Efficacy of oral 20-hydroxyecdysone (BIO101), a MAS receptor activator, in adults with severe COVID-19 (COVA): a randomized, placebo-controlled, phase 2/3 trial

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Summary

Background SARS-CoV-2 binding to ACE2 is potentially associated with severe pneumonia due to COVID-19. The aim of the study was to test whether Mas-receptor activation by 20-hydroxyecdysone (BIO101) could restore the Renin-Angiotensin System equilibrium and limit the frequency of respiratory failure and mortality in adults hospitalized with severe COVID-19.

Methods Double-blind, randomized, placebo-controlled phase 2/3 trial. Randomization: 1:1 oral BIO101 (350 mg BID) or placebo, up to 28 days or until an endpoint was reached. Primary endpoint: mortality or respiratory failure requiring high-flow oxygen, mechanical ventilation, or extra-corporeal membrane oxygenation. Key secondary endpoint: hospital discharge following recovery (ClinicalTrials.gov Number, NCT04472728).

Findings Due to low recruitment the planned sample size of 310 was not reached and 238 patients were randomized between August 26, 2020 and March 8, 2022. In the modified ITT population (233 patients; 126 BIO101 and 107 placebo), respiratory failure or early death by day 28 was 11.4% lower in the BIO101 (13.5%) than in the placebo (24.3%) group, (p = 0.0426). At day 28, proportions of patients discharged following recovery were 80.1%, and 70.9% in the BIO101 and placebo group respectively, (adjusted difference 11.0%, 95% CI [–0.4%, 22.4%], p = 0.0586). Hazard Ratio for time to death over 90 days: 0.554 (95% CI [0.285, 1.077]), a 44.6% mortality reduction in the BIO101 group (not statistically significant). Treatment emergent adverse events of respiratory failure were more frequent in the placebo group.

Interpretation BIO101 significantly reduced the risk of death or respiratory failure supporting its use in adults hospitalized with severe respiratory symptoms due to COVID-19.

Funding Biophytis.

Keywords: SARS-CoV-2; COVID-19; Renin-angiotensin-system (RAS); MasR; 20-Hydroxyecdysone; Respiratory failure

Introduction

Despite massive vaccination campaigns, and the availability of preventive antiviral treatment used as prophylaxis, such as (Paxlovid™), hospitalization and mortality due to coronavirus disease 2019 (COVID-19) remains substantial worldwide (https://covid19.who.int/), particularly in countries where vaccination rates are lower and in which prophylactic antivirals are less available, as well as in specific patient populations such as the elderly with comorbidities or those non-responsive to vaccines.[1–3] Treatment of hospitalized patients with severe COVID-19 symptoms generally includes supportive oxygenation, corticosteroids administration when viral replication has declined,[4,5] remdesivir and immunomodulators. However, antibodies targeting inflammatory cytokines show heterogeneous results[6] and antivirals are of limited efficacy in patients hospitalized with severe COVID-19.[7,8] Thus, new therapeutic options are still required to limit respiratory failure and mortality in this indication.

The renin-angiotensin system (RAS) encompasses many peptides (angiotensins), peptidases, and receptors representing two functional pathways in a balance whose equilibrium regulates respiratory functions, among others.[9] The angiotensin-converting enzyme 2 (ACE2) regulates RAS equilibrium by converting angiotensin-II (Ang-II), a ligand of the angiotensin receptor one (AT1R), into angiotensin-(1-7), a ligand of the Mas receptor (MasR).[9,10] The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) uses ACE2 as the receptor to infect lung epithelial and endothelial cells.[11–13] Binding of SARS-CoV-2's spike protein to ACE2 may lead to a dysregulation of the RAS.[14] Accordingly, abnormal plasma levels of Ang-II and angiotensin-(1–7) have been reported in COVID-19

Research in context

Evidence before this study

Between January 2020 up to this day (November 2023). An extensive review of relevant articles published in English were identified through searches in the authors' personal files, in PubMed and Research Gate. The search terms were "COVID-19", or "SARS-CoV-2", and "Remdesivir", or "Hydroxychloroquine/Chloroquine", or "Lopinavir", or "Ritonavir", "Favipiravir", or "Molnupiravir", or "viral load", or "viral shedding", or "Convalescent plasma (CP)", or "Neutralizing antibodies", or "Tocilizumab", or "Dexamethasone", or "cytokine storm", or "IL-6", or "IL-1", or "CRP", or "inflammation", or "angiotensin converting enzyme-2 (ACE2)", or "Angiotensin-II", or "Angiotensin-(1-7)", or "Mas receptor", or "Renin-angiotensin system" and "aging".

