)-2 infection confirmed by polymerase chain reaction (PCR) test or radiological confirmation with chest CT scan
3. Participants with stable oxygen saturation (>=94%) on supplementary oxygen
4. CRP >= 30 mg/L.
5. Participants will have given their written informed consent to participate in the study and are able to comply with instructions and nebuliser.

Exclusion criteria

1. Females who are pregnant, planning pregnancy or breastfeeding.
2. Concurrent and/or recent involvement in other research or use of another experimental investigational medicinal product that is likely to interfere with the study medication within the last 3 months before study enrolment.
3. Serious condition meeting one of the following:
    I. respiratory distress with respiratory rate >=40 breaths/min
    II. oxygen saturation <=93% on high-flow oxygen
4. Require mechanical invasive or non-invasive ventilation at screening
5. Concurrent severe respiratory disease such as asthma, COPD and/or ILD.
6. Any major disorder that in the opinion of the Investigator would interfere with the evaluation of the results or constitute a health risk for the study participant.
7. Terminal disease and life expectancy <12 months without COVID-19.
8. Known allergies to the dornase alfa and excipients.
9. Participants who are unable to inhale or exhale orally throughout the entire nebulisation period.

Statistical methodology and analysis:

Full details of the planned statistical analysis will be presented in the Statistical Analysis Plan (SAP). All baseline data, demographics, endpoints, safety and tolerability will be summarised overall and by treatment group and by day. In general, continuous data will be summarised using the mean (standard deviation), median (1st and 3rd quartiles), minimum

and maximum, and categorical data will be represented as frequency counts (percentages).

For analyses relating to the primary objective, group comparisons will be performed using a repeated measures mixed model, adjusted for baseline factors and with treatment as the main effect. Prior to analysis, all endpoints will be assessed for conformance to normality assumptions and the appropriate transformation will be conducted if necessary. Some exploratory endpoints may only be available in the active treatment group. In this case, a within group analysis will be conducted to compare baseline and post-baseline measurements.

An interim analysis will be conducted when 12 participants have been randomised. The results of the interim analysis will be used to re-estimate the sample size if necessary.

3 Background and rationale

Clinical background

COVID-19 is a heterogeneous disease caused by infection with SARS-CoV-2 and although the majority of patients (80%) have mild disease, 15% will require oxygen and of these 25% will require ICU, of which 47 – 71% require ventilatory support. Risk factors for severe disease include older age, male sex, obesity and comorbid disease. There are no specific cures for COVID-19 and current care is supportive. A key challenge is to intercept patients early in the course of their disease to prevent deterioration and reduce the numbers that need ventilatory support, a life-saving treatment that is currently only available for a minority of patients.

SARS-CoV-2 is able to directly infect nasal, bronchiolar and alveolar epithelial cells resulting in lung inflammation, characterised, in severe cases, by an over-exuberant inflammatory response, shortness of breath and hypoxaemia. Once SARS-CoV-2 infection progresses to the stage of pneumonia, key pathogenic drivers result in symptoms characteristic of the acute respiratory distress syndrome (ARDS) with a high mortality.

In patients with confirmed COVID-19 pneumonia, 50% developed dyspnoea at 8 days after illness onset (range: 5–13 days). Mortality of these patients is around 4-15%, as a result of ARDS, coagulation dysfunction, and secondary infection that may result in septic shock and multi-organ failure (Zhou et al., Lancet 2020). Cytokines are thought to be key drivers of these pathological events and in particular levels of IL-6 are found to be raised in COVID-19 patients and to correlate with mortality (Gong et al., medRχiv 2020). Anti-IL-6 is seen as a potential therapy in COVID-19 following favourable reports from a clinical study in China.

There is an urgent need to intervene and reduce the inflammatory response of patients with COVID-19 early in the course of their disease and so prevent progression to ICU.

Scientific background

Our hypothesis is that in the COVID-19 lung, the release of neutrophil extracellular traps (NETs) by neutrophils promotes lung damage and the induction of pathogenic pro-inflammatory cytokines, such as IL-6 and IL-1. This inflammatory cascade recruits additional neutrophils leading to a pathogenic feedback amplification loop. High neutrophil infiltration is prominent in the lungs of COVID-19 patients and evidence of NET components in the circulation and lung biopsies has been reported in clinical study of COVID-19 patients (Betsy J. Barnes et al., JEM 2020l; Zuo et al. medRxiv preprint doi:https://doi.org/10.1101/2020.04.09.20059626). Based on this evidence, blocking or clearing NETs to treat severe COVID-19 symptoms has now been proposed by an international consortium (Betsy J. Barnes et al., JEM 2020).

NETs are composed of a backbone of decondensed chromatin fibres coated with antimicrobial granular and cytoplasmic proteins, such as myeloperoxidase, neutrophil elastase (NE) and α- defensins. Double-stranded DNA is a major component of NETs, which prevents their degradation. This extracellular DNA is normally broken down by endogenous deoxyribonucleases (DNases) but in severe inflammation these DNases may become overwhelmed by a massive release of NETs and are unable to completely degrade NETs. Our recent work suggest incomplete NET degradation would lead to amplification rather than reduction of inflammation.

While sensing of microbes and viruses promotes inflammation, the release of endogenous host molecules during infection, known as damage-associated molecular patterns (DAMPs), can amplify cytokine induction. Work in Dr Venizelos Papayannopoulos' lab at the Francis Crick institute has identified specific DAMPs that are critical for the induction of pathogenic hyperinflammation during infection. Free circulating histones are key pathogenic factors in microbial sepsis. We recently found that extracellular chromatin acts as a potent pro-inflammatory signal, allowing extracellular chromatin structures called neutrophil extracellular traps (NETs) to induce IL-1β and IL-6, via Toll-like receptor 4 (TLR4) (Tsourouktsoglou et al. *in press*).

Consistently, NET-mediated pathology causes death in murine models of severe pulmonary *Influenza* infection (Pillai et al., Science 2016) and the presence of NETs correlates with flu severity in humans (Zhu et al., J. Infect. Dis. 2018). DNase treatment significantly delayed mortality in severe flu in immune-compromised mice (Pillai et al., Science 2016) and cleared NETs and lowered airway obstruction in severe bovine respiratory syncytial virus (RSV) infection (Cortjens et al., Thorax 2018). Finally, mice that are genetically pre-disposed to NET overproduction following pulmonary infection with a virulent fungal mutant strains can be rescued from lethality with NET formation inhibitors (Papayannopoulos, Nat. Rev. Immunol. 2018). NETs are major drivers of coagulation during sepsis, particularly in the absence of endogenous DNAses, and are regulated by the complement cascade (Jimenez-Alcazar et al., Science 2017).

Rationale for the trial

Dornase alfa is a recombinant human DNase enzyme indicated in conjunction with standard therapies for the management of cystic fibrosis (CF) to improve pulmonary function. Dornase alfa degrades extracellular DNA, and so promotes the clearance of NETs and lead to a significant improvement in lung function for treated CF patients by facilitating mucus clearance in the lung. Dornase alfa is approved worldwide as a nebulised formulation, with an excellent safety profile and is well tolerated. The most

common side effect is a hoarse voice. Moreover, dornase alfa could be administered in addition to effective antiviral therapy and should not interfere with antiviral drugs that could be used for COVID-19.

By facilitating the clearance of NETs, dornase alfa not only facilitates sputum clearance in CF patients, but has additional anti-inflammatory activity. Dornase alfa has been shown to reduce NETs in the bronchoalveolar lavage (BAL) and sputum of participants with CF (Konstan et al 2012). In the Bronchoalveolar Lavage for the Evaluation of Anti-inflammatory Treatment (BEAT) study, the percentage of neutrophils in bronchoalveolar lavage fluid significantly increased in untreated CF patients ($P<0.02$) while remaining constant in the dornase alfa-treated group. Levels of elastase and IL-8 also significantly increased from baseline in the untreated group ($P<0.007$ and $P<0.02$ for elastase and IL-8, respectively), but remained stable in patients receiving dornase alfa (Konstan and Ratjen, J. Cyst. Fibros. 2012).

There is scientific evidence to support the potential benefits of dornase alfa in COVID-19 infection. Viral sepsis driven by a hyperinflammation is thought to be a major cause of mortality in COVID-19 infection. Interleukin-1β (IL-1β), IL-6 and TNFα are key cytokines in microbial sepsis. Positive outcomes with Roche's Actemra (tocilizumab), an antibody that blocks the pro-inflammatory cytokine interleukin-6 (IL-6), in COVID-19 treatment has led to several anti-inflammatory trials.

