Dornase alfa in COVID-19 (The COVASE trial)

Name of drug:	Pulmozyme®, recombinant human dornase alfa (2.5 mg nebulised BID)
Indication studied:	COVID-19 pneumonia
Protocol number:	132333
EudraCT no.	2020-001937-11
Phase of study:	IIa

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 pneumonia.

Study initiation date:	Date of early termination:	Study competition date:
16th June.2020	(Not applicable)	15th November.2021

Sponsor representative:	Chief Investigator:
Joint Research Office, UCL, 1st Floor Maple House, 149 Tottenham Court Road, London W1T 7NF Postal address: Joint Research Office, UCL Gower Street, London WC1E 6BT	Professor Joanna Porter UCL Respiratory The Rayne Building 5 University Street London WC1E 6JF

Name of company/sponsor signatory: Professor Joanna Porter

Telephone number: [redacted]

Email address: [redacted]

The study was performed in compliance with Good Clinical Practices (GCP), including the archiving of essential documents.

Date of report: (Identify any earlier reports)	9th February 2023 version 2.0 following minor adverse event reporting analysis errors in version 1.0 4th February 2022

1 SYNOPSIS

<table>
<tr><td>Name of Sponsor/Company:
Joint Research Office, UCL, 1st Floor Maple House</td><td></td><td rowspan="3">(For National Authority
Use only)</td></tr>
<tr><td>Name of Finished Product:
Pulmozyme ®</td><td>Volume:
Each ampoule contains 2500 U (corresponding to 2.5 mg) of dornase alfa per 2.5 mL corresponding to 1000 U/mL or 1 mg/mL</td></tr>
<tr><td>Name of Active Ingredient:
Recombinant human dornase alfa</td><td></td></tr>
<tr><td colspan="3">Title of Study:
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 pneumonia</td></tr>
<tr><td colspan="3">Investigators: Professor Joanna Porter</td></tr>
<tr><td colspan="3">Study centre(s): University College London Hospital</td></tr>
<tr><td colspan="3">Publication (reference)
Anti-inflammatory therapy with Nebulised Dornase-Alfa in Patients with Severe COVID-19 Pneumonia- manuscript in preparation</td></tr>
<tr><td>Studied period (years):
1 year and 2 months</td><td colspan="2">Phase of development: IIa</td></tr>
</table>

First patient first visit: 17th June.2020 Last patient last visit: 12th August 2021	

Objectives:

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

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

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

Methodology:

Participants were screened, consented, enrolled and randomised up to 3 days after they were admitted to hospital. They were randomised in a 3:1 ratio to receive best available care (BAC) + dornase alfa or BAC alone. A total of 39/40 participants were enrolled (30 received BAC plus dornase alfa and 9 received BAC). On Day1 to Day7 of the trial, participants randomised to the active arm, received 2.5mg BID nebulised dornase alfa in addition to BAC. On Day1, Day3, Day5 and Day7, blood samples were drawn in both trial arms in order to test pharmacodynamic endpoints (PD), biomarkers and clin labs. Clinical assessments were undertaken daily (as per UCLH clinical guidelines). Participants were followed until discharge or death or a maximum of 28 days follow-up.

A planned sample size re-estimation was conducted when 12 participants had been randomised. This analysis ensured that the assumptions made in the sample size calculation remained valid. The variability of the primary endpoint (C-reactive protein [CRP]) was as predicted and no additional subjects were required.

CRP was 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.

Historic control group: Comparator data from UCLH was used as historic controls for the Primary and Secondary Endpoints. CRP is routinely measured daily (or on alternate days) in all participants admitted to UCLH and was used to control for the Primary Endpoint. All of the Secondary Endpoints are also routinely measured and were available in the database.

Number of participants (planned and analysed):

30/30 participants randomised to receive BAC plus dornase alfa

9/10 participants randomised to receive BAC alone 60/60 matched historic controls (extracted and matched from the UCLH database of COVID-19 admissions during the study period)
Diagnosis and main criteria for inclusion: • Male and female participants, aged ≥ 18 years • Participants who are hospitalised for suspected Coronavirus (SARS-CoV)-2 pneumonia confirmed by polymerase chain reaction (PCR) test and radiological confirmation • Participants with stable oxygen saturation (>=94%) on supplementary oxygen • CRP >= 30 mg/L. Before treatment with dornase alfa • Participants will have given their written informed consent to participate in the study and are able to comply with instructions and nebuliser
Test product, dose and mode of administration, batch number: 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. One 2.5 mg single-use ampule inhaled twice daily using a recommended nebulizer jet nebulizer/compressor system or eRapid™ Nebulizer System.
Duration of treatment: Up to 7 days
Reference therapy, dose and mode of administration, batch number Participants in the open-label COVASE trial received BAC per UCLH guidelines. After the finalisation of the protocol but before the first participant was recruited, BAC consisted of symptomatic relief: antipyretics, analgesics and intravenous fluids if needed. In addition, participants received supplemental oxygen and/or mechanical ventilation if required. Dexamethasone was added to BAC before the first participant was recruited. All participants and historic controls received dexamethasone (6mg for 10 days or to hospital discharge whichever was sooner) as part of BAC. Towards the end of the study, antivirals, anti-interleukin (IL)-6, were added to BAC.
Criteria for evaluation: **Efficacy** Primary endpoint: ● Changes in acute phase reactant (C-Reactive Protein (CRP)) Secondary endpoints may include, but are not limited to: ● 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 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

Safety

- Physical examination
- Vital signs (Blood pressure, heart rate, temperature, respiration rate)
- Clinical Laboratory assessments
- Pregnancy test (urine)
- Recording and reporting of adverse events

Statistical methods:
There were multiple analysis populations:

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

2. Per protocol population - as above but excluding protocol violations.
3. Safety population - all enrolled participants receiving at least one dose of dornase alfa and the comparator groups.
4. Comparator population - the matched historical controls, participants randomised to BAC and historical records linked to biobanked samples.
5. Exploratory analysis population - all evaluable participants randomised to dornase alfa or to BAC plus historical participant data from biobanked samples.

The primary analysis was conducted using the primary analysis population and was based on the ITT (intention to treat) principle.

The key baseline data that was used to compare the groups and the analysis populations were age, gender, BMI, baseline CRP and the presence/absence of comorbidities. In general, continuous data was summarised using the mean, standard deviation, median, minimum and maximum and categorical data was represented as frequency counts and percentages.

An interim analysis was conducted when 12 participants had been randomised. The results of the interim analysis were used to re-estimate the sample size if necessary. The interim analysis was conducted by an independent statistician in a secure, password protected environment. The analysis involved the production of least square means from the primary endpoint analysis, a listing of AEs and descriptive statistics for baseline characteristics by study population and by treatment. No formal statistical test between the treatment groups was performed. The sample size re-estimation did not result in the recruitment of more subjects than originally planned.

Summary – Conclusions

Efficacy Results:

The COVASE study met its primary endpoint to show a reduction in CRP due to administration of dornase alfa for 7 days in participants hospitalised for COVID-19. This reduction was robust, as it was consistently observed in all sensitivity analyses, including stratification by type of BAC received.

Several important secondary endpoints also showed statistically significant effects of dornase alfa.

A time-to-event analysis of baseline to discharge from hospital showed a 63% greater chance of discharge from hospital at any given time over 35 days follow-up in the dornase alfa group ($p = 0.03$) compared to the BAC group, and a median time to discharge of 6 days in the dornase alfa group compared to 7 days in the BAC group. This result was supported by non-significant trends in other endpoints related to hospitalisation (for example length of hospitalisation).

Dornase alfa had no detectable effect on any endpoint related to ICU (length of stay over 7 and 35 days; proportion of participants admitted to ICU during 7 and 35 day of follow-up).

Dornase alfa treatment resulted in a significant increase in blood lymphocyte count and a significant decrease in PCT and d-dimer.

Dornase alfa had no detectable effect on WHO ordinal scale, survival, mechanical ventilation or the proportion of participants with secondary bacterial pneumonia.

Safety Results:

Dornase alfa was safe and well-tolerated. Adverse events were unremarkable and no treatment-related SAEs were detected.

Conclusion:

In conclusion, dornase alfa treatment resulted in a significant reduction in CRP. This effect was supported by significant clinical effects on a selection of secondary endpoints, including hospitalisation, differential blood count (specifically lymphocytes) and relevant biomarkers, procalcitonin and d-dimer. Dornase alfa was safe and well-tolerated in hospitalised COVID-19 participants. These data may be used to optimally design a subsequent efficacy trial of dornase alfa in COVID-19.

Date of report:

PRINCIPAL OR COORDINATING

INVESTIGATOR(S) SIGNATURE(S)

OR SPONSOR'S RESPONSIBLE MEDICAL OFFICER

Study title:

Dornase alfa in COVID-19 (The COVASE trial)

Study author(s): Pauline Lukey Jamie Ins haw Joanna Porter	
I have read this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the study	
Investigator: Professor Joanna Porter	Signature(s): 

Affiliation: UCL Respiratory The Rayne Building 5 University Street London WC1E 6JF	
Date:	16th May 2023
Statistician: Dr. Jamie Inshaw Affiliation: Exploristics Ltd. Floor 4, 24 Linenhall Street, Belfast, BT2 8BG	
Date:	18th May 2023

2 TABLE OF CONTENTS FOR THE INDIVIDUAL CLINICAL STUDY REPORT

Table of Contents

3 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Meaning/Definition
AEs	Adverse events
APACHE score	Acute physiology score + age points + chronic health points
BAC	Best available care
BAL	Bronchoalveolar lavage
BID	Twice per day
BMI	Body mass index
CF	Cystic fibrosis
cfDNA	Cell-free DNA
CI	Chief investigator
CI	Confidence interval
CIP	Confidential Participant Information
COPD	Chronic obstructive lung disease
COVID-19 pneumonia	Defined by +ve PCR, radiological changes and hypoxia
CRF	Clinical report form
CRP	C-reactive protein
CSR	Clinical study report

DA	Dornase alfa
DMC	Data Monitoring Committee
EMA	European medicines agency
FDA	Federal drug administration
GCP	Good clinical Practice
GDPR	Guide to data protection regulation
ICU	Intensive care unit
IL	Interleukin
ILD	interstitial lung disease
ITT	Intention to treat
L	Litre
mg	milligram
mL	millilitre
MV	Mechanical Ventilation
NET	Neutrophil extracellular traps
ng	nanogram
PCR	Polymerase chain reaction
PCT	procalcitonin
PD	Pharmacodynamic
PIS	Participant information sheet
RBC	Red blood cell
SAEs	Seious adverse events
SAP	Statistical analysis plan
SmPC	Summary of product characteristics
SOFA	Sepsis-related Organ Failure Assessment
SOP	Standard operating procedure
TFLs	Tables, figures and listing s
TMG	Trial Management Group
TNF	Tumour necrosis factor
UCLH	University college London hospital
ug	microgram
UIN	Unique identification number
VAP	Ventilator-Associated Pneumonia
VAP	Ventilator-associated pneumonia
WHO	World health organisation

4 ETHICS

4.1 Independent Ethics Committee (IEC) or Institutional Review Board (IRB)

The COVASE trial was reviewed by an Independent Ethics Committee or Institutional Review Board.

Name and Chair of the IEC : Professor Vincenzo Libri, South Central - Hampshire B Research Ethics Committee Level 3 Block B Whitefriars Lewins Mead Bristol BS1 2NT

4.2 Ethical Conduct of the Study

The COVASE trial was conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki.

4.3 Participant Information and Consent

It was 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 who obtained consent was GCP trained, suitably qualified and experienced, and had been delegated this duty by the CI on the Staff Signature and Delegation of Tasks.