SARS-CoV-2 spike protein uses ACE2 as its main receptor, suggesting that the Renin-Angiotensin System (RAS) could play a role in COVID-19 pathology. Indeed, SARS-CoV-2 infection may increase the levels of angiotensin-II (Ang-II), known for its role in vasoconstriction, thrombosis, inflammation, and respiratory distress symptoms, through activation of its AT1R receptor. Conversely, it was hypothesized that SARS-CoV-2 by blocking ACE2's activity may reduce levels of angiotensin-(1-7), which generally opposes the effects of Ang-II by activating the Mas receptor (MasR).

ACE inhibitors or AT1R blockers (ARBs) inhibiting the deleterious arm of the RAS (ACE/Ang-II/AT1R) or AT2R or MasR activators stimulating the protective arm of the RAS (ACE2/angiotensin 1-7 (Ang-1-7)/MasR axis) could potentially prove beneficial in COVID-19 patients.

BIO101 (20-hydroxyecdysone (20E)), is a non-peptidic MasR activator that reduces the severity of lung injury induced by an intratracheal instillation of lipopolysaccharide in mice. In August 2020, we launched a multicentre double-blind, randomized placebo-controlled phase 2/3 clinical trial (COVA). In this trial we evaluated the safety and efficacy of 350 mg BID daily oral administration of BIO101 vs. placebo in the setting of standard of care (including glucocorticoid therapy) in hospitalized patients with severe COVID-19.

Added value of this study

Compared to placebo, BIO101 significantly lowers the proportion of patients experiencing respiratory failure or early death by day 28. The proportion of patients recovered was higher in the BIO101 group than placebo and the proportion of patients who died was lower, however these differences did not reach statistical significance.

Moreover, the anticipated good safety profile of BIO101 previously reported during BIO101's phase-1 and in a phase-2 clinical trial on sarcopenic patients was confirmed with similar frequencies of treatment emergent adverse events (TEAEs) in patients assigned to the placebo group than to the BIO101 group, although respiratory failure events were more frequent in the placebo group.

Implications of all the available evidence

The findings reported here show that BIO101 modulating the RAS via activation of the Mas receptor, reduces the frequency of respiratory failure and death associated with COVID-19. Thus, these results are important as they make BIO101 a treatment candidate in the armamentarium of drugs for patients hospitalized with severe respiratory symptoms due to COVID-19.

patients.[15–17] Increased levels of Ang-II and reduced production of Ang-1-7 due to the lower ACE2 activity could provoke multiple COVID-19 symptoms, including vasoconstriction, thrombosis, inflammation, and potentially fatal respiratory failure.[14,18–21] We and others hypothesized that RAS modulation might thus limit the frequency of respiratory failure and mortality in hospitalized COVID-19 patients.[10,18,19]

Restoring RAS balance could be achieved using classical antihypertensive drugs such as ACE inhibitors or AT1R blockers (ARBs) that inhibit the classical (deleterious) arm of the RAS (ACE/Ang-II/AT1R) or alternatively with AT2R or MasR activators that stimulate the protective arm of the RAS (ACE2/angiotensin 1-7/MasR axis). However, the exact value of RAS modulation as a treatment option for COVID-19 and the optimal strategy to apply (either inhibiting the harmful arm or activating the protective arm of the RAS) remain a subject of debate. Indeed, results of a phase 2 clinical trial early in the pandemic showed improvement of respiratory function in patients with severe COVID-19 treated with an agonist of the AT2R (C21),[22] but these observations were not confirmed in phase 3, and recently clinical trials testing this therapeutic approach using a biased agonist of AT1R (TRV-027) and a synthetic Angiotensin-1-7 (TXA-127) did not show clinical improvement in this patient population.[23]