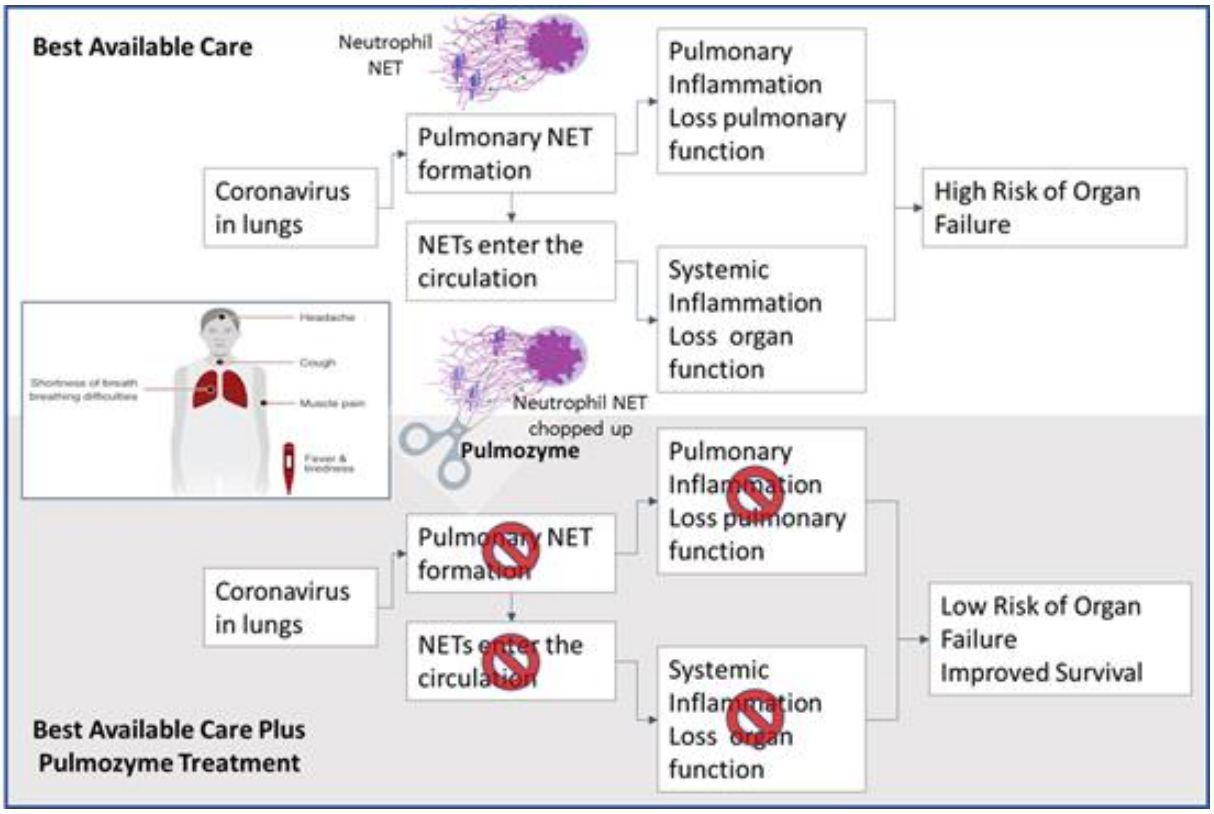
Our hypothesis is that nebulised dornase alfa will break down the DNA backbone of NETs in the COVID-19 lung which will promote the degradation of pro-inflammatory extracellular histones and prevent the amplification of the inflammatory response and the resultant lung damage.

Positive data will enable rapid testing into a large clinical trial in the UK and prevent ICU capacity issues faced today. Dornase alfa is a cost-effective drug and is currently available for prescription.

We propose to test this hypothesis with this COVASE Phase IIa trial. We propose that all people with COVID-19 who are admitted to hospital for supplementary oxygen, who showed evidence of systemic inflammation but did not immediately require intubation and ventilation, would be eligible for nebulised dornase alfa, a safe and cost-effective treatment, twice daily for 7 days.

The hypothesis to be tested is illustrated in Figure 1.

Figure 1: Schematic Representation of the Hypothesis to be tested in the COVASE trial



Potential Real-World evidence

It is theoretically possible that the CF population could provide Real-World evidence on the effect of dornase alfa on COVID-19. Some participants with CF may develop COVID-19 and may or may not be on dornase alfa. An epidemiological study could determine outcomes in the two groups (CF + COVID-19 not taking dornase alfa and CF + COVID-19 taking dornase alfa). However, currently, the numbers are too low. Considering participants with CF across London, there are a few adults with COVID-19, none of whom have severe disease or have been admitted to ITU. However, they are likely to have been 'shielding' since before the term was coined. So far, no paediatric cases have been reported. However, given that much of the infected paediatric age group elsewhere is asymptomatic or mild, they may not be effectively documented. Therefore, it is not possible at this stage to consider the effect of dornase alfa on the progression of COVID-19 in the setting of CF. All cases of COVID-19 infection in participants with CF will be captured through the national patient registry. This also identifies patients on dornase alfa, so retrospectively these cohorts can be assessed. Data will not be available in high enough numbers for this to be useful during the current pandemic.

Dornase alfa dosing rational

The dose to be administered in this trial is 2.5 mg dornase alfa BID (approved) administered with the eRapid nebuliser (or equivalent jet nebuliser connected to an air compressor with an adequate air flow and equipped with a mouthpiece as recommended for dornase alfa-see section 8.3 Table 1). This is twice the standard daily dose (2.5mg QD) and is recommended for older/refractory people with CF. It has been shown to be safe and well tolerated in children and adults with CF. Doses of 2.5mg QD have been

administered for years (since 1994) to thousands of people with CF. Higher doses up to 10mg BID have been used in short-term studies with a similar safety and tolerability profile. Dornase alfa has been shown to be safe and well-tolerated during acute exacerbations of CF. Dornase alfa is administered in addition to other treatments for CF (FDA label and EMA SmPC).

Thus, dornase alfa at 2.5 mg BID for seven days is expected to be safe and well-tolerated in hospitalised participants with COVID-19. It is well tolerated and has the potential to block the production of not one but several pro-inflammatory cytokines and acts enzymatically. Moreover, it has been shown not to interfere with anti-viral immune defence (Cortjens et al., Thorax 2018).

3.1 Assessment and management of risk

The table below summarises the risks, frequencies and mitigations of COVASE trial

Name of treatment	**Potential risk**	**Risk Frequency**	**Risk Management**
Recombinant human DNase 1 (dornase alfa) administered by nebulisation	voice alteration pharyngitis rash laryngitis chest pain conjunctivitis	≥3%	DMC configured to mitigate participant safety and data integrity risks
Recombinant human DNase 1 (dornase alfa) administered by nebulisation	rhinitis decrease in FVC of ≥10%* fever dyspnoea	less than 3%	DMC configured to mitigate participant safety and data integrity risks
Recombinant human DNase 1 (dornase alfa) administered by nebulisation	There is a low potential immunogenicity risk and antibodies to dornase alfa will not be measured in this study	Low (2-4%)	There have been no reports of anaphylaxis attributed to the administration of dornase alfa. Urticaria, mild to moderate, and mild skin rash have been observed and have been transient. Within all of the studies, a small percentage (average of 2-4%) of people treated with dornase alfa developed serum antibodies to dornase alfa. None of these people developed anaphylaxis, and the clinical significance of serum antibodies to dornase alfa is unknown.

	Failure to recruit patients	unlikely	Feasibility data suggests that sufficient participants can be recruited over the time specified. However, additional sites are available through the NOCRI respiratory TRP that represents 10 of the UK BRCs
	Delay in dornase alfa supply	unlikely	UCLH pharmacy to check and put a PO as soon as the grant is awarded and REC/MHRA approval received.
	Delay in eRapid supply in the UK	unlikely	UCLH pharmacy to check and put a PO as soon as the grant is awarded and MHRA/REC approval received.
	Competing clinical trials	unlikely	There are trials currently ongoing at UCLH that will compete. Trials at UCLH are prioritised and overseen by the COVID-19 committee to ensure equitable recruitment of hospitalised participants to all ongoing trials. Deliverability of trials is also under their management.
	Staff sickness due to COVID-19	unlikely	Additional nursing support is available to cover levels of 25-50% due to COVID-19 infection and time off work.

*Single measurement only, does not reflect overall FVC changes.

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

Intervention	**Potential risk**	**Risk Management**
Nebulisation of dornase alfa to participants with COVID-19	As dornase alfa is approved for the treatment of children and adults with CF, we will be using the drug 'off-label' for the first time in participants with COVID-19. There is a low risk that the safety and tolerability in this population may be different to that expected from CF	We will therefore use 'sentinel' dosing for the first three participants enrolled. This means that the three sentinel participants will each commence dosing and safely complete 2 days of treatment individually, before another participant is dosed. Thereafter (participant 4 and onwards) enrolment and dosing may occur in parallel.

