Research Paper

Seven days treatment with the angiotensin II type 2 receptor agonist C21 in hospitalized COVID-19 patients; a placebo-controlled randomised multi-centre double-blind phase 2 trial

Göran Tornling, Rohit Batta, Joanna C. Porter, Bryan Williams, Thomas Bengtsson, Kartikeya Parmar, Reema Kashiva, Anders Hallberg, Anne Katrine Cohrt, Kate Westergaard, Carl-Johan Dalsgaard, Johan Raud

Respiratory Medicine Division, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden
Vicore Pharma AB, Gothenburg, Sweden
UCL Respiratory, University College London and Department of Thoracic Medicine, University College Hospital, London, UK
Institute of Cardiovascular Science, University College London and National Institute for Health Research (NIHR) University College London Hospitals Biomedical Research Centre, London, UK
StatMind AB, Lund, Sweden
BJ Medical College and Department of Medicine, Civil Hospital, Asarwa, Ahmedabad Gujarat, India
Department of Medicine, Noble Hospitals Pvt. Ltd, Hadapsar, Pune, Maharashtra, India
Department of Medicinal Chemistry, Uppsala University, Uppsala, Sweden
Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

ABSTRACT

Background: COVID-19 morbidity and mortality remains high and the need for safe and effective drugs continues despite vaccines.

Methods: Double-blind, placebo-controlled, multi-centre, randomised, parallel group phase 2 trial to evaluate safety and efficacy of oral angiotensin II type 2 receptor agonist C21 in hospitalized patients with COVID-19 and CRP/C21 > 50-150 mg/L conducted at eight sites in India (NCT04452435). Patients were randomly assigned 100 mg C21 bid or placebo for 7 days in addition to standard of care. Primary endpoint: reduction in CRP. The study period was 21 July to 13 October 2020.

Findings: 106 patients were randomised and included in the analysis (51 C21, 55 placebo). There was no significant group difference in reduction of CRP, 81% and 78% in the C21 and placebo groups, respectively, with a treatment effect ratio of 0.85 [90% CI 0.57, 1.26]. In a secondary analysis in patients requiring supplemental oxygen at randomisation, CRP was reduced in the C21 group compared to placebo. At the end of the 7-day treatment, 37 (72.5%) and 30 (54.5%) of the patients did not require supplemental oxygen in the C21 and placebo group, respectively (OR 2.20 [90% CI 1.12, 4.41]). A post hoc analysis showed that at day 14, the proportion of patients not requiring supplemental oxygen was 98% and 80% in the C21 group compared to placebo (OR 12.5 [90% CI 2.9, 126]). Fewer patients required mechanical ventilation (one C21 patient; four placebo patients), and C21 was associated with a numerical reduction in the mortality rate (one vs three in the C21 and placebo group, respectively). Treatment with C21 was safe and well tolerated.

Interpretation: Among hospitalised patients with COVID-19 receiving C21 for 7 days there was no reduction in CRP compared to placebo. However, a post-hoc analysis indicated a marked reduction of requirement for oxygen at day 14. The day 14 results from this study justify further evaluation in a Phase 3 study and such a trial is currently underway.

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1. Introduction

In January 2020, SARS-CoV-2 was identified as the causative agent of an outbreak of the new viral pneumonia disease COVID-19, with the first cases reported in December 2019 [1-3]. The disease...
The AT2R is mainly expressed in embryonic tissue and, under normal conditions, only at low levels in most tissues in healthy adults [17,18]. However, it has recently been described that the AT2R is highly expressed in alveolar type 2 (AT2) progenitor cells in the adult human lung and can be upregulated during repair and regeneration [14,19]. Moreover, the AT2 cells are the primary site of SARS-CoV-2 replication in the dilat airways which is likely to contribute to the alveolar dysfunction and impaired gas exchange caused by the virus [20].

A few recent COVID-19 trials with angiotensin-converting enzyme inhibitors (ACEi) or angiotensin AT1 receptor blockers (ARBs) have shown mixed results. Briefly, one open-label study [21] and one blinded placebo-controlled study [22] showed no clinically significant impact of losartan therapy, while one open-label study with high dose telmisartan showed reduced CRP, morbidity, and mortality in hospitalized COVID-19 patients [23]. In studies addressing whether or not RAS modulating anti-hypertensive medications are beneficial or harmful in patients with COVID-19, it has been found that ACEi and/or ARBs do not have significant beneficial (or harmful) effects on COVID-19 and do not differ significantly from a calcium channel blocker with or without a beta-blocker [24,25].

Compound 21 (C21) is a first-in-class, low molecular weight, orally available, specific, high-affinity AT2R agonist currently in clinical development in idiopathic pulmonary fibrosis [26,27]. In a recent Phase 1 study, 100 mg C21 twice daily was found to be safe and well tolerated [28]. The clinical pharmacokinetics following a single oral dose of 100 mg C21 are as follows: Tmax 40 min, Cmax 2004 ng/mL, T1/2 5.4 h and AUC(0-24h) 2438 h*ng/mL [29], with an apparent clearance (CL/F) of 42 L/h and an apparent volume of distribution (Vz/F) of 37 L [28], suggesting distribution of C21 to extra- and intra-cellular compartments. After repeated oral administration of this dose, the pharmacokinetics are very similar, suggesting little accumulation over time [28].