Biophytis is developing BIO101, a pharmaceutical grade formulation of 20-Hydroxyecdysone (20E), which is a MasR activator[24] that reduces the severity of lung injury induced by an intratracheal instillation of lipopolysaccharide in mice.[25] The aim of the "COVA" study, a double-blind, randomized placebo-controlled phase 2/3 clinical trial, launched in August 2020, was to evaluate BIO101's safety and efficacy in hospitalized patients with severe COVID-19.[26]

Methods

Study design and participants

This was a double-blind, placebo-controlled, group sequential study in 2 parts. After part 1 a first interim analysis (IA) was undertaken by an independent data monitoring committee to determine safety and to obtain preliminary indication of BIO101 efficacy in preventing further respiratory deterioration in 50 hospitalized COVID-19 patients. A second IA (IA2) was performed on results of the first 155 randomized patients to assess safety, futility and re-estimate the sample size for final analysis. Enrollment began on August 26, 2020, and ended on March 8, 2022 following sponsor decision to halt the study due to low recruitment. There were 37 sites, in Brazil (14), the United States (13), France (7) and Belgium (3).

Inclusion criteria: patients aged 45 years (55 years in France) or above, hospitalized, or planned to be hospitalized with evidence of pneumonia due to COVID-19 confirmed within the last 28 days prior to randomization, as determined by polymerase chain reaction or another approved commercial assay. Respiratory symptoms: either tachypnoea (≥25 breaths per minute), or arterial oxygen saturation ≤92% or both, should have started not more than 7 days before first study medication administration. The exclusion criteria included 1) patients' inability to take medication by oral route and 2) being in a moribund condition not expected to survive for more than 7 days due to other non-COVID-19 related conditions. All standard supportive care and any other treatment for COVID-19 including antivirals, anti-inflammatory or experimental drugs were allowed except products extracted from *Leuzea carthamoides*; *Cyanotis vaga* or *Cyanotis arachnoidea* containing 20E. Subjects remained eligible while receiving low flow oxygen supplementation but were excluded if they required invasive mechanical ventilation, extracorporeal membrane oxygenation (ECMO) or high flow oxygen (HFO_2). This last category was excluded in protocol versions 1–11 only. From protocol version 12, as more patients were on HFO_2 immediately upon arrival at hospital due to the modification of medical practices in some sites, patients needing HFO_2 could be recruited, and requirement for HFO_2 was no longer computed as a negative event for these patients.). The trial protocol was approved by national regulatory agencies in each country and by institutional review board at each site or by a centralized national ethics committee. Written informed consent was obtained from each patient or from their legally authorized representative if the patient was unable to provide consent. This report is based on the final protocol version 13.0, and the final SAP version 1.0, dated 06 May, 2022 that was finalized prior to unblinding.

Randomisation and masking

In Part 1 of the study, randomization was stratified for RAS pathway modulators use and comorbidities (no vs. ≥1) only. In Part 2, randomization was additionally stratified by center, gender, and use of CPAP/BiPAP/HFO_2 (Yes/No/Missing). During the study, the participants, investigators, and the sponsor were kept blind to which treatments arms each patient was allocated.

Procedures

BIO101 (350 mg of 20E) or matching placebo was administered orally twice daily (BID) up to 28 days or until a pre-defined endpoint was reached (hospital discharge, start of HFO_2 (until protocol version 12), mechanical ventilation, ECMO, or death). Patients' clinical status during hospitalization between day 1 and 28 was assessed four times (once each between 2 and 4, 6–8, 12–16 and at day 28, or when a clinical endpoint was reached). Post-intervention evaluations were carried out three times over the phone at day 28 (for patients discharged before that day), 14 days after last on-treatment visit and 90 days post-randomization.

Outcomes

The primary measure of the primary outcome was the proportion of subjects with 'negative' events as the first event (among negative or positive ones) up to day 28. "Negative events" as a composite outcome were defined as either mortality or respiratory failure requiring mechanical ventilation (including cases that were not intubated due to resource restrictions and triage), ECMO or (until protocol V12) HFO_2 use. Positive events were defined as official discharge from hospital care due to improvement in condition. The key secondary outcome measure was the proportion of subjects with 'positive' events as the first event (negative or positive) up to day 28. Other secondary outcome measures included the proportion of subjects with all-cause mortality at day 28, day 90 and time to death. Safety outcomes included any treatment emergent adverse event (TEAE), serious (SAEs) or non-serious (AEs), and discontinuation or temporary suspension of treatment administration.