Blood draws	Complications that can arise from venepuncture include haematoma formation, nerve damage, pain, haema-concentration, extravasation, iatrogenic anaemia, arterial puncture, petechiae, allergies, fear and phobia, infection, syncope and fainting, excessive bleeding, oedema and thrombus	Experienced hospital staff will be drawing the blood and will minimise these potential risks

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

4 Objectives and endpoints

Primary objective: to assess the effect of nebulised dornase alfa on the inflammatory/immune responses in hospitalised participants with COVID-19

- Primary endpoint:
  - Changes in acute phase reactant (C-Reactive Protein (CRP))

Secondary objective: to assess the effect of nebulised dornase alfa on clinical responses in hospitalised participants with COVID-19 compared to control group.

- Secondary endpoints:
  - Physical exam and vital signs
  - Whole blood count and differential count
  - Incidence of Mechanical Ventilation (MV)
  - Time on MV
  - ProCalcitonin (PCT)
  - D-dimer
  - Oxygen requirement (oxygen flow or oxygenation index)
  - Length of ICU stay [hours]
  - Length of stay in the hospital [days)
  - Incidence of multi-organ failure according to SOFA (Sepsis-related Organ Failure Assessment)
  - Incidence of Ventilator-Associated Pneumonia (VAP) or hospital acquired pneumonia
  - Acute physiology score + age points + chronic health points (APACHE score)
  - Ordinal score (WHO scoring tool)
  - Survival at Day35

Exploratory objective: to assess the effect of nebulised dornase alfa on inflammation, biomarkers of NETs, coagulation, complement activation and haemolysis in hospitalised participants with COVID-19

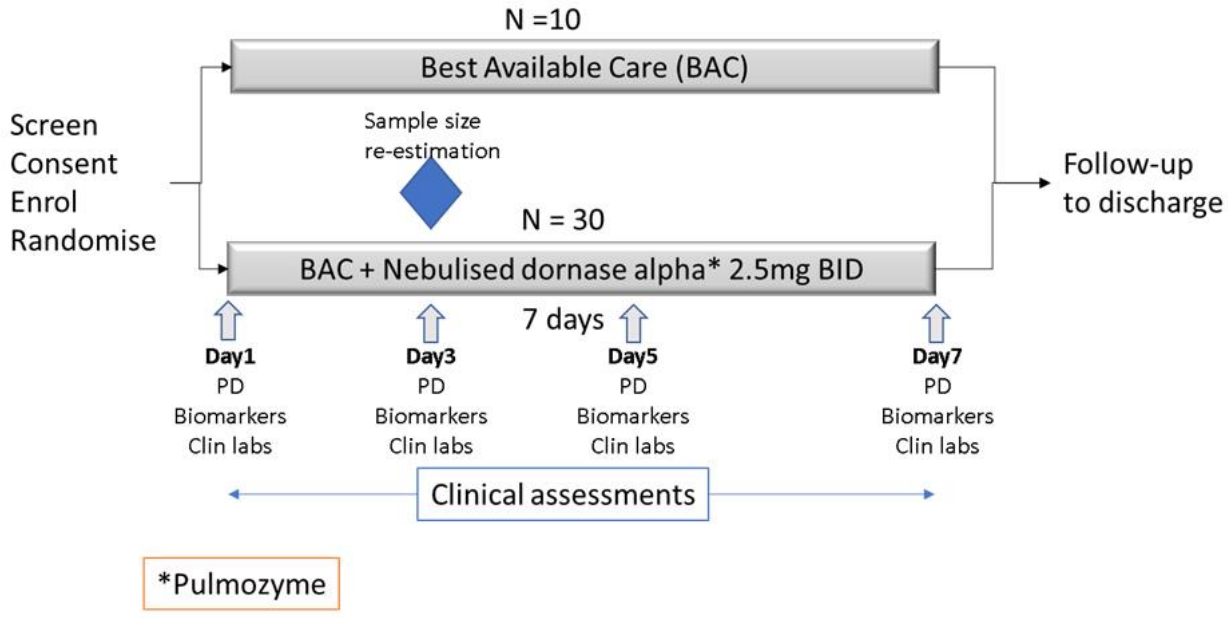
- Exploratory endpoints may be measured in the circulation (blood) and, when these are available, in bronchial secretions (spontaneous expectorant or routine bronchoscopy during MV). They may include, but are not limited to:
  - Circulating pro-inflammatory cytokines (e.g. IL-6, TNFα, IL-1β, IL-8)
  - Cell-free DNA (cfDNA)
  - Circulating histone
  - Citrullinated H3
  - NET Elisa assay
  - NET formation assay
  - Coagulation (e.g fibrin, tissue factor, Von Willebrand factor, thrombin, thromboxane A2)
  - Complement cascade (e.g C1q)
  - Haemolysis (e.g RBC lysis)
  - Expression profiling of white blood cells by RNA seq

5 Trial design

5.1 Overall design

A single-site, randomised, controlled, parallel design, open-label investigation of an approved nebulised recombinant human DNase enzyme (dornase alfa) to reduce hyperinflammation in hospitalised participants with COVID-19 (the COVASE Trial: Figure 2).

Figure 2: COVASE Trial Schematic



Participants will be screened, consented, enrolled and randomised up to 3 days after they are admitted to the hospital. They will be randomised in a 3:1 ratio to receive BAC + dornase alfa or

BAC alone. A total of 40 participants will be enrolled (30 to receive BAC plus dornase alfa and 10 to receive BAC). On Day1 to Day7 of the trial, participants randomised to the active arm, will receive 2.5mg BID nebulised dornase alfa in addition to BAC. On Day1, Day3, Day5 and Day7, blood samples will be drawn in both trial arms in order to test pharmacodynamic endpoints (PD), biomarkers and clin labs. Clinical assessments will be undertaken daily (as per UCLH clinical guidelines). Participants will be followed until discharge or death or a maximum of 28 days follow-up.

A sample size re-estimation is planned when 12 participants have been randomised. This analysis will ensure that the assumptions made in the sample size calculation remain valid. However, if the variability is higher than expected then up to an additional 10 participants will be enrolled and treated with dornase alfa (up to 50 participants in total).

CRP has been chosen as the Primary Endpoint because it is a clinically important marker of inflammation and is used to make clinical treatment decisions. In addition, it is induced by the over-exuberant inflammation mediated by the NETs and inflammatory histones. CRP is a prognostic marker and correlates with clinical symptoms and response to therapy. Thus, CRP is at the centre of the COVID-19 disease pathway: from NETS to CRP to clinical disease progression.

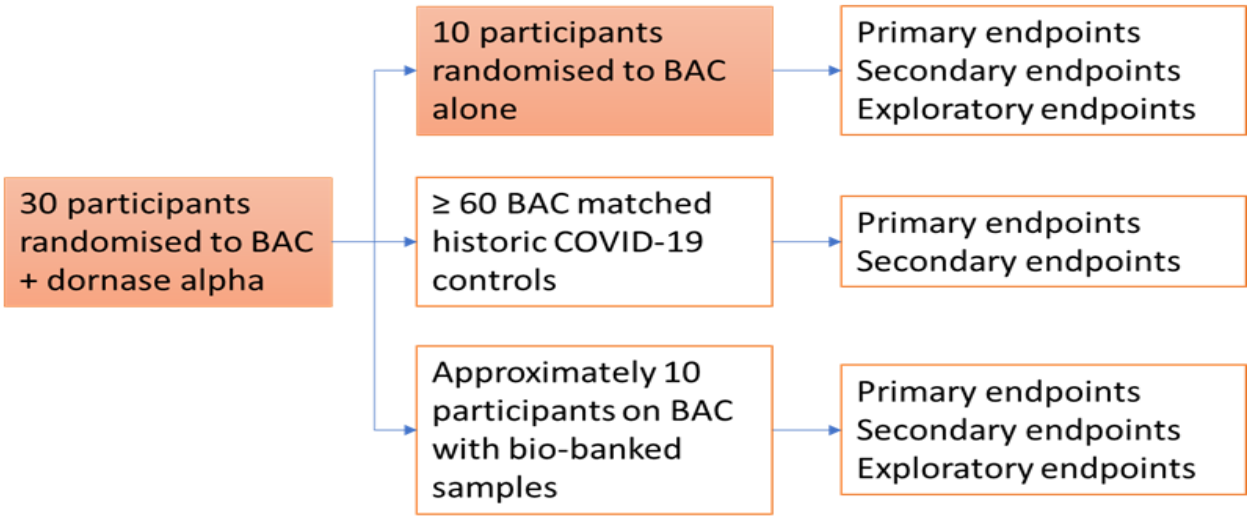
Based on clinical judgement, it may be decided to keep some participants on treatment for up to 14 days. In particular, if participants have significant benefits from therapy, but show relapses of the COVID-19 inflammatory state (rising CRP and increasing oxygen requirements in the absence of bacterial infection), on completing 7 days of treatment, then the medical team have the choice of reinstating dornase alfa treatment for up to 7 further days (14 days in total). Blood sampling, as specified, will continue until the last day of dosing with dornase alfa.