Based on the established safety and tolerability of C21 in healthy subjects and the unprecedented imperative for new and effective treatments of patients with moderate COVID-19, we investigated whether C21 could have beneficial effects in this disease and further characterised its safety profile. To our knowledge, this is the first trial of an AT2R agonist in any human disease.

2. Methods

2.1. Study design

The ATTRACT trial (Angiotensin II Type Two Receptor Agonist COVID-19 Trial) had a randomised, parallel group, double-blind design, and was performed at eight hospitals in India. The protocol, patient information, patient consent form and other documents, as required, were approved by properly constituted IECs (Supplement) and by the national regulatory authorities. The study protocol is available online at ClinicalTrials.gov.

2.2. Patients

The trial enrolled patients aged 19 to 69 years who were hospitalised with SARS-CoV-2 infection confirmed by polymerase chain reaction (PCR) test <4 days before enrolment and with signs of an acute respiratory infection but not requiring invasive or non-invasive mechanical ventilation on the day of randomisation. To be eligible for inclusion in the trial, the patient should have a C-reactive protein (CRP) value of >50 and <150 mg/L. Concomitant medication and supportive care as per standard of care at the trial site was permitted, although patients should not have received any previous experimental treatment for COVID-19. Full inclusion and exclusion criteria are presented in the Supplement. Written informed consent was obtained from each patient prior to any trial-related procedure.
2.3. Randomisation and masking

The patients were sequentially randomly assigned by the Investigator in a 1:1 ratio to receive either C21 or placebo in blocks of four, with randomisation stratified by trial site. The randomisation sequence was generated by a statistician not involved in the rest of the trial, and the randomisation codes were kept in sealed envelopes at the trial sites. C21 and placebo capsules were identical in size, colour, smell, and appearance. Patients, care providers, those assessing outcomes and Sponsor staff were kept blinded until database lock had been performed for the complete trial.

2.4. Procedures

Twice daily doses of 100 mg C21 or matching placebo were administered orally for 7 days. A final visit (at the hospital or by phone call) was performed 7-10 days after the last dose of the trial drug. During the hospitalisation, patients were assessed daily by physical examination, vital signs, need for supplemental oxygen, body temperature and routine blood and urine analyses, including CRP. Other biomarkers were measured in one central laboratory using a chemiluminescence immunoassay on a Siemens Immulite 2000 XPI instrument (IL-6, IL-10, TNF) or a chemiluminescent micro-particle immunoassay on an Abbott Alinity instrument (CA125 and ferritin). The trial drug was withdrawn if the patient needed mechanical invasive or non-invasive ventilation or was discharged early from the hospital. Patients were withdrawn from the trial if the Investigator judged it necessary due to medical reasons (e.g. adverse events), if lost to follow-up, or at the patient’s own decision. Full withdrawal criteria are presented in the Supplement.

2.5. Outcomes

The primary endpoint of the trial, change in CRP from baseline to end-of-treatment, was selected based on early data in glucocorticoid naïve patients demonstrating that CRP predicts severe outcomes. Secondary endpoints were change from baseline in body temperature, IL-6, IL-10, TNF, CA125 and ferritin, number of subjects not in need of oxygen supply or mechanical ventilation, time to need of mechanical invasive or non-invasive ventilation, and time on oxygen supply. Safety was assessed by recording of all adverse effects and associated grading (e.g. seriousness, expectedness, causality etc) with a special emphasis on SAE’s with maintenance of a database for collection, analysis, reporting, and submission of adverse events in accordance with regulatory requirements. Signal management, signal detection, signal prioritisation, and signal assessment were performed on an ongoing basis during the course of the study.

2.6. Sample size

The sample size calculation was based on the primary endpoint, the difference in reduction of CRP in C21 treated patients compared to placebo. Based on the limited understanding of the natural course of COVID-19 infections at the time the study was initiated, the original target for the difference was set to 30 mg/L. Due to the abundant use of steroids in the treatment of COVID-19 and the consequent lower likelihood to detect a difference in the reduction in CRP, the Sponsor changed the target to 25 mg/L without unblinding the data. This was documented in a protocol amendment, which was approved by the Indian authorities (31 August 2020) and was classified as a non-substantial amendment by the MHRA. Based on the new target, the sample size calculation indicated that 75 patients per group (compared to 50 patients per group in the initial calculation) were needed to achieve 80% power to detect the target difference in reduction of CRP in C21 treated patients compared to placebo using a two-sided t-test at 10% significance level. However, following recruitment challenges outside India, the trial was halted by the Sponsor for feasibility reasons after 106 patients had been randomised.