“Adequate time” was given for consideration by the participant before taking part. Due to the rapidly escalating pandemic situation, consent was sought at least 12 hours after being given the study documentation. It was recorded in the medical notes when the participant information sheet (PIS) had been given to the participant.

The Investigator or designee explained 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 were conducted prior to the participant giving consent by signing the Consent form. Consent did not denote enrolment into the trial.

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

The PIS and consent form were reviewed throughout the trial and no changes were required.

Representative written information for the participant (if any) and a sample participant consent form should be provided in appendix 16.1.3.

Consent for the historic controls was covered by the Health Service (Control of Participant Information) Regulations 2002 that allows the processing of Confidential Participant Information (CPI) for specific purposes. Regulation 3 provides for the processing of CPI in relation to communicable diseases and other threats to public health and in particular allows the Secretary of State to require organisations to process CPI for purposes related to communicable diseases. The COVID-19 pandemic is covered by this legislation which allows a range of purposes related to diagnosing, managing, and controlling the spread of COVID-19. Purposes could include but are not limited to:

- understanding COVID-19 and risks to public health, trends in COVID-19 and such risks, and controlling and preventing the spread of COVID-19 and such risks
- identifying and understanding information about participants or potential participants with or at risk of COVID-19

- delivering services to participants, clinicians, the health services
- research and planning in relation to COVID-19

The notice covers confidential participant information so any data regardless of its identifiability, which is being used for the purposes set out above is covered. It will all be treated in line with the principles of GDPR i.e., fairly, lawfully and securely.

5 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

The COVASE trial is a single-centre trial with one chief investigator (Professor Joanna Porter) and University College London Hospital.

A Trial Management Group (TMG) was involved in the running of the study. This consisted of the Chief Investigator, Statistician, Biologist, Clinical Scientist, Project Leader. A quorum of three members was required for a meeting to take place. Agenda and Minutes were produced for each meeting.

An Independent Data Monitoring Committee (DMC) oversaw the conduct of the study. This consisted of a consultant respiratory physician, a statistician and a consultant rheumatologist, all with experience in clinical trials. Meetings of the DMC were held *ad hoc* to review emerging data as well as the results of the interim analysis. The DMC did not recommend stopping the study at the interim analysis (or at any stage of the study). The TMG decision to stop recruitment in September 2021 was endorsed by the DMC. Only one participant was required to replace another participant randomised to BAC. Difficulty in recruiting a suitable replacement resulted in the TMG recommendation to stop recruitment (endorsed by DMC).

6 INTRODUCTION

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. 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.

Our hypothesis was 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 neutrophil extracellular traps (NETs) 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).

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 leads to a significant improvement in lung function for treated CF patients by facilitating mucus clearance from 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 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 tested this hypothesis in the COVASE Phase IIa trial. All people with COVID-19 pneumonia who were admitted to hospital for supplementary oxygen, who showed evidence of systemic inflammation but did not immediately require intubation and ventilation, were eligible for nebulised dornase alfa, a safe and cost-effective treatment, twice daily for 7 days.

The COVASE trial was designed when BAC was supportive only and there was no vaccine available. Before recruitment began, dexamethasone was added to supportive therapy as BAC ('Dexamethasone in Hospitalized Patients with Covid-19' 2020). Therefore, all subjects included in the COVASE Trial were receiving dexamethasone or equivalent. Because of the impact of dexamethasone on CRP, participants were only included if CRP was still elevated (>= 30 mg/L) after dexamethasone. No participant in the COVASE Trial was vaccinated.

7 STUDY OBJECTIVES

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)) in the blood

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 include, but are not limited to:

- Levels of acute phase reactant CRP over 35 days follow-up
- Length of hospitalisation from baseline (days)
- Survival at Day35, mortality data collected from EPIC database for both HCs and randomised individuals
- White blood cell count over 7 days follow-up
- Neutrophil count over 7 days follow-up
- Lymphocyte count over 7 days follow-up
- Monocyte count over 7 days follow-up
- Eosinophil count over 7 days follow-up
- Basophil count over 7 days follow-up
- Procalcitonin over 7 days follow-up
- D-dimer count over 7 days follow-up
- Blood pressure over 7 days follow-up
- Pulse rate over 7 days follow-up
- Temperature over 7 days follow-up
- Respiratory rate over 7 days follow-up
- Oxygen index over 7 days follow-up
- Time on Oxygen over 7 days follow-up
- Time on Oxygen over 35 days follow-up
- Incidence of Ventilator-Associated Pneumonia (VAP) over 7 days follow-up
- Ordinal score (WHO scoring tool) over 7 days follow-up
- Incidence of Mechanical Ventilation (MV) over 7 days follow-up
- Time on MV over 7 days follow-up
- Length of ICU stay (hours) over 7 days follow-up
- Incidence of Ventilator-Associated Pneumonia (VAP) over 35 days follow-up
- Incidence of Mechanical Ventilation (MV) over 35 days follow-up
- Time on MV over 35 days follow-up
- Length of ICU stay (hours) over 35 days follow-up

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

Exploratory endpoints will not be reported here but will be reported separately.

8 INVESTIGATIONAL PLAN

8.1 Overall Study Design and Plan-Description

A single-site, randomised, controlled, parallel, 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 1).

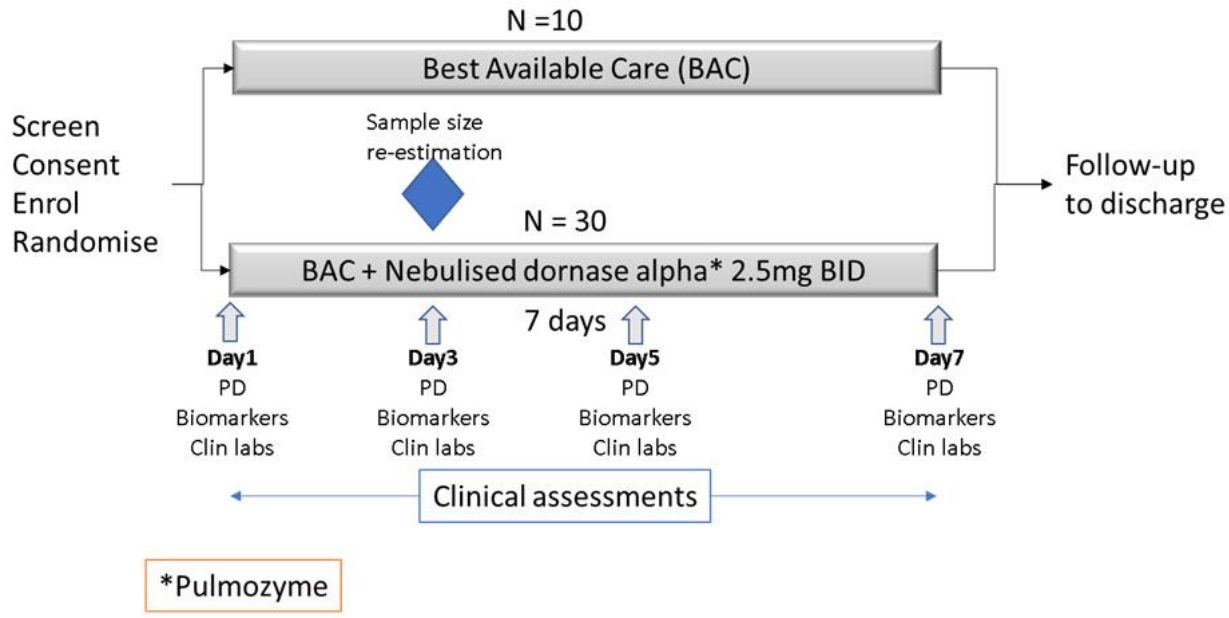

Figure 1: The COVASE trial design

Participants were screened, consented, enrolled and randomised up to 3 days after they were admitted to the hospital. They were randomised in a 3:1 ratio to receive BAC + dornase alfa or BAC alone. A total of 39 participants out of a planned total of 40 participants were enrolled (30 received BAC plus dornase alfa and 9 received BAC). On Day1 and for up to Day7 of the trial, participants randomised to the active arm received 2.5mg BID nebulised

dornase alfa in addition to BAC. On Day1, Day3, Day5 and Day7, blood samples were drawn in both trial arms in order to test pharmacodynamic endpoints (PD), biomarkers and clin labs. Clinical assessments were undertaken daily (as per UCLH clinical guidelines). Participants were followed until discharge or death or approximately of 28 days follow-up.

A sample size re-estimation was conducted as planned when 12 participants had been randomised. This analysis did not identify any reason to alter the planned sample size . The outcome of the interim was that the planned sample size was sufficient, and no additional participants were required. This was endorsed by the DMC.

No participants received treatment with dornase alfa for longer than 7 days.

8.2 Discussion of Study Design, including the Choice of Control Groups

CRP was 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 (Póvoa 2008). Thus, CRP is at the centre of the COVID-19 disease pathway: from NETS to CRP to clinical disease progression.

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

The data derived from the 9 participants who were randomised to the BAC arm of the study who did not receive dornase alfa provided control data for all of the study endpoints.

Additionally, 60 matched controls from a database of people with COVID-19 who received BAC at UCLH were also used as comparator data for the primary and secondary endpoints. Participants in the database were selected (2 controls for each of the 30 participants who received dornase alfa) to act as controls as follows:

- Apply the inclusion and exclusion criteria of the COVASE study
- Additional selection to identify closest matches using a propensity score based on age, gender, BMI, baseline CRP and the presence/absence of comorbidities.

8.3 Selection of Study Population

The intent was to enrol participants who had the hyper-inflammatory form of COVID-19 but did not require mechanical ventilation. Therefore, hospitalised people with COVID-19 were enrolled.

8.3.1 Inclusion criteria

1. Male and female participants, aged ≥ 18 years
2. Participants who are hospitalised for suspected Coronavirus (SARS-CoV)-2 pneumonia confirmed by polymerase chain reaction (PCR) test or radiological confirmation
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

8.3.2 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:
    a. Respiratory distress with respiratory rate >=40 breaths/min
    b. 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

8.3.3 Removal of participants from therapy or assessment

A participant could be withdrawn from trial treatment whenever continued participation was no longer in the participant's best interests. The decision to withdraw a participant from treatment should be recorded in the CRF and medical notes.

The decision of the participant to withdraw from treatment or follow-up had to 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.

40 evaluable participants were required to meet the primary endpoint, Therefore, if a participant withdrew or was withdrawn, a replacement participant may have been enrolled to the same treatment arm.

8.4 Treatments

Participants were randomised to receive BAC plus dornase alfa or BAC alone.

8.4.1 Treatments administered

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 supplied directly from the manufacturer (Roche) as required. Handling and management of dornase alfa was subject to standard procedures of the pharmacy. The dornase alfa was not modified in any way but administered as approved.

The eRapid Nebulizer System from PARI is approved to deliver dornase alfa and to reduce treatment times (three minutes to deliver 2.5mg).

8.4.2 Identity of investigational product(s)

Batch numbers

- N0293B01
- N0005B13
- N0007B11
- N0008B15
- N0009B08

8.4.3 Method of assigning participants to treatment groups

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

Following participant consent, and confirmation of eligibility the randomisation procedure described in appendix 15.1.7 was carried out.

8.4.4 Selection of doses in the study

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

8.4.5 Selection and timing of dose for each participant

Twice daily dosing was administered in the COVASE Trial to maximise the potential to observe an effect.

8.4.6 Blinding

This was an open-label trial.

8.4.7 Prior and concomitant therapy

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

BAC consisted of symptomatic relief: antipyretics, analgesics and intravenous fluids if needed. Dexamethasone (6ng for 10 days or until hospital discharge whichever was sooner) was included in BAC at the commencement of the Trial. In addition, some participants needed supplemental oxygen and/or mechanical ventilation.

