

Nebulised dornase alfa reduces inflammation and improves clinical outcomes in severe COVID-19: a randomised clinical trial

Reviewed Preprint

Revised by authors after peer review.

About eLife's process

Reviewed preprint version 2
November 2, 2023 (this version)

Reviewed preprint version 1
June 13, 2023

Posted to medRxiv
April 22, 2023

Sent for peer review
March 17, 2023

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Abstract

Background

Cell-free (cf)-DNA, from cellular sources, including Neutrophil Extracellular Traps (NETs), is found in the circulation of COVID-19 patients and may contribute to immune dysregulation. This study determined whether pulmonary administration of the endonuclease, dornase alfa, reduced systemic inflammation by degrading local and systemic cf-DNA.

Methods

Eligible patients were randomized (3:1) to receive twice-daily nebulised dornase alfa in addition to best available care (BAC) or BAC alone for seven days or until discharge. A 2:1 ratio of matched contemporary controls (CC) provided additional comparators. The primary endpoint was improvement in C-reactive protein (CRP) over time, analysed using a repeated-measures mixed model, adjusted for baseline factors.

Results

Between June 2020–October 2021 we recruited 39 evaluable patients: 30 randomised to dornase alfa; 9 randomised to BAC; with 60 CC. Dornase alfa reduced CRP by 33% compared

to BAC. Least squares (LS) mean post-dexamethasone CRP fell from 101.9mg/L to 23.23 mg/L in the BAC+ dornase alfa group versus a fall from 99.5mg/L to 34.82 mg/L in the BAC group at 7 days; $P=0.01$. This effect of dornase alfa on CRP was confirmed with subgroup and sensitivity analyses. Dornase alfa increased the chance of live discharge by 63% (HR 1.63, 95% CI 1.01 to 2.61, $P=0.03$), increased lymphocyte counts (LSmean 1.08 vs 0.87, $P=0.02$) and reduced circulating cf-DNA and the coagulopathy marker D-dimer (LS mean: 570.78 vs 1656.96 $\mu\text{g/mL}$, $P=0.004$). Dornase alfa was well-tolerated.

Conclusions

We provide proof-of-concept evidence that dornase alfa reduces pathogenic inflammation in hospitalised patients with COVID-19 pneumonia.

eLife assessment

This small-sized clinical trial comparing nebulized dornase-alfa to the best available care in patients hospitalized with COVID-19 pneumonia is **valuable**, but in its present form the paper is **incomplete**.

Introduction

SARS-CoV-2 pneumonia can lead to hyperinflammation, coagulopathy, respiratory failure, and death (Siddiqi and Mehra, 2020 [↗](#); Zhou et al., 2020 [↗](#)), even with less pathogenic variants and in immunised patients (2000 [↗](#)). In severe COVID-19 neutrophil activation drives neutrophil extracellular trap (NET) formation (Radermecker et al., 2020 [↗](#); Zuo et al., 2020 [↗](#)). NETs, are composed of DNA-histone complexes and have been associated with coagulopathy and endothelial dysfunction (Papayannopoulos, 2018 [↗](#)). Moreover, extracellular histones promote inflammation, immune dysfunction and lethality in sepsis (Ioannou et al., 2022 [↗](#); Tsourouktsoglou et al., 2020 [↗](#); Xu et al., 2011 [↗](#); Xu et al., 2009 [↗](#)). Digestion of chromatin DNA by endonucleases suppresses the proinflammatory activity of histones and enables their clearance from the circulation (Ioannou et al., 2022 [↗](#); Tsourouktsoglou et al., 2020 [↗](#)). Consistently, DNase I treatment reduces pathology in murine pulmonary viral infections (Cortjens et al., 2018 [↗](#); Pillai et al., 2016 [↗](#)). Endogenous DNase activity and NET clearance capacity are defective in severe COVID-19 pneumonia (Aramburu et al., 2022b [↗](#)), and the extent of these defects correlates with mortality. Hence, supplementation with exogenous DNases may facilitate extracellular chromatin degradation and increase survival.

Pulmozyme, dornase alfa, is a recombinant human DNAase approved since 1993 for patients with cystic fibrosis (CF) (Konstan and Ratjen, 2012 [↗](#); Lazarus and Wagener, 2019 [↗](#)). Dornase alfa solubilizes NETs, reduces inflammation and improves pulmonary function in chronic and acute exacerbations of CF (Konstan and Ratjen, 2012 [↗](#); Papayannopoulos et al., 2011 [↗](#)). Pulmozyme is safe and well-tolerated in children and adults with CF at doses up to 10mg BD. Nebulized pulmozyme does not increase circulating endonucleases. Hence, it remained unclear whether the degradation of extracellular chromatin in the lungs would reduce circulating chromatin and systemic hyper-inflammation.

To probe the effectiveness of DNase I as an anti-inflammatory therapy in severe COVID-19 pneumonia, we measured as our primary endpoint C-reactive protein (CRP). CRP is a reliable marker of systemic inflammation in severe infection (McArdle et al., 2004 [↗](#)). In patients infected

with COVID-19, a high CRP of ≥ 40 mg/L carried a poor prognosis with a lower cut-off of ≥ 35 mg/L in the elderly (Villoteau et al., 2021 [↗](#)), particularly when combined with lymphopenia and coagulopathy marked by elevated D-dimer (Fisher et al., 2022 [↗](#); Liu et al., 2020 [↗](#); Luo et al., 2020 [↗](#); Smilowitz et al., 2021 [↗](#); Tornling et al., 2021 [↗](#); Ullah et al., 2020 [↗](#)). Poor outcomes are associated with an early rise (Mueller et al., 2020 [↗](#)), higher peak and delayed reduction in CRP (Cui et al., 2021 [↗](#)). Moreover, elevated IL-6 and CRP can predict the need for mechanical ventilation (Herold et al., 2020 [↗](#)). In addition, CRP correlated well with levels of DNase and NET degradation activity in COVID-19 patient plasma suggesting a link between CRP and the capacity to degrade cf-DNA in the circulation (Aramburu et al., 2022b [↗](#)). CRP became a reliable primary endpoint in subsequent trials demonstrating the effectiveness of other systemic therapies such as nanimulab or infliximab that target inflammatory cytokines (Fisher et al., 2022 [↗](#)).

In this proof-of-concept study we evaluated the effect of dornase alfa on inflammation, as measured by the impact on circulating CRP in patients with COVID-19, compared with best available care (BAC). The trial was initiated in June 2020 and was completed in October 2021. At the start of the trial only dexamethasone had been proven to benefit hospitalized COVID-19 pneumonia patients and was thus included in both arms of the trial. To increase the chance of reaching significance under challenging constraints in patient access, we opted to increase our sample size by using a combination of randomized individuals and available CRP data from matched contemporary controls (CC) hospitalized at UCL but not recruited to a trial. These approaches demonstrated that when combined with dexamethasone, nebulized DNase treatment was an effective anti-inflammatory treatment in randomized individuals with or without the implementation of CC data.

Methods

Sponsor and location

The trial was sponsored by University College London (UCL) and carried out at University College London Hospital UCLH with ethical (REC: 20/SC/0197, Protocol: 132333, RAS ID:283091) and UK MHRA approvals. All randomized participants provided informed consent. Consent for CC was covered by Health Service (Control of Participant Information) Regulations 2002. Safety and data integrity were overseen by the Trial Monitoring Group and Data Monitoring Committee. All data was collected at UCLH. Additional CRP data is reported from the Pa-COVID-19 study, Charité Universitätsmedizin Berlin with ethical approval, Berlin (EA2/066/20). Both Pa-COVID-19 and COVASE studies were carried out according to the Declaration of Helsinki and the principles of Good Clinical Practice (ICH 1996).

Trial design

The COVASE trial was a single-site, randomised, controlled, parallel, open-label investigation. The primary endpoint was change in CRP. Screening was performed within 24 hours prior to the administration of dornase alfa (**Figure 1A** [↗](#)). Eligible, consented participants were randomly assigned (3:1) to either BAC plus nebulised dornase alfa (BAC+DA) or BAC alone. On Day 1 a baseline sample was collected. From Day 1-7 participants randomised to active arm received 2.5mg BD nebulised dornase alfa in addition to BAC. In all cases BAC included dexamethasone (6 mg/day) for 10 days or until discharge, whichever was shorter as per RECOVERY (Group et al., 2021 [↗](#)). Participants received additional treatments at the discretion of their physicians. The primary analysis was performed on samples up to Day 7. The final trial visit occurred at day 35.

