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An Inhaled Galectin-3 Inhibitor in COVID-19 Pneumonitis (DEFINE)

A Phase Ib/IIa Randomised Controlled Trial

An inhaled galectin-3 inhibitor in COVID-19 pneumonitis (DEFINE): a phase Ib/IIa randomised controlled trial

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Our research paper highlights the pharmacokinetics/pharmacodynamics of GB0139, an inhaled galectin-3 inhibitor, in COVID pneumonitis. Importantly, we demonstrate target engagement and modulation of key biological pathways involved in COVID-19 immunobiology (inflammation, coagulation and monocyte/macrophage biology).

Author contributions: KD was the chief investigator of the study. TQ, EG were the clinical delivery team. AB, JA were the clinical management team. DH, ROC, FL, CB, AV, MB, PE, BM, GR, GH, RM, & ES were the laboratory team. TS, BL, AB, JA, DD, TW, JN, RP, OC, KT, MSH, AA, NH & KD contributed to trial protocol development.

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At a Glance Commentary:

What is the current scientific knowledge on this subject?

High circulating galectin-3 is associated with poor outcomes in patients with COVID-19. We hypothesised that GB0139, a potent inhaled thiodigalactoside galectin-3 inhibitor with anti-inflammatory and antifibrotic actions, would be safely and effectively delivered in COVID-19 pneumonitis.

What does this study add to the field?

In COVID pneumonitis, inhaled GB013 was well-tolerated, achieved clinically relevant plasma concentrations with target engagement. The data support larger clinical trials to determine clinical efficacy.

Some of the results of these studies have been previously reported in the form of a preprint (medRxiv 10 January 2022 https://www.medrxiv.org/content/10.1101/2021.12.21.21267983v2)

This article has an online data supplement, which is accessible from this issue's table of content online at www.atsjournals.org.

Abstract

Rationale: High circulating galectin-3 is associated with poor outcomes in patients with COVID-19. We hypothesised that GB0139, a potent inhaled thiodigalactoside galectin-3 inhibitor with anti-inflammatory and antifibrotic actions, would be safely and effectively delivered in COVID-19 pneumonitis.

Objectives: Primary outcomes were safety and tolerability of inhaled GB0139 as an add-on therapy for patients hospitalised with COVID-19 pneumonitis.

Methods: We present the findings of two arms of a phase Ib/IIa randomised controlled platform trial in hospitalised patients with confirmed COVID-19 pneumonitis. Patients received standard of care (SoC) or SoC plus 10 mg inhaled GB0139 twice daily for 48 hours, then once daily for up to 14 days or discharge.

Results: Data are reported from 41 patients, 20 of which were assigned randomly to receive GB0139. Primary outcomes: the GB0139 group experienced no treatment-related serious adverse events. Incidences of adverse events were similar between treatment arms (40 with GB0139+SoC *vs* 35 with SoC). Secondary outcomes: plasma GB0139 was measurable in all patients after inhaled exposure, and demonstrated target engagement with decreased circulating galectin (overall treatment effect post-hoc ANCOVA over days 2–7: p=0.0099 *vs* SoC). Plasma biomarkers associated with inflammation, fibrosis, coagulopathy and major organ function were evaluated.

Conclusions: In COVID pneumonitis, inhaled GB013 was well-tolerated, achieved clinically relevant plasma concentrations with target engagement. The data support larger clinical trials to determine clinical efficacy.

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the disease it causes (coronavirus disease 2019 [COVID-19]) has put major stress on the world's healthcare services, and despite vaccination, is likely to remain a problem for the foreseeable future.[1] Severe morbidity and mortality from COVID-19 is predominantly a consequence of immunopathology in response to viral infection,[2] characterised by aberrant monocyte and macrophage-driven lung injury, T cell depletion[3], cytokine excess, inflammation-driven thrombosis, and fibrotic lung damage.[4-7] Amongst the many interventions tested[8], treatments that target this immunopathology such as corticosteroids[9] and interleukin-6 antagonists[10], have been shown to improve outcomes in hospitalised patients with COVID-19, with the greatest effect observed in those requiring respiratory support. Despite the approval of these agents as standard of care (SoC) for COVID-19, there is still an urgent need to identify further effective and tolerable therapies, particularly those that target COVID-19 immunopathology[11], alongside antiviral effects.

Galectin-3, a mammalian ß-galactoside-binding lectin, is an important regulator of immune homeostasis, highly upregulated following lung injury (i.e., biomarker for lung damage). [12-15] There is an illness severity dependent increase in galactin-3 concentration in COVID-19[16,17] and given immunopathology of COVID-19, galectin-3 blockade is considered a plausible therapeutic target.[7,18] Galectin-3 drives a systemic and pulmonary pro-inflammatory cytokine profile following co-infection of pneumococcus and H1N1 avian influenza virus,[19] via upregulation of macrophage IL-1β production[20] and NLRP3 inflammasome activation.[19,20] Therefore, anti-galectin-3 therapy can reduce macrophage and dendritic cell secretion of cytokines IL-1, IL-6, and TNF-α, and attenuate other inflammatory COVID-19-associated

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consequences[16,18,21] including inflammation-driven thrombosis.[22] Galectin-3-binding protein[23] (LGALS3BP) is an interaction partner of SARS-CoV-2 spike glycoprotein, enhancing syncytia formation. Furthermore, inhaled GB0139, a potent thiodigalactoside galectin-3 inhibitor, in a phase 1/2a trial, suppressed galectin-3 expression and decreased key plasma biomarkers associated with idiopathic pulmonary fibrosis (IPF) disease progression.[24] These initial findings support the hypothesis that inhaled GB0139 may counteract the SARS-CoV-2-associated pathobiology enhanced by galectin-3, thereby reducing the severity of COVID-19, and potentially the development of post-viral pulmonary fibrosis.

Therefore, we sought to investigate the safety, pharmacokinetics (PK) and pharmacodynamics (PD) of inhaled GB0139 in hospitalised patients with COVID-19 pneumonitis, prior to evaluating clinical efficacy in larger trials. Biological and clinical indices of efficacy were also explored.

Some of the results of these studies have been previously reported in the form of an abstract.[25]

Methods

Study design

Define (University of Edinburgh COVID-19 Define trial, 2020; ClinicalTrials.gov identifier: NCT04473053, EurdraCT number: 2020-002230-32) was a randomised, controlled, open-label, parallel-group, experimental medicine platform study evaluating safety and PK and PD of GB0139 and Nafamostat (data presented separately[26]) in patients with COVID-19.

Following eligibility screening, patients were randomly assigned (1:1:1) to receive GB0139 plus SoC, nafamostat plus SoC, or SoC alone. Separate statistical analysis

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plans were used for each active treatment versus SoC comparison. Treatment arms have been reported separately. Additional information regarding randomisation and SoC treatment is included in an online supplement. For this analysis, we report data from the GB0139+SoC and SoC cohorts.

GB0139 was administered as an inhaled formulation of 10mg twice daily via a dry powder inhaler (Plastiape[TM], Berry Bramlage, Lohne, Germany) for the first 48 hours, followed by 10 mg once daily for up to 14 days, or until discharge from hospital or withdrawal from the trial.

The dose of GB0139 is based on previous experience in idiopathic pulmonary fibrosis (IPF) patients receiving 10 mg of GB0139 daily for 14 days. In IPF patients, a near-total attenuation of Gal-3 levels in lung macrophages was observed, showing near-total target engagement in the target organ[27]. A loading dose strategy was implemented in Define for the first two days of treatment (10 mg twice-daily) to reach the desired lung levels of GB0139 faster.

Independent ethics committee approval (Scotland A Research Ethics Committee: 20/SS/0066) was obtained prior to initiation of the study. The independent Data Monitoring Committee scrutinised accumulating data.

Study participants

The trial protocol has been previously reported[28]. Eligible patients were aged ≥16 years, had polymerase chain reaction-confirmed COVID-19 infection, and required oxygen or had evidence of pneumonitis on x-ray. Inclusion and exclusion criteria are provided in table 1.

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Objectives

The primary objective was to evaluate the safety of inhaled GB0139 as add-on therapy in patients with COVID-19 pneumonitis. Secondary objectives included assessments of PK and PD properties of GB0139. Secondary exploratory biomarkers were assessed during treatment, as was potential for clinical efficacy. Safety was assessed daily using daily observations and blood parameters (table E1 in supplementary materials). Adverse events (AE) and concomitant medications were recorded from the screening visit until discharge, withdrawal, or death, or 90 day follow up.

Pharmacokinetics

Blood plasma samples for PK assessments were drawn pre-dose on days 1, 3, 5, 8, and 11 (+/− 1 day) for the determination of trough plasma GB0139 concentrations. Sample analysis is detailed in online supplementary material.

Flow cytometry

Inflammatory cell phenotypes were analysed by fluorescence-activated cell sorting of peripheral blood. Methods are described in the Supplementary Appendix, and gating strategies and list of antibodies used are available in figure E1 and table E2, respectively.

Cytokine analysis

Blood samples were collected daily for biomarker and cytokine measurement, subject to patient tolerance. Details of biomarkers selected and analysis can be found in online supplementary materials.

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Viral load

Viral load was determined as previously described[26].

Statistical analysis

Note that this study is an early phase clinical trial with small sample size and therefore we only have sufficient power to detect large differences between groups. An indicative sample size calculation suggested that 20 patients per group provides 80% power to detect an effect size of 0.7 using a two group t-test with a one sided 10% one-sided significance level (equivalent to a two-sided 20% level) and assuming 5% missing data, for the difference of means in a biomarker between GB0139 and control groups. For ease of presentation and to reduce confusion for the reader we chose to present the usual two-sided 95% credible intervals. Non-significant results should be interpreted cautiously.

Bayesian Generalised Linear Mixed Effects models (GLMM) were fitted to continuous safety outcomes, with baseline and trial arm as explanatory variables, and a random effect for patient. Day of measurement post-randomisation was also included as a categorical factor variable. Non-informative flat priors were used. Results were reported as posterior mean differences and highest posterior density (HPD) 95% credible intervals. Further details are provided in the online methods supplement. Note that whenever we use the phrase "statistically significant" in the post hoc analysis of trends, this means that the credible intervals do not contain zero and so the posterior probability of a treatment benefit (or harm) will be at least 97.5%.

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Results

Patient disposition and baseline characteristics

The study population, recruited between September 2020 and February 2021, comprised of 43 patients aged 27–87 years admitted to the Royal Infirmary of Edinburgh and Western General Hospital, Edinburgh. Twenty-two patients were randomised to receive GB0139+SoC and 21 to receive SoC (figure 1). Two patients assigned to the GB0139+SoC arm did not receive any study drug due to clinical deterioration following randomisation but prior to administration, and were excluded from the analyses as per the predefined statistical analysis plan. One patient missed a loading dose. Full baseline demographics and disease characteristics are provided in table 2, with a patient level breakdown of co-morbidities in table E3. Details of SoC treatment on an individual basis are provided in table E4.

It was noted that in the GB0139+SoC arm, 30% of patients had a NEWS2[29] of ≤3 at trial entry, and 28.6% of patients had a NEWS2 of ≥6 at baseline. In the SoC arm, 50% of patients had a baseline NEWS2 of ≤3 and 9% of patients had a NEWS2 of ≥6. A post-hoc subgroup analysis of patients with a baseline NEWS2 ≥4 was therefore undertaken. Please see the online supplement for these additional results.

Safety and tolerability of GB0139

There were no safety or tolerability concerns associated with inhaled administration of GB0139, and no statistically significant differences observed in haematological and biochemical safety laboratory outcomes or vital signs (table 3). The GB0139+SoC group experienced 40 adverse events (AEs) compared with 35 in the SoC group. The total number of patients who experienced at least one AE was 14 (70.0%) in the GB0139+SoC group versus 12 (57.1%) in the SoC group (table 4).

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The odds ratio (95% confidence interval [CI]) of ≥1 AE occurring between trial arms was 1.83 (0.48–6.81) and the rate ratio (95% CI) between arms for the number of AEs per patient was 1.20 (0.75–1.89). There were three serious AEs recorded from one patient in the GB0139+SoC group after hospital discharge (small bowel obstruction and paracetamol overdose both requiring hospital admission, and worsening of hyponatraemia). These were considered unrelated to study treatment. Amongst patients who received GB0139+SoC, five reported an AE considered by the clinical study team to be possibly related to treatment. These comprised of an isolated instance of prolonged QTc on ECG with no arrhythmia that spontaneously resolved, nausea, sore throat, oral thrush, and hair loss (all mild severity). Over 400 IPF patients have been treated with GB0139 (ClinicalTrials.gov Identifiers: NCT02257177 and NCT03832946) and none of these adverse events have previously been reported with treatment. Table E5 in the online supplement details all AEs.

Plasma concentrations of GB0139

After an initial peak of 19.94 ng/mL on day 3, consistent with the higher 10 mg BID dosing for the first 2 days, geometric mean trough plasma concentrations of GB0139 decreased to 7.81 ng/mL on day 5 and had reached steady-state by day 8 (6.01 ng/mL). Similar trough concentrations were measured at the end of the PK assessment period on day 11 (5.50 ng/mL). The apparent decrease of plasma concentrations between days 3 and 5 was due to the two-fold lower daily dose administered from day 3 onwards (10 mg QD from day 3 *vs* 10 mg BID for the first 48 hours). Ranges of individual values overlapped between days 5, 8, and 11, and interindividual variability on trough plasma concentrations was moderate, with

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coefficients of variation ranging across days from 39% to 57%, indicating consistent exposure between patients and across days (table 5). Inhaled GB0139 achieved plasma concentrations in patients with COVID-19 were comparable to that previously observed in patients with IPF[14,24] , with individual ranges overlapping between the two populations (figure E2).

GB0139 effect on biomarkers

There was a decrease in the mean serum concentration of galectin-3 over time with GB0139+SoC treatment (overall treatment effect post-hoc ANCOVA *vs* SoC over days 2–7: p=0·0099), indicating target engagement activity (figure 2). Further post hoc analysis, using Bayesian GLMM, showed a significantly greater rate of decline of galectin-3 levels between patients receiving GB0139 compared with those receiving GB0139+SoC (Table E6.1, Table E6.2).

Biomarkers of inflammation

All biomarkers were assayed as planned. Although GB0139+SoC-treated patients had higher baseline CRP levels than those receiving SoC (115 *vs* 45 mg/L, respectively), a post hoc analysis showed that the rate of decline of CRP was significantly greater in patients receiving GB0139+SoC compared with those receiving SoC (figure 3A, Table E6.1). Similarly, post hoc analysis showed the rates of decline of lactate dehydrogenase (LDH) and neutrophil to lymphocyte ratio (NLR) were also significantly greater in patients receiving GB0139+SoC compared with those receiving SoC (figure 3B and C, Table E6.1).

In post hoc analysis, the rate of decline of CXCL10 was significantly greater in those receiving GB0139+SoC rather than SoC (figure 3D, Table E6.1). Cytokine IL-1b and

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granulocyte-macrophage CSF are not reported due to low or undetectable levels (below the LLOQ). IL-1rα, IL-8, IL-17, and amphiregulin were measured but no differences were noted between treatment arms.