Study controls

Due to the evolving situation with hospitalised COVID-19 participants, the burden on the NHS and the availability of other COVID-19 trials, it is considered inappropriate to conduct a placebo-controlled study. Therefore, a randomised, controlled, open-label approach where dornase alfa is administered on top of BAC and compared to BAC alone has been adopted.

The data derived from the 10 participants who are randomised to the BAC arm of the study who do not receive dornase alfa will provide control data for all of the study endpoints.

Figure 3 Comparator Data.



The 30 participants randomized to BAC + dornase alfa will be compared to 10 randomised controls from the COVASE study. In addition, 10 participants in other clinical trials on BAC (bio-banked samples) and 60 participants from a historic database of the first 120 people with COVID-19 treated at UCLH will also be used as comparator data.

However, to enhance the control group, a hybrid approach will be applied such that additional data will be combined with the randomised controls, illustrated in Figure 3. The sample size calculation indicates that 90 evaluable participants are required (60 control:30 active). These control data may include: data from the randomised BAC arm, historic COVID-19 UCLH database, biobanked samples from other ongoing trials and observational trials in COVID-19.

This hybrid strategy allows two things:

- provides comparators for all of the study objectives (including limited data for the exploratory objectives)
- demonstrates that the historic controls are representative/similar to the study population

Comparator data from UCLH is available as historic controls for the Primary and Secondary Endpoints. A database of the first 120 participants with COVID-19 admitted to UCLH is available and being analysed currently. CRP is routinely measured daily (or on alternate days) in all participants admitted to UCLH and will be used to control for the Primary Endpoint. All of the Secondary Endpoints are also routinely measured and will be available in the database. Participants in the database will be selected to act as controls as follows:

1. Apply the inclusion and exclusion criteria of the COVASE study
2. Additional selection to identify closest matches using a propensity score based on age, gender, BMI, baseline CRP, oxygen requirements and the number of comorbidities.

At least 60 control participants are expected to be available to act as controls.

Other ongoing and planned trials may be a source of cytokine data as well as biobanked samples that could be used to provide control information on the exploratory endpoints (e.g. ISARIC and RECOVER Trials) assuming suitable consent is available.

6 Off-label use of an Approved Medicinal Product (Pulmozyme)

6.1 Recombinant Human DNase (dornase alfa)

Genentech (a wholly owned subsidiary of Roche) and Chugai Pharmaceutical developed and launched an inhalation solution (Pulmozyme) of dornase alfa, a highly purified recombinant human deoxyribonuclease (dornase alfa), for daily administration in conjunction with standard therapies. The product is indicated for the management of people with cystic fibrosis (CF) to improve pulmonary function. Dornase alfa is safe and well tolerated in adults and children.

Dornase alfa was launched in the US for the management of mild-to-moderate CF in conjunction with standard therapies in January 1994; in December 1996, the FDA expanded approval for use in CF participants with advanced CF. In April 1994, the drug was launched in the UK and Ireland, and by October 1994, it had been launched in France and Germany. In June 2012, dornase alfa was launched in Japan for the improvement of pulmonary function in participants with CF.

In December 2014, the FDA approved the eRapid Nebuliser System from PARI to deliver dornase alfa and to reduce treatment times (three minutes to deliver 2.5mg).

The recommended dosage is one 2.5 mg single-use ampule inhaled once daily using a recommended nebuliser jet nebuliser/compressor system or eRapid™ Nebuliser System. Some participants (older/refractory) benefit from twice daily administration. (FDA label and EMA SmPC).

The most common adverse reactions (occurring in ≥ 3% of participants treated with dornase alfa over placebo) seen in clinical trials in CF participants were: voice alteration, pharyngitis, rash, laryngitis, chest pain, conjunctivitis, rhinitis, decrease in FVC of ≥10%, fever, and dyspnoea.

6.2 Source of dornase alfa, manufacture and distribution

Dornase alfa inhalation solution is a sterile, clear, colourless solution supplied in 30 unit cartons containing 5 foil pouches of 6 single-use ampules. Each 2.5 mL ampule contains 2.5mg of dornase alfa (1 mg/mL): NDC 50242-100-40.

Dornase alfa will be prescribed by the CI (or designee) and dispensed to patients via hospital stock. Alternatively, dornase alfa will be supplied directly from the manufacturer (Roche) as required. Handling and management of dornase alfa will be subject to standard procedures of the pharmacy. The dornase alfa will not be modified in any way, but administered as approved.

6.3 Storage and handling of dornase alfa

Dornase alfa is stored under refrigeration (2°C to 8°C/36°F to 46°F) in their protective foil to protect from light. Dornase alfa should not be used beyond the expiration date stamped on the ampule. Unused ampules must be stored in their protective foil pouch under refrigeration. Dornase alfa must be refrigerated during transport and not exposed to room temperatures for a total time of 24 hours.

6.4 Accountability of dornase alfa

The Drug Accountability Log must be completed to record each dose of dornase alfa 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 ampules maybe returned to site pharmacy, to be then updated in the drug accountability log in the pharmacy site file. Following authorisation by the sponsor, drug destruction will be conducted in accordance to local practice, and this will be documented in the drug destruction log in the hospital pharmacy file.

Detailed instructions are contained in the summary of drug arrangements.

6.5 Concomitant medication

Participants in the COVASE trial will continue to receive best available care (BAC) per UCLH guidelines. Dornase alfa will be administered in addition to BAC.

BAC currently consists of symptomatic relief: antipyretics, analgesics and intravenous fluids if needed. In addition, patients may need supplemental oxygen and/or mechanical ventilation.

There is no basis to support a drug-drug interaction risk, as this is a recombinant human protein that is administered directly to the lungs. Systemic exposure is very low and dornase alfa is cleared by proteinases present in the lungs.

There is a potential risk that other medications administered as BAC may affect the endpoints in the study e.g. decrease CRP. However, this cannot be avoided and will be considered in the analysis plan for the data. Furthermore, a sample-size re-estimation is planned in order to take this potential source of unexpected variability into account.

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

6.6 Post-trial IMP arrangements

No specific arrangements required.

7 Selection of participants

People who are at high probability of COVID-19 and admitted to hospital will be identified from inpatient lists and approached, pending COVID PCR results (12 hours). They will be given a patient information sheet (PIS) with details of the clinical trial to read before deciding on entry into the COVASE trial.

7.1 Eligibility of trial participants

7.1.1 Trial participant inclusion criteria

1. Male and female participants, aged ≥ 18 years

2. Participants who are hospitalised for suspected Coronavirus (SARS-CoV)-2 infection confirmed by polymerase chain reaction (PCR) test or radiological confirmation with chest CT
3. Participants with stable oxygen saturation (>=94%) on supplementary oxygen
4. CRP >= 30 mg/L
5. Participants will have given their written informed consent to participate in the study and are able to comply with instructions and nebuliser

7.1.3 Trial participant exclusion criteria

1. Females who are pregnant, planning pregnancy or breastfeeding
2. Concurrent and/or recent involvement in other research or use of another experimental investigational medicinal product that is likely to interfere with the study medication within (specify time period e.g. last 3 months) of study enrolment
3. Serious condition meeting one of the following:
    i. Respiratory distress with respiratory rate >=40 breaths/min
    ii. oxygen saturation<=93% on high-flow oxygen
4. Require mechanical invasive or non-invasive ventilation at screening
5. Concurrent severe respiratory disease such as asthma, COPD and/or ILD
6. Any major disorder that in the opinion of the Investigator would interfere with the evaluation of the results or constitute a health risk for the trial participant
7. Terminal disease and life expectancy <12 months without COVID-19
8. Known allergies to dornase alfa and excipients
9. Participants who are unable to inhale or exhale orally throughout the entire nebulisation period

7.2 Recruitment

Participants will be recruited from inpatients at UCLH.

Participant recruitment will only commence when the trial has been issued with the 'Open to Recruitment' letter by the Sponsor.

Recruitment rate is estimated to range from 3 to 6 participants per week. Recruitment is estimated to take 12-15 weeks.

7.3 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 before taking part. Due to the rapidly escalating situation, consent will be sought after giving the participant adequate time

to consider their decision after being given the study documentation. Patients will be given additional time up to 18 hours if needed. It must be recorded in the medical notes when the participant information sheet (PIS) has been given to the participant.

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.