In addition to the recruited randomised group, we included contemporary controls (CC) to increase the sample size due to practical difficulties in recruiting patients in the UK. For every COVASE participant randomised to active treatment, 2 matched CC were included. CC were admitted to UCLH over the same time as randomised patients and treated with the same BAC,

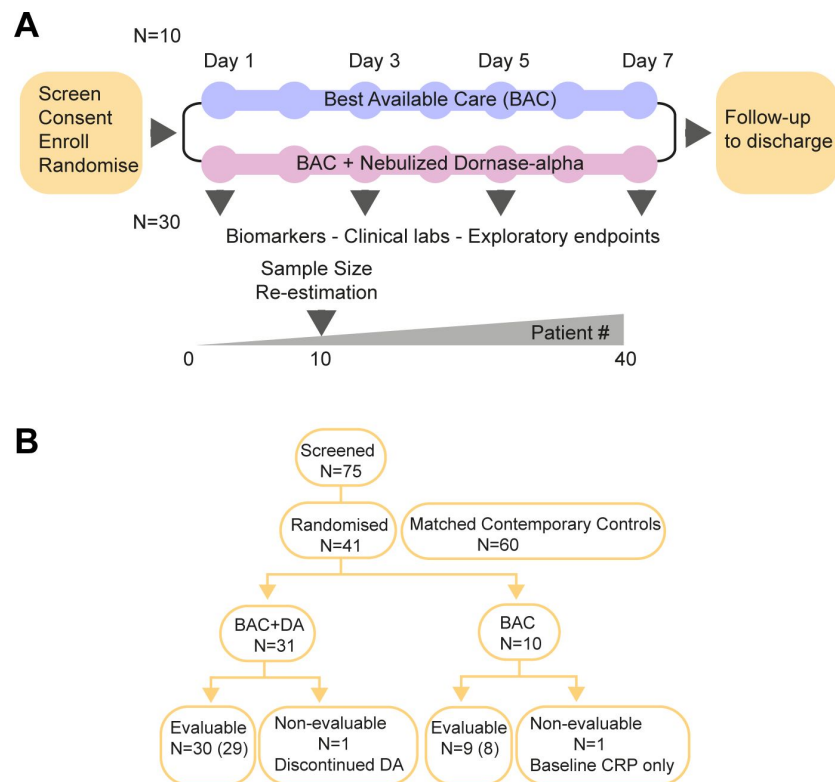


Figure 1.

Trial design and Consort diagram

A. COVASE Trial Design.

B. Consort diagram summary. Numbers not in parentheses indicate the participants in the intention-to-treat population and the numbers in parentheses indicate the number of participants in the per-protocol population. A complete consort diagram is shown in Supplementary figure 1.

including dexamethasone. CCs fulfilled the inclusion and exclusion criteria of the COVASE study and were matched for age, gender, BMI, comorbidities and CRP. CRP matching was based either on admission CRP (CC1), or matched based on first CRP after starting dexamethasone (CC2).

Eligibility

Trial participant inclusion criteria

1. Male and female participants, aged ≥ 18 years Dornase alfa in COVID-19: Sponsor code: 132333 Protocol Version 2.0, 25.4.2020 Page 26 of 51
2. Participants who are hospitalised for suspected Coronavirus (SARS-CoV)-2 infection confirmed by polymerase chain reaction (PCR) test or radiological confirmation with chest CT
3. Participants with stable oxygen saturation ($\geq 94\%$) on supplementary oxygen
4. CRP ≥ 30 mg/L
5. Participants will have given their written informed consent to participate in the study and are able to comply with instructions and nebuliser

Exclusion criteria

1. Females who are pregnant, planning pregnancy or breastfeeding
2. Concurrent and/or recent involvement in other research or use of another experimental investigational medicinal product that is likely to interfere with the study medication within (specify time period e.g. last 3 months) of study enrolment
3. Serious condition meeting one of the following:
 1. Respiratory distress with respiratory rate ≥ 40 breaths/min
 2. oxygen saturation $\leq 93\%$ 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

So briefly

Patients were excluded if they were:

- 1. pregnant, planning pregnancy or breastfeeding
- 2. Serious condition meeting one of the following:
 1. Respiratory distress with respiratory rate ≥ 40 breaths/min
 2. oxygen saturation $\leq 93\%$ on high-flow oxygen
- 4. Require ventilation at screening

- 5. Concurrent severe respiratory disease such as asthma, COPD and/or ILD
- 6. 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”

Participants

Adults (≥ 18 years of age) admitted to UCLH with confirmed SARS-Cov2 infection by RT-PCR and radiologically confirmed COVID-19 pneumonia on chest radiograph or CT-scan; oxygen saturation < 94% requiring supplemental oxygen; and evidence of hyperinflammation (CRP ≥ 30 mg/L, after administration of dexamethasone) were eligible. While all participants had CRP > 30 mg/L at screening, on two occasions the baseline CRP concentrations fell below

30 mg/L after the patients had already agreed to participate. For full inclusion and exclusion criteria see Protocol: Supplementary Appendix 1.

Outcomes

The primary outcome was the least square (LS) mean CRP up to 7 days or at hospital discharge whichever was sooner. Data beyond 7 days was included in the model to estimate the slope. Subsequently, the estimated slope in each group was used in order to calculate the LS mean at 7 days. Pre-specified secondary outcomes included days on oxygen; time to hospital discharge; mortality by day 35; and changes in clinically relevant biomarkers including lymphocyte count and D-dimer levels. Day 35 was chosen as being likely to include most early mortality due to COVID-19 being 4 weeks after completion of a week of treatment. (i.e. d7 of treatment +28 (4 x 7 days)).

Efficacy assessments of primary and secondary outcomes in the intention-to-treat (ITT) population were performed on all randomised participants who had received at least one dose of dornase alfa if randomized to treatment. For full details see Statistical Analysis Plan. The ITT was adjusted to mitigate the following protocol violations where one participant in the BAC arm and one in the DA arm withdrew before they received treatment and provided only a baseline CRP measurement available. The participant in the DA arm was replaced with an additional recruited patient. Exploratory endpoints were only available in randomised participants and not in the CC. In this case, a *post hoc* within group analysis was conducted to compare baseline and post-baseline measurements.

Sample size calculation

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 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.

This study used existing data collected at UCLH from contemporary controls (CC) admitted with COVID-19. This gave a ratio of active versus comparator of 1:2. The required power, would result in 30 participants in the active treatment group and at least 60 in the control. An additional 10 participants were recruited as a control for exploratory objectives and to compare the characteristics of enrolled participants with CC. This gave a total of 40 participants enrolled in the study and 60 CC.

Randomisation

A closed envelope method was implemented to randomize the participants into the control and active treatment groups.

Results

Patient characteristics

From June 2020–October 2021, 41 participants were recruited and randomised. 1 participant in BAC group was discharged before a second CRP measurement, and was excluded from all, except safety, analyses (CONSORT Diagram: **Figure 1B** and **S1**). One participant withdrew consent prior to receiving dornase alfa and was replaced and excluded from all analyses. 39 participants were included in the intention-to-treat analysis set, 30 BAC+DA and 9 BAC. One participant withdrew due to side-effects. This participant was removed from the per protocol population (PPP). All 39 participants were followed up for 35 days or death whichever was sooner. Two participants were excluded from the PPP. One from BAC as this was the only patient in whom randomisation occurred prior to dexamethasone being widely used in COVID-19 and a second participant who withdrew after one dose of dornase alfa (**Figure 1B** and **S1**). The trial ended when 40 eligible participants had been recruited, although thereafter 1 had participant in the BAC arm had to be excluded from the primary and secondary endpoint analysis.

Baseline characteristics were well balanced across groups (**Table 1**). Selection of 60 matched CC participants via propensity score matching was successful in ensuring the means of the characteristics included in propensity score matching were similar (**Table 1** and **Figure S2**). The overall mean age was 56.8 years (56.8 years BAC+DA, 56.8 years BAC). The percentage of males was 75.8% (76.7% BAC+DA, 75.4% BAC). The most prevalent ethnicity was “White British”, with 30.3% of participants identifying in that category (33.3% BAC+DA, 29.0% BAC). The mean BMI was 28.0kg/m² (27.8kg/m² BAC+DA, 28.2kg/m² BAC). The mean baseline CRP (post dexamethasone) was 100.2mg/L (101.9mg/L BAC+DA, 99.5mg/L BAC). The proportion of participants with a comorbidity, defined as one or more of hypertension, diabetes, or cardiovascular disease, was 52.5% (46.7% BAC+DA, 55.1% BAC). All randomised participants except one received dexamethasone prior to randomisation, and 48 of the total 99 participants also received remdesivir or tocilizumab in addition to dexamethasone within the first 7 days. The average duration of dexamethasone treatment prior to dornase alfa was 1.13+/-0.79 days (**Table S1**). The last pre-dexamethasone CRP was also similar between groups, mean of 125.0mg/L (mean 128.1mg/L BAC+DA, 122.7mg/L BAC). The number of days between dexamethasone initiation and baseline was 1.2 days (0.7 days BAC+DA, 1.3 days BAC). There were imbalances noted at baseline between the groups in white blood cell count, neutrophil count, procalcitonin count and D-dimer (**Table S2**).