Biomarkers of coagulopathy

Patients receiving GB0139+SoC compared with patients receiving SoC alone had consistently low levels of D-dimer (figure 4A) and in post hoc analysis, the rate of decline of plasma levels of FPR(Fibrinogen/platelet ratio) was significantly greater in patients receiving GB0139+SoC compared with those receiving SoC (figure 4B, Table E6.1). We observed an increase in platelet count and aPTT in GB0139+SoC-treated patients, compared with patients receiving SoC alone (figure 4C and D).

Biomarkers of fibrosis

GB0139+SoC-treated patients had lowered levels (from days 4–7) of YKL-40 from baseline compared with SoC (figure E4 A). Furthermore, flow analyses of peripheral blood in the patient subgroup with a NEWS2 ≥4 suggest that GB0139 may change monocyte phenotype from profibrotic transitional to antifibrotic classical and decrease transitional monocytes (figure E4 C).

Patient outcomes and mortality rates

Seven deaths occurred in the overall population of 41 randomised patients: 4 deaths in 20 patients in the GB0139+SoC arm (where patients received a median of two GB0139 doses) and 3 deaths in 21 patients in the SoC group. In the NEWS2≥4 group, six deaths occurred, 3/11 deaths in SOC compared to 3/14 deaths in GB0139+SOC (full details are available in table E7). No deaths were attributed to

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GB0139. There were no patients who received invasive ventilation or renal replacement therapy whilst taking part in the trial. The median duration of hospital stay for GB0139+SoC patients was 3 days longer on average compared to the SoC arm (SoC: 3 days, minimum 1 day, maximum 21 days; GB0139+SoC: 6 days, minimum 2 days, maximum 35 days). This was non-significant.

The GB0139 arm had a significantly lower number of oxygen free days compared to standard care (19/123 versus 29/86; rate ratio 0.45, 95% HPD credible interval 0.25 to 0.82), after taking into account the number of times oxygen status was recorded in each arm. There was a significantly greater reduction in FiO_2, observed in patients treated with GB0139+SoC compared with SoC (Figure E4).

Viral data

Nasopharyngeal and saliva samples were taken at baseline, day 3 and day 5 for RT PCR analysis. Viral load decreased over time in both groups (Fig.E5). During the course of the study the virus strain dominant in the community was initially alpha for the first half and then beta.

Discussion

Define is the first reported clinical trial of an inhaled galectin-3 inhibitor for the treatment of COVID-19. GB0139 had an acceptable safety and tolerability profile in hospitalised patients with COVID-19 pneumonitis. Despite being breathless and requiring oxygen, patients were able to inhale and achieve consistent exposure of GB0139. The 10 mg BID dose used for the first 2 days is higher than the top dose used in an ongoing study in IPF (NCT03832946), and was well tolerated. Inhaled

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GB0139 caused a significant reduction of galectin-3 levels in patients with COVID-19 and may reduce plasma levels of other key prognostic biomarkers associated with severe disease.

High galectin-3 levels correlate with severity of COVID-19, and it performs as a biomarker for the severity of ARDS in COVID-19.[30] Patients with high galectin-3 serum levels also have markedly higher risk of intensive care unit admission or death.[31]

Despite a small sample size, this study adds further weight to the therapeutic potential of galectin-3 inhibition in the treatment of patients with COVID-19. Whilst the trial was not powered to assess efficacy, GB0139 treatment showed a greater decrease in the rate of inflammatory and physiological biomarkers associated with COVID-19 severity in patients on GB0139 + SOC compared to SOC. CRP and NLR are measures of systemic inflammation, with increased levels indicative of severe of COVID-19 infection. CXCL10 has been identified as a cardinal chemokine in driving COVID-19 immunopathogenesis.[32,33] Raised CXCL10 levels are often observed in both plasma and bronchoalveolar lavage of patients who are critically ill with the disease.[34] We have shown a greater rate of decline of CRP , NLR and CXCL10 in patients treated with GB0139+SoC compared with SoC. This suggests that GB0139, as add-on to SoC, may have the potential to reduce the severity of systemic inflammation and the cytokine excess in patients with COVID-19. Lymphocyte exhaustion is also a feature of both infection and progression of severe COVID-19.[35] In the NEWS2 ≥4 subgroup, we observed that while the level of B cells and T cells may have been higher in the GB0139+SoC-treated group compared with SoC, exhaustion markers for CD4 and CD8 T cells still had a greater decrease with

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GB0139+SoC treatment. These observations suggest that GB0139 therapy may possess a role in enhanced immunological responses to COVID-19 infection and improve recovery compared with SoC.

A high incidence of micro/intravascular thrombosis and thromboembolic events are notable features of severe COVID-19 infection.[36] D-dimer is a fibrin degradation product and elevated concentrations (>1000 ng/mL^{-1}) are strongly linked to the rate of disease, poor outcomes, and death.[37-41] In the overall population, low D-dimer levels were maintained with GB0139+SoC treatment, in contrast to SoC where a steady increase was observed. The observations in additional markers of coagulopathy (FPR, platelets and aPTT) also suggest that inhibition of galectin-3 by GB0139 may impact upon the COVID-19 induced coagulopathy.

Lung injury, with subsequent respiratory failure is a key feature of COVID-19,[42,43]. The reduction in FiO_2, observed in patients treated with GB0139+SoC compared with SoC, requires further investigation.

Recovery from COVID, is an emerging healthcare burden,[44] with studies demonstrating persistent pulmonary dysfunction[45]. We showed that inhaled GB0139 leads to systemic exposure in COVID-19 patients comparable to that previously observed in IPF patients who subsequently had reduced levels of biomarkers associated with fibrotic disease progression.[24] YKL-40 is secreted by activated macrophages and neutrophils in response to IL-1 and IL-6, and associated with extracellular tissue remodelling and fibrosis. PAI-1 is a positive regulator of inflammation, clotting, and fibrosis.[46-48] These drivers of lung fibrosis were both

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consistently lower following GB0139+SoC treatment versus SoC in the current analysis, particularly in patients with baseline NEWS2 ≥4. Transitional monocytes are associated with fibrosis and classical monocytes are associated with antimicrobial effects.[49,50] In the NEWS2 ≥4 subgroup, we observed low levels of transitional monocytes with GB0139+SoC versus SoC (<100% *vs* 100–300%) but higher overall levels of classical monocytes in the former group. These potentially beneficial effects on monocyte subsets may augment the immune response to infection and decrease fibrotic tendency in patients with moderate-to-severe COVID-19. '

In the post hoc analysis of the NEWS2 ≥4 subpopulation, there was an imbalance in clinical severity between the 2 arms (NEWS2 ≥6 9% in SOC and 28.6% on GB0139). In this NEWS2 ≥4 subgroup there were 3/14 (21.4%) deaths amongst patients who received GB0139+SOC versus 3/11 (27.3%) in patients receiving SOC. These numbers are too small to draw any definitive conclusion and this warrants further investigation in a larger clinical study.

Limitations

This study aimed to evaluate the safety and tolerability of inhaled GB0139 in hospitalised COVID-19 patients requiring oxygen and was limited by a small sample size, particularly as patients left the trial towards the end of the 16 day trial period. Given the small number of patients, differences in secondary outcomes such as length of hospital stay become difficult to interpret, as they are liable to skewing by individual patients. The numbers in the study are too small to evaluate if biomarker changes translate into improved clinical outcomes. The numbers of patients in the post hoc analysis of NEWS >4 is therefore smaller again, and whilst the observations

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are of interest, they should be interpreted with caution. When analysing results, the duration of symptoms or treatment was not taken into account. Investigator knowledge of treatment allocation in this open label trial may have biased the provision of inspired oxygen, however the investigators were not part of the clinical team caring for the patient. The use of NEWS2 score in the post hoc analysis may have been skewed due to underlying comorbidities.

Conclusions

This is the first trial of a galectin-3 inhibitor in COVID-19. It has demonstrated that hospitalised patients with COVID-19 can safely and effectively inhale GB0139 and achieve plasma concentrations known to induce biomarker changes. Our data indicate that inhaled GB0139 decreases levels of circulating galectin-3 and may reduce inflammation. Our early phase trial data support the need for further investigation in larger clinical trials for GB0139 in treating hospitalised patients with COVID-19.

Declaration of interests

EG, TQ, AMB, FL, RO'C, RAP, JN, JD, ARA, OK,, MSH, TW, DD, JA, KD: no conflicts of interest to disclose. TS, AMcK, VA, JW-J, RJS, LG, BL, DH, SP, HS, DP, BL: employees of Galecto Inc. NH: received grants from Galecto Inc. AM: employee of Exploristics and received funding from Galecto Inc.

Data sharing

Ownership of the data arising from this study resides with the study team. Scientific publications and the sharing of clinical data generated as part of this trial is crucial to

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better understanding COVID-19 and developing new treatments. As such, the results
will be submitted for publication in a peer-reviewed journal. Data will be shared, with
appropriate data sharing agreements, upon request.

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TABLES

Table 1: Inclusion and exclusion criteria for patient eligibility for study participation

Inclusion criteria	Exclusion criteria
- COVID-19 positive test result within the last 14 days	- Current or recent history, as determined by the Investigator, of severe, progressive, and/or uncontrolled cardiac disease (NYHA class IV), uncontrolled renal disease (eGFR <30 mL/min/1·73 m^2), severe liver dysfunction (ALT >5x ULN) or anaemia (Hb <80 g/L)
- Hospitalised with breathlessness requiring oxygen and evidence of pneumonitis on x-ray	- Women who are pregnant or breastfeeding
- Provision of informed consent from the patient or representative	- Participation in another clinical trial of an investigational medicinal product
- Aged at least 16 years	- Known hypersensitivity to the IMP or excipients (eg, lactose)
- If the patient is of child-bearing potential, the patient, and their partner(s), agree to use medically accepted double-barrier methods of contraception during the study and for at least 90 days after termination of study therapy	- Concomitant use of treatments for COVID-19 that are not recognised as locally approved standard care
	- Significant electrolyte disturbance (hyperkalaemia K+ >5.0 mmol/L or hyponatraemia Na+ <120 mmol/L)
	- Currently receiving potassium sparing diuretics that cannot be reasonably withheld
	- Currently receiving anticoagulation or antiplatelet agents that cannot be reasonably withheld if randomised to receive nafamostat
	- Patients (or their partners) planning on donating sperm/eggs during the trial period
	- Ongoing dialysis
	- History of serious liver disease (Child Pugh score >10)
	- Severe uncontrolled diabetes mellitus
	- In the Investigator's opinion, patient is unwilling or unable to comply with drug administration plan, laboratory tests or other study procedures

ALT=alanine aminotransferase. AST=aspartate transaminase. COVID-19=coronavirus disease 2019. eGFR=estimate glomerular filtration rate.

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Hb=haemoglobin. IMP=investigational medicinal product. NYHA=New York Heart

Association. ULN=upper limit of normal.

Table 2: **Patient demographics and disease characteristics at study entry**

Characteristic	Overall population		Subgroup with baseline NEWS2 ≥4	
	GB0139+SoC (n=20)	SoC (n=21)	GB0139+SoC (n=14)	SoC (n=10)
Male sex; n (%)	11 (55.0)	12 (57.1)	10 (71.4)	5 (50·0)
Age, years				
Mean (SD)	65.2 (13.9)	65.0 (16.4)	65.1 (14.8)	64.5 (19.0)
Median (range)	66.5 (29.0–87.0)	65.0 (27.0–29.0)	66.5 (29.0–86.0)	67.5 (27.0–87.0)
BMI, kg/m^2; mean (SD)	32.1 (5.5)	32.4 (6.3)	32.8 (5.4)	35.9 (5.4)
Ethnicity; n (%)				
White	19 (95.0)	19 (90·5)	13 (92.9)	10 (100)
Asian	1 (5.0)	1 (4.8)	1 (7.1)	0
Black	0	1 (4.8)	0	0
Smoking status; n (%)				
Non-smoker	12 (60·0)	11 (52.4)	9 (64.3)	5 (50·0)
Ex-smoker	8 (40·0)	10 (47.6)	5 (35.7)	5 (50·0)
No. of days since first symptoms; mean (SD)	8.5 (3.8)	8.6 (3.7)	9.8 (4.4)	9.4 (3.7)
Any co-morbidities			12 (85.7)	9 (90·0)
Chronic cardiac disease	5 (26.3)	5 (23.8)	3 (28.6)	3 (30·0)
Hypertension	8 (42.1)	8 (38.1)	7 (50·0)	4 (40·0)
Chronic pulmonary disease	6 (31.6)	5 (23.8)	5 (35.7)	3 (30·0)
Asthma	3 (15.8)	3 (14.3)	2 (14.3)	3 (30·0)

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Chronic kidney disease	2 (10·5)	1 (4.8)	1 (7.1)	1 (10·0)
Chronic liver disease	1 (5.3)	0	0	0
Chronic neurological disorder	2 (10·5)	4 (19.0)	2 (14.3)	2 (10·0)
Diabetes	4 (21.1)	3 (14.3)	4 (28.6)	3 (30·0)
Malignancy	1 (5.3)	3 (14.3)	1 (7.1)	2 (20·0)
Immunocompromised	0	2 (9.5)	0	1 (10·0)
Other	8 (42.1)	11 (52.4)	5 (35.7)	4 (40·0)
NEWS2				
Mean (SD)	4(1.8)	4 (1.7)	5 (1.5)	5(1.6)

NEWS2=National Early Warning Score 2. SD=standard deviation. SoC=standard of care.
Mean NEWS2 rounded to whole integers to represent the scale.
A full breakdown comorbidities at an individual participant level, please see table E3

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Table 3: Primary outcome of safety assessments. Continuous outcome variable results: haematological and biochemical safety laboratory outcomes and vital signs

Variable	Mean	95% HPD interval	
		Lower limit	Upper limit
Haemoglobin (g/L)	−0·495	−7·21	5·963
Haematocrit (ratio)	−0·206	−5·04	4·996
Red cell count (109/L)	−0·128	−4·87	4·641
Mean cell volume (fl)	−0·136	−5·73	5·546
Mean cell Hb (pg)	−0·344	−5·36	4·608
Mean cell Hb concentration (g/L)	0·050	−6·94	7·375
White cell count (10^9/L)	−0·489	−6·04	5·255
Neutrophil count (10^9/L)	0·031	−5·80	6·003
Lymphocytes (10^9/L)	−0·030	−5·64	5·422
Monocyte count (10^9/L)	0·112	−5·93	5·751
Basophil count (10^9/L)	−0·640	−5·88	4·626
Eosinophil count (10^9/L)	0·691	−10·8	12·99
Platelet count (10^9/L)	17·76	−25·5	59·05
Random glucose (micromole/L)	−0·536	−6·45	4·911
Urea (mmol/L)	0·533	−5·08	6·125
Sodium (mmol/L)	−0·203	−5·25	5·327
Potassium (mmol/L)	0·144	−4·96	5·791
Chloride (mmol/L)	−1·05	−7·05	4·631
Magnesium (mmol/L)	−0·090	−5·95	6·038
Bicarbonate (mmol/L)	0·856	−4·65	6·472
Creatinine (micromole/L)	−1·77	−8·39	4·721
Total protein (g/L)	−0·896	−6·93	5·036
Albumin (g/L)	−1·05	−6·37	4·081
AST (U/L)	−10·3	−28·4	7·814
Total bilirubin (micromole/L)	0·741	−4·83	6·798
GGT (U/L)	−15·2	−44·2	15·82
ALT (U/L)	−2·97	−26·2	20·77
Alkaline phosphatase (U/L)	−1·37	−9·75	6·990