There is a potential risk that other medications (e.g. remdesivir and tocilizumab) administered as BAC may affect the endpoints in the study e.g. decrease CRP. However, this cannot be avoided and was considered in the analysis plan for the data.

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

8.4.8 Treatment compliance

Treatment with dornase alfa was under direct supervision. Monitoring (e.g. watching participant inhale dornase alfa) and recording this appropriately.

8.5 Efficacy and Safety Variables

8.5.1 Efficacy and safety measurements assessed and flow chart

The following trial specific procedures were 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

The following assessments and procedures were conducted Day1, Day3, Day5 and Day7 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 were recorded if/when they occurred

- 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)

8.5.2 Appropriateness of measurements

All measurements were appropriate for the clinical setting and objectives of the study.

8.5.3 Primary efficacy variable(s)

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 (Sharifpour et al. 2020). Thus, CRP is at the centre of the COVID-19 disease pathway: from NETS to CRP to clinical disease progression.

8.5.4 Drug concentration measurements

No pharmacokinetic measurements were undertaken because this is an approved drug with well-documented exposure parameters and it is inhaled with very little systemic availability. In the dornase alfa label, no increase in serum DNase concentration greater than 10ng/ml was observed, following administration of 2500 U (2).

8.6 Data Quality Assurance

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

Data was collected on Trial specific case report forms (CRFs). The Case Report Forms (CRFs) have 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 were pseudo-anonymised (according to standard operating procedures) prior to sending data externally for analysis. This was clearly explained to the participant in the Participant information sheet.

Source data were contained in source documents and were 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 was implemented prior to the start of the trial to identify:

- which data was to be recorded directly onto the CRF;
- which data was recorded firstly into source documents, such as medical notes, and then transcribed into the CRF; and
- which data was not to be recorded in the CRF, but only recorded in source documents, e.g., participant questionnaires and diary cards.

A trial specific data management SOP was in place for the trial. This contained details of the software 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 were transferred electronically in accordance with the UK Data Protection Act 1998 as well as UCL Information Security Policy and Trust Information Governance Policy. There is a documented record of data transfer and measures in place for the recovery of original information after transfer.

8.7 Statistical Methods Planned in the Protocol and Determination of Sample Size

8.7.1 Statistical and analytical plans

Statistical methods:

There were multiple analysis populations:

1. Primary analysis population - all evaluable participants randomised to dornase alfa + matched historical comparators. (ITT)
2. Per protocol population - as above but excluding protocol violations.
3. Safety population - all enrolled participants receiving at least one dose of dornase alfa and the comparator groups.
4. Comparator population - the matched historical controls, participants randomised to BAC and historical records linked to biobanked samples.
5. Exploratory analysis population - all evaluable participants randomised to dornase alfa or to BAC plus historical participant data from biobanked samples.

The primary analysis was conducted using the primary analysis population and was based on the ITT principle.

The key baseline data that was used to compare the groups and the analysis populations were age, gender, BMI, baseline CRP and the presence/absence of comorbidities. In general, continuous data was summarised using the mean, standard deviation, median, minimum and maximum and categorical data was represented as frequency counts and percentages.

An interim analysis was conducted when 12 participants had been randomised. The results of the interim analysis were used to re-estimate the sample size if necessary. The interim analysis was conducted by an independent statistician in a secure, password protected environment. The analysis involved the production of least square means from the primary endpoint analysis, a listing of AEs and descriptive statistics for baseline characteristics by study population and by treatment. No formal statistical test between the treatment groups was performed. The sample size re-estimation did not result in the recruitment of more subjects than originally planned.

8.7.2 Determination of sample size

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 participants, this difference would be achievable and clinically relevant.

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

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

This gave the correct ratio of active versus comparator (ratio of 1:2). To achieve the required power, 30 participants in the active treatment group and at least 60 in the control were required. An additional 9 out of 10 planned participants were recruited as a control for the exploratory objectives and to compare the characteristics of enrolled participants with the historical controls. This gave a total of 39 out of the planned 40 participants enrolled in the study and 60 historical controls.

Participants who dropped out of the study were expected to be replaced so the sample size related to the number of evaluable participants required.

A re-estimation of the sample size was carried out following an interim analysis when 12 participants had been randomised.

8.8 Changes in the Conduct of the Study or Planned Analyses

One participant, randomised to receive BAC alone, had no CRP measurement after dosing (they were discharged prior to blood draw). It proved impossible to replace this participant in a reasonable time frame (several weeks) due to competition from other trials as well as the availability of vaccination. Therefore, the TMG recommended stopping recruitment without replacing this participant. This recommendation was endorsed by the DMC.

Therefore, 30/30 evaluable participants received dornase alfa plus BAC, 9/10 participants received BAC alone and 60/60 comparator controls were identified.

Some changes to the planned analyses took place:

The following changes were made to the Tables, Figures and Listings (TFLs) in the outputs compared to the planned Tables, Figures and Listings:

Table/Figure/Listing	Change	Reason for change
Table 1	Changed the first protocol violation which defines the per-protocol population from: "Initiated dornase-alfa prior to Dexamethasone To:	If we follow the initial definition, only the BAC + dornase-alfa group could be excluded, but in the interest of treating both groups as similarly as possible, in the BAC arm, randomisation date was used as a proxy for dornase-alfa initiation date.

	"Initiated dornase-alfa/randomised prior to Dexamethasone"	
Table 2	Removed summary of "Days between diagnosis and hospitalisation"	COVID-19 diagnosis date not available for COVASE participants.
Table 2	Removed summary "Days between hospitalisation and baseline"	Hospitalisation admission date not available for COVASE participants.
Listing 1a	Included relationship to study drug in listing 1a	This is an important aspect and was an oversight in the mock TFLs.
Table 26a	Included a Table which includes only SAEs that were treatment related.	There were inconsistencies with SAE reporting with regards to Type 1 and Type 2 respiratory failure. By definition, all participants randomised to COVASE were in Type 1 respiratory failure due to their diagnosis of COVID-19, and some may progress to Type 2 respiratory failure as part of expected disease progression. During the course of the COVASE study, SAE reporting was discussed with the sponsor, and Type 1 and Type 2 respiratory failure were not considered to be SAEs and were not reported to the sponsor. However, this decision was not reflected in the database. Therefore, Type 1 and Type 2 respiratory failure were included as SAEs in the locked database. However, they were not treatment-related SAEs. The team decided to keep the database unchanged and report the treatment related SAEs that occur in >5% of the population in a separate Table: Table 26a.

The following details of analyses were implemented in the analysis scripts that were not stated in/changed from the SAP.

Table/Figure/Listing	**Change**	**Reason for change**
Table 10	Imputed missing pre-dexamethasone CRPs, by fitting a linear regression model, with log(pre-Dexamethasone CRP) as the outcome, and age, BMI, key comorbidity and	To maximise available data, imputation was performed to generate pre-Dexamethasone CRP measurements for the COVASE participants who did not have them (COV001 and COV019). Then all individuals could be included in the sensitivity analysis where the

	sex as covariates. Used the predictions from this model to impute pre-Dexamethasone CRPs for those individuals with missing pre-Dexamethasone CRPs.	matching was performed based on pre-Dexamethasone CRP. These imputed values were used for both the propensity score matching, and as covariates in the model for the sensitivity analysis corresponding to Table 10.
Any tables with baseline CRP included as part of the analysis. (Tables 2, 4, 5, 6, 7, 8, 9, 11, 13, 14, 15, 16, 17, 21, 22, 25)	For COVASE participants, "baseline CRP" was taken to be the Day 1 CRP measurement from the database, as opposed to the last CRP prior to randomisation.	The SAP states that the baseline CRP value should be taken as "the last CRP measurement prior to randomisation, or the first CRP following Dexamethasone for historical controls". However, some of the COVASE participants did not have CRP measurements prior to randomisation and after starting Dexamethasone, as their screening CRP is the same as their Day 1 CRP. Thus, it was appropriate to make the baseline CRP value the Day 1 value from the database, as opposed to a measurement prior to randomisation. This was appropriate as, although it was measured after randomisation, it was measured prior to dornase-alfa initiation and after dexamethasone initiation, which was the intention of using the last measurement prior to randomisation when the SAP was written.
Table 11a	Strata defined by treatments received from day of baseline to 7 days post-baseline.	In the initial analysis (Table 11) there were 4 participants who were on either Remdesivir or Toculizumab prior to randomisation, but came off these treatments before starting dornase alfa. It was felt it was more appropriate to define the strata based on the time period from randomisation to 7 days post-randomisation. Therefore Table 11a was generated and takes precedence over Table 11.
Tables 18 & 19	Only values measured on Day 1, 3, 5, 7 and 35 in the Historical controls included in the analyses.	This was not specifically stated in the SAP but, in order to ensure the data from the Historical controls and the COVASE participants was as comparable as possible, the blood markers collected on days other than day 1, 3, 5, 7 and 35 were removed from the analysis. This is because only these values would be included from the COVASE participants.

All Tables including Historical controls.	In addition to applying the inclusion and exclusion criteria (where possible) to the historical control dataset prior to propensity score matching, the following additional filtering was applied: 1) those who had oxygen saturation at Dexamethasone initiation was <90%. 2) those Historical Controls that were on the ICU ward at the start of their Dexamethasone treatment, or in the ICU ward on the same day as starting Dexamethasone.	Historical Controls matching criteria 1) were used as a proxy for the exclusion criteria that states: Serious condition meeting one of the following: I. respiratory distress with respiratory rate >=40 breaths/min II. oxygen saturation <=93% on high-flow oxygen Historical Controls matching criteria 2) would be very unlikely to be randomised into COVASE in reality, so were excluded.
Potentially any Table including CRP as part of the analysis: (Tables 2, 4, 5, 6, 7, 8, 9, 11, 13, 14, 15, 16, 17, 21, 22, 25)	Anomalies from CRP measurements were removed prior to matching or analyses. Anomalies were initially identified by applying the following criteria: Any 5-fold increase that comes back down within 48 hours of the increase. Then manually inspected and agreed or otherwise by Principal Investigator.	These anomalies would have been removed from the COVASE participants, so in the interest of treating the two datasets in the same way, the same criteria was applied to the Historical Controls to minimise any bias.

9 STUDY PARTICIPANTS

9.1 Disposition of Participants

Thirty-one participants were randomised to BAC + dornase-alfa, with ten randomised to BAC. The 60 historic controls were all treated at the same site and during a similar timeframe as the randomised participants.

One participant (COV026) from the BAC + dornase alfa group withdrew consent prior to receiving any treatment and were therefore replaced by participant COV126. Thus, there were thirty evaluable participants in the BAC + dornase-alfa group

One participant (COV010) from the BAC group had a baseline CRP measurement and no more, hence were unevaluable. Thus, there were nine evaluable participants randomised to the BAC group.