Clinical outcomes

Longitudinal CRP correlates with disease outcomes

To ascertain the clinical value of CRP as the COVASE trial's primary endpoint, we first examined the correlation of CRP with mortality in a cohort of 63 hospitalized patients with severe COVID-19 (Aramburu et al., 2022b; Kurth et al., 2020; Messner et al., 2020). All participants who reached a WHO severity ordinance scale 7 were recruited at the Charité Hospital in Berlin in spring 2020 and did not receive anti-inflammatory therapies such as dexamethasone or anti-IL-6 antibody treatments. Plasma CRP measurements in 465 samples were segregated by disease outcome. This analysis revealed that deceased participants had significantly higher CRP than participants in the survivor group (**Figure 2A**). Clustering the 63 participant according to their longitudinal average CRP indicated that the frequency of mortality increased as the average CRP

	Random ised to BAC + dornase -alfa (N=30)	Random ised to BAC (N=9)	Contem porary controls (N=60)	All BAC (N=69)	Total (N=99)
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/m²)					
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)

Table 1.

Patient baseline characteristics

per patient increased (**Figure 2B**). Consistently, Mantel Cox survival analysis of the participants segregated into three groups with average longitudinal CRP ranges of 0-100 mg/L, 100-200 mg/L and 200-450 mg/L, showed that survival decreased significantly as the average CRP increased (**Figure 2C**). This results highlighted the strong correlation between CRP and survival in hospitalized individuals with severe COVID-19 pneumonia, suggesting that the blood CRP concentration was a strong predictor of clinical outcomes such as the probability of survival.

Primary outcome

Next, we analysed the results from our COVASE participants whose individual CRP traces over time are shown in **Figure S3A**. Blood collection for both BAC and BAC+DA groups occurred at similar times and comparable frequencies (**Figure S3B**). For the ITT group, the LS mean log (CRP) over 7 days follow-up was 3.15 (95% confidence interval [CI] 2.87 to 3.42) BAC+DA (n=30), and 3.55 (95% CI, 3.35 to 3.75) in BAC (n=69) (**Table 2**, **3** and **Figure 3A**), $p=0.01$. This indicates a reduction in mean CRP of approximately 33% in the BAC+DA (23.23 mg/mL) compared to BAC (34.82 mg/mL) at mean follow-up over 7 days. This effect of dornase alfa on CRP was confirmed in various other subgroup analyses (**Table 2**): the per-modified-protocol population only; the participants who were randomised into the COVASE trial, excluding the CC (**Figure 3B**); participants who were randomised to BAC+DA in the COVASE trial, and CC excluding those randomised to BAC only. In addition, to ensure that the CC did not have a significantly different CRP trajectory to those randomised to BAC, we compared participants who were randomised to BAC with CC by excluding those randomised to BAC+DA and found no significant differences (**Table 2**). Sensitivity analyses supported the observed effect on CRP. These included the log(CRP) as an area under the curve; the CC matched for their last pre-dexamethasone CRP measurement as opposed to their first CRP after starting dexamethasone; and the effect of remdesivir or tocilizumab (**Table 2**).

To better understand the effects of dornase alfa on the CRP of the treated population we also performed a frequency distribution analysis of the change in CRP over time. Instead of fitting a slope on the CRP values of the individual samples of each participant, we plotted the fraction between the CRP measurement of the baseline and final sample collected for each participant during the 7 day treatment period. We then plotted the distribution of participants within the different ranges of change in CRP (**Figure 3C**). Compared to BAC, the BAC+DA treatment group exhibited a distribution towards larger ranges in CRP reduction with better suppression of the fraction of patients that did not respond to treatment as indicated by an increase in CRP (fraction: 1 to 6). These results indicated that the addition of dornase alfa to dexamethasone cause a significant and sustained reduction in CRP in a greater number of COVID-19 infected participants.

Secondary outcomes

Length of hospitalisation was analysed as a time-to-event outcome of alive discharge from hospital censored at 35 days. The hazard ratio observed in the Cox proportional hazards model was 1.63 (95% CI, 1.01 to 2.61), $p=0.03$ (**Table 4**, **Figure 3D** and **S3C**). Showing that throughout 35 days follow-up, there was a 63% higher chance of discharge alive at any given time-point in BAC+DA compared to BAC. Although the rate of discharge was similar in 50% of patients, 80% discharge occurred by 8 days in BAC+DA whereas, whereas the same proportion was reached at 30 days in BAC. This trend was also seen when only the randomised participants were considered, although not powered to reach significance and with a smaller HR of 1.18 (95% CI, 0.52-2.69), $p=0.62$, (**Table S3** and **Figure S3D**).

Over 7 days of follow up there was no significant difference between BAC+DA versus BAC alone in either the fraction of participants admitted to ICU (23.3% versus 21.74%), $p=0.866$, or the length of ICU stay, LS mean 21.25 (95% CI, 4.65 to 37.84) hours versus 19.85 (95% CI, 8.00 to 31.70) hours,

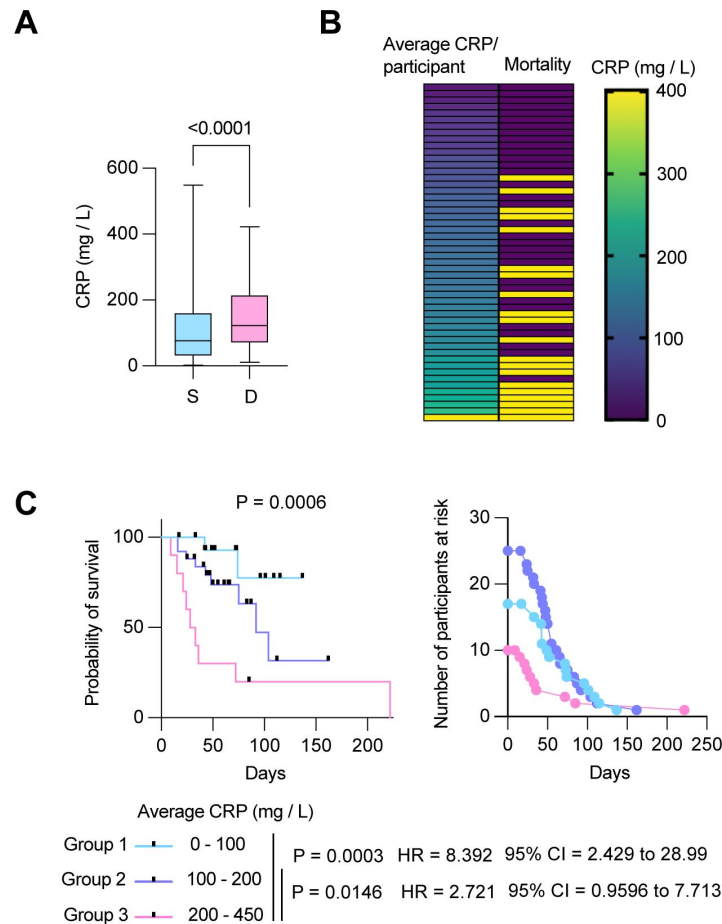


Figure 2.

Longitudinal CRP predicts survival probability in severe COVID-19 pneumonia. Data from the Berlin COVID-19 study

A. Individual CRP concentrations in 465 plasmas from 63 participants with maximum WHO severity grade 7 COVID-19 pneumonia segregated into survivor and deceased groups.

B. Participants ordered by their longitudinal average CRP concentrations shown in the left column. Mortality is depicted in yellow in the right column.

C. Kaplan Meier survival probabilities (left panel) and numbers at risk (right panel) for patients segregated into three categories of longitudinal average CRP ranges: 0-100, 100-200, and 200-450 mg / L. Statistical significance (P), Hazard ratios (HR) and 95% confidence intervals (95% CI) for group 1 against group 3 and group 2 against group 3 are shown below the survival plot.

Statistics by Mann-Whitney and Mantel-Cox log rank tests.

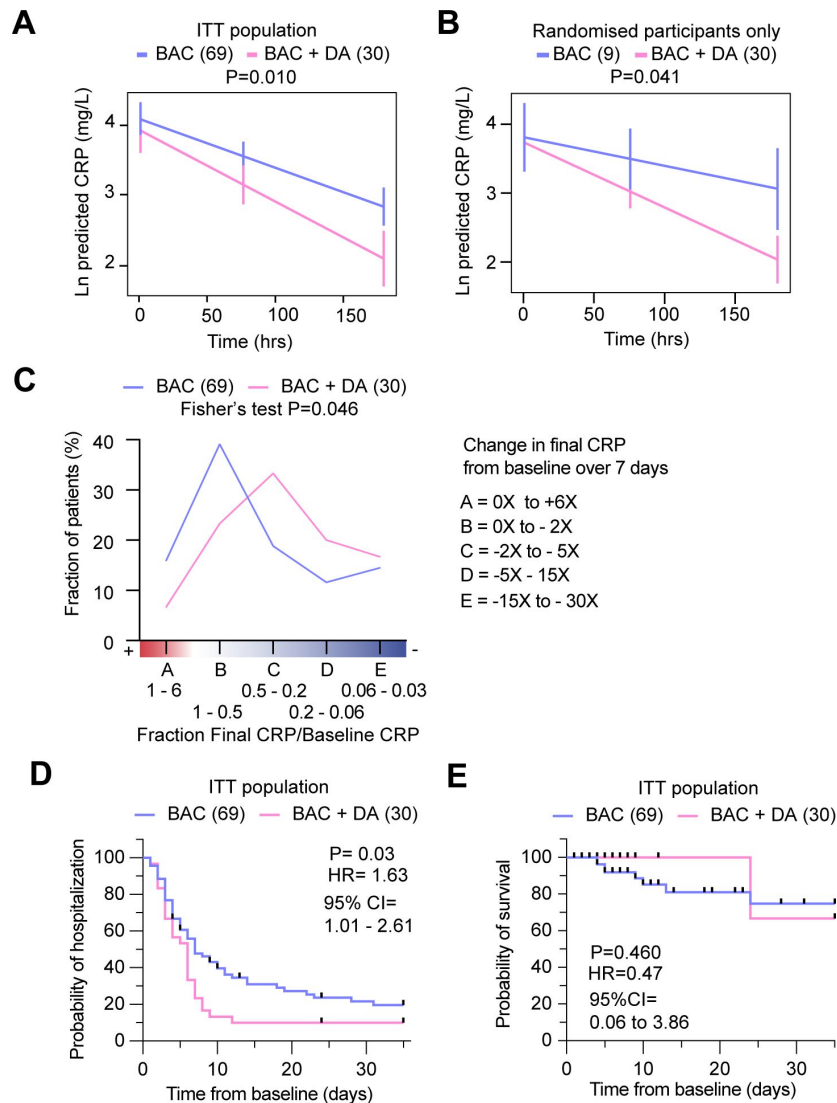


Figure 3.