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LDH (U/L)	−18·8	−62·7	25·53
Ferritin (micromole/L)	63·66	−96·9	220·1
CRP (mg/L)	0·168	−27·1	27·82
Troponin (ng/L)	−0·469	−10·5	10·06
Triglycerides (mmol/L)	−0·696	−6·69	4·959
D-dimer (ng/ml)	5·609	−208	209·1
Creatinine kinase (U/L)	−23·1	−76·8	25·12
INR (ratio)	−0·444	−5·43	4·268
Prothrombin time (seconds)	−0·484	−5·42	4·740
Fibrinogen (g/L)	−0·071	−6·14	5·822
aPTT (seconds)	0·226	−6·06	6·674
Protein C (IU/ml)	−0·134	−5·78	5·671
Antithrombin (IU/ml)	−0·131	−6·16	5·718
O_2 saturation (%)	0·616	−3·49	4·848
Respiratory rate (bpm)	0·774	−5·06	6·715
Systolic Blood Pressure (mm Hg)	−3·62	−13·6	6·819
Diastolic Blood Pressure (mm Hg)	−0·773	−7·81	6·425
Temperature (degrees C)	0·196	−4·78	5·035
Pulse (bpm)	5·242	−4·52	14·52
ECG rate	2·143	−8·11	12·86
ECG QTc	−4·37	−22·5	13·09

All of the 95% HPD credible intervals include zero, and therefore none are statistically significant. ALT=alanine aminotransferase. aPTT=activated partial thromboplastin time. AST=aspartate aminotransferase. CRP=c-reactive protein. ECG=electrocardiogram. GGT=gamma-glutamyltransferase. Hb=haemoglobin. HPD=highest posterior density. INR=international normalised ratio. LDH=lactate dehydrogenase. QTc=corrected QT interval.

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Table 4: **Adverse events in the safety population**

Study group	Total population		Subgroup with baseline NEWS2 ≥4	
	GB0139+SoC (n=20)	SoC (n=21)	GB0139+SoC (n=14)	SoC (n=10)
Any AE, n (%)	14 (70·0)	12 (57.1)	10 (71.4)	4 (40·0)
Any serious AE, n (%)	1 (5.0)	0 (0·0)	0 (0·0)	0 (0·0)

AE=adverse event. NEWS2=national early warning score 2. SoC=standard of care.

*Three serious adverse events were reported for one patient in the GB0139 study group; small bowel obstruction, paracetamol overdose and worsening of hyponatraemia.

Table 5: **Trough (pre-dose) plasma GB0139 concentrations (ng/mL) in patients with COVID-19**

	Baseline	Day 3	Day 5	Day 8	Day 11
n	18	20	12	9	4
Arithmetic mean (SD)	BLQ	22.91 (12.03)	8.58 (4.60)	6.66 (3.80)	5.85 (2.30)
CV, %	–	53	54	57	39
Geometric mean	–	19.94	7.81	6.01	5.50
Median (min–max)	BLQ (BLQ–BLQ)	18.28 (7.45–51.21)	7.32 (3.94–21.62)	5.27 (3.87–15.99)	5.70 (3.22–8.81)

BLQ=below the limit of quantitation (0·5 ng/mL). CV=coefficient of variation.

SD=standard deviation.

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FIGURES
Figure 1 **Consort diagram patient disposition**

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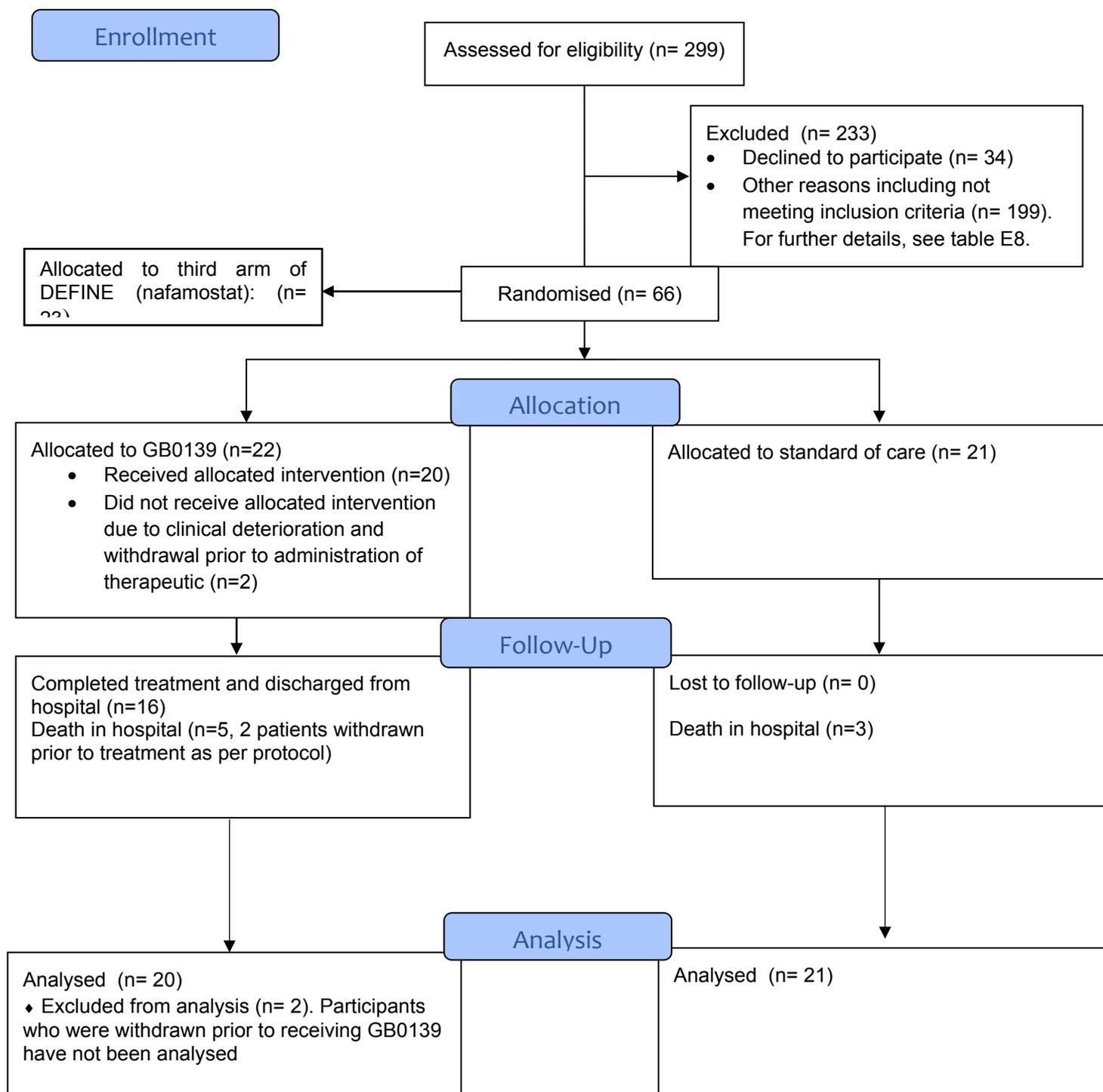
Figure 2 Individual patient serum galectin-3 concentrations from all patients receiving GB0139+SoC (red) or SoC (blue)
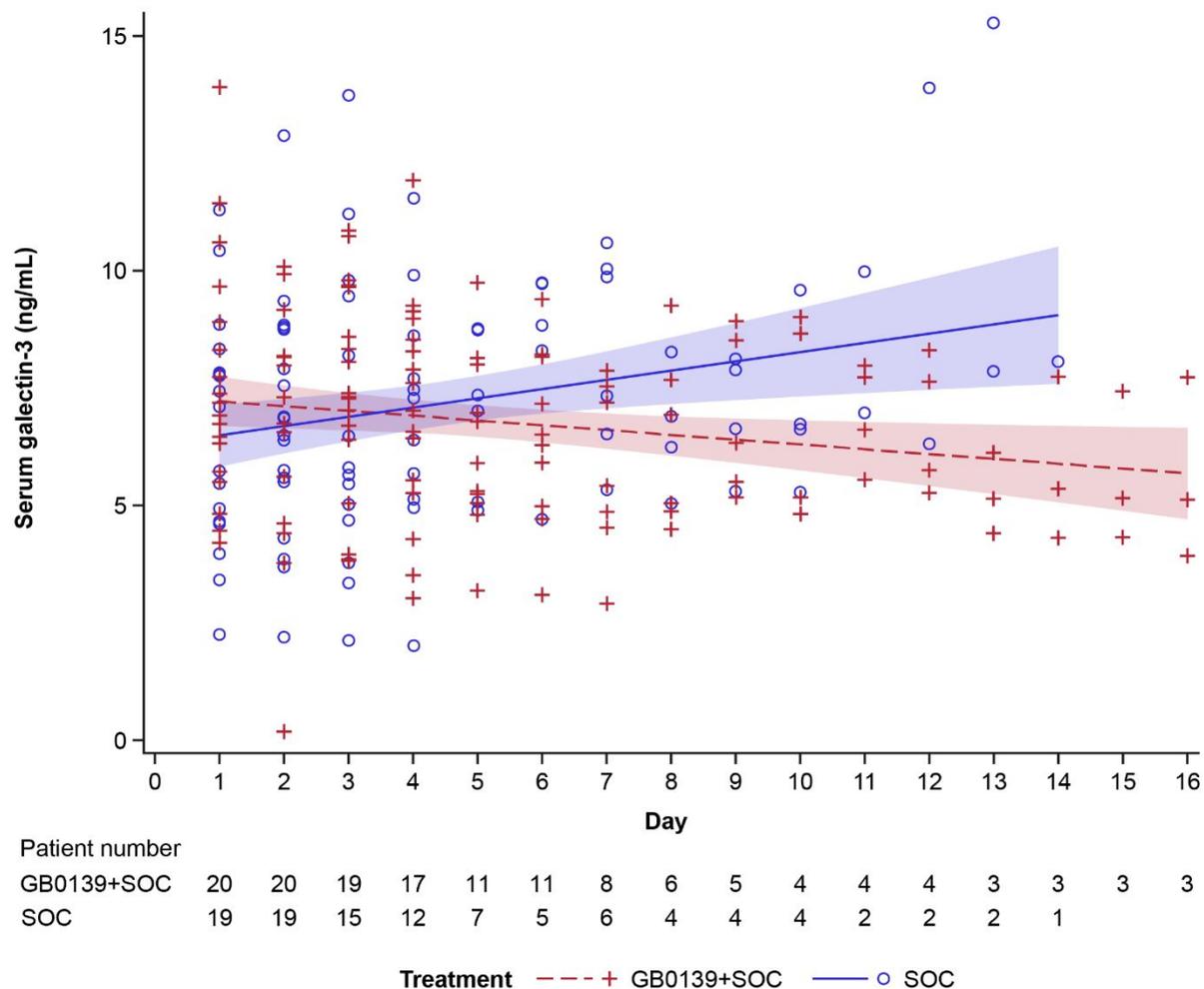
Linear regression analysis showing line of best fit with 95% CI (shaded area).

CI=confidence interval. SoC=standard of care. Of note, overall treatment effect,

using all available data to day 16 was also significant for GB0139+SoC versus SoC

(post-hoc ANCOVA over days 2-16: p=0·0001) however the last day for data

availability in the SoC arm was day 14.

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Figure 3 **Absolute change from baseline in markers of inflammation in the**

overall population: (A) C-reactive protein, (B) lactate dehydrogenase, (C)

neutrophil/lymphocyte ratio, and (D) CXCL10
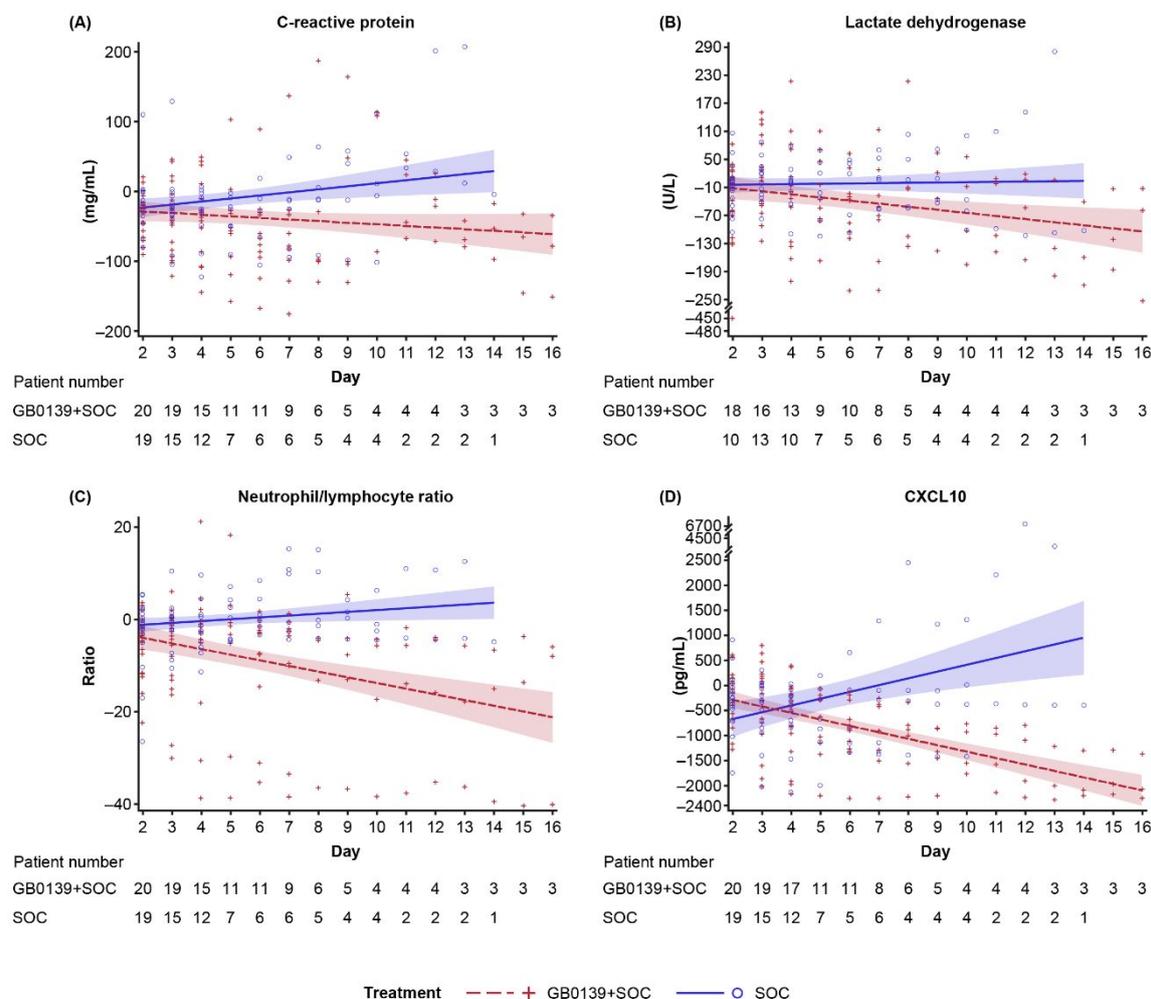
Individual patient-level data from all those who received GB0139+SoC (red) or SoC

(blue): linear regression analysis showing line of best fit with 95% CI (shaded area).