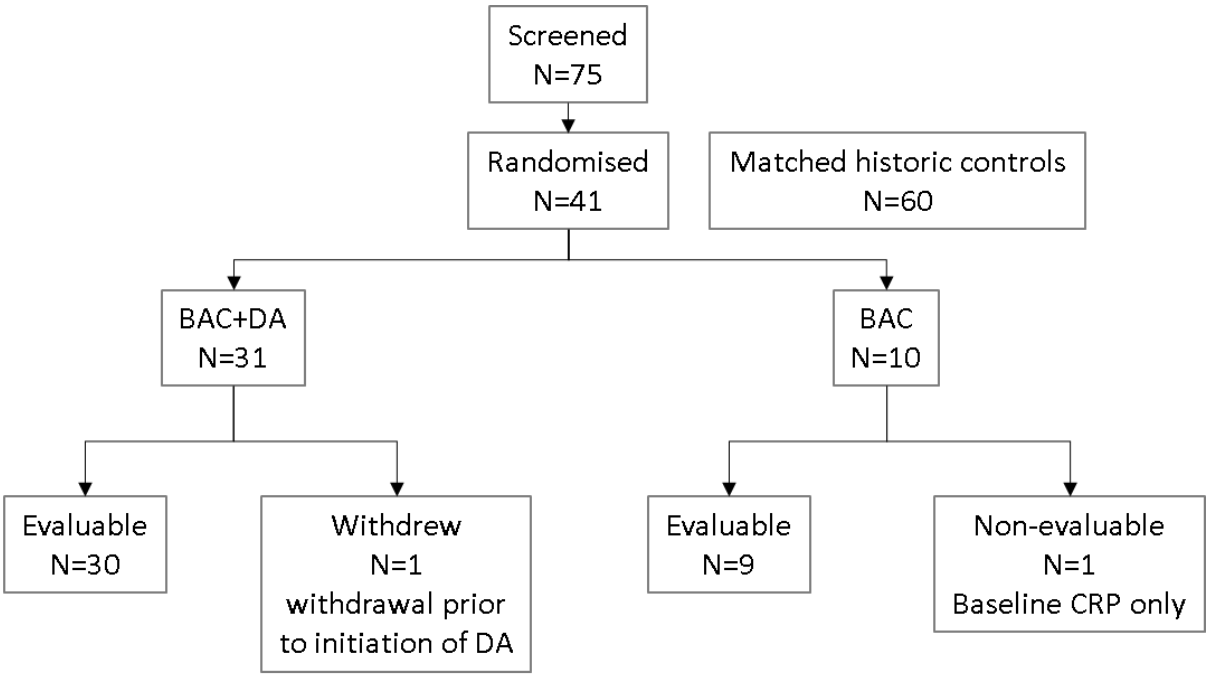

Figure 1: Disposition of participants
All evaluable participants completed follow-up. Abbreviations: DA, dornase alfa.

Source: TFLs Table 1.

9.2 Protocol Deviations

Protocol deviations that defined the per-protocol population are listed below:

- One participant, COV001, had not initiated Dexamethasone at the time of initiation of dornase alfa. Whilst strictly not a protocol violation, this participant was excluded from the per-protocol population so the conclusions could be interpreted in the context of everyone in the analysis being on Dexamethasone as part of their best available care.
- One participant, COV002, withdrew from the study prior to 7 days follow up, due to an adverse event, “tingling of the mouth, cough, shortness of breath”.

10 EFFICACY EVALUATION

10.1 Data Sets Analysed

Intention-to-treat analysis set

In total, 39 participants were randomised and included in the intention-to-treat analysis set, 30 in the BAC + dornase-alfa group and 9 in the BAC group. The two participants excluded from the ITT populations:

- One participant in the BAC group had a baseline CRP measurement, then was discharged from hospital prior to having a second CRP measurement. This makes this participant non-evaluable according to the SAP analysis populations. Therefore, this participant was excluded from all analyses, except for the safety analyses.
- One participant withdrew consent prior to receiving any dose of dornase-alfa. Therefore, this participant was replaced, and excluded from all analyses.

Per protocol population

A further two participants were excluded from the per-protocol population, one from the BAC + dornase-alfa group, and one from the BAC group.

- The exclusion from the BAC + dornase-alfa group was due to a treatment discontinuation after one dose of dornase-alfa.
- The exclusion from the BAC group was due to the participant never starting Dexamethasone treatment, and therefore they did not get randomised prior to initiation of Dexamethasone. This participant was randomised prior to this treatment being widely used in the treatment of COVID-19 and was the only person in the analysis set not to be on Dexamethasone at the start of follow-up.

10.2 Demographic and Other Baseline Characteristics

Propensity score matching

The propensity score matching of each covariate is similar between the COVASE participants and the historical controls. These are summarised in CSR Table 1.

Table 1 Covariates used in propensity score

Covariates	BAC + dornase alfa	Historic controls
Baseline CRP (mean mg/L)	101.9	100.7
Age (mean years)	56.8	57.3
Sex (% Male)	76.7	75.0
BMI (mean kg/m^2)	27.8	27.8
Comorbidity (% with key comorbidity)	46.7	53.3

Source: TFLs Tables 2 and 3, Figures 1-10

These results imply that the propensity score matching was effective at balancing the characteristics included in the matching in the COVASE participants randomised to BAC + dornase-alfa to the historical controls.

Table 2 Baseline Characteristics

	Randomised to BAC + dornase-	Randomised to BAC (N=9)	Historical controls (N=60)	All BAC (N=69)	Total (N=99)

	alfa (N=30)				
Age (years)					
N	30	9	60	69	99
Mean	56.8	53.3	57.3	56.8	56.8
SD	12.5	13.7	14.5	14.3	13.7
Median	58.0	53.0	57.0	57.0	57.0
Min	32.0	31.0	23.0	23.0	23.0
Max	77.0	76.0	86.0	86.0	86.0
Gender					
Female N (%)	7 (23.3)	2 (22.2)	15 (25.0)	17 (24.6)	24 (24.2)
Male N (%)	23 (76.7)	7 (77.8)	45 (75.0)	52 (75.4)	75 (75.8)
BMI (kg/m^2)					
N	30	9	60	69	99
Mean	27.8	30.8	27.8	28.2	28.0
SD	4.7	7.8	5.6	6.0	5.6
Median	26.5	28.9	27.9	28.2	27.7
Min	20.7	22.6	16.3	16.3	16.3
Max	41.7	48.4	43.8	48.4	48.4
Baseline CRP (mg/L)					
N	30	9	60	69	99
Mean	101.9	91.9	100.7	99.5	100.2
SD	52.2	68.1	68.3	67.8	63.3
Median	86.3	74.6	75.8	75.3	79.6
Min	25.2	18.9	30.8	18.9	18.9
Max	261.5	221.6	336.4	336.4	336.4
Key Comorbidity					
No N (%)	16 (53.3)	3 (33.3)	28 (46.7)	31 (44.9)	47 (47.5)
Yes N (%)	14 (46.7)	6 (66.7)	32 (53.3)	38 (55.1)	52 (52.5)

Source: TFLs Table 2

Baseline characteristics were generally well balanced across groups (Source: TFLs Table 2).

- The overall mean age was 56.8 years (mean in BAC + dornase-alfa group=56.8 years, mean in BAC group=56.8 years).

- The percentage of Males was 75.8% overall (76.7% BAC + dornase-alfa group, 75.4% BAC group).
- The most prevalent ethnicity was "White British", with 30.3% of participants identifying in that category overall (33.3% BAC + dornase-alfa group, 29.0% BAC group).
- The overall mean BMI was 28.0kg/m2 (mean in BAC + dornase-alfa group=27.8kg/m2, mean in BAC group=28.2kg/m2).
- The mean baseline CRP as defined in the primary analysis was 100.2mg/L (mean in BAC + dornase-alfa group=101.9mg/L, mean in BAC group=99.5mg/L).
- The overall proportion of individuals with a key comorbidity, defined as one or more of hypertension, diabetes or cardiovascular disease, was 52.5% (46.7% BAC + dornase-alfa group, 55.1% BAC group).

The last pre-dexamethasone CRP means were also similar between groups, with an overall mean of 125.0mg/L (mean in BAC + dornase-alfa group=128.1mg/L, mean in BAC group=122.7mg/L). The days between Dexamethasone initiation and baseline was 1.2 days overall (mean in BAC + dornase-alfa group=0.7 days, mean in BAC group=1.3 days).

There were imbalances noted in means at baseline between the groups in white blood cell count, neutrophil count, procalcitonin count and D-dimer (Source: TFLs Table 3).

10.3 Measurements of Treatment Compliance

Treatment was directly observed during nebulisation in hospital and each treatment was noted in the patient medication administration records (MAR).

10.4 Primary Efficacy Results

The primary objective was to assess the effect of dornase alfa on CRP in hospitalised participants with COVID-19.

The least squares mean (95% CI) on the log scale in the BAC + dornase-alfa group was 3.15 (2.87, 3.42), and in the BAC group was 3.55 (3.35, 3.75). This corresponds to a treatment effect two-sided p-value of 0.010, which is statistically significant at an alpha of 0.05. On the real scale, the ratio of the least squares mean in the BAC + dornase-alfa group to the BAC group is 0.67, indicating a reduction in mean CRP of approximately 33% in the BAC + dornase-alfa group compared to the BAC group at the mean follow-up time over 7 days follow-up (CSR Table 3 and CSR Figure 2).

Table 3 CRP over 7 days follow-up by treatment: ITT population including all individuals (BAC + dornase-alfa, BAC & historical controls).

CRP (mg/L)	Randomised to BAC + dornase-alfa (N=30)	All BAC (N=69)	Difference between BAC + dornase-alfa and BAC	p-value*
N	30	69		
Least-squares mean log(CRP)*	3.15	3.55	-0.4	0.010
Lower bound of 95% CI of least squares mean log(CRP)*	2.87	3.35	-0.71	
Upper bound of 95% CI of least squares mean log(CRP)*	3.42	3.75	-0.10	

Least-square mean CRP**	23.23	34.82	0.67
Lower bound of 95% CI of least squares mean CRP**	17.71	28.55	0.49
Upper bound of 95% CI of least squares mean CRP**	30.46	42.47	0.91

Source: TFLs Table 4.

**From linear repeated measures model, adjusted for log(baseline CRP), age, sex, BMI, serious condition, time, treatment, a treatment*time interaction, and subject as a random effect. Least squares means compared at mean follow-up time.*

***Antilog of estimates from *. Ratio of BAC + dorna-alfa: BAC shown in the difference column.*

These data re illustrated (Figure 2).

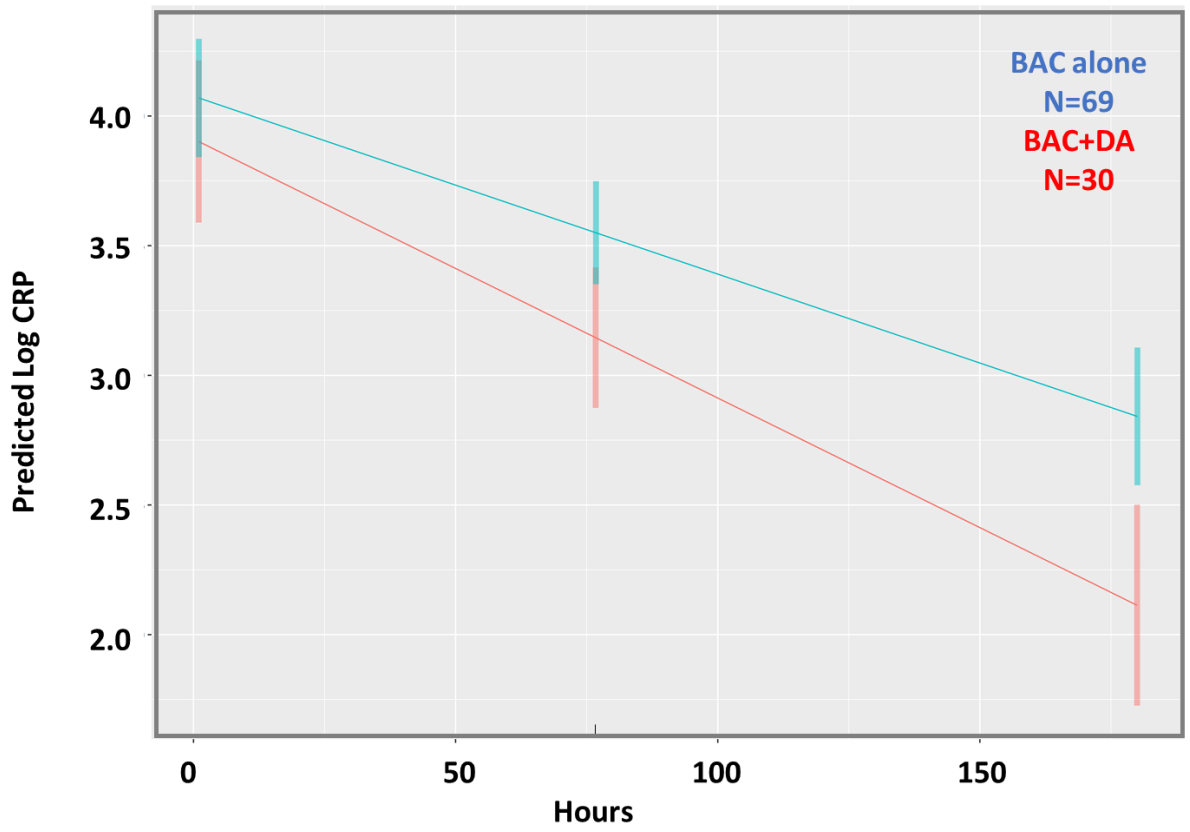

Figure 2 CRP in BAC vs BAC+DA (ITT population)
Source: TFLs Table 4

10.4.1 Sensitivity analysis

In order to test the robustness of this positive result, various sensitivity/supplementary analyses were conducted.