Analysis of primary and clinical endpoints

A and B. Fitted mean (95% confidence interval) from mixed model, with natural log (CRP) over 7 days follow-up as the outcome, adjusting for natural log baseline CRP, age, sex, BMI, serious comorbidity (Diabetes, Cardiovascular disease or hypertension), time and a treatment $\times$ time interaction. P-value generated by comparing least-square means between arms. (A) Intention to treat (ITT) population (Blue: CC and participants randomised to BAC, N=69; Pink: participants randomised to BAC+DA, N=30) and (B) randomised participants only: (Blue: participants randomised to BAC, N=9; Pink: participants randomised to BAC+DA, N=30).

C. Distribution of participants based on the change in CRP measured as a ratio of the final CRP reading within the 7 day treatment period over the baseline CRP reading per patient. Statistical analysis by Fisher's test.

D. Kaplan-Meier plot showing time to discharge from hospital from baseline. ITT population. Hazard ratio from Cox proportional hazards model adjusted for baseline CRP, age, sex, BMI, serious comorbidity (diabetes, cardiovascular disease or hypertension). P-value from log-rank test. (Blue: CC and participants randomised to BAC, N=69. Pink: participants randomised to BAC+DA, N=30).

E. Kaplan-Meier plot showing time to death over 35 days follow up. ITT population. Hazard ratio from Cox proportional hazards model adjusted for baseline CRP, age, sex, BMI, serious comorbidity (Diabetes, Cardiovascular disease or hypertension). P-value from log-rank test. (Blue: CC and participants randomised to BAC, N=69. Pink: participants randomised to BAC+DA, N=30). Abbreviations: BAC-best available care, CRP-C-reactive protein, DA-dornase alfa, ITT-intention-to-treat.

CRP (mg/L)	Randomised to BAC + dornase- alfa	BAC	Difference between BAC + dornase-alfa and BAC	p-value*
Primary analysis of Primary Endpoint: ITT population including all individuals: (BAC + dornase-alfa, BAC & historical controls).				
N	30	69		
Least-squares mean	3.15	3.55	-0.4	0.010
log(CRP) * (95% CI)	(2.87 to 3.42)	(3.35 to 3.75)	(-0.71 to -0.10)	
Least-square mean	23.23	34.82	0.67	
CRP ** (95% CI)	(17.71 to 30.46)	(28.55 to 42.47)	(0.49 to 0.91)	
Sensitivity Analyses				
PP population including all individuals: (BAC + dornase-alfa, BAC & historical controls)				
N	29	68		
Least-squares mean	3.12	3.55	-0.43	0.006
log(CRP)* (95% CI)	(2.85 to 3.39)	(3.36 to 3.74)	(-0.73 to -0.13)	
Least-square mean	22.64	34.82	0.65	
CRP** (95% CI)	(17.35 to 29.54)	(27.7 to 42.21)	(0.48 to 0.88)	
ITT population including randomised individuals only				
N	30	9		
Least-squares mean	3.1	3.59	-0.5	0.041
log(CRP)* (95% CI)	(2.84 to 3.35)	(3.13 to 4.06)	(-0.97 to -0.02)	
Least-square mean	22.12	36.34	0.61	
CRP** (95% CI)	(17.16 to 28.5)	(22.79 to 57.94)	(0.38 to 0.98)	
ITT population including randomised individuals to BAC + dornase-alfa and contemporary controls only.				
N	30	60		
Least-squares mean	3.18	3.56	-0.37	0.019
log(CRP)* (95% CI)	(2.91 to 3.45)	(3.35 to 3.76)	(-0.68 to -0.06)	

Table 2.

Primary endpoint and sensitivity analysis

Least-square mean	24.09	35.03	0.69	
CRP**	(18.36 to 31.6)	(28.44 to 43.15)	(0.5 to 0.94)	
(95% CI)				
ITT population including randomised individuals to BAC and contemporary controls only, i.e. the comparator population				
N	9	60		
Least-squares mean	3.78	3.53	0.24	
log(CRP)*	(3.23 to 4.33)	(3.3 to 3.77)	(-0.32 to 0.8)	0.386
(95% CI)				
Least-square mean	43.69	34.23	1.28	
CRP**	(25.18 to 75.8)	(27.11 to 43.24)	(0.73 to 2.23)	
(95% CI)				
Area under the log(CRP), standardised by days followed up, over 7 days follow-up: ITT population including all individuals (BAC + dornase-alfa, BAC & contemporary controls).				
N	30	69		
Least-squares mean	3.45	3.72	-0.27	0.043
area ^a	(3.22 to 3.68)	(3.55 to 3.88)	(-0.53 to -0.01)	
(95% CI)				
ITT population including all individuals: (BAC + dornase-alfa, BAC & contemporary controls matched including last pre-Dexamethasone CRP value).				
N	30	69		
Least-squares mean	3.16	3.69	-0.53	0.007
log(CRP)*	(2.83 to 3.5)	(3.44 to 3.93)	(-0.91 to -0.14)	
(95% CI)				
Least-square mean	23.57	39.92	0.59	
CRP**	(16.85 to 32.970)	(31.32 to 50.89)	(0.4 to 087)	
(95% CI)				
ITT population including all individuals: (BAC + dornase-alfa, BAC & contemporary controls). Stratified by BAC treatment				
No Remdesivir or Tocilizumab				
N	12	39		
Least-squares mean	3.29	3.75	-0.45	0.079
log(CRP)*	(2.83 to 3.76)	(3.45 to 4.04)	(-0.96 to 0.05)	
(95% CI)				
Least-square mean	26.97	42.35	0.64	
CRP**	(16.87 to 43.11)	(31.44 to 57.04)	(0.38 to 1.06)	

Table 2. (continued)

(95% CI)

Remdesivir no

Tocilizumab

N	16	23		
Least-squares mean	3.16	3.5	-0.35	0.123
log(CRP)*	(2.79 to 3.53)	(3.18 to 3.83)	(-0.79 to 0.1)	
(95% CI)				
Least-square mean	23.53	33.26	0.71	
CRP**	(16.29 to 33.99)	(23.97 to 46.15)	(0.45 to 1.1)	
(95% CI)				

Tocilizumab no

Remdesivir

N	1	5
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Remdesivir and

Tocilizumab

N	1	2
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* From linear repeated measures model, adjusted for natural 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.

^a From linear model, adjusted for natural log(baseline CRP, age, sex, BMI, serious condition, and treatment.

Table 2. (continued)

Days from baseline	Mean BAC + DA	SD BAC + DA	N BAC + DA	Mean BAC	SD BAC	N BAC
1	4.058	0.591	21	4.029	0.734	42
2	3.538	0.658	33	3.852	0.937	52
3	3.332	0.816	24	3.839	1.037	53
4	2.917	1.017	17	3.573	1.132	37
5	2.52	1.118	15	3.872	1.157	24
6	3.059	1.508	12	3.547	1.516	26
7	3.386	1.824	9	3.504	1.451	27
8	3.428	-	1	2.478	1.393	9

Table 3.

Primary endpoint by day

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

Secondary Endpoints	Randomised to BAC + dornase- alfa	BAC	Difference between BAC + dornase-alfa and BAC	p-value*
Time to discharge from hospital (days)				
Number discharged	27	51		
Median time to discharge ^a	6	7	-1	
(95% CI)	(4 to 7)	(6 to 12)		
Hazard ratio ^b			1.63	
(95% CI)			(1.01 to 2.61)	0.030
D-dimer (ug/L) FEU ^d				
N	28	11		
Least-square mean ^c	570.78	1656.96	0.34	
(95% CI)	(384.51 to 847.3)	(876.93 to 3130.81)	(0.17 to 0.69)	0.004
Lymphocyte count (×10⁹/L)**				
N	30	61		
Least-square mean ^c	1.08	0.87	1.25	
(95% CI)	(0.92 to 1.27)	(0.76 to 0.98)	(1.03 to 1.51)	0.021
Procalcitonin levels (ng/mL)				
N	26	7		
Least-square mean ^c	0.18	1.31	-1.13	
(95% CI)	(-0.2 to 0.56)	0.56 to 2.05)	(-1.88 to -0.37)	0.005

a Estimated from Kaplan-Meier curve.

b From Cox proportional hazard model, adjusting for age, baseline CRP and treatment

c 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.

d 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.