Statistical analysis

Initial sample size was 310 patients which gave the study 80% power to detect a 15% absolute difference in proportion of subjects with primary endpoint (negative events), estimated at 40% in the placebo group and 25% in the BIO101 group, corresponding to a 37.5% relative risk (RR) reduction. Results of the second interim analysis (IA2) were promising in term of efficacy and the sample size of at least 310 was updated to 375 based on the method described by Mehta and Pocock.[27] The modified intention to treat (mITT) analysis set was defined as all patients randomized in the clinical trial who received at least one dose of the study drug or placebo. Primary analysis of the primary endpoint and of the key secondary endpoint were performed using the Cochran-Mantel-Haenszel (CMH) test stratified by RAS pathway modulator use (Yes/No), gender, co-morbidities

(no vs. ≥1) and receiving CPAP/BiPAP/HFO_2 at study entry (Yes/No/Missing) and with placebo-based multiple imputation for missing data (3 [1.3%] subjects) based on an imputation model that included the stratification factors, fitted to data for placebo subjects only. Reporting was done in terms of adjusted difference and associated 95% CI and p-value. Multiplicity was controlled by evaluating the key secondary endpoint only if the primary endpoint gave a statistically significant result. All p-values are reported as two-sided. Other secondary endpoints were not controlled for multiplicity and only descriptive statistics and 95% confidence (CI) intervals for treatment differences are provided except in the case of mortality at day 28 where the p-value is presented as "nominal".

Sensitivity analyses were conducted to evaluate the robustness of the results for the primary endpoint including requiring HFO_2 excluded from the definition of the primary endpoint. Occurrence of all negative events up to day 28 (including requiring HFO_2 and ignoring the occurrence of a prior positive event) was evaluated by logistic regression with the stratification factors described above, except center. Age and immunosuppressants usage at baseline were included as additional covariates in the model because of their clinical relevance (age) and due to the observed imbalance of immunosuppressants usage at baseline between treatment arms. Finally, proportions of subjects with negative events at day 28 were evaluated using the Kaplan–Meier method by censoring subjects lost to follow-up, to avoid imputation for missing data. Heterogeneity of treatment effects was evaluated using the CMH method in subgroups to determine the influence of stratification factors on response. The Overall OR for this display used the CMH method adjusted by RAS pathway modulator use (Yes/No), gender, Comorbidities (None/1 and above) and receiving CPAP/BiPAP/HFO2 at study entry (Yes/No/Missing).

As secondary analysis, mortality up to day 28 was also evaluated using the CMH methodology. In this analysis patients with missing outcomes were assumed to have survived. Kaplan–Meier curves plotted for time to death were also used to estimate the mortality rates at day 28. Kaplan–Meier curves plotted for time to death and respiratory failure up to day 90 were also plotted. To avoid imputation for missing data, subjects alive at the time of the end of study visit or lost-to-follow up were censored at the time of the last assessment/measurement. A hazard ratio together with a 95% CI for time to death through 90 days was estimated using a Cox model with the stratification factors (except center), and age and immunosuppressants use at baseline included as covariates.

Role of the funding source

Biophytis SA provided funding and personnel from Biophytis were involved in study activities including study design, data collection, data analysis, data interpretation, and writing of the report. Data management and statistical analyses were done in a masked manner by a Contract Research Organization paid by the funding source. Biophytis declares that potential commercial interests had no impact on the scientific conduct of the study nor on the analysis/interpretation of data.

Results

A total of 275 participants were enrolled including 37 screening failures (Fig. 1). There were 238 subjects randomized: (129 assigned to BIO101 and 109 receiving placebo). From these, consistent with ITT principles, three subjects in the BIO101 group and 2 in the placebo group that did not receive any study medication were not included in the mITT analysis set (n = 233). A total of 194 (83.3%) subjects in the mITT analysis group completed the study treatment. Thirty-nine subjects (16.7%) discontinued from study treatment, the reasons being: AE/SAE (24 [10.3%] subjects), other/unspecified reasons (8 [3.4%] subjects), withdrawal of consent (6 [2.6%] subjects), and noncompliance of 1 subject (0.4%) (Fig. 1). A total of 13 (5.6%) subjects were reported to have at least 1 major protocol deviation. The most common major protocol deviations were related to inclusion criteria (9 [3.9%] subjects) and missing endpoint assessments (3 [1.3%] subjects).