No clinical trial procedures will be conducted prior to the participant 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.

8 Trial procedures

8.1 Pre-treatment Assessments

The following trial specific procedures will be carried out after consent and within 3 days of treatment to assess the participant's eligibility:

- Informed consent
- Medical history
- Physical examination
- Vital signs
- Pregnancy test (urine)
- Whole blood count and differential
- Oxygen saturation and record oxygen delivery device if applicable (can be repeated if necessary)
- Oxygen requirement
- Blood draw for PD
- Blood draw for biomarkers
- Clinical Laboratory assessments including CRP, d-dimer and PCT (can be repeated if necessary)
- Concomitant medications

All pre-treatment procedures will be carried out as specified in the schedule of assessments (Appendix 1).

8.2 Randomisation Procedures

Participant randomisation will be undertaken centrally by an independent statistician (ie not the trial statistician) using SAS PROC PLAN according to SOPs. The randomisation schedule will be maintained in a secure, password protected environment, inaccessible to others supporting the trial.

Following participant consent, and confirmation of eligibility (see section 8.1 for pre-treatment assessments) the randomisation procedure described below will be carried out.

Participants are considered to be enrolled into the trial following: consent, pre-treatment assessments (see section 8.1), confirmation of eligibility, completion of the randomisation process, allocation of the participant trial number and treatment by the central coordinating team.

8.3 Treatment Schedule

Dornase alfa will be nebulised at 2.5mg twice per day (12 ± 3 hours apart) for seven days using either:

- the recommended eRapid Nebuliser System, consisting of the eRapid™ Nebuliser Handset with eBase™ Controller OR
- a jet nebuliser connected to an air compressor with an adequate air flow and equipped with a mouthpiece (Table 1).

Table1 Recommended Jet Nebulisers/Compressors

Jet nebuliser	Compressor
Hudson T Up-draft II with	Pulmo-Aide
Marquest Acorn II with	Pulmo-Aide
PARI LC Plus with	PARI PRONEB
Durable Sidestream with	MOBILAIRE™
Durable Sidestream with	Porta-Neb

8.3.1 Dose modifications

Based on clinical judgement, it may be decided to extend the dosing period for some participants. For example, a change from 2.5mg BID for 7 days to 2.5mg BID for up to 14 days. In particular, if participants have significant benefits from therapy, but show relapses of the COVID-19 inflammatory state (rising CRP and increasing oxygen requirements in the absence of bacterial infection), on completing 7 days of treatment, then the medical team have the choice of reinstating dornase alfa treatment for up to 7 further days (14 days in total) at 2.5mg BID. Blood sampling will continue until the last day of dosing with dornase alfa.

8.4 Subsequent assessments and procedures

8.4.1 Schedule of assessments

The following assessments and procedures will take place on Day1, Day3, Day5 and Day7 of dosing. In participants who remain on treatment beyond Day7, assessments will occur on Day9, Day11 and Day14 of dosing.

- Eligibility confirmation (at Day1 only)
- Physical examination

- Vital signs (Blood pressure, heart rate, temperature, respiration rate)
- Whole blood count and differential
- Oxygen saturation and record oxygen delivery device if applicable
- Oxygen requirement (oxygen flow or oxygenation index)
- Blood draw and bronchial secretions (when available) for PD
- Blood draw and bronchial secretions (when available) for biomarkers
- Clinical Laboratory assessments including CRP, d-dimer and PCT (can be repeated if necessary)
- Multi-organ failure according to SOFA (Sepsis-related Organ Failure Assessment)
- Acute physiology score + age points + chronic health points (APACHE score) data that has been collected to calculate this score.
- Ordinal score (WHO scoring tool)
- Adverse Events review
- Concomitant Medication review

Other assessments to be recorded if/when they occur:

- Length of ICU stay (hours)
- Length of stay in the hospital (days)
- Length of time on mechanical ventilation (days)
- Ventilator-associated pneumonia (VAP) or hospital-acquired pneumonia
- Survival (days)

Assessments/procedures at follow-up. These assessments and procedures will occur before the participant is discharged from the hospital or Day35, whichever comes first.

- Physical examination
- Vital signs (Blood pressure, heart rate, temperature, respiration rate)
- Pregnancy test (urine)
- Whole blood count and differential
- Oxygen saturation and record oxygen delivery device if applicable
- Oxygen requirement (oxygen flow or oxygenation index)
- Blood draw for PD
- Blood draw for biomarkers
- Clinical Laboratory assessments including CRP, d-dimer and PCT (can be repeated if necessary)
- Multi-organ failure according to SOFA (Sepsis-related Organ Failure Assessment)
- Acute physiology score + age points + chronic health points (APACHE score) data that has been collected to calculate this score.
- Ordinal score (WHO scoring tool)
- Adverse Events review (daily)
- Concomitant Medication review

Other assessments to be recorded if/when they occur:

- Length of ICU stay (hours)
- Length of stay in the hospital (days)
- Length of time on mechanical ventilation (days)
- Ventilator-associated pneumonia (VAP) or hospital-acquired pneumonia

- Survival (days)

In participants who are discharged before Day35, a telephone call will occur at Day35 to ask them about their breathing e.g. Are you short of breath? Has your breathing returned to the same level as previously?

A schedule of all trial assessments and procedures is set out in Appendix 1.

8.5 Laboratory Assessments and Procedures

Local laboratories will be used for the primary and secondary assessments and procedures. These include CRP, whole blood count and differential count, proCalcitonin (PCT), and D-Dimer. The samples will be taken as per hospital standard procedures as part of routine clinical care.

The following tests will be carried out at Local Laboratories:

Laboratory test	Parameters
BLOOD	
Haematology	leukocytes, erythrocytes, haemoglobin, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), platelets, neutrophils, eosinophils, basophils, lymphocytes, monocytes
Serum chemistry	glutamate pyruvate transaminase (GPT / ALAT), glutamic-oxaloacetic transaminase (GOT / ASAT), gamma-glutamyl transferase (gamma-GT), alkaline phosphatase, total bilirubin, creatinine, chloride, potassium, sodium, total protein, albumin, Lactate, Renal function: (Creatinine, Urea and Na, K, Cl), Clotting screen including Prothrombin time, Bone profile (ca2+), High-sensitivity troponin, Ferritin, LDH, Creatinine
Biomarkers	CRP, PCT, D-dimer

PD and biomarkers (exploratory) samples

The volume of blood required for PD and biomarkers exploratory endpoints is 10ml.

When it is available, bronchial secretions may be collected to measure PD and biomarkers. The bronchial secretion will be obtained due to spontaneous expectoration or route clinical bronchoscopy during MV.

All samples for exploratory endpoints (biomarkers and PD) will be labelled with the unique identifying number (UIN) prior to transfer to The Francis Crick Institute. Samples will be stored in secure UCL research facilities with restricted access under the custodianship of the Chief Investigator or designee until the samples are shipped by courier.

Transfer of samples for exploratory endpoints (biomarkers and PD) to The Francis Crick Institute will be subject to a laboratory agreement in which the Francis Crick Institute will be responsible for restricting use of the samples and data to agreed purposes, maintenance of confidentiality and data security, reporting publications and other outputs to the chief investigator of this trial and restricting onward transfer of samples to a third party.

PD and biomarker samples will be collected under the supervision of the Chief Investigator (or designee) and send to The Francis Crick Institute with courier to a dedicated person in the laboratory of Dr Veni Papayannopoulos. Blood (up to 10 mL – vacutainers- heparin or EDTA) and bronchial secretions will be transferred to The Francis Crick institute. Samples will be processed and stored for further analysis in the Francis Crick Institute Freezer Farm. Tracking of samples will be done with Freezerpro data base. Research samples will be processed in the Crick laboratories by designated research staff within an SOP (COVASE SOP) under the supervision of Dr Veni Papayannopoulos.

Exploratory samples (blood draw and bronchial secretion for PD and biomarkers – Appendix I)

Sample processing:

1) Leukocyte pelleting for RNA extraction and sequencing (biomarker).

2) Plasma collected for: Haemolysis (biomarker); cytokine analysis by ELISA and multiplex panel for 67 markers of inflammation (biomarker); coagulation markers such fibrin, tissue factor, Von Willebrand factor, thrombin, thromboxane A2 (biomarker), complement cascade markers such as C1q, (biomarker), quantification of NETs by NET ELISA (PD) , quantification of cell-free DNA (PD) and in vitro NET formation assays (biomarker).

3) Plasma samples denatured and boiled in SDS for Western immunoblotting to assess neutrophil markers such as neutrophil elastase; myeloperoxidase; histones; citrullinated H3. (PD).

4) Bronchial secretions will be processed according to local procedures for the measurement of biomarkers.

These samples will be handled at containment level 2 according to local guidelines.