2.7. Statistical analysis

The efficacy analyses were conducted by intention to treat on the Full Analysis Set (FAS), which included all randomised patients, unless otherwise specified. Patients were included by randomised treatment. For CRP and other biomarkers, the mean of the last two assessments in the treatment period was used for analysis. Data was compared between treatments using ANCOVA with treatment as factor and baseline as covariate. Since the model assumptions for normal distribution were not met, data were log-transformed prior to analysis as specified in the Statistical Analysis Plan. Patients with no baseline or no post-treatment data were excluded from analysis. Patients not in need of supplementary oxygen were compared using logistic regression with treatment as factor. Patients requiring mechanical ventilation or who died were considered to be in need of oxygen from the start of the event. For other withdrawals, the last value assessed was carried forward. In post hoc analyses, data on oxygen supplementation at the end of 14-day follow-up period and sub-analyses based on disease severity at randomisation as assessed by need for supplemental oxygen were compared using logistic regression. Descriptive statistics was used for adverse event analysis.

Data are presented as 90% confidence intervals in line with a 10% significance level. There was no adjustment for multiplicity, and the results for secondary endpoints should thus be regarded as indicative. All analyses were performed by SAS version 9.4. The Statistical Analysis Plan is available at ClinicalTrials.gov. There was no data monitoring committee. The study was registered at ClinicalTrials.gov with identifier NCT04452435.

2.8. Role of the funding source

Employees of Vicore Pharma (listed as authors) played a role in the design and conduct of the study. This included collection, analysis, and interpretation of the data, and preparation, review, and approval of the manuscript as well as the decision to submit the manuscript for publication. To ensure independent interpretation of clinical study results, Vicore Pharma grants all external authors access to all relevant study material needed for them to fulfil their role and obligations as authors under the ICMJE criteria. All authors had access to the collated data and took the decision to submit for publication. Five authors had access to the full data set (GT, RB, TB, CJD, JR) and initiated the drafting of the manuscript. LifeArc had no role in study design, data collection, data analysis, data interpretation, or writing of the manuscript.

3. Results

During 21 July to 29 September 2020, 206 patients were assessed for eligibility. Those 106 who fulfilled the eligibility criteria underwent randomisation: 51 were assigned to the C21 group and 55 to the placebo group (Figure 1). The main reason (90 out of 96) for not fulfilling eligibility criteria was CRP outside the inclusion criterion. Six patients had C21 treatment discontinued before completion of all doses due to need for mechanical ventilation (1), withdrawal of consent (1), or discharge from hospital (4). Twelve patients had placebo treatment discontinued before completion of all doses due to need for mechanical ventilation (4), withdrawal of consent (4), or discharge from hospital (4). One additional patient in the placebo group was discharged from hospital after completion of the treatment and withdrew consent for follow-up assessments. The study period was 21 July to 13 October 2020.

As shown in Table 1, the treatment groups were well balanced regarding age (mean 52.6 years) and gender (75.5% males).
Approximately half of the patients had one or more coexisting medical condition. Hypertension and overweight/obesity were more common in the C21 group compared with the placebo group, but there was no difference in the presence of diabetes mellitus. Supplemental oxygen supply was required in 71.7% of the patients on the day of randomisation with no major difference between the treatment groups. Concomitant medication according to local standard of care was permitted in the trial. A large majority of the patients received glucocorticoids and antiviral compounds (most often remdesivir) prior to randomisation and during the double-blind period, with no major differences between the treatment groups (Table 2). The use of glucocorticoids from randomisation to end of study in the two groups is shown in Table S5.

The primary endpoint, change in CRP from randomisation to end of treatment, was not met. In both the C21 and the placebo group, CRP decreased rapidly between screening and randomisation/base-line, and from randomisation to end of treatment there was a further continued reduction with 81% and 78% in the C21 and placebo group, respectively (ratio of adjusted treatment means 0.85; 90% CI 0.57, 1.26, Table 3 and Figure 2). Nevertheless, in the more severely ill patients, i.e. those who needed oxygen therapy at baseline (57.5%), the difference was more pronounced with a CRP reduction of 84% in the C21 group and 72 % in the placebo group at the end of the treatment period (treatment ratio 0.59; 90% CI 0.35, 0.98, Supplement Table S2).

Of the secondary endpoints, there was no difference in effect on other biomarkers (IL-6, IL-10, TNF, CA125, and ferritin) between C21