CI=confidence interval. CXCL10=C-X-C motif chemokine ligand 10. SoC=standard of

care.

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Figure 4 **Effect of GB0139+SoC (blue) and SoC (red) on coagulopathy:**

individual patient-level data for (A) D-dimer levels, (B) fibrinogen/platelet ratio,

(C) platelets, and (D) activated partial thromboplastin time
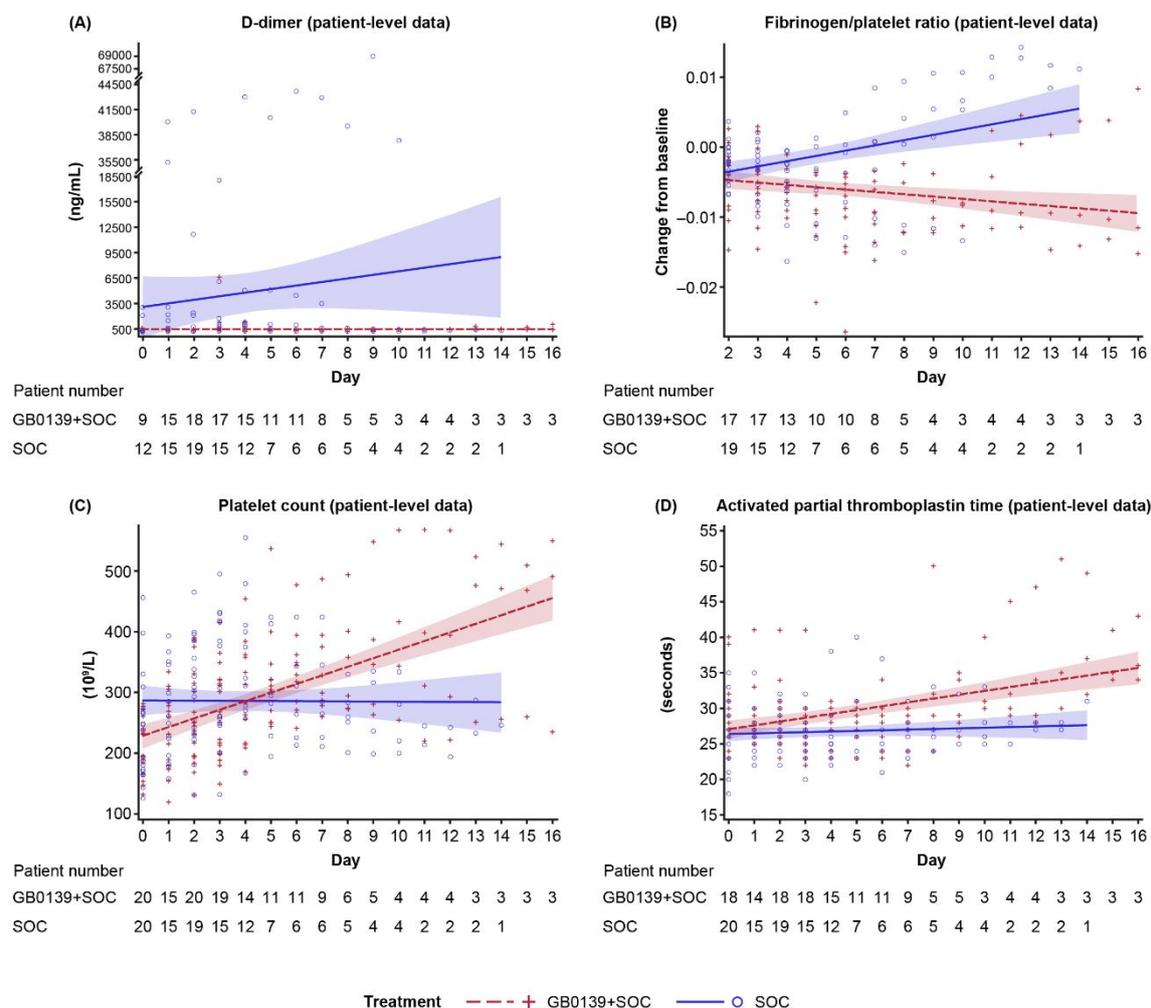
Parts A and B display linear regression analysis showing line of best fit with 95% CI (shaded area). CI=confidence interval. LLN=lower limit of normal. SoC=standard of care. ULN=upper limit of normal.

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An inhaled galectin-3 inhibitor in COVID-19 pneumonitis: a phase Ib/IIa randomised controlled trial

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Online Data Supplement

Supplementary methods

Supplementary methods

Study design

Randomisation was carried out by a member of the research team using a centralised web-based service provided by the Edinburgh Clinical Trials Unit (Usher Institute, University of Edinburgh, Edinburgh, UK). A minimisation procedure based on sex, age, body mass index, and a history of diabetes was used for randomisation and incorporated a 20% random element, meaning that the treatment arm selected by the minimisation procedure was switched with a probability of 20%. Investigators, patients and treating clinicians were unaware of treatment allocation in advance, however were aware of individual allocations following assignment. SoC included dexamethasone, tocilizumab, antibiotics, low molecular weight heparin, and remdesivir, and was determined by the treating physician.

Pharmacokinetics

Samples were analysed by Simbec Orion (Merthyr Tydfil UK) using a validated high performance liquid chromatography method with tandem mass spectrometry detection. The lower limit of quantitation (LLOQ) was 0.5 ng/mL.

Cytokine analysis

Pre-selected relevant biomarkers of COVID-19 (IL-1β, IL-6, IL-8, TNF-α, C–C motif chemokine ligand 2, IL-17A, IL-10, C-X-C motif chemokine ligand 10 [CXCL10], granulocyte-macrophage colony-stimulating factor (CSF), amphiregulin and IL-1rα)(3) were analysed using the ELLA platform. Lymphocyte and monocyte

phenotype determined by flow cytometry. Plasma biomarkers known to be involved

in pulmonary fibrosis(4) were analysed by ELISA (chitinase-3-like protein 1 [YKL-40]

and plasminogen activator inhibitor-1 [PAI-1]) (R&D Systems, MN, USA).

Flow cytometry

All staining and processing were performed in a class II microbiological safety

cabinet (MSCII). Centrifugation steps used capped tubes in a biosafety bucket,

which were loaded and unloaded within the MSCII. Fluorescence-activated cell

sorting (FACS) tubes containing fragment crystallisation (Fc) block were prepared

with 5 µL of Trustain FcX (Biolegend 422301) and 5 µL of monocyte blocker

(Biolegend0426102). Antibody cocktails were prepared in FACS staining buffer

(phosphate buffered saline 2% flow cytometry staining buffer [Gibco]) containing

Brilliant violet plus buffer (BD 566385). Five minutes after addition of 100 µL of whole

blood/EDTA, 50 µL of antibody staining cocktail was added to each tube before

incubation in the dark at room temperature for 20 minutes. Red blood cells were

lysed using BD FACS lyse and washed twice in staining buffer before fixation

(Biolegend Fixation buffer). After 20 minutes, fixed samples were moved from the

MSCII to cold storage. All samples were collected on a 5 laser BD LSR Fortessa at

the FACS facility at Queens Medical Research Institute within 24 hours of staining.

Freshly prepared 8 peak calibration beaks were ran daily prior to sample collection.

Gating strategies

After exclusion of debris and selection of lymphocytes on FSC (forward scatter)

versus SSC (side scatter) doublets were excluded and CD3+ T cells selected. CD4+

and CD8+ T cell subsets were selected and subdivided into naïve versus memory subsets according to expression of CD45RA and CCR7 as shown. Expression of T-cell receptor $\gamma\delta$ on double negative cells expressing CD3 but neither CD4 nor CD8 was used to identify gamma-delta-T cells. Treg were gated within the CD4+ population as CD25+CD127-low cells.

After exclusion of debris and doublets CD45+ cells were selected and subdivided into lymphocytes, monocytes and granulocytes according to CD45 expression level and side scatter properties. Within the lymphocyte gate B cells were selected as CD19+ and antibody secreting cells identified as CD27+CD38+. Lineage negative monocytes (CD3/CD19/CD66b/CD56−) were subdivided to classical, transitional and non-classical subsets according to expression of CD14 and CD16. Granulocytes were subdivided into neutrophils and eosinophils on the basis of CD16 expression.

After exclusion of debris and doublets SSC-H high, lineage negative (CD3/CD19/CD56/Siglec-8) events were gated and CD15+CD66b+ cells selected as neutrophils. Immature neutrophils were gated as CD10 low.

Statistical analysis

Sample size

Note that this study is an early phase clinical trial with small sample size and therefore we only have sufficient power to detect large differences between groups.

 An indicative sample size calculation suggested that 20 patients per group provides 80% power to detect an effect size of 0.7 using a two group t-test with a one sided 10% one-sided significance level (equivalent to a two-sided 20% level) and assuming 5% missing data, for the difference of means in a biomarker between

GB0139 and control groups. For ease of presentation and to reduce confusion for the reader we chose to present the usual two-sided 95% credible intervals. Non-significant results should be interpreted cautiously.

Bayesian methods

Results were reported as posterior mean differences and highest posterior density (HPD) intervals, derived from the posterior distributions of the parameters generated from the Bayesian models. When Bayesian analyses are conducted, we combine prior information (in this case, non-informative prior information) with the observed data using Bayes Theorem to form a posterior distribution based on the parameter of interest (in this case the true underlying mean difference)(1).

Highest posterior density intervals were chosen because they ensure that all parameter values contained inside the interval are more likely (i.e. have highest probability density) compared to all those outside, even if the posterior distribution is non-symmetric(2).

Statistical analysis of key secondary variables

To evaluate the change from baseline values for key secondary variables (namely FiO_2) a Bayesian GLMM was fitted including the following explanatory variables: baseline, patient (as a random effect), day of measurement post-randomisation (as a continuous variable), trial arm (GB0139+SoC/SoC), and an interaction term for day of measurement and trial arm. We assessed whether changes in secondary variables over time significantly differed by treatment arm by means of the interaction term. Pearson correlation was used to evaluate whether there was statistical

evidence for a linear relationship among galectin-3 levels and biomarkers of interest. The p-value for target engagement was calculated using an Analysis of Covariance model adjusting for treatment and baseline galectin-3. This was done using information from days 2–7 and with assessment day as a repeated measure. The p value assessed the significance of the variable treatment in the model.

For AEs, a Bayesian logistic regression model was used to compare the odds of "at least one" AE between trial arms; and a Bayesian Poisson model was fitted to the total number of AEs per patient to compare rates of AEs. Trial arm was the only explanatory variable in the models, with no other covariates. All statistical models were fitted using SAS software (SAS Institute Inc., Cary, NC, USA).

Post hoc analysis using a combination of ANCOVA and Bayesian GLMM (Generalized Linear Mixed Model) has been performed on selected biomarkers and clinical parameters. The ANCOVA assessment looked to determine the presence of an overall treatment effect, with change from baseline as the dependent variable, adjusting for treatment and baseline value, including time as a repeated measure statement across subjects. The Bayesian GLMM analysis looked to evaluate the change in the treatment groups across time. Fixed effects included baseline value, treatment, time (as a continuous variable) and a treatment by time interaction. Subject was fitted as a random effect. The assessment used the interaction term (treatment and time) to determine whether the linear trend significantly varied by treatment group by way of the 95% HPD (credible interval) interval excluding zero.

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Supplementary results

Biomarkers of organ function

Despite initial FiO_2 being higher on admission in the GB0139+SoC arm than the SoC arm (44.05% *vs* 34.05%, respectively), the rate of FiO_2 (% per day) decline was significantly greater with GB0139+SoC, with a posterior mean difference of −1.51 (95% HPD −2.90, −0.189) versus SoC (figure E3A).

The SpO2/FiO2 improved over time in the GB0139+SoC arm with the converse in SoC (Figure E3B).

Post hoc analysis of NEWS2>4 subgroup

In the current study, 40% of patients had mild disease (defined as NEWS2 ≤3) but there was a marked difference in the clinical severity between GB0139+SoC and SoC groups, with 30% of patients randomised to GB0139+SoC identified as having low clinical risk at baseline (NEWS2 ≤3) compared with 50% in the SoC group. Similarly, 20% of patients in the GB0139+SoC arm were of high clinical risk at baseline (NEWS2 ≥6), versus 5% in the SoC arm. As patients with severe disease may benefit more from lowered galectin-3, a post hoc subgroup analysis of patients with a baseline NEWS2 ≥4 was undertaken. The decrease in cytokines, CXCL-10, IL-10, IL-6, and TNF were particularly evident in this subgroup (figure E6 A–D), and analysis of lymphocytes by flow cytometry showed that while total lymphocyte numbers remained steady in the SoC arm, there was an increase with GB0139+SoC. Markers of CD4 T-cell activation were similar across groups. Activation markers on CD8 T cells appeared stable to day 4 in both groups, at day 7 were higher with SoC versus GB0139+SoC. There was a consistent decrease in exhaustion markers for both CD4 and CD8 in patients treated with GB0139+SoC

compared with an increase seen in those receiving SoC. There was a higher percentage of B cells with GB0139+SoC treatment compared with SoC (figure E7).

In the subgroup with baseline NEWS2 ≥4, YKL-40 levels remained consistently low with GB0139+SoC, and PAI-1 was reduced over time in both treatment arms, with levels remaining lower with GB0139+SoC treatment versus SoC (figure E4 A, B and C).