Table 4.

Secondary Endpoints

* From linear repeated measures model, adjusted for natural 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.

Table 4. (continued)

$p=0.883$. The same was seen over 35-day follow-up, LS mean 55.21 95% CI, -23.59 to 134.00) hours versus 60.60 (95% CI, 4.34 to 116.86) hours, $p=0.905$. At any point during the 35 days follow-up, 23% of BAC+DA were admitted to ICU compared to 23.19% BAC, $p=0.983$ (**Table S4**).

There was no significant difference in time requiring oxygen between the two groups, at either 7 days, LS mean 94.32 (95% CI, 72.8 to 115.79) hours, versus 88.96 (95% CI, 73.64 to 104.29) hours, $p=0.662$, or 35 days, LS mean 133.22 (95% CI, 52.01 to 214.43) hours versus 156.35 (95% CI, 98.36, 214.33) hours, $p=0.618$. At 35 days follow up, there were only 9 randomised participants to evaluate, but mean oxygen use tends to a reduction of 123 hours with BAC+DA, versus 241 hours for BAC, $p=0.187$ (**Table S4**)

The time to event data was censored at 28 days post last dose (up to d35) for the randomised participants and at the date of the last electronic record for the CC. Over 35 days follow up, 1 person amongst the 30 patients in BAC+DA died, compared to 8 of the 69 participants in BAC. The hazard ratio observed in the Cox proportional hazards model was 0.47 (95% CI, 0.06 to 3.86), indicating a trend towards a reduced chance of death at any given time-point in BAC+DA compared to BAC, but this did not reach significance $p=0.460$ (**Figure 3E** and **S3D**). 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.

There was no significant difference at either 7-or 35-days follow-up, in the number of participants that required mechanical ventilation in BAC+DA compared with BAC (16.67% vs 13.04%), $p=0.628$. Amongst participants that were ventilated, the mean length of mechanical ventilation at 7 days follow-up in BAC+DA was 76.8 hours, compared to 88.78 in BAC. At 35 days follow-up, the mean length of mechanical ventilation in BAC+DA was 76.8 hours compared to 411.17 hours in BAC (**Table S4**). There was no significant difference in superadded bacterial pneumonia at either 7-or 35-days follow-up: 7 days, 1 (3.33%) participant in BAC+DA compared to 3 (4.35%) participants in BAC, $p=0.934$; 35 days, 2 (6.67%) participants in BAC+DA had bacterial pneumonia, compared to 3 (4.35%) participants in BAC, $p=0.548$ (**Table S4**).

Blood analysis with no adjustment for multiple testing showed a significant treatment effect in BAC+DA vs. BAC group for three parameters: lymphocyte counts, D-dimer, and procalcitonin (PCT). First, BAC+DA exhibited higher lymphocyte counts with LS mean of 0.87 (95% CI, 0.76-0.98) in the BAC group vs. 1.08 (95% CI, 0.92-1.27) in BAC+DA, $p=0.02$ (**Table 4** and **Table S3**). Patients with lymphopenia at baseline ($<1 \times 10^9$ lymphocytes/L) exhibited a greater increase in blood lymphocyte numbers in BAC+DA than in BAC during the entire length of treatment (**Figure 4A**). Furthermore, D-dimer levels were lower in BAC+DA compared to BAC, with LS mean D-dimer difference of 1657 (95% CI, 3131-877) (**Table 4**, **Table S3**, **Figure 4B** and **Figure S4A**). Our analysis indicated lower levels of PCT in participants that received BAC+DA, mean 0.18 ng/mL (95% CI, -0.2-0.56) compared to BAC alone, mean 1.31 ng/mL (95% CI, 0.56-2.05), $p=0.005$ (**Table 4**). Repeat analysis excluding the CC population replicated these results, and changes in all 3 parameters were significant (**Table S3**).

Exploratory outcomes

Given the role of circulating cf-DNA in pathology, we examined whether the pulmonary administration of dornase alfa influenced systemic cf-DNA levels. Compared to the plasma of anonymized healthy donor volunteers at the Francis Crick institute (HD), plasma cf-DNA levels were elevated in both BAC and DA-treated COVASE participants. There was no difference in baseline plasma cf-DNA levels between the two groups. However, during the treatment period cf-DNA was reduced in participants randomised to BAC+DA compared to BAC alone (**Figure 4C**

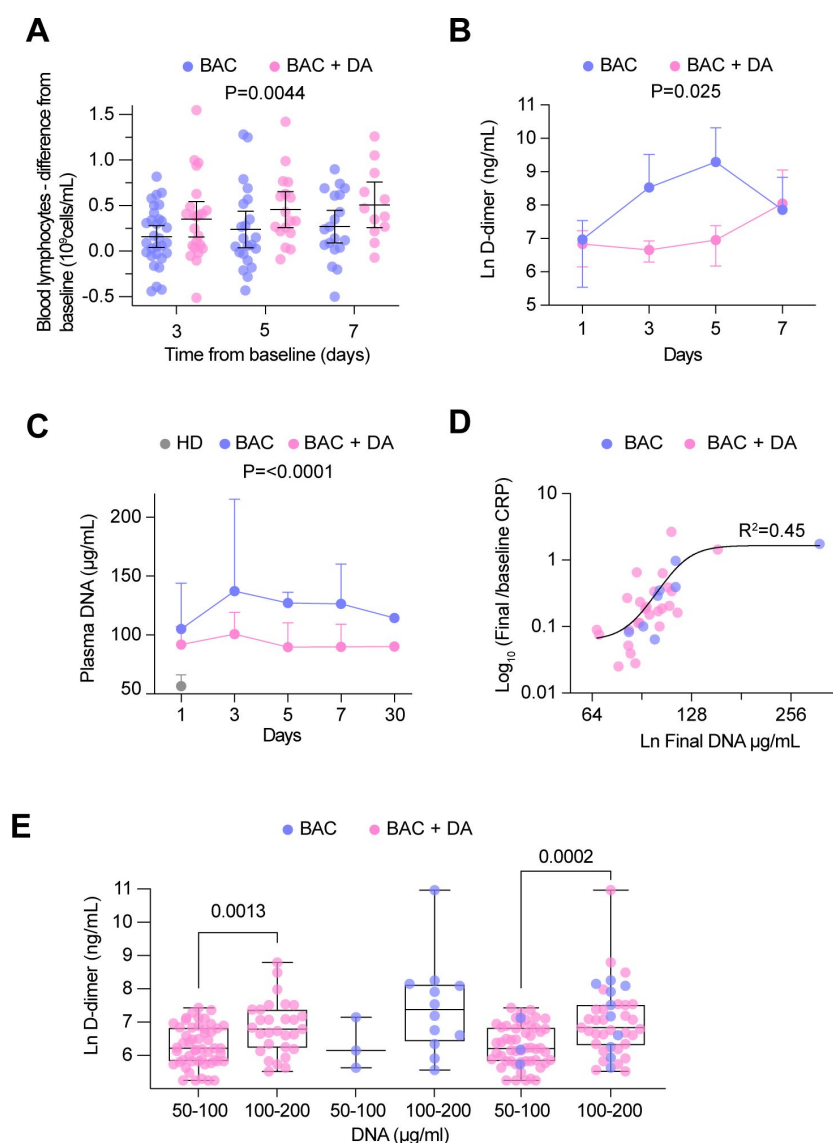


Figure 4.

Analysis of secondary and exploratory endpoints in blood

- A. Difference between the lymphocyte count for each day of the treatment period and the baseline in each patient who exhibited lymphopenia at baseline ($<1 \times 10^9$ lymphocytes/mL). Mean and 95%CI interval is shown with statistical analysis by two-way Anova.
- B. Mean D-dimer levels per day in randomised BAC (blue) and BAC+DA (pink) participants with error bars depicting 95% CI in randomised BAC (blue) and BAC+DA (DA) participants (pink). Statistical difference by mixed effects Anova analysis.
- C. Mean cf-DNA levels per day in randomised BAC (blue) and BAC+DA (pink) participants, with error bars depicting standard deviation. Statistical analysis by mixed effects Anova.
- D. Correlation between the final cf-DNA levels and ratio of CRP at day-7 normalized to the baseline CRP ($CRP_{final}/CRP_{baseline}$) per participant. Fitting by non-linear regression.
- E. Correlation between D-dimer and cell-free (cf) DNA levels in the blood of participants randomised to BAC (blue) or to BAC+DA (DA) (pink), where samples have been segregated depending on whether the corresponding levels of cf-DNA were below or above 100μ g/mL. Statistical analysis by unpaired parametric t-test.

and Figure S4B). There was a positive correlation between the levels of cf-DNA in the final sample collected during the treatment period and the ratio of final/baseline CRP ($\text{CRP}_{\text{final}}/\text{CRP}_{\text{baseline}}$) (Figure 4D [↗](#)). Moreover, samples with cf-DNA $>100\mu\text{g/mL}$ had significantly higher D-dimer levels compared to samples with cf-DNA levels $<100\mu\text{g/mL}$ (Figure 4E [↗](#)). Hence, dornase alfa treatment was associated with a suppression of circulating cf-DNA that correlated with a more sustained reduction in CRP.