### Table 1

Demographic and Clinical Characteristics at Baseline for included patients

| Characteristic                  | C21 (N=51) | Placebo (N=55) | All (N=106) |
|--------------------------------|------------|----------------|-------------|
| Age; mean (SD)                 | 54.3 (9.1) | 51.1 (11.2)    | 52.6 (10.3) |
| Male sex, N (%)                | 38 (74.5)  | 42 (76.4)      | 80 (75.5)   |
| Ethnicity                      | Asian      | 51 (100)       | 106 (100)   |
| Coexisting conditions          | Diabetes mellitus; N (%) | 17 (33.3) | 19 (34.5) | 36 (34.0) |
|                               | Hypertension; N (%) | 38 (75.3) | 14 (25.5) | 32 (30.2) |
|                               | Body mass index (kg/m²); mean (SD) | 25.4 (4.0) | 25.1 (3.4) | 25.2 (3.7) |
|                               | Overweight or obese (BMI≥25.0); N (%) | 26 (51.0) | 24 (43.6) | 50 (47.2) |
|                               | Overweight (BMI≥30.0); N (%) | 22 (43.1) | 18 (32.7) | 40 (37.7) |
|                               | Obese (BMI≥35.0); N (%) | 4 (7.8) | 6 (10.9) | 10 (9.4) |
|                               | Respiratory rate; mean (SD) | 20.7 (2.1) | 20.7 (2.3) | 20.7 (2.2) |
|                               | Pulmonary artery systolic pressure; mean (SD) | 80.2 (7.3) | 81.5 (9.9) | 82.8 (8.8) |
|                               | Systolic blood pressure; mean (SD) | 121.4 (5.7) | 123.5 (8.1) | 122.5 (8.9) |
|                               | Diastolic blood pressure; mean (SD) | 77.1 (5.8) | 77.8 (6.2) | 77.5 (6.0) |
|                               | Respiratory rate; mean (SD) | 20.7 (2.1) | 20.7 (2.3) | 20.7 (2.2) |
|                               | Supplemental oxygen randomisation day; N (%) | 37 (72.5) | 39 (70.9) | 76 (71.7) |
|                               | Body temperature; mean (SD) | 37.0 (0.9) | 37.0 (0.7) | 37.0 (0.8) |
|                               | CRP; mean (SD) | 49.8 (38.7) | 61.5 (47.6) | 55.8 (43.7) |
|                               | IL-6; mean (SD) | 51.1 (114.4) | 34.9 (39.8) | 42.4 (82.8) |
|                               | IL-10; mean (SD) | 8.4 (8.9) | 10.1 (12.8) | 9.3 (11.1) |
|                               | TNFα; mean (SD) | 17.0 (18.7) | 18.3 (16.7) | 17.7 (17.6) |
|                               | Ferritin; mean (SD) | 464.2 (324.6) | 707.5 (564.3) | 588.5 (476.3) |
|                               | CA125; mean (SD) | 14.3 (12.9) | 20.5 (38.8) | 17.5 (29.3) |
|                               | Neutrophil/Lymphocytes; mean (SD) | 8.0 (4.3) | 9.1 (7.4) | 8.6 (6.1) |

Values are given as Number (%)

### Table 2

Prior and concomitant medication (Safety Analysis Set)

| Medication                      | Prior medication C21 (N=51) | Placebo (N=55) | All (N=106) | Concomitant medication C21 (N=51) | Placebo (N=55) | All (N=106) |
|--------------------------------|----------------------------|----------------|-------------|-----------------------------------|----------------|-------------|
| Glucocorticoids                | 45 (88.2)                  | 42 (76.4)      | 87 (82.1)   | 45 (88.2)                        | 45 (81.8)      | 90 (84.9)   |
| Dexamethasone                  | 17 (33.3)                  | 11 (20.0)      | 28 (26.4)   | 19 (37.3)                        | 16 (29.1)      | 35 (33.0)   |
| Antiviral compounds            | 40 (78.4)                  | 40 (72.7)      | 80 (75.5)   | 42 (84.3)                        | 46 (83.6)      | 89 (84.0)   |
| Remdesivir                     | 28 (54.9)                  | 24 (45.5)      | 52 (51.9)   | 34 (66.7)                        | 37 (67.3)      | 71 (67.0)   |
| Insulins and analogues         | 15 (29.4)                  | 18 (32.7)      | 33 (31.1)   | 23 (41.8)                        | 43 (40.6)      | 23 (41.8)   |
| Oral glucose lowering drugs    | 17 (33.3)                  | 17 (30.9)      | 34 (32.1)   | 13 (23.6)                        | 29 (27.4)      | 13 (23.6)   |
| Heparin                        | 39 (76.5)                  | 38 (69.1)      | 77 (72.6)   | 42 (76.4)                        | 81 (76.4)      | 42 (76.4)   |
| Platelet aggregation inhibitors excl. heparin | 14 (27.5) | 11 (20.0) | 25 (23.6) | 21 (41.2) | 22 (40.0) | 43 (40.6) |
| Pirenidone                     | 3 (5.9)                    | 2 (3.6)        | 5 (4.7)     | 16 (31.4)                        | 12 (21.8)      | 28 (26.4)   |
| Hydroxychloroquine             | 8 (15.7)                   | 9 (16.4)       | 17 (16.0)   | 7 (13.7)                         | 7 (12.7)       | 14 (13.2)   |
| Angiotensin II receptor blockers | 7 (13.7)               | 6 (10.9)       | 13 (12.3)   | 8 (15.7)                         | 6 (10.9)       | 14 (11.2)   |
| Serotonin (5-HT2) antagonists  | 34 (66.7)                  | 34 (67.3)      | 71 (67.0)   | 30 (58.8)                        | 37 (67.3)      | 67 (63.2)   |
| Proton pump inhibitors         | 48 (94.1)                  | 50 (90.9)      | 98 (92.5)   | 51 (100)                         | 52 (94.5)      | 103 (97.2)  |