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Supplementary figures

Figure E1 Gating strategies for T cell subsets
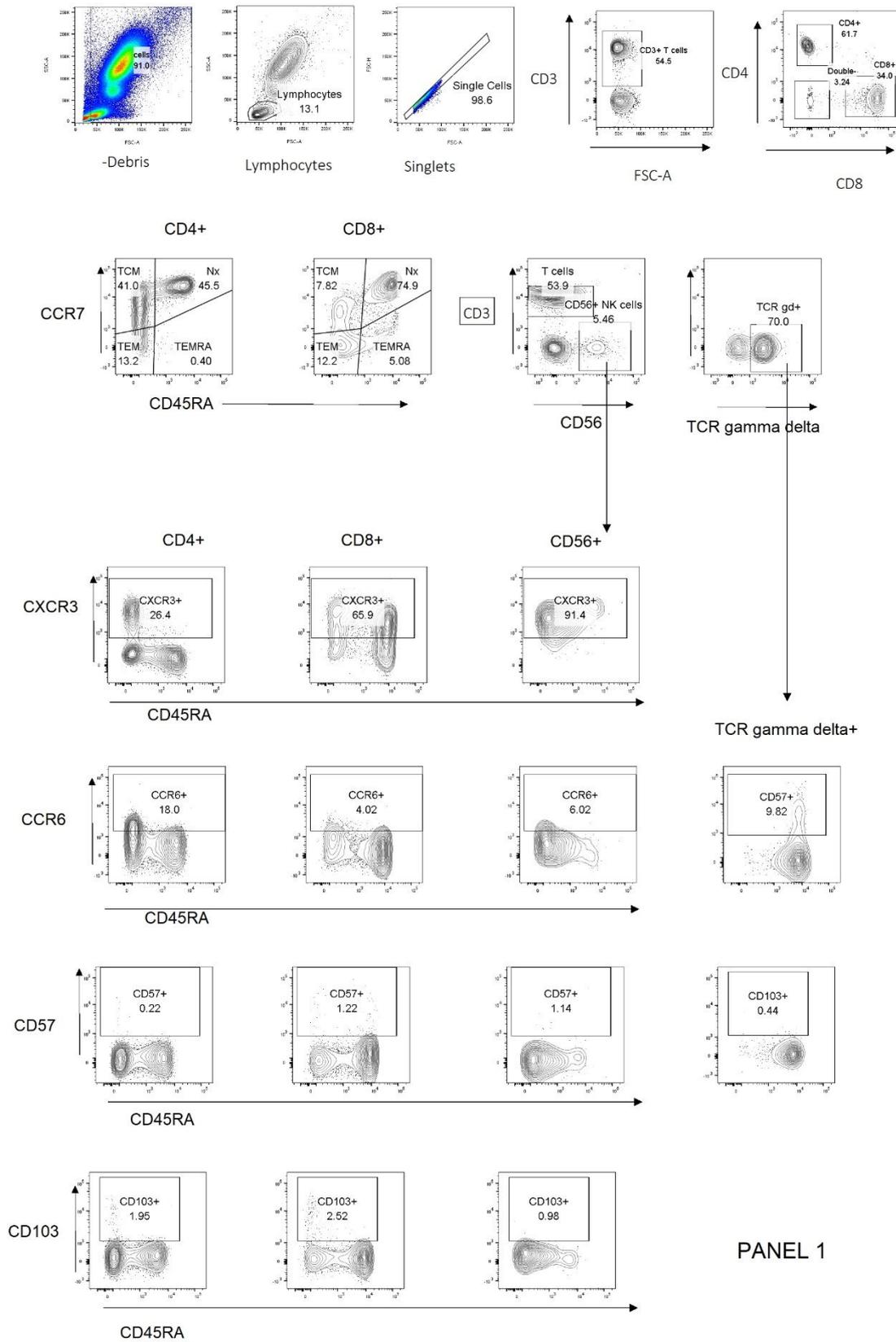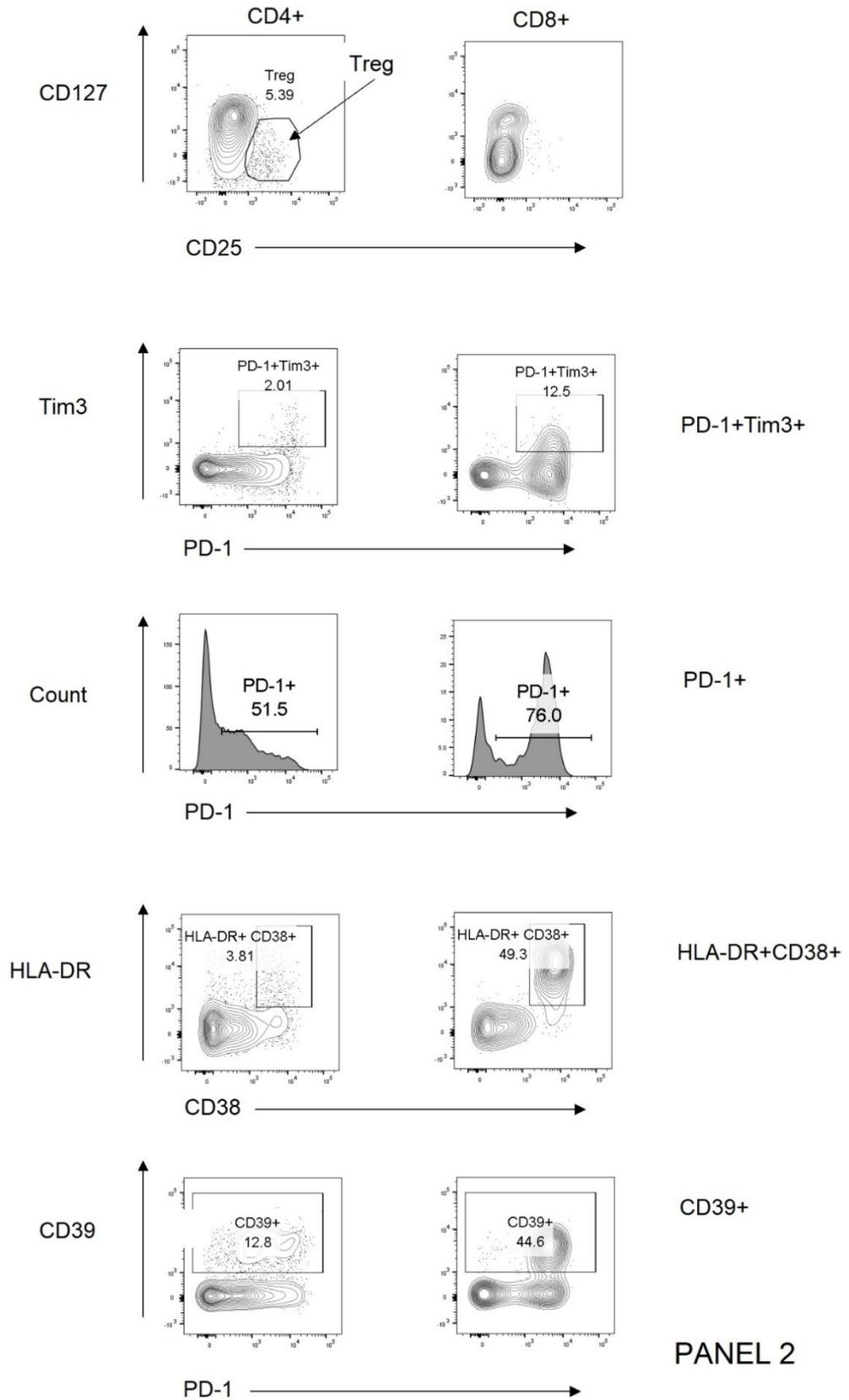
PANEL 1

PANEL 2

Gating strategy for B cells and monocytes

PANEL 3
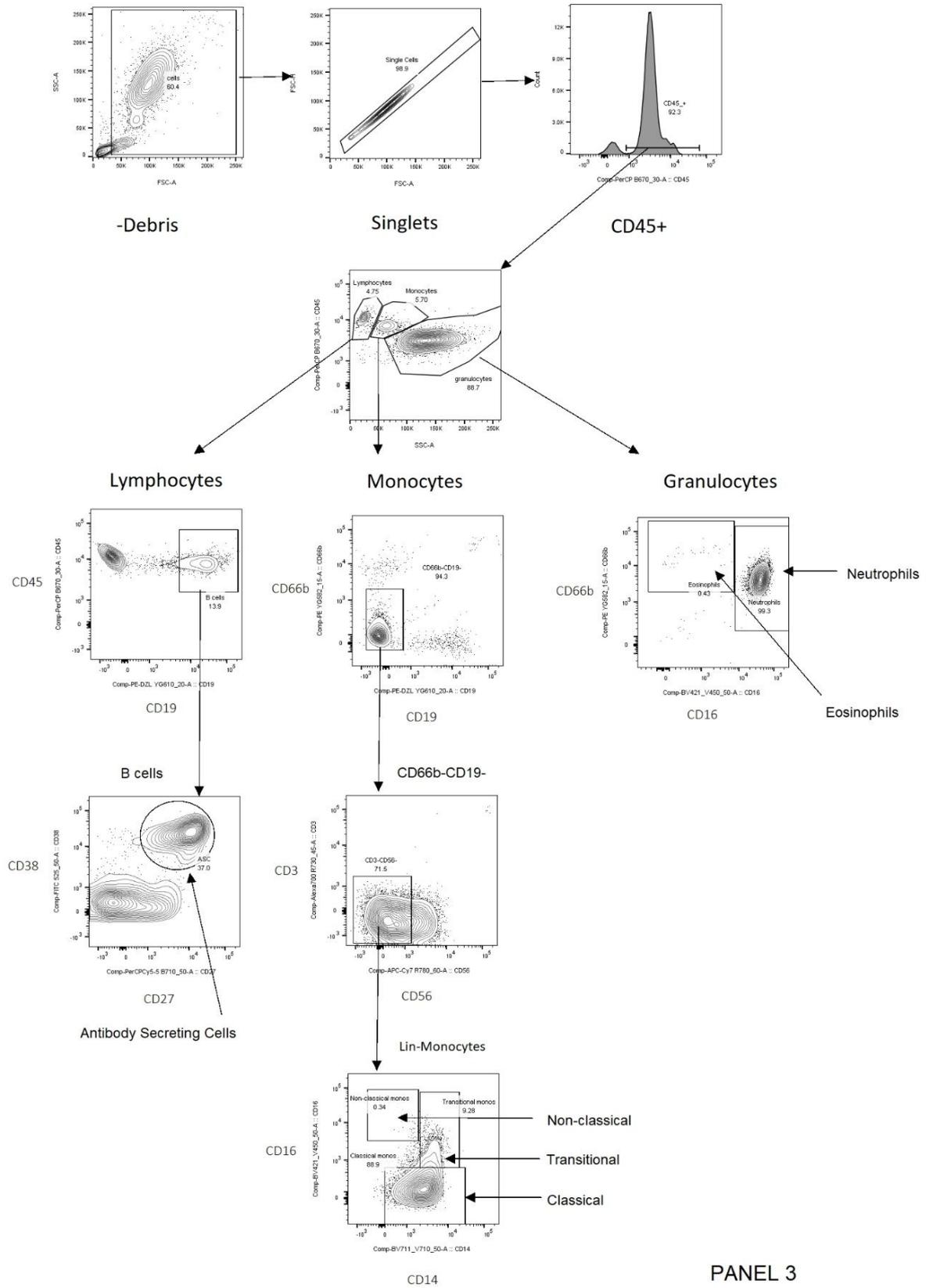
Gating strategy for neutrophils
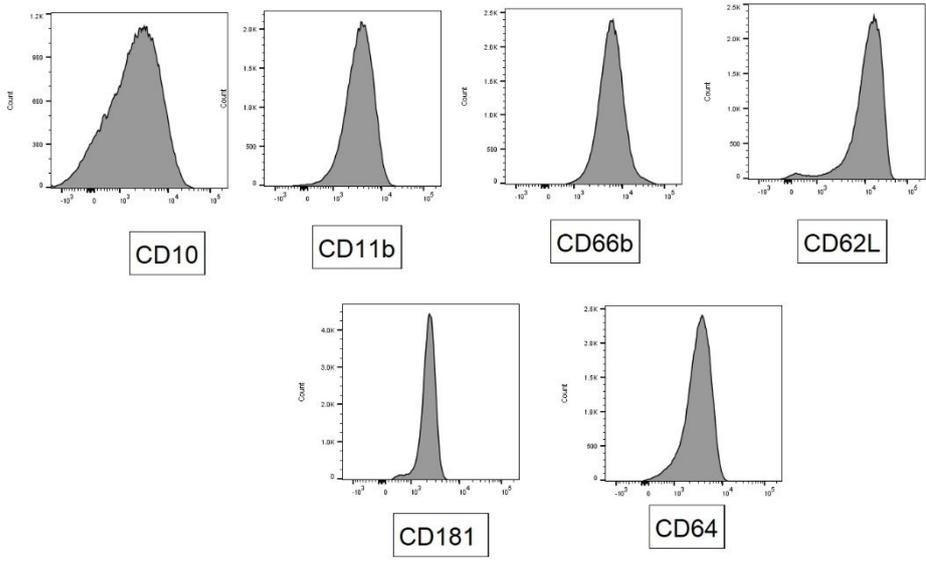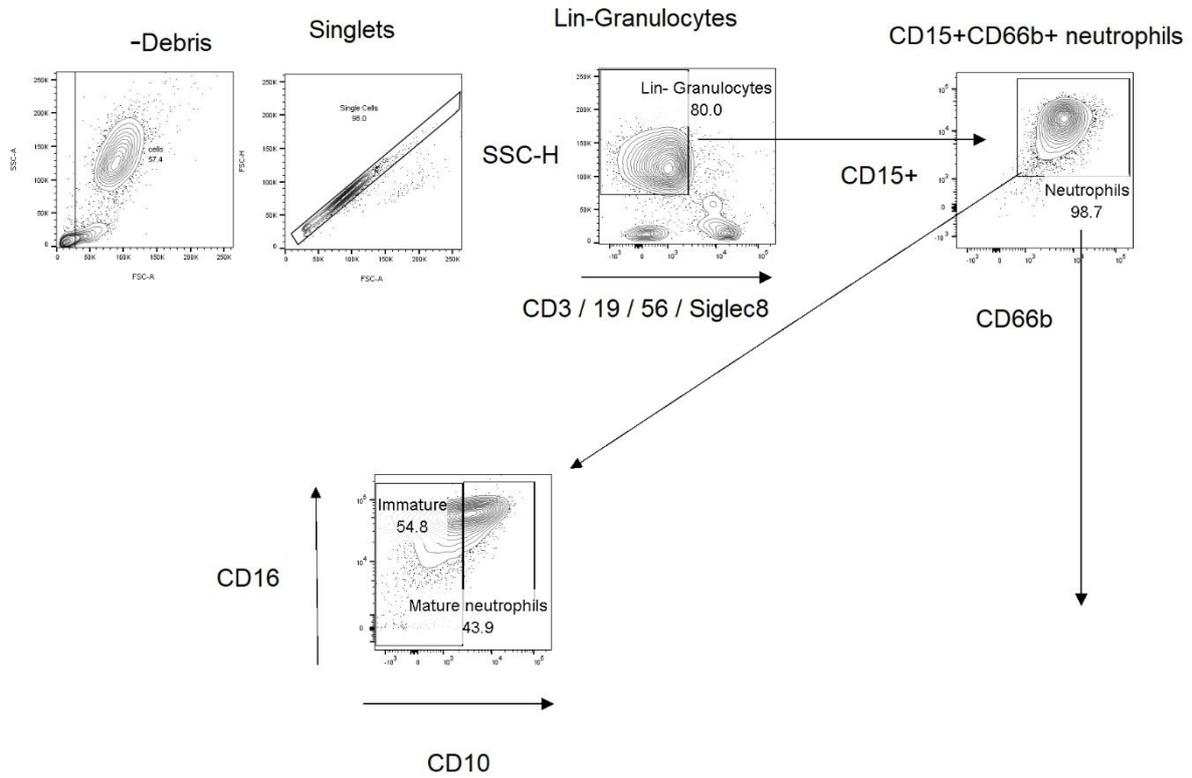
Figure E2: GB0139 plasma concentrations in patients with COVID-19 and patients with IPF
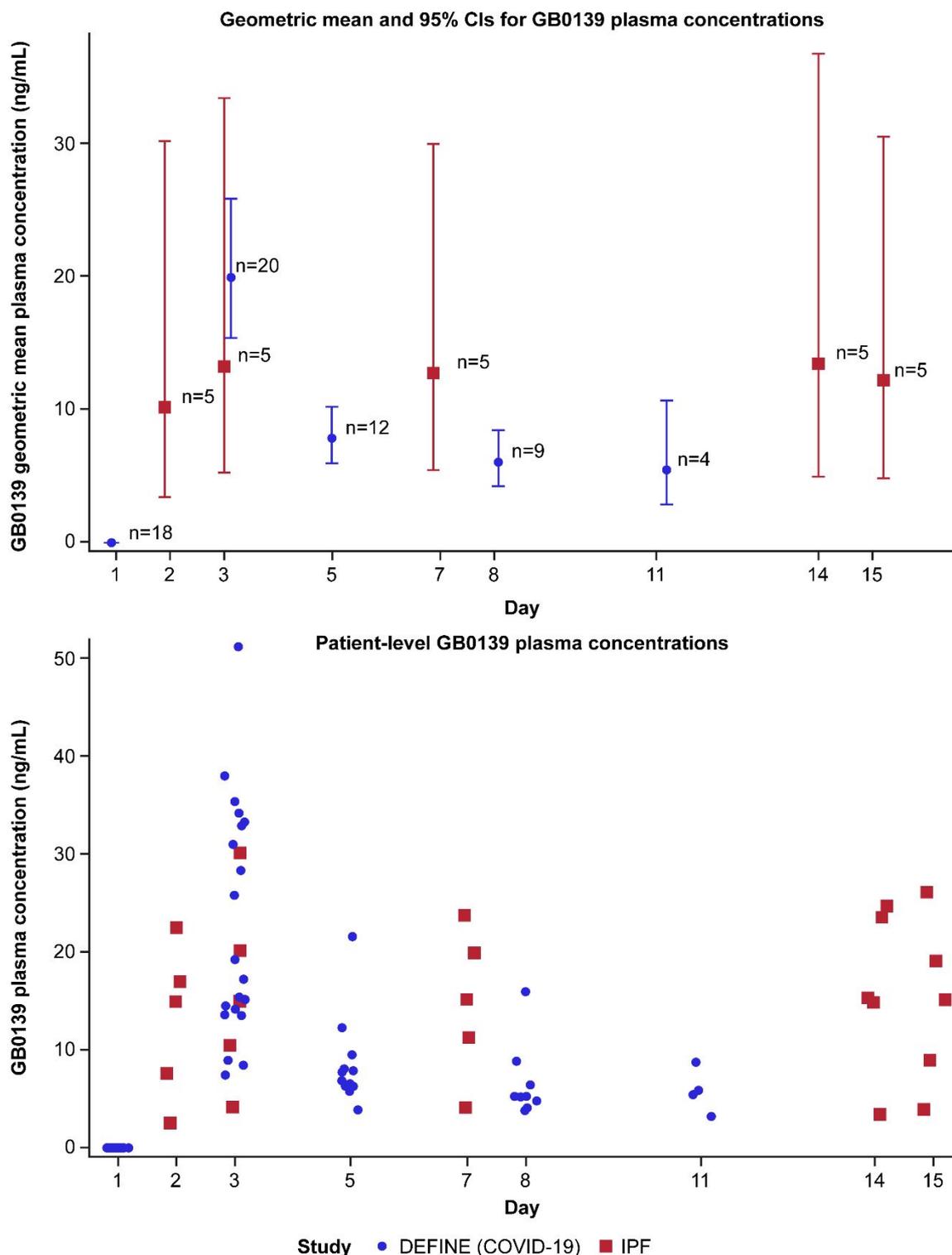
Figure E2: GB0139 plasma concentrations in patients with COVID-19 and patients with IPF