Sixty percent of patients were enrolled in Brazil, 27% in Europe and 13% in North America. Race/ethnicity was not reported in 23.2% of patients but overall, 64.8% of the patients were Caucasian/White, 9.4% Black, and below three percent were Asian or designated as other. One hundred and thirty participants (55.8%) were Hispanic or Latino. The mean age of the global population was 62.8 years (SD 9.21 years) and was not different between the placebo and BIO101 groups. Overall, 63.5% were males but with a higher proportion (66.7%) in the BIO101 than in the placebo group (59.8%). Most patients had at least one (92.3%) coexisting condition at enrollment, which were well balanced between the two groups, the most frequents being hypertension (51.5%), obesity (22.7%), and type 2 diabetes mellitus (19.7%). The median time between first respiratory symptoms and inclusion was 8.5 days in both groups. Importantly, at enrolment the percentages of patients needing oxygen supplementation were equivalent and SARS-CoV-2 viral load were similar in both groups. During the study, concomitant medications including corticosteroids usage were also equally distributed except immunosuppressants (mainly baricitinib and tocilizumab) which were used more frequently in the placebo group (9 subjects, [8.4%]) than in the BIO101 group (4, [3.2%]) (Table 1). Overall, 92.7% of patients achieved 80–100% treatment compliance and the total number of doses administered, mean daily doses and the mean number of days on treatment (BIO101; 8.9 ± 6.13 days vs.