Values are given as Number (%)
and placebo (Table 3). At the end of the 7-day treatment, 37 (72.5%) and 30 (54.5%) of the patients did not require supplemental oxygen in the C21 and placebo group, respectively (OR 2.20 [90% CI 1.12, 4.41]). Time on oxygen supply for those not needing mechanical ventilation did not differ between the treatment groups (median 5 days in both groups). One patient (2.0%) in the C21 group and four (7.3%) patients in the placebo group needed mechanical ventilation (OR 3.92 [90% CI 0.61, 25.4]). Analysis of time to need of mechanical ventilation did not differ between the treatment groups (median 5 days in both groups). One patient (2.0%) in the C21 group and eleven (20.0%) patients in the placebo group still required supplemental oxygen (OR 12.5 [90% CI 2.9, 126], p<0.002).

A post hoc analysis demonstrated that extended need for oxygen therapy was more frequent in the placebo group than in the C21 group (Figure 3 and Supplement Table S4). At day 14 after start of treatment, one (2.0%) patient in the C21 group and 11 (20.0%) patients in the placebo group still required supplemental oxygen (OR 12.5 [90% CI 2.9, 126], p<0.002).

### Table 3
Results of effect on primary and secondary endpoints (Full Analysis Set)

| Endpoint | C21 N=51 | Placebo N=55 | Treatment Effect |
|----------|----------|--------------|-----------------|
| C-Reactive Protein (mg/L) | 49.81 (38.73) | 61.51 (47.62) | 0.85 (0.57, 1.26) |
| Interleukin 10 (pg/mL) | 8.41 (8.90) | 10.06 (12.81) | 0.90 (0.68, 1.19) |
| Interleukin 6 (pg/mL) | 51.13 (114.41) | 34.85 (39.79) | 1.00 (0.61, 1.66) |
| Tumor Necrosis Factor (pg/mL) | 17.02 (18.70) | 18.29 (16.65) | 0.90 (0.72, 1.14) |
| Ferritin (ng/mL) | 464.2 (324.6) | 707.5 (564.3) | 1.00 (0.85, 1.19) |
| Body temperature (°C) | 36.97 (0.86) | 37.01 (0.74) | 0.23 (0.04, 0.42) |
| Not in need of oxygen supply, N (%) | 22 (43.1) | 23 (41.8) | 2.20 (1.11, 4.41) |
| Not in need of mechanical ventilation, N (%) | 0 (43.1) | 0 (43.1) | 1.89 (0.25, 14.52) |

Table effect is expressed as ratio in adjusted treatment means for biomarkers, difference for body temperature and odds ratio for patients not in need for oxygen supply or mechanical ventilation. Values are Mean (SD) unless otherwise specified. Treatment Effect [90% confidence interval].

Details of the analyses are presented in the Supplement.

![Figure 2](image2.png)

**Figure 2.** CRP in patients treated with C21 and placebo. Geometric mean of CRP at screening (Scr), randomization (Ran) and during the treatment period in all randomized patients. No differences between the groups were statistically significant.

![Figure 3](image3.png)

**Figure 3.** Patients not in need of supplemental oxygen therapy during the treatment and follow-up period. Daily estimates of the proportion of patients not in need of supplemental oxygen therapy. The figure shows the total full analysis set (C21 N=51, placebo N=55; p=0.055 after 7-day treatment, p=0.003 at 14-day follow-up).
4. Discussion

This double-blind, randomised, phase 2, placebo-controlled trial, evaluated the effectiveness of oral treatment with C21, an AT2R agonist, in patients hospitalised with COVID-19. Although the primary endpoint (reduction in CRP) was not different between C21 and placebo treated patients after 7 days of treatment, secondary analyses of clinical outcomes suggest that C21 treatment may be beneficial in reducing the extended need for supplemental oxygen therapy. Although the numbers are necessarily small in a phase 2 trial, the data also suggest that treatment with C21 may have reduced progression to more severe respiratory disease, as shown by the lower proportion of need for mechanical ventilation or death due to respiratory failure. Importantly, C21 was given on top of standard of care, with a vast majority of patients receiving glucocorticoids and two thirds receiving remdesivir, commencing prior to randomisation. This trial, the first study of an AT2R agonist in any human disease, also demonstrated that C21 was safe and well tolerated in patients hospitalised with COVID-19, with a favourable benefit-risk profile.