Total blood draw/participant

treatment arm	blood at each timepoint	number of blood draw timepoints	Number of days in the trial	total blood over the trial
BAC for 7 days	30mL	6	35 days	180mL
Dornase alfa for 7 days	30mL	6	35 days	180mL
Dornase alfa for 14 days	30mL	9	35 days	up to 270mL

8.6 Clinical Procedures and Data Collection

All study procedures will be carried out by UCLH medical staff in the hospital and as specified in UCLH guidelines.

Medical examination: The following sites will be examined: head, neck, ears, nose, throat, eyes, chest, lungs, heart, abdomen, skin, and lymph nodes; and the following systems will be assessed: musculoskeletal and neurological.

8.7 Assessment of dornase alfa compliance

Monitoring (e.g. watching participant inhale dornase alfa) and recording this appropriately.

8.8 Discontinuation/withdrawal of participants

In consenting to participate in the trial, participants are consenting to trial treatment, assessments, follow-up and data collection.

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. Reasons for discontinuing treatment may include:

- Alternative clinical diagnosis emerges (no longer considered to have primary COVID-19 lung disease).
- Disease progression whilst on therapy.
- Unacceptable toxicity.
- Intercurrent illness which prevents further treatment.
- Patients withdrawing consent to further trial treatment.
- Any alterations in the participant's condition which justifies the discontinuation of treatment in the CI's or designee's opinion.
- Persistent non-compliance to protocol requirements.
- Participant is put on end of life pathway and dies prior to dosing.

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.

Participant withdrawal from trial treatment or follow-up

If a participant expresses their wish to withdraw from trial treatment or 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.

Withdrawal of consent to data collection

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

8.9 Replacements

40 evaluable participants are required to meet the primary endpoint, Therefore, if a participant withdraws or is withdrawn, a replacement participant may be enrolled to the same treatment arm.

8.10 Stopping rules

The trial may be stopped before completion on the recommendation of the sponsor and CI and following guidance from the DMC following an interim analysis or at any stage during the study.

8.11 Definition of end of trial

Recruitment rate is estimated to range from 3 to 6 participants per week. Recruitment is estimated to take 12-15 weeks.

The expected duration of the trial is 3 – 4 months from recruitment of the first participant to last follow-up visit.

The end of trial is the date of the last visit of the last participant.

9 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).

9.1 Definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a participant administered dornase alfa 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 dornase alfa 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 dornase alfa 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 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).

9.2 Recording adverse events

All adverse events will be assessed every day and recorded in the medical records in the first instance.

All adverse events will be recorded with clinical symptoms and accompanied with a simple, brief description of the event, including dates as appropriate.

Non-serious Adverse Events (AEs) and Serious Adverse Events (SAEs) related to COVID-19 will not be collected in the CRFs or reported to Sponsor for this trial.

The following events listed below describe anticipated Covid-19 related AEs:

Worsening respiratory failure, fever, venous thrombo-embolic disease, worsening respiratory failure, hospital-acquired secondary infection, organ-failure, lymphopenia, rising CRP, death.

9.3 Assessments of Adverse Events

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

9.3.1 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

9.3.2 Causality

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

The differentiated causality assessments will be captured in the trial specific CRF/AE Log and/or SAE 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.

9.3.3 Expectedness

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

* This includes listed events that are more frequently reported or more severe than previously reported

The RSI to be used to assess expectedness is section 4.8 of the SmPC for Pulmozyme (dornase alfa).

9.3.4 Seriousness

All events are assessed for seriousness as defined for an SAE in section 9.1.

9.4 Procedures for recording and reporting Serious Adverse Events

All serious adverse events (SAEs/SARs/SUSARs) will be recorded in the medical records in the first instance. Serious Adverse Events (SAEs) related to COVID-19 will not be collected in the CRFs or reported to Sponsor for this trial.

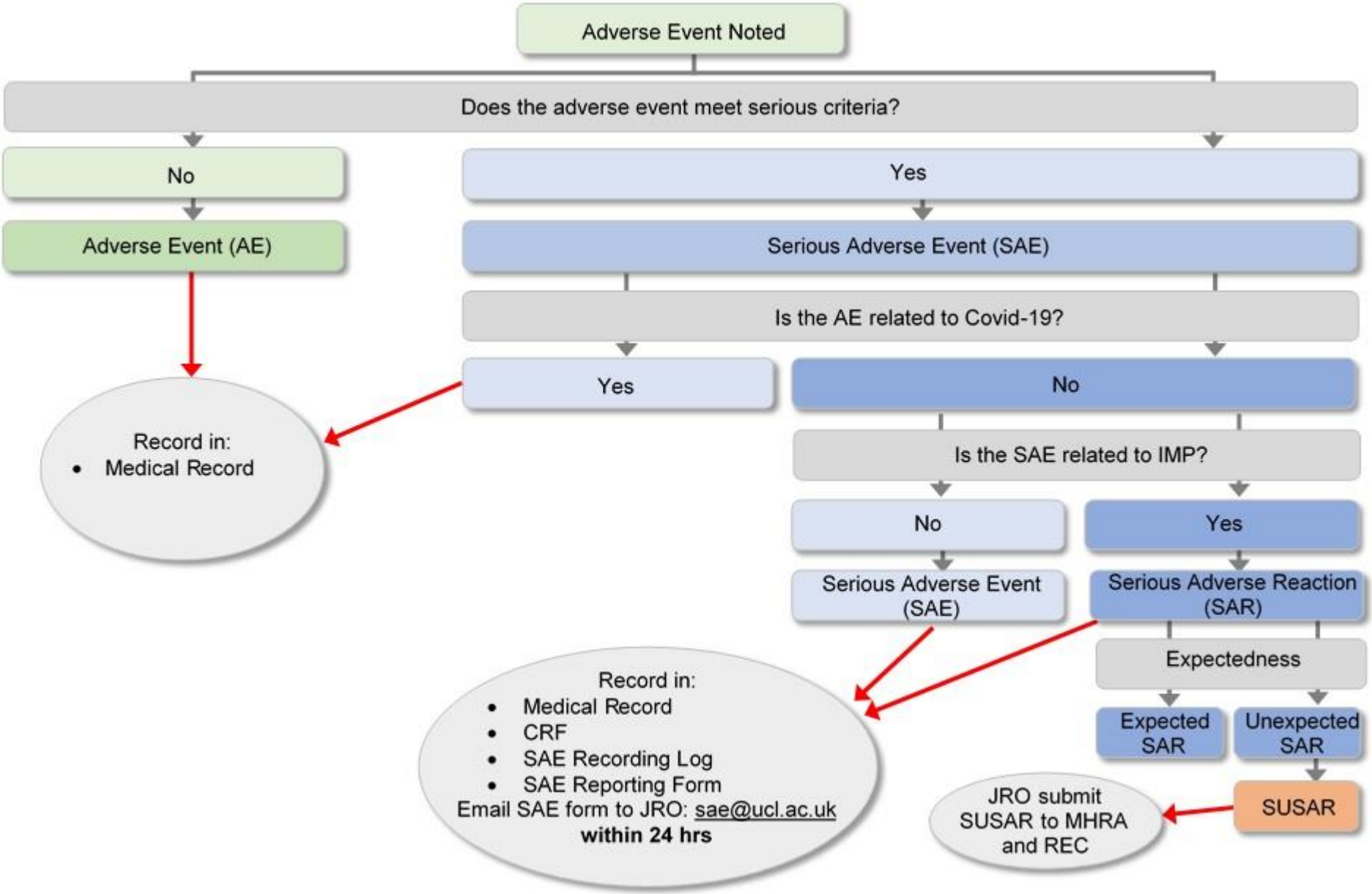
All other SAEs will be recorded in the CRF, and the sponsor's SAE Recording Log and SAE Reporting Form.

All SAEs will be recorded from randomisation until 24h after last study dose.

All SAEs (except Covid-19 related SAEs specified in section as not requiring reporting to the Sponsor), must be recorded on a serious adverse event (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, within 24 h of his/her becoming aware of the event. The Chief or Principal Investigator or designee 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

Flow Chart for SAE reporting



9.5 Serious Adverse Events which do not require immediate reporting

The following events listed below describe anticipated COVID-19 related AEs:

Worsening respiratory failure, fever, venous thrombo-embolic disease, worsening respiratory failure, hospital-acquired secondary infection, organ-failure, lymphopenia, rising CRP, death.

These SAEs will be RECORDED in the participants' medical notes. They will not be recorded in the CRF or SAE Recording Log and SAE Reporting Forms will not be completed and sent to the sponsor.

If the frequency or severity of these events is not consistent with the COVID-19 , the event must be reported to the sponsor as an SAE in the normal way.

9.6 Reporting 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.

9.7 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.