Per-protocol population analysis

Source: TFLs Table 5.

In participants who were included in the per-protocol population, the treatment effect was a 35% reduction in CRP (p=0.006) at the mean follow-up time over 7 days follow-up.

Randomised participant only analysis

Source: TFLs Table 6

In participants who were randomised to the COVASE Study (excluding the historic controls) , the treatment effect was a 39% reduction in CRP (p=0.041) at the mean follow-up time over 7 days follow-up.

BAC + dornase-alfa group and historical control groups only analysis

Source: TFLs Table 7

Comparing the randomised participants in the BAC + dornase alfa group with the historic controls (excluding the randomised BAC controls) , the treatment effect was a 31% reduction in CRP (p=0.019) at the mean follow-up time over 7 days follow-up.

Area under the log(CRP) curve analysis

Source: TFLs Table 9

Using the log(CRP) AUC, standardised by number of days follow-up, the least squares mean log(CRP) AUC was lower in the BAC + dornase alfa group compared to the BAC group (p=0.043) over 7 days follow-up.

Propensity score matching using pre-Dexamethasone CRP

Source: TFLs Table 10

In the main analysis, the Day 1 CRP measurement was used in the propensity score matching, which was usually after initiation of Dexamethasone but prior to the initiation of dornase alfa. In order to determine the sensitivity of this definition of "baseline", the same analysis as the primary analysis was conducted, but the last pre-Dexamethasone CRP was used in the matching and as the baseline CRP to adjust for in the model. The magnitude and direction of treatment effect was reasonably consistent with the primary analysis, with the least squares mean being 41% lower in the BAC + dornase alfa group compared to the BAC group (p = 0.007) at the mean follow-up time over 7 days follow-up.

Analysis stratified by type of BAC received

Source: TFLs Table 11a

The primary analysis was stratified by the type of BAC the participants were receiving at any point during follow-up, from baseline to 7 days post baseline. The most important changes in BAC were the introduction of remdesivir and tocilizumab to BAC during the COVASE study.

In the 51 participants who did not receive either remdesivir or tocilizumab (12 BAC + dornase alfa group, 39 BAC group) the treatment effect was a 36% reduction in CRP (p=0.079) in the BAC + dornase alfa vs BAC alone at the mean follow-up time over 7 days follow-up.

In the 39 participants (16 BAC + dornase-alfa group, 23 BAC group) who were on remdesivir but not tocilizumab the treatment effect was a 29% reduction in CRP (p=0.123) in the BAC + dornase alfa vs BAC alone at the mean follow-up time over 7 days follow-up.

Given the small number of participants that were either on Tocilizumab and not Remdesivir, or who were on both Tocilizumab and Remdesivir, the least squares mean estimates were unable to be estimated due to failure of convergence in the mixed models.

In summary, the changes in BAC during the COVASE study did not materially affect the primary conclusion.

10.5 Secondary Efficacy Results

10.5.1 CRP over 35 days follow-up

Source: TFLs Table 13

Analysing the CRPs up to 35 days after randomisation showed that the treatment effect was a 16% reduction in CRP (p=0.358) at the mean follow-up time over 35 days follow-up.

10.5.2 Length of hospitalisation over 35 days follow-up

Source: TFLs Table 14

Length of hospitalisation after 35 days follow up from baseline was analysed. The least squares mean (95% CI) in the BAC + dornase-alfa group was 6.40 (2.39, 10.42) days, and in the BAC group was 10.75 (7.88, 13.61). This corresponds to a treatment effect two-sided p-value of 0.061, which does not reach statistical significance.

Source: TFLs Table 14a

In order to assess whether that trend towards reduced hospitalisation length in the BAC + dornase-alfa group was driven by the historical control population only, a sensitivity analysis was performed, excluding the historical controls from the analysis set. The least squares mean (95% CI) in the BAC + dornase-alfa group was 6.23 (1.66, 10.79) days, and in the BAC group was 12.73 (4.51, 20.95). The trend towards reduced hospitalisation length observed in the full ITT population was consistent with the direction of effect in the randomised participants only analysis (p = 0.135).

Source: TFLs Table 15

The length of hospitalisation was also analysed as a time-to-event outcome, defined as time from baseline to hospital discharge, censored at 35 days follow-up or death. The hazard ratio observed in the Cox proportional hazards model (95% CI) was 1.63 (1.01, 2.61), which estimates that throughout 35 days follow-up, there was a 63% higher chance of discharge at any given time point in the BAC + dornase-alfa group compared to the BAC group (p = 0.030).

Source: TFLs Table 15a

In order to assess whether the hazard ratio was driven by the historical control population only, a sensitivity analysis was performed, excluding the historical controls from the analysis set. The hazard ratio observed in the Cox proportional hazards model (95% CI) was 1.18 (0.52, 2.69), indicating that the effect direction is consistent with Table 15.

10.5.3 Length of ICU stay over 7 and 35 days of follow-up

Source: TFLs Table 16

Four analyses were conducted, none of which reached statistical significance.

1. The length of ICU stay over 7 days follow-up was analysed. The least squares mean (95% CI) in the BAC + dornase-alfa group was 21.25 (4.65, 37.84) hours, and in the BAC group was 19.85 (8.00, 31.70) hours. This corresponds to a treatment effect two-sided p-value of 0.883, which does not reach statistical significance at an alpha of 0.05.
2. The proportion of participants in each group that were on the ICU at any point during 7 days follow-up was compared. In the BAC + dornase-alfa group 7 (23.3%) participants went onto the ICU at any point over 7 days follow-up, compared to 15 (21.74%) in the BAC group. This corresponds to a treatment effect two-sided p-value of 0.866, which does not reach statistical significance at an alpha of 0.05.
3. The length of ICU stay over 35 days follow-up was investigated. The least squares mean (95% CI) in the BAC + dornase-alfa group was 55.21 (-23.59, 134.00) hours, and in the BAC group was 60.60 (4.34, 116.86) hours. This corresponds to a treatment effect two-sided p-value of 0.905, which does not reach statistical significance at an alpha of 0.05.
4. The proportion of participants in each group that were on the ICU at any point during 35 days follow-up. In the BAC + dornase-alfa group 7 (23.3%) participants went onto the ICU at any point over 35 days follow-up, compared to 16 (23.19%) in the BAC group. This corresponds to a treatment effect two-sided p-value of 0.983, which does not reach statistical significance at an alpha of 0.05.

10.5.4 Time on oxygen over 7 and 35 days of follow-up

Source: TFLs Table 17

Time on oxygen between groups, at 7 days follow-up was compared. The least squares mean (95% CI) in the BAC + dornase-alfa group was 94.32 (72.86, 115.79) hours, and in the BAC group was 88.96 (73.64, 104.29). This corresponds to a treatment effect two-sided p-value of 0.662, which does not reach statistical significance at an alpha of 0.05.

Time on oxygen between groups, at 35 days follow-up was compared. The least squares mean (95% CI) in the BAC + dornase-alfa group was 133.22 (52.01, 214.43) hours, and in the BAC group was 156.35 (98.36, 214.33), corresponding to a treatment effect two-sided p-value of 0.618, which does not reach statistical significance at an alpha of 0.05.

Source: TFLs Table 17a

A supplementary analysis was performed, excluding the historical controls from the analysis set and including only randomised participants.

Time on oxygen between groups, at 7 days follow-up was compared. The least squares mean (95% CI) in the BAC + dornase-alfa group was 92.93 (69.32, 116.55) hours, and in the BAC group was 82.78 (40.26, 125.30). This corresponds to a treatment effect two-sided p-value of 0.646, which does not reach statistical significance at an alpha of 0.05.

Time on oxygen between groups, at 35 days follow-up was compared. The difference between least squares means is greater, with the least squares mean (95% CI) in the BAC + dornase-alfa group being 123.52 (29.13, 217.90) hours, and in the BAC group was 241.69 (71.76, 411.61), corresponding to a treatment effect two-sided p-value of 0.187, which does not reach statistical significance at an alpha of 0.05.

10.5.5 Whole blood counts and differential, procalcitonin and d-dimer over 7 days follow-up

Source: TFLs Table 18

With no adjustment for multiple testing, a significant difference in means between the two groups at the mean follow-up time at a two-sided alpha of 0.05 was observed in three blood counts:

1. Lymphocyte count (increase in BAC + dornase-alfa group vs. BAC group),
2. Procalcitonin levels (decrease in BAC + dornase-alfa group vs. BAC group)
3. D-dimer levels (decrease in BAC + dornase-alfa group vs. BAC group).

Table 4 Whole blood count and differential plus procalcitonin and D-dimer over 7 days follow-up by treatment: ITT population including all individuals (BAC + dornase-alfa, BAC & historical controls).

	Randomised to BAC + dornase-alfa (N=30)	**All BAC (N=69)**	**Difference between BAC + dornase-alfa and BAC**	**p-value**
Lymphocyte count ($\times 10^9$/L)**				
N	30	61		
Least-square mean*	1.08	0.87	1.25	0.021
Lower bound of 95% CI of least squares mean*	0.92	0.76	1.03	
Upper bound of 95% CI of least squares mean*	1.27	0.98	1.51	
Procalcitonin levels (ng/mL)				
N	26	7		
Least-square mean*	0.18	1.31	-1.13	0.005
Lower bound of 95% CI of least squares mean*	-0.2	0.56	-1.88	
Upper bound of 95% CI of least squares mean*	0.56	2.05	-0.37	

D-dimer (ug/L) FEU**				
N	28	11		
Least-square mean*	570.78	1656.96	0.34	0.004
Lower bound of 95% CI of least squares mean*	384.51	876.93	0.17	
Upper bound of 95% CI of least squares mean*	847.3	3130.81	0.69	

** From linear repeated measures model, adjusted for baseline endpoint, age, sex, BMI, serious condition, time, treatment, a treatment*time interaction, and subject as a random effect. Least squares means compared at mean follow-up time.*
***Modelled by log transforming the outcome. Estimates shown are the antilog of the estimates from the fitted model. Ratio of BAC + dornase-alfa: BAC shown in the difference column.*

There was no significant treatment effect at the mean follow-up time over 7 days in whole blood count, neutrophil count, monocyte count, eosinophil count and basophil count.

Source: TFLs Table 18a

After supplementary analysis, excluding the historical control population, the effect of dornase alfa on these three blood markers still reached statistical significance.

10.5.6 WHO ordinal scale over 7 days follow-up (including COVASE participants only)

Source: TFLs Table 20

The least-squares mean WHO ordinal score at the mean follow-up time over 7 days in each group was estimated. In the BAC + dornase-alfa group the mean (95% CI) was 4.86 (4.40, 5.33) and in the BAC group was 4.91 (4.05, 5.77). This corresponds to a two-sided p-value of 0.916, which does not reach statistical significance at an alpha of 0.05. This analysis included only randomised participants, as longitudinal WHO data were not available for historical controls.