CRP was initially selected as the primary endpoint due to reports at the time this trial was designed suggesting that CRP predicted progression to severe disease and mortality in patients with COVID-19 [6,30,31]. However, we observed a rapid and substantial decrease in CRP in both the C21 and the placebo group during the screening process which occurred up to four days before randomisation. While some of the CRP reduction in the placebo group may have been natural variation, a possible contributing factor is that a large proportion of patients were already receiving glucocorticoids during the screening period. This is supported by findings that, in COVID-19 patients not treated with glucocorticoids, CRP remains elevated for more than a week [30]. In a recent open-label study of telmisartan in COVID-19, CRP was not reduced at day 8 after randomization in the control group, within which 41% of patients received dexamethasone [23]. We nevertheless believe that, in our study, the influence of glucocorticoids on baseline levels of CRP provides a reasonable explanation for the reduced capacity to discriminate between CRP effects of C21 and placebo with respect to the primary outcome, i.e. change in CRP levels from baseline. Furthermore, it was seen that, in the patients with more pronounced respiratory distress, i.e. those in need of supplemental oxygen at baseline, the reduction of CRP was more pronounced in the C21 than in the placebo group (p=0.088). Further assessment of whether C21 has an effect on suppressing proinflammatory cytokines such as IL-6 and TNF (which were not different between groups) may also have been obscured by the widespread use of glucocorticoids.

The suggestion in exploratory secondary analyses that there may be reduced need for extended oxygen supplementation in those treated with C21 hints that treatment with C21 may improve gas exchange at the alveolar level. This is interesting because AT2 progenitor cells, apparently the only cells in the lungs that express the AT2R, are the primary site for viral replication in the distal airways, prompting the hypothesis that C21 could restore lung function by a direct action on these cells [19,20].

Viral pneumonias can result in long-standing complications, including pulmonary fibrosis [32,33]. In a follow-up investigation of 133 patients 100 days after the diagnosis of COVID-19, lung function impairment was seen in 36% and pathological CT findings in 63% [34]. Severity and duration of the initial phase of the disease seem to be important, since days on oxygen supplementation during the acute phase of COVID-19 was identified as a risk indicator for decreased diffusion capacity and increased total CT score at 12 weeks after the initial infection [35]. COVID-19 shares characteristics with the severe acute respiratory syndrome coronavirus (SARS-CoV), and the Middle East respiratory syndrome coronavirus (MERS-CoV), and long-term follow-up studies in both these diseases have demonstrated a high frequency of pulmonary fibrosis [36,37]. However, the experience from the SARS and MERS cannot be directly transferred to COVID-19 due to the differences in demography as COVID-19 more severely affects an older population which may lead to a higher frequency of long-term sequelae. Anti-fibrotic therapies, primarily nintedanib and pirfenidone, have been suggested for the prevention of fibrosis after SARS-CoV-2 infection [38]. C21 has shown efficacy in pre-clinical studies of pulmonary fibrosis, which suggests that it may have a broader use in longer term treatment of COVID-19 pneumonia, its sequelae and/or so-called ‘long COVID’ [39,40].

We acknowledge the limitations of this study. Although the trial was typical in size for an exploratory proof-of-concept trial, the reduction in subject numbers during the study reduced the power to detect the predefined endpoints. However, the study was well balanced regarding risk factors for poor prognosis such as overweight (which was more prevalent in the C21 group), diabetes, and hypertension. Moreover, the baseline severity was similar between the two treatment groups based on the need for oxygen supplementation at randomisation. Another limitation was that the CRP levels were similar in the two groups at screening but had decreased more in the C21 group at the time of randomisation. On the other hand, the IL-6 levels were numerically higher in the C21 group. The size of the trial did not allow subgroup stratification for potentially important baseline characteristics/risk factors, and the power for the predefined subgroup analyses was limited. However, in patients with more severe disease indicated by oxygen use at baseline, a more pronounced effect of C21 on CRP was observed. Another limitation is that the trial was restricted to a population of hospitalised patients not in need of mechanical ventilation, and it is difficult to extrapolate the results to other patient populations. Almost 30% of the patients did not need supplemental oxygen at randomisation and the reason for hospitalisation of these patients was not documented. However, it seems clear that the patients not requiring oxygen were not driving the observed results. Further, the amount of supplemental oxygen required was not recorded due to logistic challenges in capturing frequent changes of oxygen supply during acute circumstances, which is similar to other trials, e.g. RECOVERY [8].

In conclusion, among hospitalised patients with moderately severe COVID-19 receiving 100 mg bid of C21 for 7 days on top of standard of care, including glucocorticoids and remdesivir, there was no difference in rate of decline of CRP compared to placebo. In a post hoc analysis, there was however a marked reduction of requirement for oxygen at day 14 in those randomised to C21. The day 14 results in this study justify further evaluation in a Phase 3 study and such a trial (NCT04880642) is currently underway.