Safety

Dornase alfa was well tolerated with no systemic effects, consistent with its short half-life (Table S5). There were 10 reported adverse events (AEs) in 9 participants with BAC versus 30 AEs in 30 participants with BAC+DA (Table S6). Of these, one was reported by the clinical team as definitely related and one as unlikely to be related to study drug (Table S5). No treatment-related serious AEs were reported.

Discussion

Our findings show that nebulised dornase alfa significantly reduced CRP in patients with severe COVID-19 pneumonia and systemic inflammation even when already receiving dexamethasone. This was robust with several sensitivity analyses including analysis of only randomised participants (i.e excluding CC). The secondary outcomes were consistent with the same directionality. Despite not being formally powered for length of stay, dornase alfa reduced time to discharge over 35 days.

Identification of novel/repurposed treatments effective for COVID-19 were hampered by patient recruitment to competing studies during a pandemic. This resulted in small studies with inconclusive or contrary findings. Our study design took advantage of frequent repeated measures of CRP in each patient, to allow a smaller sample size to determine efficacy/futility. A similar CRP-based approach was applied in CATALYST (Fisher et al., 2022 [↗](#)) and ATTRACT studies (Tornling et al., 2021 [↗](#)). In addition, we used contemporary controls as additional comparators to efficiently use limited resources.

Following the RECOVERY trial, dexamethasone became standard care in patients with COVID-19 pneumonitis requiring oxygen. We recruited participants with $\text{CRP} \geq 30 \text{ mg/L}$, on the day after receiving dexamethasone to minimise steroid-dependent effects on CRP. The finding that dornase alfa can significantly reduce CRP (by average 33%) in participants receiving dexamethasone suggests a complementary mechanism of action. Dornase alfa may provide a treatment choice for patients with mild COVID-19 pneumonia not requiring oxygen, in whom dexamethasone may be harmful (Group et al., 2021 [↗](#)).

Our primary effect was consistently reflected in secondary outcomes. Dornase alfa increased the chance of live discharge by 63% at any time up to 35 days, with a trend to reduce hospital stay. This impact on hospital occupancy during a pandemic cannot be overestimated. In addition, dornase alfa significantly increased lymphocyte counts and reduced D-dimer. CRP levels are associated with venous thromboembolic disease in COVID-19 with the worst outcomes seen in patients with high CRP and D-dimers (Smilowitz et al., 2021 [↗](#)). In a metaanalysis of 32 studies involving 10 491 COVID-19 patients, elevated CRP (OR 4.37, $p<0.00001$), lymphopenia (3.33, $p<0.00001$), elevated D-dimer (3.39 ($p<0.00001$)) and elevated PCT (6.33, $p<0.00001$) were independent markers of poor outcomes (Malik et al., 2021 [↗](#)).

Our study design offered a solution to the early screening of compounds for inclusion in larger platform trials. The study took advantage of frequent repeated measures of quantifiable CRP in each patient, to allow a smaller sample size to determine efficacy/futility than if powered on

clinical outcomes. We applied a CRP-based approach that was similar to the CATALYST and ATTRACT studies (Fisher et al., 2022 [↗](#); Tornling et al., 2021 [↗](#)). CATALYST showed in much smaller groups (usual care, 54, namilumab, 57 and infliximab, 35) that namilumab that is an antibody that blocks the cytokine GM-CSF reduced CRP even in participants treated with dexamethasone whereas infliximab that targets TNF- α had no significant effect on CRP. This led to a suggestion that namilumab should be considered as an agent to be prioritised for further investigation in the RECOVERY trial. A direct comparison of our results with CATALYST is difficult due to the different nature of the modelling employed in the two studies. In general, Dornase alfa exhibited comparable significance in the reduction in CRP compared to standard of care as described for namilumab at a fraction of the cost. Furthermore, endonuclease therapies may prove superior to cytokine blocking monotherapies, as they are unlikely to increase the risk for microbial co-infections that have been reported for antibody therapies that neutralize cytokines that are critical for immune defence such as IL-1 β , IL-6 or GM-CSF.

In addition, Dornase alfa reduced circulating cf-DNA levels, suggesting that this nebulized enzyme exerts systemic effects on circulating chromatin and inflammatory mediators. The reduction in cf-DNA suggests that by stripping the DNA from chromatin, dornase alfa suppresses the proinflammatory properties of histones and potentiates their degradation (Aramburu et al., 2022a [↗](#); Papayannopoulos et al., 2011 [↗](#)). The inverse correlation between final circulating cf-DNA and change in CRP during treatment highlights the link between circulating chromatin and systemic inflammation. The correlation of d-dimer with cf-DNA and the reduction in d-dimer with dornase alfa is consistent with the reported pro-thrombotic role of NETs in the alveoli (Radermecker et al., 2020 [↗](#)). Moreover, the difference in the recovery rates for lymphopenia is in line with a link between extracellular chromatin and lymphocyte death in sepsis (Ioannou et al., 2021 [↗](#)). Another reason why recombinant endonuclease treatment may be warranted in patients with acute infections is the reduction in endogenous chromatin degradation capacity that correlates with a high risk for mortality (Aramburu et al., 2022a [↗](#)).

Nebulized dornase alfa treatment has several advantages. Whilst immunisation has reduced COVID-19 hospital admissions, a substantial population remain unvaccinated or immunocompromised. Hence, there will always be a need for virally agnostic therapies that retain efficacy as viruses mutate. Nebulised dornase alfa controls immune pathology in a range of infections. Moreover, it can be administered safely and effectively outside the health-care setting. Three other small trials of dornase alfa (totalling 18 patients) in COVID-19 have reported improved oxygenation (Holliday et al., 2021 [↗](#); Okur et al., 2020 [↗](#); Weber et al., 2020 [↗](#)). One small study indicated improvements in plasma and sputum proteomic profiles (Fisher et al., 2021 [↗](#)). Despite differing study designs, patient populations and endpoints, the overall trend suggests an improvement in clinical outcomes.

There are limitations in this study. This single centre open-label study was designed to powerfully report futility or efficacy despite including just under 40 patients and 60 CC. However, the trial was not powered to report mortality nor to overcome confounders, such as the use of antivirals and tocilizumab, an IL-6 inhibitor recognised to reduce CRP (Galvan-Roman et al., 2021 [↗](#)), or the impact of an open-label study on influencing other therapeutic/ discharge decisions. Although underpowered, we demonstrate a trend to a reduction in CRP with dornase alfa in participants that had received tocilizumab and/or remdesivir, and those that had not. Moreover, the open-label nature of the study could potentially introduce bias. Nevertheless, we tried to minimize this by applying a standard testing schedule and discharge criteria.

In conclusion, we demonstrate that nebulised dornase alfa significantly reduces inflammation in hospitalised patients with severe COVID-19 pneumonia leading to improved clinical profiles and earlier discharge from hospital. These results confirm the role of cf-DNA in promoting systemic inflammation, coagulopathy and immune dysfunction in acute respiratory infections and suggest that recombinant DNases provide an additional mode of anti-inflammatory action that can

broaden the effectiveness and sustainability of anti-inflammatory regimens. These encouraging data warrant further investigation in other respiratory infections characterized by hyperinflammation in the setting of cell lysis and/ or NET formation.

Data Availability

All data produced in the present work are contained in the manuscript

Acknowledgements

We thank the patients, caregivers, and families who participated in the trial; and acknowledge the help of the following: Additional BRC Contributors: Margaret Duku, Gulten Geneci, Farah Islam, Ciprian-Ionut Matei, Marta Merida, Eleni Nastouli, Marivic Ricamara, Anisa Tariq. Pharmacy: Matthew Baker, Nina Bason, Chi Yee Chung, Zoila Gilham-Fernandez, Temi Olusi. Sponsors/UCL: Liam Banks, Helen Cadiou, Novin Fard, Farhat Gilani, Vince Greaves, Yusuf Jaami, Pushpsen Joshi, Misha Ladva, David Lomas, Catherine Maidens, Anthea Mo, Anisha Nayar, Nick McNally, Samim Patel. Data Monitoring Committee: Balaji Ganeshan, Maria Leandro, Kay Roy. COVID Clinical Consultants: Diana Ayoola, Robin Bailey, David Brealey, Mike Brown, Anna Checkley, Charlie Coughlan, Philip Gothard, Robert Heyderman, Sarah Logan, Nicky Longley, Jessica Manson, Michael Marks, David Moore, Neil Stone, Emma Wall. T8 Nursing Staff: Adam Cureton-Griffiths, Amy Mann, Laura Nichols, Pantelis Savvides. NOCRI Respiratory Translational Research Collaboration: Chris Brightling, Jane Davies, Ratko Djukanovic, Liam Heeney, Ling-Pei Ho, Alex Horsley, Tracy Hussell, Stefan Marciniak, Lorcan McGarvey, Thomas Wilkinson. Pari/Roche Products Limited/LifeArc: Mal Apter, Ruth Davies, Ciara O'Brien, Pauline Stasiak, Davia Viellec.