10.5.7 Survival at Day 35

Source: TFLs Table 21

Over 35 days follow up, 1 person in the BAC + dornase-alfa group died, compared to 8 in the BAC group. The hazard ratio observed in the Cox proportional hazards model (95% CI) was 0.47 (0.06, 3.86), which estimates that throughout 35 days follow-up, there was a 53% reduced chance of death at any given timepoint in the BAC + dornase-alfa group compared to the BAC group, though the confidence intervals are wide due to a small number of events. The p-value from a log-rank test was 0.460, which does not reach statistical significance at an alpha of 0.05. CSR Figure 3 shows the Kaplan-Meier curves.

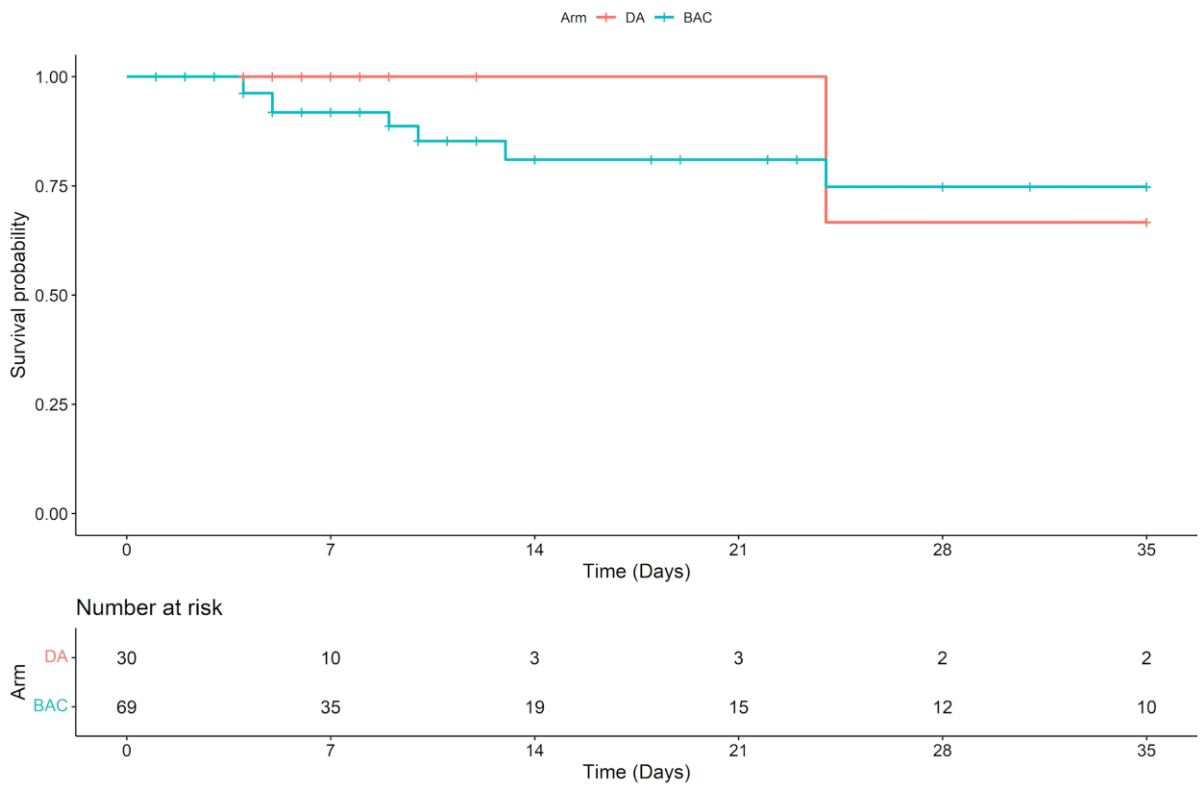

Figure 3 Kaplan-Meier plot showing survival probability over time: ITT population including all individuals (BAC + dornase-alfa, BAC & historical controls).
Source: TFLs Figure 15.

10.5.8 Mechanical ventilation over 7 and 35 days of follow-up

Source: TFLs Table 22

The number of participants that required mechanical ventilation in the BAC + dornase-alfa group was 5 (16.67%), compared to 9 (13.04%) in the BAC group. This corresponds to a two-sided treatment effect p-value of 0.628, which is not statistically significant at an alpha of 0.05. The result is the same over both 7 days follow-up and 35 days follow-up

Source: TFLs Table 23

The mean length of mechanical ventilation at 7 days follow-up in the BAC + dornase-alfa group was 76.8 hours, compared to 88.78 in the BAC group. At 35 days follow-up, the mean length of mechanical ventilation in the BAC + dornase-alfa group was 76.8, compared to 411.17 in the BAC group.

10.5.9 Proportion of participants with pneumonia over 7 and 35 days of follow-up

Source: TFLs Table 25

Over 7 days follow-up, 1 (3.33%) participant in the BAC + dornase-alfa group had pneumonia, compared to 3 (4.35%) participants in the BAC group. This corresponds to a treatment effect odds ratio (95% CI) of 0.90 (0.08, 10.21) and a two-sided p-value of 0.934, which does not reach statistical significance at an alpha of 0.05.

Over 35 days follow-up, 2 (6.67%) participants in the BAC + dornase-alfa group had pneumonia, compared to 3 (4.35%) participants in the BAC group. This corresponds to a treatment effect odds ratio (95% CI) of 1.81 (0.26, 12.61) and a two-sided p-value of 0.548, which does not reach statistical significance at an alpha of 0.05.

10.6 Additional Statistical considerations

10.6.1 Adjustments for Covariates

Covariates included in the models were age, gender, BMI, baseline CRP (defined as the Day 1 CRP value for randomised participants, or the first CRP following Dexamethasone for Historical Controls), and whether they have a key comorbidity, defined as one or more of hypertension, diabetes or cardiovascular disease.

10.6.2 Handling of Dropouts or Missing Data

Mixed models were used to analyse most endpoints; these models handle missing data naturally. Therefore, all available data will be included in all models

10.6.3 Sample Size Re-estimation

At the interim analysis, baseline demographics, subject disposition and primary endpoint analyses were carried out. This occurred after the first 12 randomised individuals completed Day7 follow-up.
A sample size re-estimation was performed based on a promising zones approach (Mehta and Pocock 2011).

At the interim stage, the following individuals were included:

- 8 individuals randomised to BAC + dornase alfa
- 4 individuals randomised to BAC only
- 18 individuals (2 × the number of individuals randomised to BAC + dornase alfa) included from the matched historical control cohorts

This gave a total of 30 individuals included in the analysis.
The recommendation after the sample size re-estimation was to continue recruiting to 40 randomised participants.

10.6.4 Multicentre Studies

COVASE was a single-centre study.

10.6.5 Multiple Comparisons/Multiplicity

This study was not powered to detect any effects relating to secondary endpoints. Therefore, the secondary analyses were not adjusted for multiple comparisons, but all results that reach statistical significance should be interpreted with caution.

10.6.6 Use of an "Efficacy Subset" of Participants

No ‘efficacy’ subsets were identified.

10.6.7 Active-Control Studies Intended to Show Equivalence

Not applicable.

10.6.8 Examination of Subgroups

No subgroups were examined. Other than the described analyses stratified by BAC.

10.6.9 Individual response data

Individual log(CRP) data are presented for 7 days (CSR Figure 4) and 35 days (CSR Figure 5).

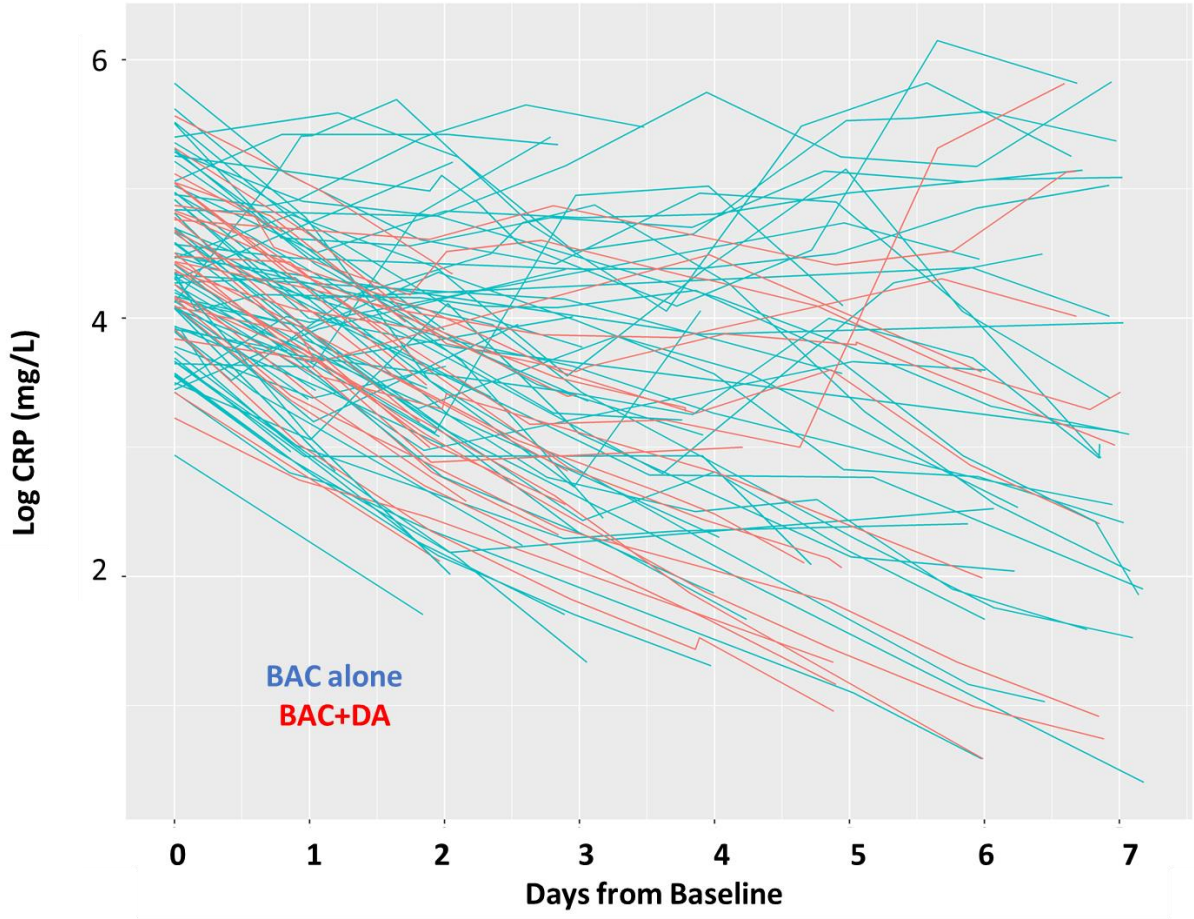

Figure 4 Individual log(CRP) over 7 days (by arm)