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**Contributors**
Study conception and design: GT, RB, JP, BW, AH, C-JD, JR
Data collection: RB, KP, RK, AKC, KW
Data analysis: GT, RB, TB, C-JD, JR
Data interpretation: GT, RB, JP, BW, TB, KP, RK, AH, AKC, KW, C-JD, JR
Writing, including literature search: GT, RB, JP, BW, TB, JR

**Data sharing**
The Sponsor will share de-identified individual participant data collected during the trial with researchers who provide a methodologically sound proposal to the corresponding author. The study protocol and statistical analysis plan are available on clinicaltrials.gov.
Declaration of Competing Interest

Dr. Tornling reports personal fees from Vicore Pharma, during the conduct of the study; personal fees from Vicore Pharma, outside the submitted work; and holding of shares and share options in Vicore Pharma. Dr. Batta reports grants from LifeArc Medical Research Charity, during the conduct of the study; personal fees from Vicore Pharma, outside the submitted work; and holding of shares in Vicore Pharma. In addition, Dr. Batta has a patent UK2004208.9 pending, a patent UK2009574.1 pending, and a patent US17/113,416 pending (patent prosecution with US Patent and Trademark Office and UK Intellectual Property Office, respectively, and all patent rights have been transferred to Vicore Pharma AB). Dr. Porter has nothing to disclose. Dr. Williams has nothing to disclose. Dr. Bengtsson reports personal fees from Vicore Pharma, during the conduct of the study; personal fees from Vicore Pharma, outside the submitted work. Dr. Parmar reports grants from Vicore Pharma, during the conduct of the study. Dr. Ballberg reports personal fees from Vicore Pharma, during the conduct of the study; personal fees from Vicore Pharma, outside the submitted work. Anne Katrine Cohrt reports personal fees from Vicore Pharma, outside the submitted work; and holding of share options in Vicore Pharma. Kate Westergaard reports personal fees from Vicore Pharma, during the conduct of the study; personal fees from Vicore Pharma, outside the submitted work. Dr. Dalsgaard reports grants from LifeArc Medical Research Charity, during the conduct of the study; personal fees from Vicore Pharma, outside the submitted work; and holding of shares and share options in Vicore Pharma. In addition, Dr. Dalsgaard has a patent UK20042089.9 pending, a patent UK2009574.1 pending, and a patent US17/113,416 pending (see above regarding patent prosecution and rights). Dr. Raud reports grants from LifeArc Medical Research Charity, during the conduct of the study; personal fees from Vicore Pharma, outside the submitted work; and holding of shares and share options in Vicore Pharma. In addition, Dr. Raud has a patent UK20042089.9 pending, a patent UK2009574.1 pending, and a patent US17/113,416 pending (see above regarding patent prosecution and rights).

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.eclinm.2021.101152.

REFERENCES

[1] Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 2020; 395(10224):565–74.
[2] Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature 2020; 579(7798):265–9.
[3] Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020; 395(10229):1054–62.

[4] WHO. Coronavirus Disease (COVID-19) Dashboard 2021 [https://covid19.who.int/; Accessed July 14, 2021].

[5] Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020; 395(10223):507–13.

[6] Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Med 2020; 46(5):846–8.

[7] Beigel JH, Tomashek KM, Dodd LE, et al. Remdesivir for the Treatment of COVID-19 - Final Report. N Engl J Med 2020; 383(19):1813–26.

[8] Group Recovery Collaborative, P Horby, Lim WS, et al. Dexamethasone in Hospi- talized Patients with Covid-19. N Engl J Med 2021; 384(18):1705–13.

[9] Solidarity Trial Consortium WHO, Pan H, Peto R, et al. Repurposed Antiviral Drugs for Covid-19 - Interim WHO Solidarity Trial Results. N Engl J Med 2021; 384(6):497–511.

[10] Rosas RD, Brau N, Waters M, et al. Tocilizumab in Hospitalized Patients with Severe COVID-19 Pneumonia. N Engl J Med 2021; 384(16):1503–16.

[11] Savova C, Schiffir EL. Inhibition of the renin angiotensin system: implications for the endothelium. Curr Diab Rep 2006; 6(4):274–8.

[12] de Man FS, Tu L, Handoko ML, et al. Dysregulated renin-angiotensin-aldosterone system contributes to pulmonary arterial hypertension. Am J Respir Crit Care Med 2012; 186(6):789–9.

[13] Summers C, Pielus A, Hauagge AH, Bertelsen JB, Steckelings UM. Anti-fibrotic mechanisms of angiotensin AT2 receptor stimulation. Acta Physiol (Oxf) 2019; 227(1):e12380.

[14] Matavelli LC, Siragy HM. AT2 receptor activators and pathophysiological implica- tions. J Cardiovasc Pharmacol 2015; 65(3):226–32.

[15] Guippabert C, de Man F, Lombe M. ACE2 as therapy for pulmonary arterial hyper- tension: the good outweighs the bad. Eur Respir J 2018; 51(6).

[16] Steckelings UM, Summers C. Correcting the imbalanced protective RAS in COVID-19 with angiotensin AT2-agonist receptors. Clin Sci (Lond) 2020; 134(22):2987–3006.

[17] Steckelings UM, Kaschina E, Unger T. The AT2 receptor – a matter of love and hate. Peptides 2005; 26(8):1401–9.