9.8 Pregnancy

Pregnancy is considered to be highly unlikely during the study as participants are hospitalised for the duration of the trial.

There are no adequate and well-controlled studies with dornase alfa in pregnant women. However, animal reproduction studies have been conducted with dornase alfa. In these studies, no evidence of foetal harm was observed in rats and rabbits at doses of dornase alfa up to approximately 600 times the maximum recommended human dose (MRHD).

It is not known whether dornase alfa is present in human milk. In a pharmacokinetic study in Cynomolgus monkeys, levels of dornase alfa detected in milk were less than 0.1% of the maternal serum concentration at 24 hours after dosing [intravenous bolus dose (0.1 mg/kg) of dornase alfa followed by an intravenous infusion (0.080 mg/kg/hr) over a 6-hour period] on post-partum day 14.

Dornase alfa is not contra-indicated in pregnancy or lactation.

In the unlikely event that a female participant or the female partner of a male participant becomes pregnant at any point during the trial, a completed trial specific Pregnancy Reporting Form will be preferably emailed to the Sponsor **SAE@ucl.ac.uk,** within 24 hours of his / her becoming aware

of the event in line with the Sponsors SOP (JRO/INV/S05). The Chief or Principal Investigator or designee will respond to any queries raised by the sponsor as soon as possible.

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

The Sponsor must be kept informed of any new developments involving the pregnancy through the completion of a follow-up Pregnancy Reporting Form. Any pregnancy that occurs in a female trial subject during a clinical trial should be followed to termination or to term.

Consent to report information regarding the pregnancy must be obtained from the pregnant participant. A trial-specific pregnancy monitoring information sheet and informed consent form for trial participants and the partners of trial participants must be used for this purpose.

With consent additional information regarding the pregnancy will be collected and reported to the Sponsor, the Sponsor will advise on the length of follow up of the pregnancy/child on a case by case basis.

9.9 Overdose

Overdose is unlikely as dornase alfa will be administered by hospital staff.

Cystic fibrosis participants have received up to 20mg BID for up to 6 days and 10mg BID intermittently (2 weeks on/2 weeks off drug) for 168 days. These doses were well tolerated.

Prescribing information

https://www.gene.com/download/pdf/pulmozyme_prescribing.pdf

9.10 Reporting urgent safety measures and other safety events

If any urgent safety measures are taken the CI/PI or designee 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.

9.11 Notification of serious breaches to GCP and/or the protocol (SPON/S15)

A “serious breach” is a breach which is likely to affect 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.

10 Data management and quality assurance

10.1 Confidentiality

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

The Case Report Forms (CRFs) will bear the participant's initials and UIN. All reports and other results are strictly confidential and access is restricted to relevant healthcare professionals. All of the participant's data will be pseudo-anonymised (according to standard operating procedures) prior to sending data externally for analysis. This will be clearly explained to the participant in the Patient information sheet.

Patient consent for this will be sought.

10.2 Data collection tools and source document identification

Data will be collected on Trial specific case report forms (CRFs) or data collection tools such as CRFs.

Source data are contained in source documents and must be accurately transcribed on to the CRF. Examples of source documents are medical records which include laboratory and other clinical reports etc.

A source document list will be implemented prior to the start of the trial to identify:

which data is to be recorded directly onto the CRF;

which data is recorded firstly into source documents, such as medical notes, and then transcribed into the CRF; and

which data is not to be recorded in the CRF, but only recorded in source documents, e.g., participant questionnaires and diary cards.

10.3 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 or designee to perform this duty. The CI/PI or designee is responsible for the accuracy of all data reported in the CRF.

10.4 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 and data transfer.

Where data are transferred electronically this will be in accordance with the UK Data Protection Act 1998 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.

11 Statistical Considerations

11.1 Outcomes

11.1.1 Primary outcomes

The primary endpoint is the change from baseline in acute phase reactant (C-Reactive Protein (CRP)). This will be measured according to the assessment schedule in section 8.4.1. This endpoint is on a continuous scale.

11.1.2 Secondary outcomes

There are multiple secondary endpoints which will be measured according to the assessment schedule in 8.4.1.

Some of these endpoints are based on change from baseline and are repeated, continuous measures:

- Whole blood count and differential count
- ProCalcitonin (PCT)
- D-dimer
- Oxygen requirement (oxygen flow or oxygenation index)

There is one survival endpoint which is censored/truncated time to event data.

Other secondary endpoints are continuous, absolute measures that are derived only at the end of the assessment period:

- Time on MV
- Length of ICU stay [hours]
- Length of stay in the hospital [days).

The remaining endpoints are on a binary or ordinal scale:

- Incidence of MV
- Incidence of multi-organ failure according to SOFA (Sepsis-related Organ Failure Assessment)
- Incidence of Ventilator-Associated Pneumonia (VAP) or hospital acquired pneumonia
- Ordinal score (WHO scoring tool).

11.2 Sample size and recruitment

11.2.1 Sample size calculation

Sample size calculations were produced using the proc power function in SAS Version 9.4. These were conducted to achieve 80% power to detect difference in the active arm versus the control group at the 5% level of significance. Based on a mean of 99mg/L in the control group and a common standard deviation of 62mg/L derived from the literature (Han et al., 2020; Zhou, 2020), a total sample size of 90 participants would provide sufficient power to detect a greater than a 40% relative difference for CRP in the dornase alfa group compared to the control group. Given the reported average values in severe and non-severe participants and on clinical observations from COVID-19 patients, this difference would be achievable and clinically relevant.

This study will use existing data collected at UCLH from participants admitted with COVID-19 as a comparator group. Participants in the database will be selected to act as controls as follows:

- Apply the inclusion and exclusion criteria of the COVASE study
- Additional selection to identify closest matches

This will give the correct ratio of active versus comparator (ratio of 1:2). To achieve the required power, this would result in 30 participants in the active treatment group and at least 60 in the control. An additional 10 participants will be recruited as a control for the exploratory objectives and to compare the characteristics of enrolled participants with the historical controls. This gives a total of 40 participants enrolled in the study and 60 historical controls.

Participants who drop out of the study will be replaced so the sample size relates to the number of evaluable participants required.

A re-estimation of the sample size will be carried following an interim analysis when 12 participants have been randomised.

11.2.2 Planned recruitment rate

The anticipated recruitment rate is 3-5 participants per week at UCLH. To obtain 40 participants, the recruitment period is likely to last between 8 and 14 weeks. Given the paucity of treatment options and the observed number of cases, the sample size should be easily attainable.

Randomisation methods

Subjects will be allocated to dornase alfa or BAC in a 3:1 ratio in accordance with the randomisation schedule. The randomisation schedule will be based on permuted block randomisation produced using SAS PROC PLAN. Block sizes will vary in multiples of 4.

11.3 Statistical analysis plan

11.3.1 Summary of baseline data and flow of participants

All subjects enrolled in the study will be accounted for in a CONSORT diagram. There will be multiple analysis populations:

1 Primary analysis population - all evaluable patients randomised to dornase alfa + matched historical comparators.

2 Per protocol population - as above but excluding protocol violations.

3 Safety population - all enrolled patients receiving at least one dose of dornase alfa and the comparator groups.

4 Comparator population - the matched historical controls, patients randomised to BAC and historical records linked to biobanked samples.

5 Exploratory analysis population - all evaluable patients randomised to dornase alfa or to BAC plus historical patient data from biobanked samples.

The primary analysis will be conducted using the primary analysis population and is based on the ITT principle. Details on subjects enrolled but not included in the analysis populations will be presented as part of the CONSORT diagram.

The key baseline data that will be used to compare the groups and the analysis populations are age, gender, BMI, baseline CRP, oxygen requirements and the number of comorbidities. In general, continuous data will be summarised using the mean, standard deviation, median, minimum and maximum and categorical data will be represented as frequency counts and percentages.

11.3.2 Primary outcome analysis

The primary outcome analysis will involve the primary endpoint and the primary analysis population. The endpoint will be summarised overall, by treatment group and by day. Data will be summarised using the mean (standard deviation), median (1st and 3rd quartiles), minimum and maximum, and 95% confidence intervals.

The primary endpoint will be compared between groups using a repeated measures mixed model, adjusted for age, gender, BMI, baseline value, oxygen requirements, time from baseline, and the number of comorbidities and with treatment as the main effect and treatment by time as an interaction effect. Prior to analysis, the primary endpoint will be assessed for conformance to normality assumptions and the appropriate transformation will be conducted if necessary. This model-based approach is likely to be more robust to missing or spurious data. Treatment effect will be declared significant at the 5% level of significance.