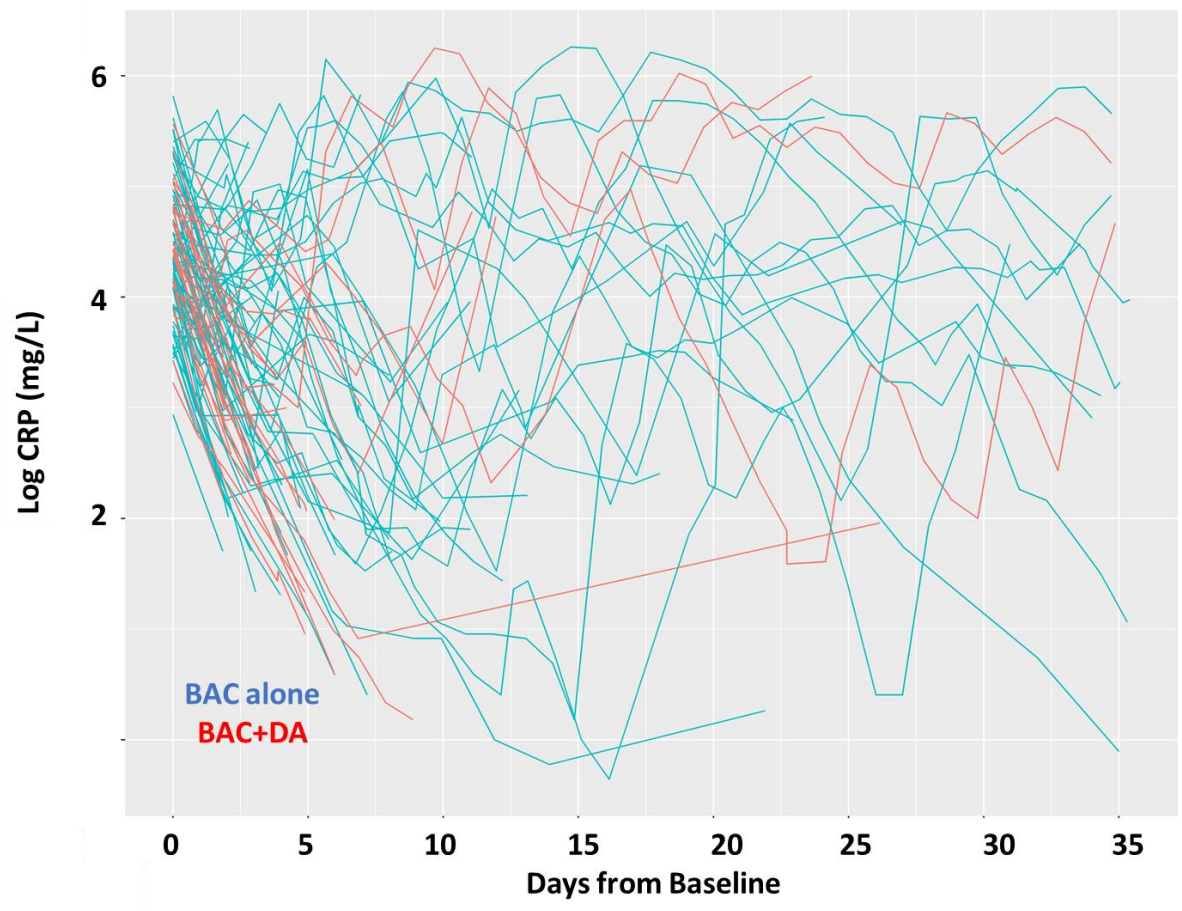

Figure 5 Individual log (CRP) over 35 days (by arm)

10.6.10 Drug dose, drug concentration, and relationships to response

No pharmacokinetic measurements were undertaken.

10.6.11 Drug-drug and drug-disease interactions

No potential drug-drug or drug-disease interactions were expected or observed.

10.6.12 By-participant displays

See CSR Figures 4 and Figure 5.

10.7 Efficacy conclusions

The COVASE study met its primary endpoint to show a reduction in CRP due to administration of dornase alfa for 7 days in participants hospitalised for COVID-19. This reduction was robust, as it was consistently observed in all sensitivity analyses.

Several important secondary endpoints also showed statistically significant effects of dornase alfa.

A time-to-event analysis of baseline to discharge from hospital showed a 63% greater chance of discharge from hospital at any given time over 35 days follow-up in the dornase alfa group ($p = 0.03$) compared to the BAC group, and a median time to discharge of 6 days in the dornase alfa group compared to 7 days in the BAC group. This result was supported by non-

significant trends in other endpoints related to hospitalisation (for example length of hospitalisation).

Dornase alfa had no detectable effect on any endpoint related to ICU (length of stay over 7 and 35 days; proportion of participants admitted to ICU during 7 and 35 day of follow-up).

Dornase alfa treatment resulted in a significant increase in blood lymphocyte count and a significant decrease in procalcitonin and d-dimer.

Dornase alfa had no detectable effect on WHO ordinal scale, survival, mechanical ventilation or the proportion of participants with pneumonia.

In conclusion, dornase alfa treatment resulted in a significant reduction in CRP. This effect resulted in significant clinical effects on a selection of secondary endpoints, including hospitalisation, differential blood count (specifically lymphocytes) and relevant biomarkers, procalcitonin and d-dimer.

11 SAFETY EVALUATION

11.1 Adverse Events (AEs)

As described in the SAP, the safety of dornase alfa was assessed by comparisons of adverse events (AEs), serious adverse events (SAEs), treatment-emergent adverse events (TEAEs) and deaths. The safety population was defined as all participants randomised to either BAC + dornase alfa or BAC only.

11.1.1 Brief summary of adverse events

The AEs are summarised (CSR Table 5).

In the BAC group, 4 out of 9 participants (44%) reported 14 AEs (CSR Table 5). None of which was listed as related to study drug.

In the BAC + dornase alfa group 19 out of 30 participants (63%) reported a total of 44 AEs (TFLs Listing 1a). Of these AEs 42 were considered not related to study drug (CSR Table 5) and 1 was considered to be definitely related to study drug (CSR Table 5). This was tingling of the mouth, struggle to sleep (CSR Table 8). One AE in this group (headache) was considered unlikely to be related to study drug (CSR Table 8).

11.1.2 Display of adverse events

Table 5 Summary of AEs

	BAC **n=9**	**BAC+DA** **n=30**
Total AEs reported	14	44
Number (%) of participants reporting at least one AE	4 (44%)	19 (63%)
Number (%) of AEs not related to study drug	14 (100%)	42 (96%)

Number (%) of AEs unlikely related to study drug	0 (0%)	1 (2%)
Number (%) of AEs definitely related to study drug	0 (0%)	1 (2%)

Source: TFLs Listing 1a

11.1.3 Analysis of adverse events

There were 14 reported AEs in the randomised BAC arm (CSR Table 6) versus 44 in the dornase alfa arm (CSR Table 7). The adverse event data reflect the clinical trial and post-marketing experience of using Pulmozyme at the recommended dose regimen. Adverse reactions attributed to Pulmozyme are rare (< 1/1000). In most cases, the adverse reactions are mild and transient in nature and do not require alterations in Pulmozyme dosing (SmPC Pulmozyme).(https://www.medicines.org.uk/emc/product/1112/smpc#UNDESIRABLE_EFFECTS

11.1.4 Listing of adverse events by participant

Table 6 Adverse Events in BAC group

Subject	BAC + dornase-alfa, or BAC only?	Adverse event	Serious ?	Relationship to study drug
COV001	BAC	Haemorrhoids - rectal bleed	No	Not related
COV001	BAC	Suspected Ephysema	No	Not related
COV001	BAC	Diarrhoea	No	Not related
COV011	BAC	Fresh blood on stool	No	Not related
COV011	BAC	Bradycardia	No	Not related
COV025	BAC	Desaturation 88%	No	Not related
COV025	BAC	Dry mouth	No	Not related
COV025	BAC	Dry nose	No	Not related
COV025	BAC	Increased confusion	No	Not related
COV025	BAC	Pulmonary embolism	Yes	Not related
COV025	BAC	Pulmonary hypertension	Yes	Not related
COV027	BAC	Large right subdural haematoma	Yes	Not related
COV027	BAC	Constipation	No	Not related
COV027	BAC	Aspiration pneumonia	Yes	Not related

Source: TFLs Listing 1a

Table 7 Adverse Events in the BAC + dornase alfa group not related to study drug

Subject	BAC + dornase-alfa, or BAC only?	Adverse event	Serious ?	Relationship to study drug
COV002	Dornase-alfa + BAC	Cough & SOB	No	Not related
COV003	Dornase-alfa + BAC	Mild depression	No	Not related
COV003	Dornase-alfa + BAC	Mild cognitive impairment	No	Not related
COV005	Dornase-alfa + BAC	Constipation	No	Not related
COV005	Dornase-alfa + BAC	Struggle to sleep	No	Not related
COV005	Dornase-alfa + BAC	Transaminitis (ALT 91 - NR 10-35 iu/L)	No	Not related

COV007	Dornase-alfa + BAC	Pulmonary Embolism	Yes	Not related
COV007	Dornase-alfa + BAC	Blood stain in sputum	No	Not related
COV012	Dornase-alfa + BAC	Type 2 Respiratory Failure	Yes	Not related
COV012	Dornase-alfa + BAC	Type 2 Respiratory Failure	Yes	Not related
COV012	Dornase-alfa + BAC	Small Pericardial Effusion	No	Not related
COV012	Dornase-alfa + BAC	Dysphonia	No	Not related
COV012	Dornase-alfa + BAC	Hypercapnia	No	Not related
COV013	Dornase-alfa + BAC	Bradycardia	No	Not related
COV013	Dornase-alfa + BAC	Ulcerative Colitis flare	No	Not related
COV015	Dornase-alfa + BAC	Mechanical Fall	No	Not related
COV015	Dornase-alfa + BAC	Dizziness	No	Not related
COV018	Dornase-alfa + BAC	Haemoptysis	No	Not related
COV018	Dornase-alfa + BAC	Dehydration	No	Not related
COV018	Dornase-alfa + BAC	Lower Respiratory Tract Infection	No	Not related
COV020	Dornase-alfa + BAC	Chest pain	No	Not related
COV021	Dornase-alfa + BAC	Pyelonephritis	Yes	Not related
COV021	Dornase-alfa + BAC	Severe respiratory distress	Yes	Not related
COV021	Dornase-alfa + BAC	Organising Pneumonia due to COVID 19	Yes	Not related
COV022	Dornase-alfa + BAC	Microcytic anaemia	No	Not related
COV022	Dornase-alfa + BAC	Type 1 respiratory failure	Yes	Not related
COV022	Dornase-alfa + BAC	Elevated Blood glucose	No	Not related
COV023	Dornase-alfa + BAC	Tachypnoea (PR 32BPM)	No	Not related
COV023	Dornase-alfa + BAC	Hyperglycaemia (BM 14.9)	No	Not related
COV031	Dornase-alfa + BAC	Chest Pain	No	Not related
COV032	Dornase-alfa + BAC	Hospital Acquired Pneumonia	Yes	Not related
COV032	Dornase-alfa + BAC	Non-occlusive Pulmonary embolism	Yes	Not related
COV035	Dornase-alfa + BAC	Type 1 respiratory failure	Yes	Not related
COV035	Dornase-alfa + BAC	Left leg spasm	No	Not related
COV035	Dornase-alfa + BAC	Rectal bleed due to haemorrhoids	No	Not related
COV037	Dornase-alfa + BAC	Chest Pain	No	Not related

COV037	Dornase-alfa + BAC	Type 1 respiratory failure	Yes	Not related
COV039	Dornase-alfa + BAC	Dizzy spells	No	Not related
COV039	Dornase-alfa + BAC	Tingling in feet	No	Not related
COV039	Dornase-alfa + BAC	Chest Tightness	No	Not related
COV040	Dornase-alfa + BAC	Type 1 respiratory failure	Yes	Not related
COV126	Dornase-alfa + BAC	Polyarthralgia after dexamethasone use	No	Not related

Source: TFLs listing 1a

Table 8 Adverse Events in the BAC + dornase alfa group definitely/unlikely related to study drug

Subject	BAC + dornase-alfa, or BAC only?	Adverse event	Serious ?	Relationship to study drug
COV002	Dornase-alfa + BAC	Tingling of the mouth	No	Definitely
COV035	Dornase-alfa + BAC	Headache	No	Unlikely

Source: Listing 1a

11.2 Deaths, Other Serious Adverse Events, and Other Significant Adverse Events

There were 4 SAEs reported in the BAC group and 12 SAEs in the Dornase-alfa + BAC group. In the BAC group the SAEs were reported by 2 participants (22%) and in the Dornase-alfa + BAC group 8 participants (27%) reported SAEs. None of these SAEs were considered to be related to study drug (CSR Table 9 and TFL Listing 1a).