Funding

This work was supported by LifeArc (UCL-UCLH132333), UCL, Breathing Matters and the Francis Crick Institute which receives its core funding from the UK Medical Research Council (FC0010129), Cancer Research UK (FC0010129) and the Wellcome Trust (FC0010129). The study was undertaken at UCLH/UCL who received a proportion of funding from the Department of Health's NIHR Biomedical Research Centres funding scheme. VJS and DB are funded by the NIHR University College London Hospitals Biomedical Research Centre. I.V.A was funded by an EMBO LTF (ALTF 113-2019). Dornase alfa was provided by Roche Products Limited and nebulizers were donated by PARI. Disclosure forms provided by the authors are available with the full text of the published article.

Author contributions

JCP, JI, ED, BW, AF, PTL, VB and VP contributed to the concept and design of the study. JCP, VJS, ED, RE, MIT, NDV, IVA, DH, DB, TC, JG, AW, HE, VJ, AL, TR, VP contributed to the acquisition of data. ETH, LL, FK designed and directed the Berlin Pa-COVID-19 study. JCP, JI, AF, PTL, VB and VP contributed to analysis, and/or interpretation of data or the creation of new software used in the work. JCP, JI, BW, AF, PTL, VB and VP drafted the manuscript. All authors have approved the submitted version (and any substantially modified version that involves the author's contribution to the study); and to have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated,

resolved, and the resolution documented in the literature. Roche Products Ltd conducted a factual accuracy check on the final version but any decision to incorporate comments was made solely at the discretion of the authors.

The authors declare no conflicts of interest.

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Joint Public Review:

In this study by Porter et al reports on outcomes from a small, open-label, pilot randomized clinical trial comparing dornase-alfa to the best available care in patients hospitalized with COVID-19 pneumonia. As the number of randomized participants is small, investigators describe also a contemporary cohort of controls and the study concludes about decrease of inflammation (reflected by CRP levels) after 7 days of treatment but no other statistically significant clinical benefit.

Suggestions to the authors:

- Please re-analyze findings by omitting from all Tables and Figures all data of comparators who were not randomized (BAC). I understand the difficulties of running this trial but the results of excess reduction of mortality do not allow the publication of a trial where comparators do not come from the randomized patient population.
- The presentation remains confusing and the manuscript should be critically revised for clarity. There is a repetition of methods (e.g. lines 176-187 repeat 160-175) and redundant results (e.g. Figure S2, Table 3). At Table 4: the authors should select one method of illustration for lab results, either Table or figure, without repetitions
- Regarding inclusion criteria, it is unclear whether high radiological suspicion is sufficient for inclusion or whether PCR based confirmation is required in all instances (differences in wording between lines 153 and 191), and under which oxygen requirements (lines 155 and 192)
- Table 1 should be merged with Table S2 and a better description of cohort baseline severity (P/F, SOFA, APACHE, organ support, number of patients in each point of the WHO severity score) and treatments should be made available

Author Response

The following is the authors' response to the original reviews.

Reviewer 1:

Public review:

In this study, Porter et al report on outcomes from a small, open-label, pilot randomized clinical trial comparing dornase-alfa to the best available care in patients hospitalized with COVID-19 pneumonia. As the number of randomized participants is small, investigators describe also a contemporary cohort of controls and the study concludes about a decrease of inflammation (reflected by CRP levels) after 7 days of treatment but no other statistically significant clinical benefit.

Suggestions to the authors:

- *The RCT does not follow CONSORT statement and reporting guidelines*

We thank you for this suggestion and have now amended the order and content of the manuscript to follow the CONSORT statement as closely as possible.

- *The authors have chosen a primary outcome that cannot be at least considered as clinically relevant or interesting. After 3 years of the pandemic with so much research, why investigate if a drug reduces CRP levels as we already have marketed drugs that provide beneficial clinical outcomes such as dexamethasone, anakinra, tocilizumab and baricitinib.*

We thank the reviewer for bringing up this central topic. The answer to this question has both a historical and practical component. This trial was initiated in June of 2020 and was completed in June of 2021. At that time there were no known treatments for the severe immune pathology of COVID19 pneumonia. In June 2020, dexamethasone data came out and we incorporated dexamethasone into the study design. It took much longer for all other anti-inflammatories to be tested. Hence, our decision to trial an approved endonuclease was based purely on basic science work on the pathogenic role of cell-free chromatin and NETs in murine sepsis and flu models and the ability of DNase I to clear them and reduce pathology in these animal models. In addition, evidence for the presence of cell-free chromatin components in COVID-19 patient plasma had already been communicated in a pre-print. Finally, several studies had reported the anti-inflammatory effects of dornase treatment in CF patients. Hence there was a strong case for a cheap, safe, pulmonary noninvasive treatment that could be self-administered outside the clinical setting.

The Identification of novel/repurposed treatments effective for COVID-19 were hampered by patient recruitment to competing studies during a pandemic. This resulted in small studies with inconclusive or contrary findings. In general, effective treatments were only picked up in very large RCTs. For example, demonstrating dexamethasone as effective in COVID-19 required recruitment of 6,425 patients into the RECOVERY study. Multiple trials with anti-IL-6 gave conflicting evidence until RECOVERY recruited 4116 adults with COVID-19 (n=2022, tocilizumab and 2094, control) similar for Baricitinib (4,148 randomised to treatment and 4,008 to standard care). Anakinra is approved for patients with elevated suPAR, based on data from one randomized clinical trial of 594 patients, of whom 405 had active treatment (PMID: 34625750). However, a systematic review analysing over 1,627 patients (anakinra 888, control 739) with COVID-19 showed no benefit (PMID: 36841793). Regarding the choice of the primary endpoint, there is a wealth of clinical evidence to support the relevance of CRP as a prognostic marker for COVID-19 pneumonia patients and it is a standard diagnostic and prognostic clinical parameter in infectious disease wards. This choice in March 2020 was supported by evidence of the prognostic value of IL-6; CRP is a surrogate of IL-6. We also provide our own data from a large study of severe COVID-19 pneumonia in figure 1, showing how well CRP correlates with survival.

In summary, our data suggest that Dornase yields an anti-inflammatory effect that is comparable or potentially superior to cytokine-blocking monotherapies at a fraction of the cost and potentially without the additional adverse effects such as the increase for co-infections.

We now provide additional justification on these points in the introduction on pg.4 as follows:

“The trial was initiated in June 2020 and was completed in September of 2021. At the start of the trial only dexamethasone had been proven to benefit hospitalized COVID-19 pneumonia patients and was thus included in both arms of the trial. To increase the chance of reaching

significance under challenging constraints in patient access, we opted to increase our sample size by using a combination of randomized individuals and available CRP data from matched contemporary controls (CC) hospitalized at UCL but not recruited to a trial. These approaches demonstrated that when combined with dexamethasone, nebulized DNase treatment was an effective anti-inflammatory treatment in randomized individuals with or without the implementation of CC data.”

We also added the following explanation in the discussion on pg. 16:

“Our study design offered a solution to the early screening of compounds for inclusion in larger platform trials. The study took advantage of frequent repeated measures of quantifiable CRP in each patient, to allow a smaller sample size to determine efficacy/futility than if powered on clinical outcomes. We applied a CRP-based approach that was similar to the CATALYST and ATTRACT studies. CATALYST showed in much smaller groups (usual care, 54, namilumab, 57 and infliximab, 35) that namilumab that is an antibody that blocks the cytokine GM-CSF reduced CRP even in participants treated with dexamethasone whereas infliximab that targets TNF- α had no significant effect on CRP. This led to a suggestion that namilumab should be considered as an agent to be prioritised for further investigation in the RECOVERY trial. A direct comparison of our results with CATALYST is difficult due to the different nature of the modelling employed in the two studies. However, in general Dornase alfa exhibited comparable significance in the reduction in CRP compared to standard of care as described for namilumab at a fraction of the cost. Furthermore, endonuclease therapies may prove superior to cytokine blocking monotherapies, as they are unlikely to increase the risk for microbial co-infections that have been reported for antibody therapies that neutralize cytokines that are critical for immune defence such as IL-1 β , IL-6 or GM-CSF. “

• Please provide in Methods the timeframe for the investigation of the primary endpoint

This information is provided in the analysis on pg. 8:

“The primary outcome was the least square (LS) mean CRP up to 7 days or at hospital discharge whichever was sooner.”

• Why day 35 was chosen for the read-out of the endpoint?