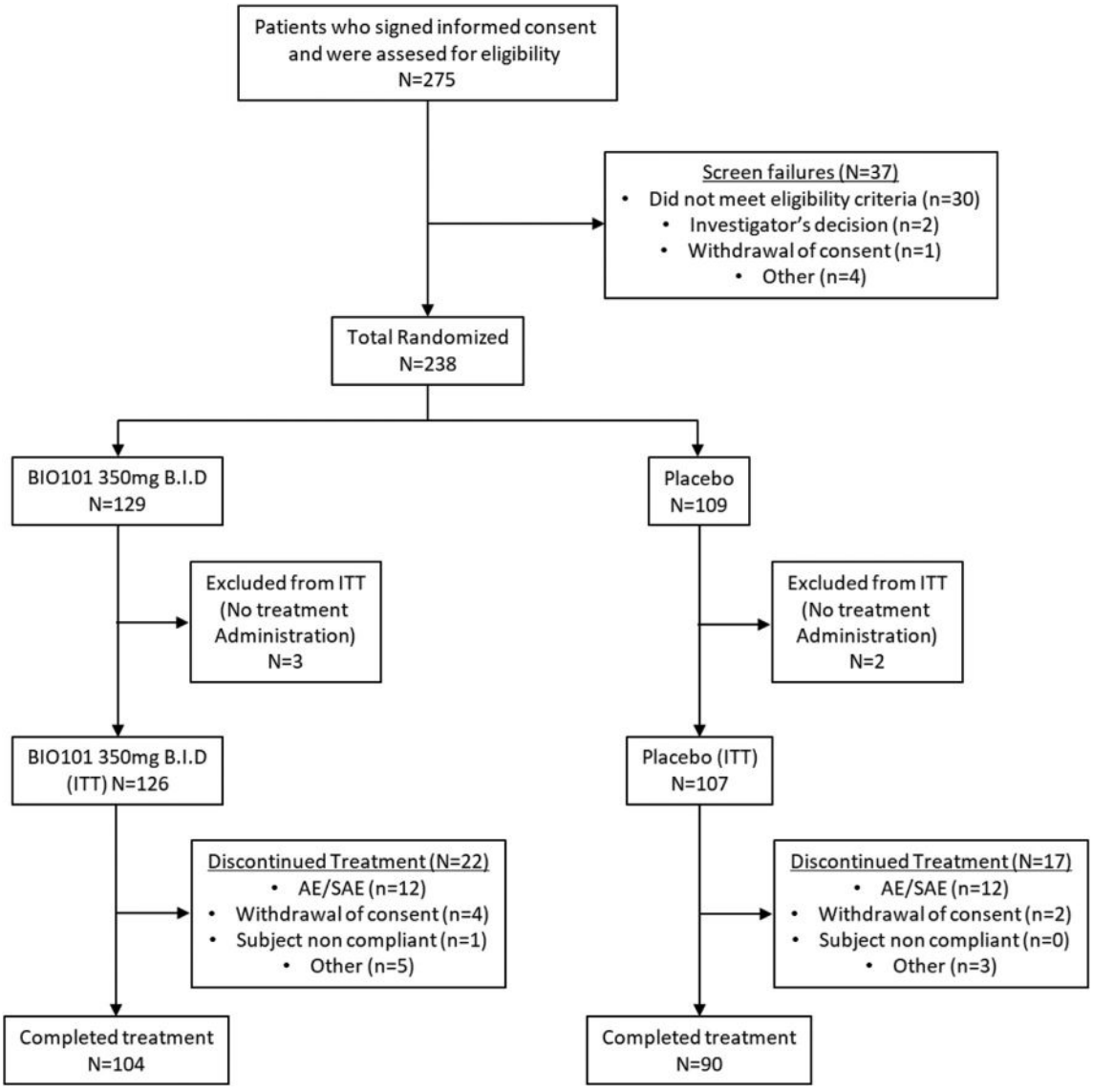


Fig. 1: *Note: Consistent with ITT principle five patients (2 in the placebo group and 3 in the BIO101 group) were excluded from the modified ITT (mITT) analysis set after randomization at the time when the trial was still blinded as these patients were not treated and had no efficacy data available.

Placebo; 7.6 ± 5.36 days) were similar between treatment arms (Supplementary Table S1).

Primary outcome

In the mITT population, using the CMH test with multiple imputation for missing data, the first negative event rate was statistically significantly lower in the BIO101 group than in the placebo group (adjusted difference: –11.4% [95% CI: –22.4%, –0.4%]; p = 0.0426) (Table 2A). The observed proportion of subjects who met the primary outcome of death or respiratory failure at or before day 28, were 13.5% vs. 24.3% (Fig. 2B). The most common negative event was respiratory failure and was numerically reduced in the BIO101 group compared to the placebo group (16 subjects, 12.7% vs. 23 subjects, 21.5% respectively) (Table 2B). The RR of having negative events was reduced by 43.8% in the BIO101 arm. Benefits of BIO101 were confirmed by sensitivity analyses by logistic regression of all negative events by day 28 (odds ratio (OR) of 0.47 (95% CI: 0.23, 0.95)) and by Kaplan–Meier proportions (difference 10.9%, 95% CI [0.6%, 21.2%]) (Fig. 2A). Treatment effects on the primary endpoint were not meaningfully different in subgroup categories. All ORs were in favour