CI=confidence interval. IPF=idiopathic pulmonary fibrosis.

Figure E3: Effect of GB0139+SoC (red) and SoC (blue) on FiO$_2$: (A) individual patient-level data from Days 1–16 and (B) SpO2/FiO$_2$ ratio Days 1-16
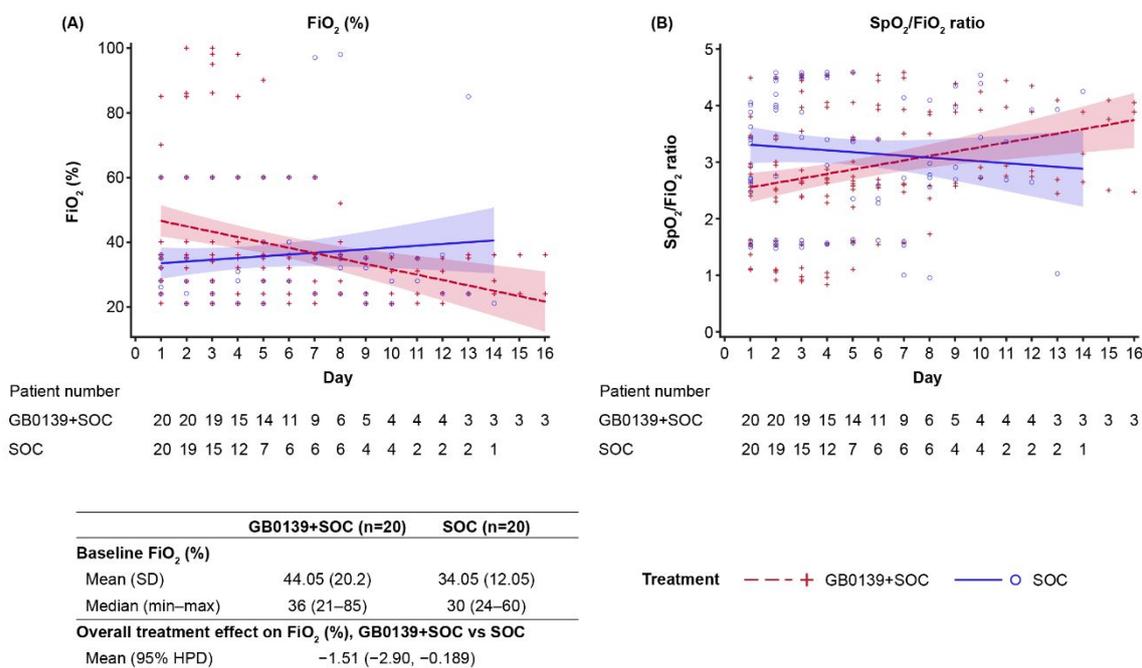
	GB0139+SOC (n=20)	SOC (n=20)
Baseline FiO$_2$ (%)		
Mean (SD)	44.05 (20.2)	34.05 (12.05)
Median (min–max)	36 (21–85)	30 (24–60)
Overall treatment effect on FiO$_2$ (%), GB0139+SOC vs SOC		
Mean (95% HPD)	−1.51 (−2.90, −0.189)	

Linear regression analysis showing line of best fit with 95% CI (shaded area).

CI=confidence interval. FiO$_2$= fraction of inspired oxygen. SpO2 = oxygen saturation

HPD=highest posterior density. max=maximum. min=minimum. SD=standard

deviation. SoC=standard of care.

Figure E4: **Effect of GB0139+SoC (red) and SoC (blue) on markers associated with fibrosis: (A) YKL-40, (B) PAI-1, and (C) monocyte subsets**
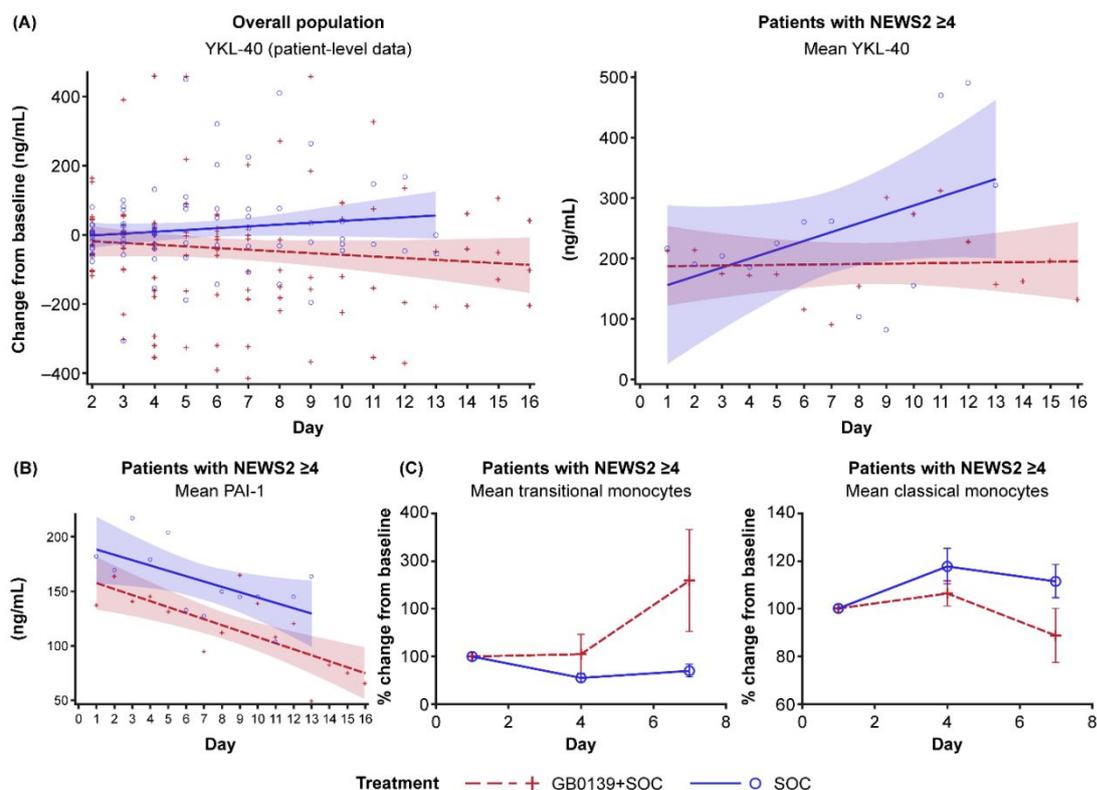
Parts A and B display linear regression analysis showing line of best fit with 95% CI (shaded area). CI=confidence interval. NEWS2=national early warning score 2. PAI-1=plasminogen activator inhibitor-1. SoC=standard of care. YKL-40=chitinase-3-like protein 1.

***Figure E5*: Viral load decreased in both groups over time**
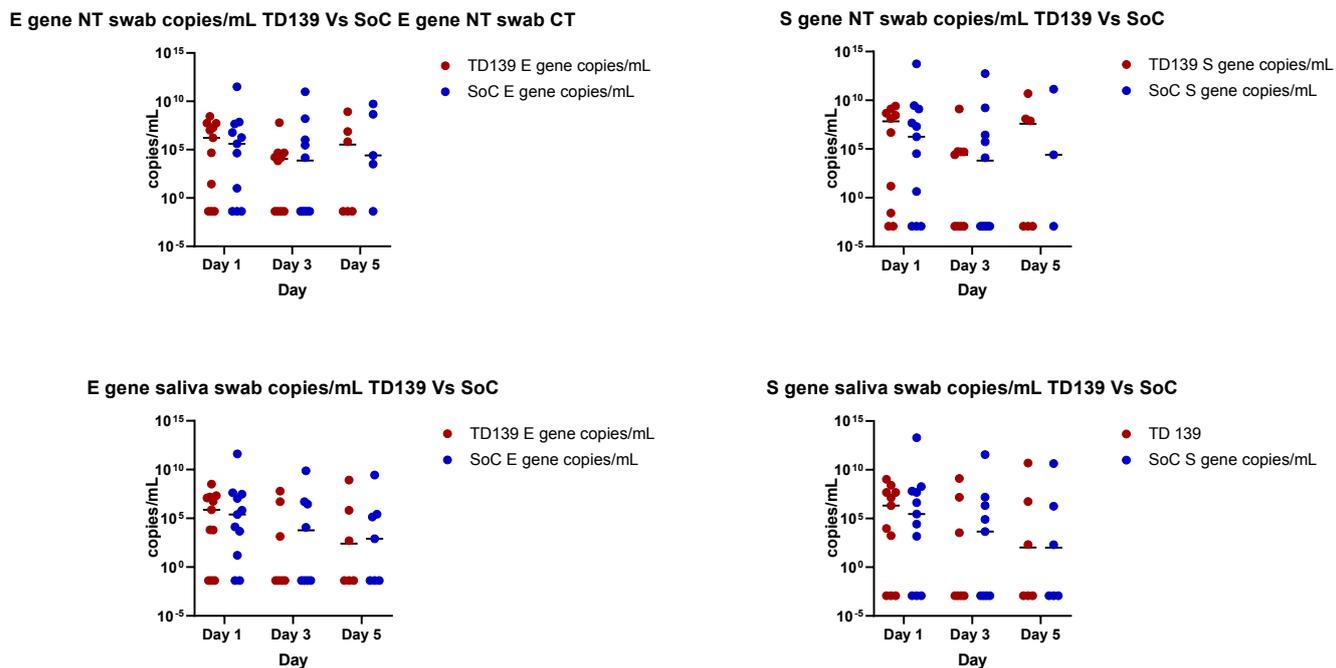
a and b – copies per mL of E and S gene respectively in nasopharyngeal and