We now state on pg. 8 that “Day 35 was chosen as being likely to include most early mortality due to COVID-19 being 4 weeks after completion of a week of treatment. (i.e. d7 of treatment +28 (4 x 7 days))”

• The authors performed an RCT but in parallel chose to compare also controls. They should explain their rationale as this is not usual. I am not very enthusiastic to see mixed results like Figures 2c and 2d.

We initially aimed at a fully randomized trial. However, the swift implementation of trial prioritization strategies towards large and pre-established trial platforms in the UK made the recruitment COVID19 patients to small studies extremely challenging. Thus, we struggled to gain access to patients. Our power calculations suggested that a mixed trial with randomized and contemporary controls was the best way forward under these restrictions in patient access that could provide sufficient power.

That being said, we also provide the primary endpoint (CRP) results in Fig. 3B as well as the results for the length of hospitalization (Fig. S3D) for the randomized subjects only.

• Analysis is performed in mITT; this is a major limitation. The authors should provide at least ITT results. And they should describe in the main manuscript why they chose mITT

We apologize if this point was confusing. We performed the analysis on the ITT as defined in our SAP: “The primary analysis population will be all evaluable patients randomised to BAC + dornase alfa or BAC only who have at least one post-baseline CRP measurement, as well as matched historical comparators.”

We understand that the reason this might be mistaken as an mITT is because the N in the ITT (39) doesn’t match the number randomised and because we had stated on pg. 8 that “Efficacy assessments of primary and secondary outcomes in the modified inten.on-to-treat popula.on were performed.”

However, we did randomise 41 participants, but:

One participant in the DA arm never received treatment. The individual withdrew consent and was replaced. We also have no CRP data for this participant in the database, so they were unevaluable, and we couldn’t include them in the baseline table even if we wanted to. In addition, 1 participant in BAC only had a baseline CRP measurement available. Hence not evaluable as we only have a baseline CRP measurement for this participant.

We have corrected the confusing statement on pg. 8 and added an additional explanation.

“Efficacy assessments of primary and secondary outcomes in the inten.on-to-treat (ITT) popula.on were performed on all randomised par.cipants who had received at least one dose of dornase alfa if randomized to treatment. For full details see Sta.s.cal Analysis Plan. The ITT was adjusted to mi.gate the following protocol viola.ons where one par.cipant in the BAC arm and one in the DA arm withdrew before they received treatment and provided only a baseline CRP measurement available. The par.cipant in the DA arm was replaced with an addi.onal recruited pa.ent. Exploratory endpoints were only available in randomised par.cipants and not in the CC. In this case, a post hoc within group analysis was conducted to compare baseline and post-baseline measurements.”

• *It is also not usual to exclude patients from analysis because investigators just do not have serial measurements. This is lost to follow up and investigators should have pre-decided what to do with lost-to-follow-up.*

Our protocol pre-specified that the primary analysis population should have at least one postbaseline CRP measurement (pg. 13 of protocol). The patient that was excluded was one that initially joined the trial but withdrew consent after the first treatment but before the first post-treatment blood sample could be drawn. Hence, the pre-treatment CRP of this patient alone provided no useful information.

• *In Table 1 I would like to see all randomized patients (n=39), which is missing. There are also baseline characteristics that are missing, like which other treatments as BAT received by those patients except for dexamethasone.*

Table 1 includes all 39 patients plus 60 CCs.

Table 2 shows additional treatments given for COVID-19 as part of BAC.

• *In the first paragraph of clinical outcomes, the authors refer to a cohort that is not previously introduced in the manuscript. This is confusing. And I do not understand why this analysis is performed in the context of this RCT although I understand its pilot nature.*

One of the main criticisms we have encountered in this study has been the choice of the primary endpoint. The best way respond to these questions was to provide data to support

the prognostic relevance of CRP in COVID-19 pneumonia from a separate independent study where no other treatments such as dexamethasone, anakinra or anti-IL6 therapies were administered. We think this is very useful analysis and provides essential context for the trial and the choice of the primary endpoint, indicating that CRP has good enough resolution to predict clinical outcomes.

- *Propensity-score selected contemporary controls may introduce bias in favor of the primary study analysis, since controls are already adjusted for age, sex and comorbidities.*

The contemporary controls were selected to best match the characteristics of the randomized patients including that the first CRP measurement upon admission surpassed the trial threshold, so we do not see how this selection process introduces biases, as it was blinded with regards to the course of the CRP measurements. Given that this was a small trial, matching for baseline characteristics is necessary to minimize confounding effects.

- *The authors do not clearly present numerically survivors and non-survivors at day 34, even though this is one of the main secondary outcomes.*

We now provide the mortality numbers in the following paragraph on pg. 13.

“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.”

- *It is unclear why another cohort (Berlin) was used to associate CRP with mortality. CRP association with mortality should (also) be performed within the current study.*

As we explained above, the Berlin cohort CRP data serve to substantiate the relevance of CRP as a primary endpoint in a cohort that experienced sufficient mortality as this cohort did not receive any approved anti-inflammatory therapy. Mortality in our COVASE trial was minimal, since all patients were on dexamethasone and did not reach the highest severity grade, since we opted to treat patients before they deteriorated further. The overall mortality was 8% across all arms of our study, which does not provide enough events for mortality measurements. In contrast the Berlin cohort did not receive dexamethasone and all patients had reached a WHO severity grade 7 category with mortality at 30%.

My other concerns are:

- *This report is about an RCT and the authors should follow the CONSORT reporting guidelines. Please amend the manuscript and Figure 1b accordingly and provide a CONSORT checklist.*

We now provide a CONSORT checklist and have amended the CONSORT diagram accordingly.

- *Please provide in brief the exclusion criteria in the main manuscript*

We have now included the exclusion criteria in the manuscript on pg. 6.

“1.1.1 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 $\leq 93\%$ on high-flow oxygen

1. Require mechanical invasive or non-invasive ventilation at screening
2. Concurrent severe respiratory disease such as asthma, COPD and/or ILD
3. 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
4. Terminal disease and life expectancy < 12 months without COVID-19
5. Known allergies to dornase alfa and excipients
6. Participants who are unable to inhale or exhale orally throughout the entire nebulisation period So briefly Participants were excluded if they were:
7. pregnant, planning pregnancy or breastfeeding 2. Serious condition meeting one of the following:

a. Respiratory distress with respiratory rate ≥ 40 breaths/min

b. oxygen saturation $\leq 93\%$ on high-flow oxygen

1. Require ventilation at screening
2. Concurrent severe respiratory disease such as asthma, COPD and/or ILD
3. Terminal disease and life expectancy < 12 months without COVID-19
4. Known allergies to dornase alfa and excipients
5. Participants who are unable to inhale or exhale orally throughout the entire nebulisation period”

• "The final trial visit occurred at day 35." "Analysis included mortality at day 35". I am not sure I understand why. In clinicaltrials.gov all endpoints are meant to be studies at day 7 except for mortality rate day 28. Why day 35 was chosen? Please be consistent.

Thank you for identifying this inconsistency. We have amended the record on clinicaltrials.gov to read “the time to event data was censored at 28 days post last dose (up to day 35) for the randomised participants and at the date of the last electronic record for the CC.”

- Please provide in Methods the timeframe for the investigation of the primary endpoint
- The authors performed an RCT but in parallel chose to compare also controls. They should explain their rationale as this is not usual. I am not very enthusiastic to see mixed results like Figures 2c and 2d.
- Analysis is performed in mITT; this is a major limitation. The authors should provide at least ITT results. And they should describe in the main manuscript why they chose mITT

analysis.

- *It is also not usual to exclude patients from analysis because investigators just do not have serial measurements. This is lost to follow up and investigators should have pre-decided what to do with lost-to-follow-up.*
- *Figure 1b as in CONSORT statement, please provide reasons why screened patients were not enrolled.*
- *In Table 1 I would like to see all randomized patients (n=39), which is missing. There are also baseline characteristics that are missing, like which other treatment as BAT received those patients except for dexamethasone.*
- *In the first paragraph of clinical outcomes, the authors refer to a cohort that is not previously introduced in the manuscript. This is confusing. And I do not understand why this analysis is performed in the context of this RCT although I understand its pilot nature.*
- *In Figure 2 the authors draw results about ITT although in methods describe that they performed an mITT analysis. Please be consistent.*

Please see answers provided to these queries above.

Reviewer #2 (Recommendations For The Authors):

1. *Suppl Figure 2B would be more informative if presented as a Table with N of patients with per day sampling*

We now provide the primary end point daily sampling table in Table 3.

1. *The numbers at risk should figure under the KM curves*

The numbers at risk for figures 1E, 2C, 2D have been added as graphs either in the main figures or in the supplement.

1. *HD in Supplementary figure 3 should be explained*

We apologize for this omission. We now provide a description for the healthy donor samples that we used in the cell-free DNA measurements in figure S3B on pg. 14:

“Compared to the plasma of anonymized healthy donors volunteers at the Francis Crick ins.tute (HD), plasma cf-DNA levels were elevated in both BAC and DA-treated COVASE par.cipants.

1. *Presentation is inappropriate for Table S4*

We thank the reviewer for pointing this issue. We have now formaxed Table S4 to be consistent with all other tables.