The primary analysis population comprises the active treatment group and historical matched controls. The matching procedure will include an initial application of the study inclusion and exclusion criteria to identify the subjects that meet the criteria within the database. Further matching will involve the use of propensity scores to select the 60 controls that most closely match with participants in the active treatment group. The propensity score model will include age, gender, BMI, baseline CRP, oxygen requirements and the number of comorbidities and two controls will be matched for each participant in the active group.

11.3.3 Secondary outcome analysis

This study was not powered to detect any effects relating to secondary endpoints. Therefore, the secondary analysis will involve descriptive statistics. In general, continuous data will be summarised using the mean (standard deviation), median (1st and 3rd quartiles), minimum and maximum, and categorical data will be represented as frequency counts (percentages).

A further comparison of the secondary endpoints will involve the appropriate general linear models for binary, continuous or ordinal data with age, gender, BMI, baseline value, oxygen requirements and the number of comorbidities as covariates and treatment as a main effect. This will provide the adjusted means and 95% confidence intervals for the endpoint in the active and control groups. The same model will be used to estimate the difference between the groups and the associated confidence interval.

Continuous endpoints will be assessed for conformance to normality assumptions and the appropriate transformation will be conducted if necessary.

A survival analysis will be conducted on the time to event data. The Kaplan-Meier method will be used to estimate the median survival times and the associated 95% confidence intervals. The time to event data will be censored at 28 days post last dose (Day35) for the randomised participants and at the date of the last electronic record for the historical control group. The survival model will include treatment as a stratification variable. A cox proportional hazards model will be used to generate a hazard ratio and associated confidence intervals, adjusting for age, gender, BMI, baseline value, oxygen requirements and the number of comorbidities as covariates and treatment as a main effect.

The secondary outcome analysis will use the primary analysis population.

11.3.4 Sensitivity and other planned analyses

All primary and secondary analyses will be repeated using the primary analysis population and the per protocol population. The use of the per protocol population is only as a sensitivity analysis to understand the impact of non-compliance.

This study utilises a combination of enrolled and randomised participants, historical controls and banked biosamples. Descriptive statistics will be produced for key baseline variables for all data sources within the comparator population. These will be used to evaluate the comparability of the source data.

There are planned subgroup analyses that relate to secondary objectives. A comparison of the secondary endpoint, time on MV, between treatment groups will be conducted only in participants that received MV. Other subgroup analyses may be performed but these are considered to be exploratory.

Further exploratory analyses are planned for the exploratory endpoints in the study. These analyses will follow the same principles and considerations with regards to the application of statistical approaches for specific data types and data structures. The exploratory analyses will use the exploratory analysis population.

Full details of the planned statistical analysis will be presented in a separate Statistical Analysis Plan (SAP). This will be finalised prior to database lock. The results from all analyses will be presented in the form of tables, figures and listings.

11.4 Interim analysis

An interim analysis will be conducted when 12 participants have been randomised. The results of the interim analysis will be used to re-estimate the sample size if necessary. The interim analysis will be conducted by an independent statistician in a secure, password protected environment. The analysis will involve the production of descriptive statistics for the primary endpoint, AEs and baseline characteristics by study population and by treatment. No formal statistical comparison between the treatment groups will be performed. The sample size re-estimation may result in the recruitment of more subjects than originally planned but not less.

11.5 Other statistical considerations

Any deviation from the original statistical plan will be described and justified in the final report, as appropriate.

12 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.

13 Oversight committees

13.1 Data Monitoring Committee (DMC)

The role of the DMC is to provide advice on data and safety aspects of the trial but not all members are independent. The members consist of the CI, a sponsor physician and a statistician or their respective designees. Meetings of the Committee will be held weekly to review emerging data as well as the results of the interim analysis (Interim Analysis section 11.4), or as necessary to address any issues. The DMC may recommend stopping the study at the interim analysis (or at any stage of the study).

14 Direct Access to Source Data/Documents

The investigator(s)/institution(s) 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.

15 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, 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/Principal Investigator or designee must receive NHS permission in writing from the Trust Research & Development (R&D). It is the responsibility of the CI/ PI or designee at each site 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 9.10 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 (the exploratory endpoints will be reported separately).

16 Monitoring requirement for the trial

The sponsor will determine the appropriate level and nature of monitoring required for the trial. Risk will be assessed on an ongoing basis and adjustments made accordingly.

The degree of monitoring will be proportionate to the objective, purpose, phase, design, size, complexity, blinding, endpoints and risks associated with the trial.

A trial specific oversight and monitoring plan will be established. The trial will be monitored in accordance with the agreed plan.

17 Finance

Funding has been sought from LifeArc and outcome is pending.

There are no financial interests by CI, PIs or trial management members in dornase alfa or the eRapid nebuliser.

18 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.

Indemnity arrangements for the eRapid nebuliser will be in place, with the manufacturer, to cover the malfunction and breakdown of the device.

19 Publication policy

There will be weekly team meetings for the staff working on the project. Data generated will be disseminated through medRxiv and bioRxiv and through the Breathing Matters newsletters and website (www.breathingmatters.co.uk and twitter feed @breathingmatter).

20 References

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21 Appendix 1 - Schedule of Assessments

Visit No.	1	2	3	4	5	6 to 8	Final visit [8,10]
	Day-1	Day1	Day3	Day5	Day7	Day9, Day11, Day14 [10]	Day35 or discharge [11]
Window of flexibility for timing of visits	±2 days		±1 day	±1 day	±1 day	±1 day	±2 day
Informed Consent	X						
Medical History	X						
Physical Examination	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X
Pregnancy test [1]	X						X
Clinical Laboratory assessments [2]	X	X	X	X	X	X	X
Eligibility confirmation		X					
CRP [3]	X	X	X	X	X	X	X
Whole blood count and differential count [4]	X	X	X	X	X	X	X
ProCalcitonin (PCT) [3]	X	X	X	X	X	X	X
D-Dimer [3]	X	X	X	X	X	X	X
Oxygen requirement (oxygen flow or oxygenation index)	X	X	X	X	X	X	X
Length of ICU stay (hours)		X	X	X	X	X	X
Length of stay in the hospital (days)		X	X	X	X	X	X
Length of time on mechanical ventilation (days)		X	X	X	X	X	X
Survival (days)		X	X	X	X	X	X

Ventilator-Associated Pneumonia (VAP) or hospital acquired pneumonia		X	X	X	X	X	X
Acute physiology score + age points + chronic health points (APACHE score)		X	X	X	X	X	X
Ordinal score (WHO scoring tool)		X	X	X	X	X	X
Blood draw for pharmacodynamics (PD) [5]	X	X	X	X	X	X	X
Blood draw for biomarkers [6]	X	X	X	X	X	X	X
Bronchial secretion for PD and biomarkers [5,6, 7]		X	X	X	X	X	X
nebulised dornase alfa administration [8]		X	X	X	X	X	
Adverse Events review [12]		X	X	X	X	X	X
Concomitant Medication review	X	X	X	X	X	X	X
Questions about breathing [9]							X

1. A urine pregnancy test
2. Clinical Laboratory assessments include: glutamate pyruvate transaminase (GPT / ALAT), glutamic-oxaloacetic transaminase (GOT / ASAT), gamma-glutamyl transferase (gamma-GT), alkaline phosphatase, total bilirubin, creatinine, chloride, potassium, sodium, total protein, albumin, Lactate, Renal function: (Creatinine, Urea and Na, K, Cl), Clotting screen including Prothrombin time, Bone profile (ca2+), High-sensitivity troponin, Ferritin, LDH, Creatinine
3. CRP, PCT and D-Dimer are measured in the clinical laboratory sample
4. Full Blood Count: leukocytes, erythrocytes, haemoglobin, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), platelets, neutrophils, eosinophils, basophils, lymphocytes, monocytes
5. Pharmacodynamics: may include but is not limited to Cell-free DNA (cfDNA), Circulating histone, Citrullinated H3, NET Elisa assay
6. Biomarkers: may include but is not limited to Circulating pro-inflammatory cytokines (e.g. IL-6, TNFα, IL-1β, IL-8), Markers of activation of the coagulation (e.g. fibrin, tissue factor, Von Willebrand factor,

thrombin, thromboxane A2) and complement cascade (e.g. C1q), haemolysis (e.g. red blood cell lysis), NET formation

7. Bronchial secretions will be collected as appropriate for clinical reasons only (e.g. spontaneous expectorant or bronchoscopy during MV)
8. Dornase alfa administered daily at 2.5mg BID by nebuliser
9. In participants who are discharged prior to Day35, a follow-up telephone call will take place to ask about their breathing.
10. In participants who receive dornase alfa (2.5mg BID) beyond Day7, additional blood draws and procedures will take place on Day9, Day11, Day14
11. Final study visit is at discharge or Day35 whichever comes first
12. Adverse events will be recorded every day and not just on study visit days