[18] de Gasparo M, Catt RJ, Ingagami T, Wright JW, Unger T. International union of pharma- cology. XXII. The angiotensin II receptors. Pharmacol Rev 2000; 52(3):415–72.

[19] Proteinatlas.org. ACTR2 2021 [https://www.proteinatlas.org/ENSG00000180727- ACTR2. Accessed January 17, 2021].

[20] Szekely L, Bozoly B, Bendeck M, et al. Pulmonary stromal expansion and intra- alveolar coagulation are primary causes of Covid-19 death. bioRxiv 2020; 2020.12.23.424172.

[21] Cerisak M, Haddad F, Kollar R, et al. Randomized Prospective Open Label Study Shows No Impact on Clinical Outcome of Adding Losartan to Hospitalized COVID-19 Patients with Mild Hypoxemia. Infect Dis Ther 2021; 10(3):1323–30.

[22] Puskarich MA, Cummings NW, Ingrahame NE, et al. A multi-center phase II random- ized clinical trial of losartan on symptomatic outpatients with COVID-19. EClini- Medicine 2021; 37:100957.

[23] Duarte M, Peloroosso F, Nicolaoli LN, et al. Telmisartan for treatment of COVID-19 patients: An open multicenter randomized clinical trial. EClinicMedicine 2021; 37:100962.

[24] Nouri-Vashek M, Kalami N, Zand R, et al. Comparison of losartan and amlopidine effects on the outcomes of patients with COVID-19 and primary hypertension: A randomised clinical trial. Int J Clin Pract 2021; 75(6):e14124.

[25] Najmeddin F, Solhjoo M, Ashraf H, et al. Effects of Renin-Angiotensin-Aldosterone Inhibitors on Early Outcomes of Hypertensive COVID-19 Patients: A Randomized Triple-Blind Clinical Trial. Am J Hypertens 2021 in press.

[26] Wang Y, Wallinder C, Plouffe B, et al. Design, synthesis, and biological evaluation of the first selective nonpeptide AT2 receptor agonist. J Med Chem 2004; 47(24):5995–6008.

[27] NCT04533022. Safety, Efficacy and Pharmacokinetics of C21 in Subjects With IPF 2021 [https://clinicaltrials.gov/ct2/show/NCT04533022. Accessed January 17, 2021].

[28] Vicore Pharma AB. Unpublished data on file 2021.

[29] Steckelings U, Lindblad L, Leisunov A, et al. Successful completion of a phase I, randomized, double-blind, placebo controlled, single ascending dose trial for the first class angiotensin AT2-receptor agonist Compound 21. Journal of Hyper- tension 2017; 35:105–66 (abstr).

[30] Tan C, Huang Y, Shi F, et al. C-reactive protein correlates with computed tomo- graphic findings and predicts severe COVID-19 early. J Med Virol 2020; 92(7):856–62.

[31] Wang L. C-reactive protein levels in the early stage of COVID-19. Med Mal Infect 2020; 50(4):332–4.

[32] Atabati E, Dehghani-Samani A, Mortazavinmoghaddam SG. Association of COVID-19 and other viral infections with interstitial lung diseases, pulmonary fibrosis, and pulmonary hypertension: A narrative review. Can J Respir Ther 2020; 56:1–9.

[33] Cheng G, Chen P, Wei Y, et al. Viral Infection Increases the Risk of Idiopathic Pulmonary Fibrosis: A Meta-Analysis. Chest 2020; 157(5):1175–87.

[34] Sonnewer T, Sahanic S, Pizzini A, et al. Cardiopulmonary recovery after COVID- 19 – an observational prospective multi-center trial. Eur Respir J 2020.

[35] Shah AS, Wong AW, Hague CJ, et al. A prospective study of 12-week respiratory outcomes in COVID-19-related hospitalisations. Thorax 2020.
[36] Hui DS, Wong KT, Ko FW, et al. The 1-year impact of severe acute respiratory syndrome on pulmonary function, exercise capacity, and quality of life in a cohort of survivors. Chest 2005;128(4):2247–61.
[37] Das KM, Lee EY, Singh R, et al. Follow-up chest radiographic findings in patients with MERS-CoV after recovery. Indian J Radiol Imaging 2017;27(3):342–9.
[38] George PM, Wells AU, Jenkins RG. Pulmonary fibrosis and COVID-19: the potential role for antifibrotic therapy. Lancet Respir Med 2020;8(8):807–15.
[39] Bruce E, Shenoy V, Rathinasabapathy A, et al. Selective activation of angiotensin AT2 receptors attenuates progression of pulmonary hypertension and inhibits cardiopulmonary fibrosis. Br J Pharmacol 2015;172(9):2219–31.
[40] Rathinasabapathy A, Horowitz A, Horton K, et al. The Selective Angiotensin II Type 2 Receptor Agonist, Compound 21, Attenuates the Progression of Lung Fibrosis and Pulmonary Hypertension in an Experimental Model of Bleomycin-Induced Lung Injury. Front Physiol 2018;9:180.