oropharyngeal swabs

c and d – copies per mL of E and S gene respectively in saliva

In both groups, the numbers of participants declined with time representing

participant discharge or withdrawal

P values shown for day 5 with 2-way ANOVA; A- 0.9758 B- 0.8177 C- >0.9999 D

>0.9999

Figure E6: **Change from baseline in markers of inflammation in the population with baseline NEWS2 ≥4: patient level data for (A) CXCL10, (B) IL-10, (C) IL-6, and (D) tumour necrosis factor**
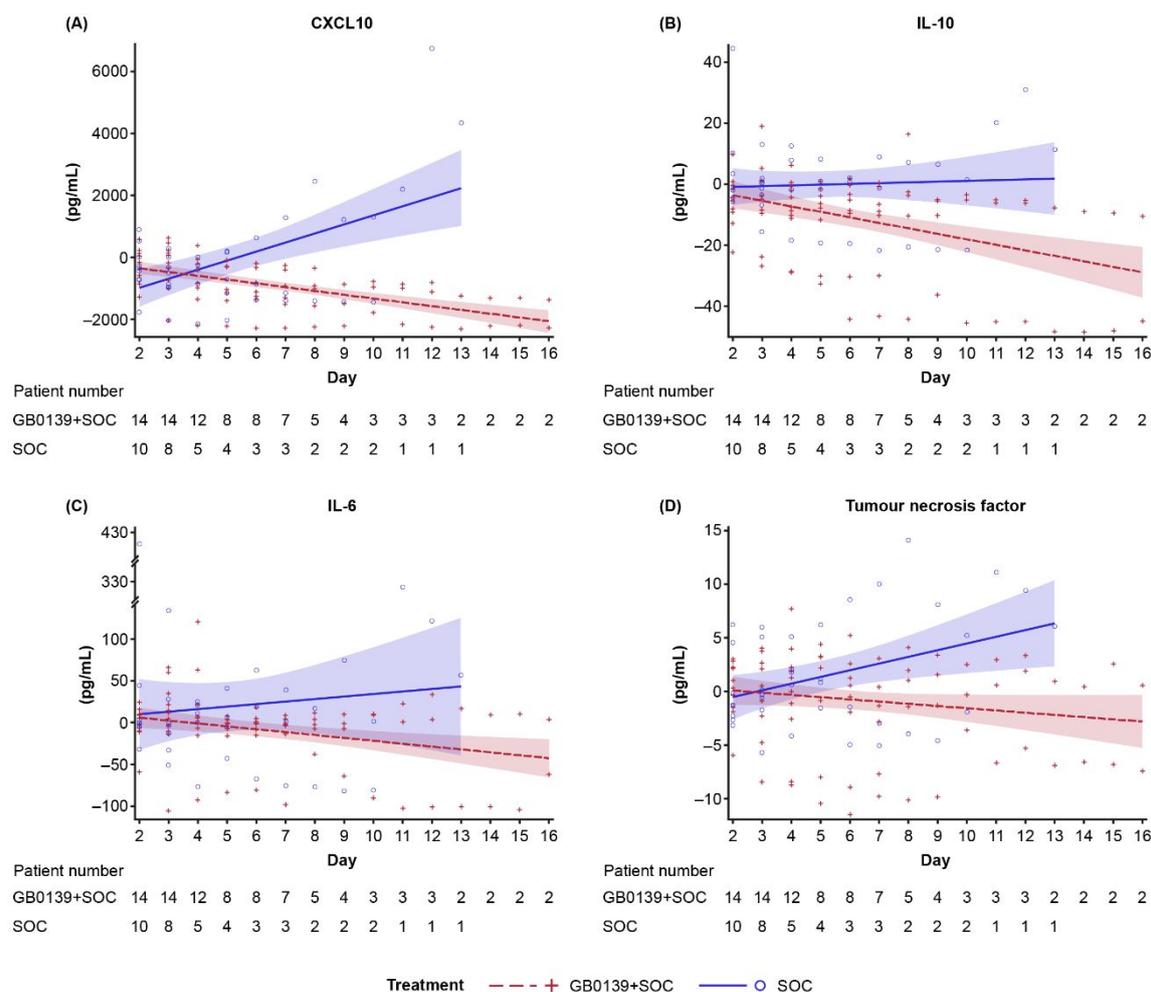
Individual patient-level data from all those who received GB0139+SoC (red) or SoC (blue): linear regression analysis showing line of best fit with 95% CI (shaded area). CI=confidence interval. CXCL10=C-X-C motif chemokine ligand 10. IL=interleukin. NEWS2=national early warning score 2. SoC=standard of care.

***Figure E7*: Effect of GB0139+SoC (red) and SoC (blue) on circulating lymphocytes in the population with baseline NEWS2 ≥4: (A) total lymphocytes, (B) CD4 T cell activation markers, (C) CD8 T cell activation markers, (D) CD4 T cell exhaustion markers, (E) CD8 T cell exhaustion markers, and (F) B cells**
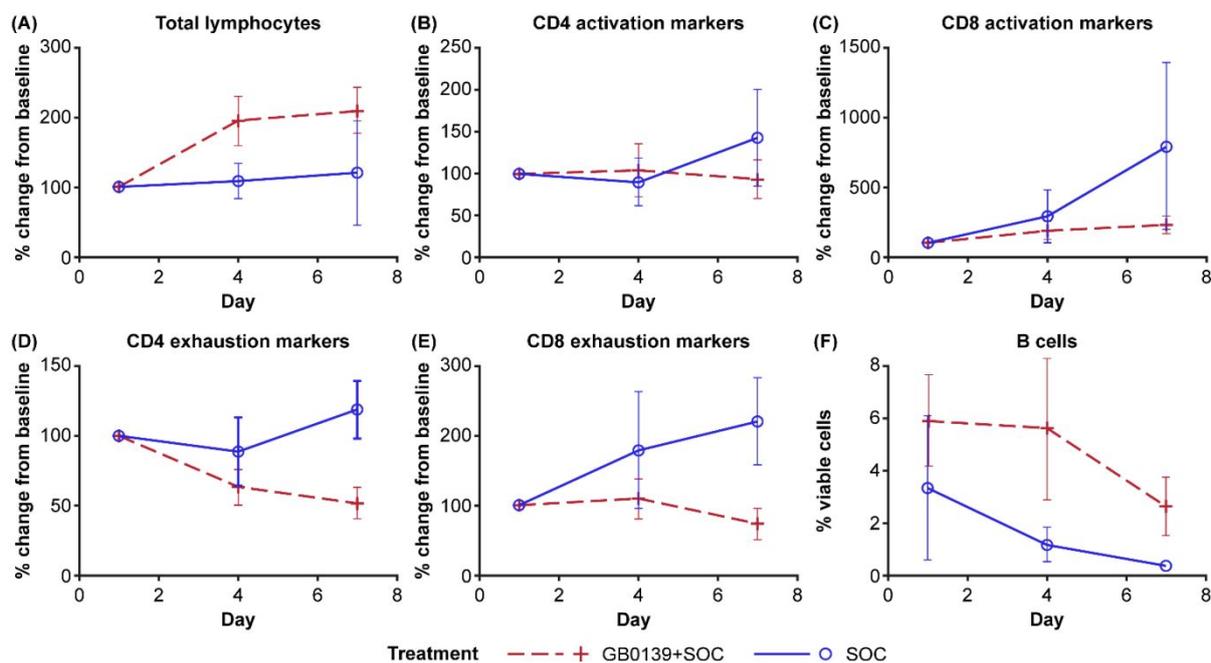
Data are expressed as mean (±SEM) % change from baseline or % viable cells.

NEWS2=national early warning score 2. SEM=standard error of the mean.

SoC=standard of care.

P values shown for day 7 with 2-way ANOVA and Bonferroni correction: A: 0.3362 B: 0.908 C: 0.0313 D: 0.0123 E: 0.0541 F: >0.9999

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Supplementary tables

Table E1 Daily assessments and blood samples.

Blood sampling	Frequency
FBC, differential WCC (excluding eosinophils), **platelets**	Daily
Urea, creatinine, sodium, potassium, bicarbonate, chloride, magnesium, calcium, **bilirubin , ALT, AST, Alkaline phosphatase**, GGT, total protein, albumin, CRP, ferritin, triglycerides, **troponin**, creatinine kinase	Daily
Activated partial thromboplastin time (APTT), Prothrombin time (PT), INR, fibrinogen, D-dimer	Daily
Glucose and/or capillary blood glucose	Daily
Biomarkers	Daily
Immune phenotyping	Daily

Safety blood assessments in bold

Table E2: List of antibodies

Target	Fluorophore	Clone	Isotype	Category number
CD3	AF700	UCHT1	ms IgG1	300424
CD4	BV711	OKT4	ms IgG2b	317440
CD8	BV421	RPA-T8	ms IgG1	301036
CD57	BV605	QA17A04	ms igG1	393304
CD45RA	FITC	HI100	ms IgG2b	304148
CCR7	PE-Dazzle	GO43H7	ms IgG2a	353236
CXCR3	PE	GO25H7	ms IgG1	353706
CCR6	PerCP-Cy5.5	G034E3	ms IgG2b	353406
CD103	APC	BerACT8	ms IgG1	350216
CD56	APC-cy7	HCD56	ms IgG1	318332
CD45RO	PerCP	UCHL1	ms IgG2a	304252
TCR gamma delta	BV510	B1	Ms IgG1	331220
CD38	FITC	HIT2	ms IgG1	303504
HLA-DR	APC	L243	ms IgG2a	307610
CD39	BV605	A1	ms IgG1	328236
PD-1	PE	EH12.2H7	ms IgG1	329905
CD25	BV650	V T0-72	ms IgG2b	302634

CD127	PerCP-cy5.5	A019D5	ms IgG1	351321
Tim3	PeDazzle	F38-2E2	ms IgG1	354034
CD45	PerCP	2D1	ms IgG1	368506
CD3	AF700	SK7	ms IgG1	344822
CD19	PE-dazzle	HIB19	ms IgG1	302252
CD56	APC-cy7	HCD56	ms IgG1	318332
CD14	BV711	63D3	msIgG1	367140
CD16	BV421	VNK80	msIgG1	302038
HLA-DR	APC	L243	ms IgG2a	307610
CD66b	PE	QA17A51	ms IgG1	392904
CD27	PerCP-cy5.5	M-T271	ms IgG1	356408
CD38	FITC	HIT2	ms IgG1	303504
CD274	BV650	29E.2A3	ms IgG2b	329740
HLA-DR	BV650	L243	ms IgG2a	307650
CCR2	PerCPcy5.5	K03632	Ms IgG2a	357204
CX3CR1	PE-dazzle	2A9-1	rat igG2b	341624
CCR5	APC-cy7	J418F1	rat igG2b	359110
CD3	APC	UCHT1	ms IgG1	300412
CD19	APC	HIB19	ms IgG1	302212
CD56	APC	HCD56	ms IgG1	318310
Siglec8	APC	7C9	msIgG1	347106
CD66b	PE	6/40c	ms IgG1	392904
CD15	APC-Cy7	W6D3	msIgG1	323048
CD11b	PerCPcy5.5	LM2	ms IgG1	393106
CD62L	PE-Dazzle	DREG-56	ms IgG1	304842
CD64	BV711	10.1	ms IgG1	305042
CD16	BV421	3g8	msIgG1	302038
CD10	BV605	HI10a	msIgG1	312222
CD54 (ICAM-1)	AF700	HA58	ms IgG1	353126
CD181 (CXCR1)	AF488	8F1 CXCR1	ms IgG2a	320616

All antibodies were supplied by Biolegend.

Table E3: Relevant comorbidity details of all patients with NEWS2 scores at enrolment.

Patient	Arm	NEWS2 at enrolment	Relevant comorbidity details
SOC 001	SoC	4	Hypertension Idiopathic interstitial pneumonia with fibrosis Type II Diabetes Mellitus
SOC 002	SoC	3	Hypertension
SOC 003	SoC	4	Mild Alzheimer's Type II Diabetes Mellitus
SOC 004	SoC	3	Atrial fibrillation Cerebrovascular disease
SOC 005	SoC	2	COPD
SOC 006	SoC	4	Hypertension Chronic kidney disease 3 Type II Diabetes Mellitus Obesity
SOC 007	SoC	4	Complete heart block with PPM Hypertension Non-Hodgkins lymphoma (in remission) Haemachromatosis
SOC 008	SoC	4	Asthma Previous severe sepsis with lower limb amputations
SOC 009	SoC	4	Ischaemic heart disease
SOC 010	SoC	1	Mild left ventricular hypertrophy Cerebrovascular disease Prostate cancer
SOC 011	SoC	2	Osteoporosis Recent cholecystitis
SOC 012	SoC	2	None
SOC 013	SoC	5	Hypertension COPD Asthma Previous PE Depression
SOC 014	SoC	3	Hypertension
SOC 015	SoC	2	None
SOC 016	SoC	4	Asthma Ulcerative colitis Previous breast cancer
SOC 017	SoC	5	Obesity
SOC 018	SoC	9	Ischaemic heart disease COPD Parkinson's disease Diverticular disease

			Prev. prolonged ICU stay following post-cholecystectomy complications involving VF arrest
SOC 019	SoC	2	Hypertension
SOC 020	SoC	3	Interstitial lung disease secondary to connective tissue disease Immunosuppressed on long term prednisolone and MMF
GB 001	GB0139	8	Vascular dementia Cerebrovascular disease
GB 002	GB0139	4	Stable chronic kidney disease (single kidney)
GB 003	GB0139	8	Congestive Cardiac Failure Hypertension COPD Moderate aortic stenosis
GB 004	GB0139	4	Hypertension Type II Diabetes Mellitus
GB 005	GB0139	1	None
GB 006	GB0139	2	Hypercholesterolaemia
GB 007	GB0139	5	Ischaemic Heart Disease COPD
GB 008	GB0139	6	Hypertension Obesity
GB 009	GB0139	3	Emphysema NAFLD (not cirrhotic) Type II Diabetes Mellitus
GB 010	GB0139	2	None
GB 011	GB0139	4	Hypertension COPD on LTOT
GB 012	GB0139	4	Ischaemic heart disease Hypertension Cerebrovascular disease
GB 013	GB0139	3	Hypertension Asthma Fibromyalgia
GB 014	GB0139	6	Undiagnosed Type II diabetes mellitus
GB 015	GB0139	4	Type II Diabetes Mellitus Ischaemic Heart Disease Chronic airways disease of unknown aetiology
GB 016	GB0139	4	None
GB 017	GB0139	4	Asthma Type II Diabetes Mellitus Obesity Chronic pain
GB 018	GB0139	4	None
GB 019	GB0139	5	Sarcoidosis with fibrosis Type II Diabetes Mellitus

GB 020	GB0139	2	Atrial fibrillation Ischaemic heart disease Osteoarthritis

Table E4 Individual treatment received for COVID-19

Patient	Arm	Treatment
SoC 001	SoC	Dexamethasone, remdesivir
SoC 002	SoC	Dexamethasone, remdesivir
SoC 003	SoC	Dexamethasone
SoC 004	SoC	Dexamethasone
SoC 005	SoC	Dexamethasone
SoC 006	SoC	Dexamethasone
SoC 007	SoC	Dexamethasone, remdesivir
SoC 008	SoC	Dexamethasone
SoC 009	SoC	Dexamethasone, remdesivir
SoC 010	SoC	Dexamethasone
SoC 011	SoC	Dexamethasone
SoC 012	SoC	Dexamethasone
SoC 013	SoC	Dexamethasone
SoC 014	SoC	Dexamethasone
SoC 015	SoC	Dexamethasone, remdesivir
SoC 016	SoC	Remdesivir (no dexamethasone- on prednsiolone for concurrent airway disease)
SoC 017	SoC	Dexamethasone
SoC 018	SoC	No dexamethasone - on prednisolone for concurrent airway disease
SoC 019	SoC	Dexamethasone
SoC 020	SoC	Dexamethasone