Table 9 Serious Adverse Events

Subject	BAC + dornase-alfa, or BAC only?	Adverse event	Serious?	Relationship to study drug
COV007	Dornase-alfa + BAC	Pulmonary Embolism	Yes	Not related
COV012	Dornase-alfa + BAC	Type 2 Respiratory Failure	Yes	Not related
COV012	Dornase-alfa + BAC	Type 2 Respiratory Failure	Yes	Not related
COV021	Dornase-alfa + BAC	Severe respiratory distress	Yes	Not related
COV021	Dornase-alfa + BAC	Pyelonephritis	Yes	Not related
COV021	Dornase-alfa + BAC	Organising Pneumonia due to COVID 19	Yes	Not related
COV022	Dornase-alfa + BAC	Type 1 respiratory failure	Yes	Not related
COV032	Dornase-alfa + BAC	Non-occlusive Pulmonary embolism	Yes	Not related
COV032	Dornase-alfa + BAC	Hospital Acquired Pneumonia	Yes	Not related
COV035	Dornase-alfa + BAC	Type 1 respiratory failure	Yes	Not related
COV037	Dornase-alfa + BAC	Type 1 respiratory failure	Yes	Not related
COV040	Dornase-alfa + BAC	Type 1 respiratory failure	Yes	Not related
COV025	BAC	Pulmonary embolism	Yes	Not related

COV025	BAC	Pulmonary hypertension	Yes	Not related
COV027	BAC	Large right subdural haematoma	Yes	Not related
COV027	BAC	Aspiration pneumonia	Yes	Not related

Source TFLs Listing 1a

Amongst randomised participants only, there were SAEs of Type 2 respiratory failure and Type 1 respiratory failure reported. These were in the Dornase-alfa + BAC group, with an incidence rate of 1.147 per patient year for Type 2 respiratory failure and 2.294 per patient year for Type 1 respiratory failure (CSR Table 9). However, they were not treatment-related SAEs.

However, there were inconsistencies with SAE reporting with regards to Type 1 and Type 2 respiratory failure during the trial. By definition, all participants randomised to COVASE were in Type 1 respiratory failure due to their diagnosis of COVID-19, and some may progress to Type 2 respiratory failure as part of expected disease progression. During the course of the COVASE study, SAE reporting was discussed with the sponsor, and Type 1 and Type 2 respiratory failure were not considered to be SAEs and were not reported to the sponsor. However, this decision was not reflected in the database. Therefore, Type 1 and Type 2 respiratory failure were included as SAEs in the locked database. However, they were not treatment-related SAEs. The team decided to keep the database unchanged and report the treatment related SAEs that occur in >5% of the population in a separate Table: Table 26a. There are no treatment related SAEs that occurred in >5% of participants, thus this Table is empty.

11.2.1 Listing of deaths, other serious adverse events, and other significant adverse events

There was one death out of 30 randomised participants in the BAC + dornase alfa group, censored at day 35. There were 8 deaths in the BAC group (8 out of 60 historic controls and 0 out of 9 randomised) over a similar timeframe.

11.2.1.1 Deaths

Table 8: Number of deaths at day 35

	Randomised to BAC + dornase-alfa (N=30)	**All BAC (N=69)**
Survival at day 35		
Number of deaths (%)	1 (3.3%)	8 (11.6%)

Source: TFLS Table 21

11.2.1.2 Other Serious Adverse Events

None observed

11.2.1.3 Other Significant Adverse Events

None observed

11.2.2 Narratives of deaths, other serious adverse events, and certain other significant adverse events

All deaths and SAEs were attributable to COVD-19.

11.2.3 Analysis and discussion of deaths, other serious adverse events, and other significant adverse events

11.3 Clinical Laboratory Evaluation

Clinical laboratory tests were not evaluated as part of the COVASE study.

11.4 Vital Signs, Physical Findings, and Other Observations Related to Safety

Vital signs and physical examinations were not evaluated as part of the COVASE study

11.5 Safety Conclusions

In general, dornase alfa was very well tolerated. There were no systemic effects which is consistent with dornase alfa having a short half-life and not absorbed from the lung. Studies in rats and monkeys after inhalation of dornase alfa show very little systemic absorption (less than 15% for rats and less than 2% for monkeys). Dornase alfa is also associated with very low accumulation with no serum concentration greater than 10ng/mL observed no matter the dose administered. The only side effects are local reactions such as tingling of the mouth as seen here or hypersensitivity/ allergic reactions which are rare and no serious allergic reactions have been described.

12 DISCUSSION AND OVERALL CONCLUSIONS

The COVASE study (open-label, randomised, controlled trial) of dornase alfa in participants hospitalised due to COVID-19, achieved its primary endpoint of reducing CRP. In fact, nebulised dornase alfa resulted in a statistically and clinically significant reduction of 33% in CRP in participants randomised to receive BAC+DA compared to participants randomised to BAC alone and historic BAC controls. The effect on CRP was robust, as it was consistently observed in all sensitivity analyses. Dornase alfa was safe and well-tolerated in this population.

The population recruited into this study consisted of participants with COVID-19 pneumonia, who had been admitted to hospital with moderate symptoms and were at risk of progression to ventilatory failure. They were randomised (3:1) to receive BAC with the addition of nebulised dornase alfa at 2.5mg twice per day (BID) for 7 days or BAC alone. The use of historic controls from the same centre, was supported by extensive matching using propensity scoring based on age, gender, BMI, baseline CRP and the presence/absence of comorbidities. This indicated that the historic controls matched the randomised participants against these criteria.

CRP was chosen as the Primary Endpoint in the COVASE study because it is a clinically important marker of inflammation and is used to make clinical treatment decisions (Sharifpour et al. 2020). It is induced by the exuberant inflammation mediated by neutrophil

extracellular traps (NETs) and inflammatory histones during COVID-19. CRP is a prognostic marker and correlates with clinical symptoms and response to therapy (Sharifpour et al. 2020). Thus, CRP is at the centre of the COVID-19 disease pathway: from NETS to CRP to clinical disease progression. A treatment-related reduction in CRP would be expected to result in clinical benefit. Calculation of the sample size, based on CRP as the primary endpoint, also resulted in a feasible trial size that was deliverable at a single center in a reasonable timeframe.

Several important secondary endpoints also showed statistically significant effects of dornase alfa. There was a 63% greater chance of discharge from hospital at any given time over 35 days follow-up in the dornase alfa group ($p = 0.03$) compared to the BAC group, and a median time to discharge of 6 days in the dornase alfa group compared to 7 days in the BAC group. This result was supported by non-significant trends in other clinical endpoints (for example length of hospitalisation). Dornase alfa treatment resulted in a significant increase in blood lymphocyte count. As the presence of lymphopaenia is associated with a nearly threefold increased risk of severe COVID-19 (Zhao et al. 2020), an increased lymphocyte count due to dornase alfa could suggest a potentially beneficial effect. Dornase alfa treatment was also associated with a significant decrease in PCT. PCT levels are used to predict disease progression and secondary bacterial infection in COVID-19 (Wang et al. 2020). In fact, PCT levels > 1.00 ng/mL are associated with secondary bacterial infections and poor prognosis (Wang et al. 2020). In the COVASE study PCT levels in the BAC control group were 1.31 ng/mL, whereas dornase alfa resulted in a reduction to 0.18 ng/mL. This observed reduction is likely to be associated with clinical benefit. Dornase alfa treatment also resulted in a significant decrease in d-dimer. D-dimer is a fibrin degradation product and is a marker of fibrinolysis in COVID-19-associated coagulopathy and disseminated intravascular coagulation (Asakura and Ogawa 2021). D-dimer on admission for COVID-19 at greater than 2000.0 µg/L may effectively predict in-hospital mortality in patients, which indicates D-dimer could be an early and helpful marker to improve management of Covid-19 patients (Zhang et al. 2020). In the COVASE study, D-dimer levels in the dornase alfa-treated group were 570.78 µg/L and in the BAC control group 1656.96 µg/L, supporting a potential clinical benefit for dornase alfa treatment.

Dornase alfa had no detectable effect on any endpoint related to ICU (length of stay), WHO ordinal scale, survival, mechanical ventilation or the proportion of participants with pneumonia. This is not surprising as the study was not powered to detect effects in these endpoints.

In conclusion, the COVASE trial met its Primary Endpoint and dornase alfa treatment resulted in a significant reduction in CRP. This effect resulted in significant clinical effects on a selection of secondary endpoints, including hospitalisation, differential blood count (specifically lymphocytes) and relevant biomarkers, procalcitonin and d-dimer. Dornase alfa was safe and well-tolerated in hospitalised COVID-19 participants These data may be used to optimally design a subsequent efficacy trial of dornase alfa in COVID-19.

13 Tables, Figures and Listings

14 REFERENCE LIST

Asakura, Hidesaku, and Haruhiko Ogawa. 2021. 'COVID-19-associated coagulopathy and disseminated intravascular coagulation', International Journal of Hematology, 113: 45-57.

Mehta, Cyrus R, and Stuart J Pocock. 2011. 'Adaptive increase in sample size when interim results are promising: a practical guide with examples', Statistics in medicine, 30: 3267-84.

Póvoa, Pedro. 2008. 'Serum markers in community-acquired pneumonia and ventilator-associated pneumonia', Current opinion in infectious diseases, 21: 157-62.

Sharifpour, Milad, Srikant Rangaraju, Michael Liu, Darwish Alabyad, Fadi B. Nahab, Christina M. Creel-Bulos, Craig S. Jabaley, Covid-Quality on behalf of the Emory, and Collaborative Clinical Research. 2020. 'C-Reactive protein as a prognostic indicator in hospitalized patients with COVID-19', PLoS one, 15: e0242400.

The Recovery Collaborative Group. 'Dexamethasone in Hospitalized Patients with Covid-19'. 2020. New England Journal of Medicine, 384: 693-704.

Wang, Dawei, Bo Hu, Chang Hu, Fangfang Zhu, Xing Liu, Jing Zhang, Binbin Wang, Hui Xiang, Zhenshun Cheng, and Yong Xiong. 2020. 'Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China', Jama, 323: 1061-69.

Zhang, Litao, Xinsheng Yan, Qingkun Fan, Haiyan Liu, Xintian Liu, Zejin Liu, and Zhenlu Zhang. 2020. 'D-dimer levels on admission to predict in-hospital mortality in patients with Covid-19', Journal of Thrombosis and Haemostasis, 18: 1324-29.

Zhao, Qianwen, Meng Meng, Rahul Kumar, Yinlian Wu, Jiaofeng Huang, Yunlei Deng, Zhiyuan Weng, and Li Yang. 2020. 'Lymphopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: A systemic review and meta-analysis', International Journal of Infectious Diseases, 96: 131-35.

15 APPENDICES

15.1 Study Information

The following information is provided

15.1.1 Protocol and protocol amendments

15.1.2 Representative written information for participant and sample consent forms

15.1.3 Randomisation scheme and codes (participant identification and treatment assigned)

15.1.4 Documentation of statistical methods

15.1.5 Documentation of inter-laboratory standardisation methods and quality assurance procedures if used

Primary and Secondary laboratory endpoints were measured by the UCLH clinical laboratory.

15.1.6 Publications based on the study

https://www.medrxiv.org/content/10.1101/2022.04.14.22272888v1