GB 001	GB 0139	Dexamethasone
GB 002	GB 0139	Dexamethasone, remdesivir
GB 003	GB 0139	Dexamethasone, remdesivir
GB 004	GB 0139	Dexamethasone, remdesivir
GB 005	GB 0139	Dexamethasone
GB 006	GB 0139	Dexamethasone, remdesivir
GB 007	GB 0139	Dexamethasone
GB 008	GB 0139	Dexamethasone, remdesivir
GB 009	GB 0139	Dexamethasone
GB 010	GB 0139	Dexamethasone, tocilizumab
GB 011	GB 0139	Dexamethasone
GB 012	GB 0139	Dexamethasone
GB 013	GB 0139	Dexamethasone
GB 014	GB 0139	Dexamethasone, remdesivir
GB 015	GB 0139	Dexamethasone
GB 016	GB 0139	Dexamethasone
GB 017	GB 0139	Dexamethasone, remdesivir
GB 018	GB 0139	Dexamethasone
GB 019	GB 0139	Dexamethasone
GB 020	GB 0139	Dexamethasone

Table E5 Individual breakdown of AEs

Patient	Arm	AE details
SOC 001	SoC	None
SOC 002	SoC	Worsening eyesight
SOC 003	SoC	None
SOC 004	SoC	None
SOC 005	SoC	None
SOC 006	SoC	Atrial Fibrillation
SOC 007	SoC	Raised D- dimer Home oxygen Foot pain
SOC 008	SoC	None
SOC 009	SoC	None
SOC 010	SoC	Urinary tract infection Fall and minor head injury Low mood Increase O2 requirements Coffee ground vomit Oral abscess UTI
SOC 011	SoC	Vomiting Abdominal pain Small bowel obstruction
SOC 012	SoC	Raised AST/ALT
SOC 013	SoC	Constipation Vomiting Nausea diarrhoea worsening pneumonitis pyrexia mouth ulcers pseudo-seizure bacterial infection
SOC 014	SoC	Urinary retention Urinary catheter placed
SOC 015	SoC	Elevated AST and ALT Anxiety and panic attacks
SOC 016	SoC	None
SOC 017	SoC	None
SOC 020	SoC	Haemopneumothorax Staph aureus bacteraemia Anaemia
SOC 018	SoC	Raised d-dimer Constipation

		Increased leg swelling (following stopping furosemide)
SOC 019	SoC	Hypokalaemia
GB 001	GB0139	Hiccup Constipation Hypotension Intermittent constipation
GB 002	GB0139	high blood glucose nausea (related) Brain fog Day hospital investigation for raised d-dimer Hair thinning
GB 003	GB0139	Prolonged QTc Sore throat Oral thrush L leg ulcer Raised blood sugar
GB 004	GB0139	fractured malleolus anaemia hair loss night sweats
GB 005	GB0139	None
GB 006	GB0139	None
GB 007	GB0139	None
GB 008	GB0139	Elevated AST/ALT
GB 009	GB0139	None
GB 010	GB0139	HFNC Elevated AST/ALT Long term breathlessness
GB 011	GB0139	Constipation Persistent cough
GB 012	GB0139	None
GB 013	GB0139	Increased Fi02 Pyrexia Anaemia Oral thrush Constipation Small bowel obstruction Paracetamol overdose Hyponatraemia Constipation
GB 014	GB0139	None
GB 015	GB0139	Bacterial LRTI
GB 016	GB0139	Widespread T wave inversion on ECG
GB 017	GB0139	Chest tightness
GB 018	GB0139	Hyperglycaemia Headaches

GB 019	GB0139	Atrial Fibrillation
GB 020	GB0139	Bacterial Pneumonia

Table E6.1: Post-hoc analysis of markers of inflammation: Continuous outcome variable results from a Bayesian GLMM assessing the difference in rate of change between treatment groups

	Mean of posterior distribution	95% HPD lower	95% HPD upper	Posterior probability of parameter < 0
Gal3	-274.56	-402.83	-159.84	1.000
LDH	-8.57	-15.16	-2.20	0.997
NLR	-0.86	-1.34	-0.44	1.000
CRP	-5.71	-10.54	-0.87	0.993
CXCL10	-185.37	-252.18	-127.05	1.000
YKL-40	-9.90	-21.21	1.20	0.963
PAI-1	0.79	-4.60	5.78	0.401
FPR	-0.0009	-0.0013	-0.0005	0.999
CXCL10 CFB	-185.81	-248.19	-120.45	1.000

Table E6.2: Estimate of slope from Bayesian GLMM for Galectin 3, by treatment group

	Slope estimate	95% HPD lower	95% HPD upper
GB0139	-58.08	-126.50	13.44
SOC	216.54	116.28	323.93

List of abbreviations: Gal3 – Galectin-3, LDH – lactate dehydrogenase, NLR – neutrophil/lymphocyte ratio, CRP – C-reactive protein, CXCL10 – CXC motif chemokine ligand 10, YKL-40 – tyrosine lysine leucine 40, PAI-1 - Plasminogen activator inhibitor-1, FPR - Fibrinogen/ plasminogen ratio, CFB – change from baseline. GLMM – Generalised Linear Mixed Model

Table E7 Mortality in patients with COVID-19 with NEWS2 ≥4 in DEFINE.

Treatment	No. of patients	Deaths (%)	Patient ID: narrative
SOC	11	3 (27.3)	SOC 01: Male; aged 81 years; BMI of 32.7; hypertension, idiopathic interstitial pneumonia with fibrosis, T2DM; admitted 24/9/2020; died 4/10/2020
			SOC 13: Female; aged 63 years; BMI of 38.6; asthma, COPD, hypertension; admitted 11/1/2020; ITU 27/1/20; died 31/1/2020
			SOC WD02: Female, aged 82 years; BMI of 28.8; hypertension, RA; short admission; died
GB0139	14	3 (21.4)	TD07: Male; aged 73 years; BMI of 29.3; comorbid CVA, AF, IHD, COPD; admitted 29/10/2020; GB0139 received 31/10/2020-1/11/2020 (2 days); died 4/11/2020
			TD12: Male; aged 86 years; BMI of 33.3; IHD, angina, HT, CVA; admitted 1/1/2021; initially treated with SOC, heparin, dexamethasone, antibiotics, GB0139 received 8–10/1/2021 (2 days); the patient was extremely unwell since admission with very high oxygen requirements; died 11/1/2021
			TD19: Male; aged 65 years; BMI of 29; sarcoidosis fibrosis, T2DM, AF; admitted 16/1/2021; GB0139 received 19–21/1/2021 (3 days; NEWS2 decreased from 5 to 3); died 5/2/2021

AF, atrial fibrillation; BMI, body mass index; CVA, cerebral vascular accident; COPD, chronic obstructive pulmonary disorder; HT, hypertension; ITU, intensive therapy unit; IHD, ischaemic heart disease; NEWS2, National Early Warning Score 2; RA, rheumatoid arthritis; SOC, standard-of-care; T2DM, type-2 diabetes mellitus.

Table E8 Details recorded for screened patients who were not enrolled to Define trial

233 total: 145 ineligible (62.5%) 34 patient refusal (14.2%)54 other

Negative swab	Patient refusal	Deranged LFT and awaiting surgical review.	Ineligible-unable to comply due to delirium	Ineligible-unable to comply due to delirium	Patient refusal
Negative swab	No O2 requirement/ asymptomatic	No O2 requirement/ asymptomatic	Awaiting surgical procedure	Ineligible-K+ >5.0	Ineligible-anticoagulation for PE
Negative swab	Patient refusal	Ineligible-uncontrolled DM	Ineligible eGFR<30	Ineligible eGFR<30	Ineligible eGFR<30

Negative swab	Patient refusal	No O2 requirement/ asymptomatic	Ineligible eGFR<30	Ineligible- unable to comply due to delirium	No O2 requirement/ asymptomatic
Patient refusal	Next of kin refusal	No O2 requirement/ asymptomatic	Palliative	Negative Swab	No O2 requirement/ asymptomatic
Negative swab	Ineligible - STEMI	Ineligible- Unable to participate due to significant mental health diagnosis.	Ineligible- unable to comply due to violence/ aggression	Ineligible- K+ >5.0	No O2 requirement/ asymptomatic
Ineligible eGFR<30	Patient refusal	Ineligible eGFR<30	No O2 requirement/ asymptomatic	Ineligible- antiplatelets for NSTEMI	Ineligible- unable to comply due to advanced cancer
Ineligible eGFR<30	Patient refusal	Ineligible eGFR<30	Ineligible- uncontrolled DM	Ineligible- unable to comply due to delirium/ neurosurgical bleed	Palliative
Negative swab	Nil recorded	Ineligible K+ >5.0	Patient refusal	Patient refusal	Unable to provide PIS in patient's language
Negative swab	Palliative	Nil recorded	Unable to provide PIS in patient's language	Awaiting surgical procedure	Ineligible- K+ >5.0
Negative swab	On another CTIMP	Nil recorded	No O2 requirement/ asymptomatic	Patient refusal	Nil recorded
Ineligible eGFR<30	Nil recorded	Nil recorded	No O2 requirement/ asymptomatic	Ineligible- unable to comply due to delirium	Active HCV infection
Palliative	Negative swab	Ongoing GI bleed	No O2 requirement/ asymptomatic	Ineligible- antiplatelets for NSTEMI	Patient refusal
Nil recorded	Negative swab	Ineligible- Hb <80	No O2 requirement/ asymptomatic	No O2 requirement / asymptomatic	Patient refusal
Ineligible eGFR<30	Ineligible- unable to comply due to delirium	Ineligible- K+ >5.0	Awaiting surgical procedure	No O2 requirement / asymptomatic/ awaiting CTPA	Patient refusal

Nil recorded	Ineligible-unable to comply due to delirium	Nil recorded	Patient refusal	Ineligible-severe liver disease	Nil recorded
Nil recorded	Bleeding risk	Nil recorded	Ineligible eGFR<30	No O2 requirement / asymptomatic	Nil recorded
No O2 requirement/ asymptomatic	Patient refusal	Ineligible-deranged LFTS and INR	Recruited to other CTIMP trial	Ineligible-unable to comply due to delirium	Nil recorded
No O2 requirement/ asymptomatic	Nil recorded	Patient refusal	Negative swab	No O2 requirement / asymptomatic/ awaiting CTPA	Nil recorded
Nil recorded	asymptomatic	No O2 requirement/ asymptomatic	Recruited to other CTIMP trial	No O2 requirement / asymptomatic/ awaiting CTPA	Nil recorded
Nil recorded	Ineligible-unable to comply due to delirium	Nil recorded	Nil recorded	Ineligible eGFR<30, K+ >5	Nil recorded
Negative swab	Ineligible-unable to comply due to severe learning difficulties	Ineligible- unable to comply due to delirium	Patient refusal	Patient refusal	Ongoing GI bleed
No O2 requirement/ asymptomatic	Ineligible-unable to comply due to delirium	Patient refusal	Ineligible-anticoagulation for PE	Refusal-Severe needle phobia	Ongoing severe haematuria
No O2 requirement/ asymptomatic	Nil recorded	Ineligible-pulmonary oedema/ severe cardiac disease	Ineligible eGFR<30	Nil recorded	Ineligible-unable to comply due to delirium
Patient refusal	No O2 requirement/ asymptomatic	No O2 requirement/ asymptomatic	Patient refusal	No capacity	Patient refusal

No O2 requirement/ asymptomatic	Ineligible eGFR<30	Patient refusal	Ineligible eGFR<30	No capacity	No O2 requirement/ asymptomatic
Ineligible-anticoagulation for PE	Nil recorded	No O2 requirement/ asymptomatic	Ineligible-unable to comply due to delirium	No O2 requirement/ asymptomatic	No O2 requirement/ asymptomatic
Ineligible-Hb <80	Ineligible-anticoagulation for PE	Active BBV infection	Ineligible-K+>5.0, Na <120	Ineligible-Severe LVSD	No capacity
Ineligible-unable to comply due to delirium	Patient refusal	No O2 requirement/ asymptomatic	Ineligible-unable to comply due to dementia	Ineligible-severe cardiac disease/ pulmonary oedmea	No O2 requirement/ asymptomatic
Recruited to other CTIMP trial	Ineligible-anticoagulation for PE	Ineligible eGFR<30	No O2 requirement/ asymptomatic	Ineligible-Hb <80	Patient refusal
Palliative	Ineligible-unable to comply due to severe learning difficulties	Ineligible- Severe cardiac disease, unable to stop antiplatelets	No O2 requirement/ asymptomatic	No O2 requirement/ asymptomatic	Ineligible-unable to comply due to delirium
Patient refusal	Ineligible-anticoagulation for PE	Ineligible-Lactose Intolerant	No O2 requirement/ asymptomatic	No O2 requirement/ asymptomatic	No O2 requirement/ asymptomatic
Patient refusal	Ineligible-unable to comply due to mental health disorders	No capacity for more patients – screened for alternative CTIMP trial	No O2 requirement/ asymptomatic	No O2 requirement/ asymptomatic	No O2 requirement/ asymptomatic
No O2 requirement/ asymptomatic	Ineligible-unable to comply due to delirium	Patient refusal	No O2 requirement/ asymptomatic	No O2 requirement/ asymptomatic	Methadone overdose
Ineligible-apixiban	Ineligible-unable to comply due to delirium	Ineligible- unable to comply due to delirium	Ineligible-unable to comply due to delirium	Ineligible-unable to comply due to delirium	Patient refusal

Ineligible-K+>5.0	Ongoing GI bleeding	Ineligible- unable to comply due to delirium	Ineligible- unable to comply due to delirium	No O2 requirement/ asymptomatic	Patient refusal
Ineligible-K+>5.0	Ineligible- anticoagulation for DVT	Ineligible- unable to comply due to delirium	Palliative	Ineligible- anticoagulation unable to be withheld	Patient refusal
No O2 requirement/ asymptomatic	Nil recorded	Nil recorded	No O2 requirement/ asymptomatic	No O2 requirement/ asymptomatic	Nil recorded
Patient refusal	No O2 requirement/ asymptomatic	Unable to provide PIS in patient's language	Unable to provide PIS in patient's language	Patient refusal	

List of abbreviations: eGFR- estimated glomerular filtration rate, STEMI- ST segment elevation myocardial infarction, LFT- liver function tests, DM- diabetes mellitus, K+- potassium, O2- oxygen, PIS- patient information sheet, NSTEMI- non ST segment elevation myocardial infarction, PE- pulmonary embolism, LVSD- left ventricular systolic dysfunction, HCV- hepatitis C virus, GI- gastrointestinal, Hb- haemoglobin, INR- international normalised ratio, CTIMP- clinical trial of investigational medicinal product, BBV- blood borne virus, Na- sodium, DVT- deep vein thrombosis