	BIO101 350 mg BID (N = 126)	Placebo (N = 107)	Total (N = 233)
Demographics			
Age (years)			
Mean (SD)	63.0 (9.82)	62.5 (8.46)	62.8 (9.21)
Median (IQR)	64.0 (40, 87)	63.0 (45, 90)	63.0 (40, 90)
Age categories (years), n (%)			
<65	69 (54.8)	62 (57.9)	131 (56.2)
≥65	57 (45.2)	45 (42.1)	102 (43.8)
Sex, n (%)			
Male	84 (66.7)	64 (59.8)	148 (63.5)
Female	42 (33.3)	43 (40.2)	85 (36.5)
Race, n (%)			
White or Caucasian	86 (68.3)	65 (60.7)	151 (64.8)
Black or African American	11 (8.7)	11 (10.3)	22 (9.4)
Asian	1 (0.8)	0	1 (0.4)
Other	2 (1.6)	3 (2.8)	5 (2.1)
Not reported	26 (20.6)	28 (26.2)	54 (23.2)
Ethnicity, n (%)			
Hispanic or Latino	70 (55.6)	60 (56.1)	130 (55.8)
Not reported	20 (15.9)	21 (19.6)	41 (17.6)
Body mass index (BMI) (kg/m^2)			
n, Median	115 (28.30)	95 (30.00)	210 (29.15)
Medical history at time of intervention, n (%)	114 (90.5)	101 (94.4)	215 (92.3)
Chronic conditions			
Metabolism and nutrition disorders	80 (63.5)	70 (65.4)	150 (64.4)
Obesity	30 (23.8)	23 (21.5)	53 (22.7)
Diabetes	23 (18.3)	23 (21.5)	46 (19.7)
Vascular disorders	66 (52.4)	59 (55.1)	125 (53.6)
Hypertension	62 (49.2)	58 (54.2)	120 (51.5)
Gastrointestinal disorders	39 (31)	29 (27.1)	68 (29.2)
Respiratory, thoracic and mediastinal disorders	25 (19.8)	28 (26.2)	53 (22.7)
Cardiac disorder	15 (11.9)	17 (15.9)	32 (13.7)
Neoplasm (benign, malignant, and unspecified)	12 (9.5)	17 (15.9)	29 (12.4)
Hepato-biliary disorders	8 (6.3)	9 (8.4)	17 (7.3)
Respiratory function and oxygen supplementation at time of intervention, n			
Time since first respiratory symptoms (days)[a]			
n	126	107	233
Mean (SD)	8.4 (3.97)	8.6 (4.22)	8.5 (4.08)
Respiratory rate (breaths/min)			
n	120	103	223
Mean (SD)	22.6 (4.97)	22.9 (5.73)	22.7 (5.32)
SpO_2(%)			
n	126	106	232
Mean (SD)	91.9 (2.96)	92.3 (3.47)	92.1 (3.20)
FiO_2 (%)			
n	118	94	212
Mean (SD)	45.83 (26.61)	45.07 (27.36)	45.49 (26.88)
Number of subjects with supplemental oxygen supply	32 (25.4)	24 (22.4)	56 (24.0)
Mode of delivery			
Low flow	29 (23.0)	25 (23.1)	54 (23.1)
High flow	5 (4.0)	2 (1.8)	7 (3.0)
Non-invasive mechanical ventilation	0 (0.0)	1 (0.9)	1 (0.4)
SARS-CoV-2 viral load (copies)			
n	46	44	
Mean (SD)	6118.78 (8870.25)	7649.26 (10311.89)	

(Table 1 continues on next page)

	BIO101 350 mg BID (N = 126)	Placebo (N = 107)	Total (N = 233)
(Continued from previous page)			
Medication on intervention period, n (%)			
Number of subjects with at least one prior and concomitant medication	126 (100.0)	107 (100.0)	233 (100.0)
Corticosteroids for systemic use	121 (96.0)	104 (97.2)	225 (96.6)
Antithrombotic agents	119 (94.4)	99 (92.5)	218 (93.6)
Drugs for acid related disorders	90 (71.4)	69 (64.5)	159 (68.2)
Analgesics	81 (64.3)	57 (53.3)	138 (59.2)
Antibacterial for systemic use	80 (63.5)	71 (66.4)	151 (64.8)
Drugs used in diabetes	50 (39.7)	52 (48.6)	102 (43.8)
Agents acting on the renin-angiotensin system	46 (36.5)	42 (39.3)	88 (37.8)
Drugs for obstructive airway diseases	37 (29.4)	34 (31.8)	71 (30.5)
Calcium channel blockers	17 (13.5)	22 (20.6)	39 (16.7)
Antivirals for systemic use	14 (11.1)	16 (15)	30 (12.9)
Remdesivir	10 (7.9)	12 (11.2)	22 (9.4)
Oseltamivir	4 (3.2)	3 (2.8)	7 (3)
Ritonavir	0 (0)	1 (0.9)	1 (0.4)
Antihistamines for systemic use	16 (12.7)	6 (5.6)	22 (9.4)
Antihypertensives	8 (6.3)	10 (9.3)	18 (7.7)
Immunosuppressants including Tocilizumab and Baricitinib	4 (3.2)	9 (8.4)	13 (5.6)
Anti-inflammatory and antirheumatic products	9 (7.1)	3 (2.8)	12 (5.2)

Note: Subjects could have more than one medication per ATC level 2 category and preferred term. At each level of subject summarization, a subject was counted once if the subject reported one or more medications. Based on their start (time) and stop date (time), prior and concomitant medications were allocated to each period during which they were administered. A medication can therefore be reported in more than one period. Prior and Concomitant medications were coded with the WHO Drug dictionary dated March 2022. [a]Time since first respiratory symptoms = first administration date — earliest of respiratory symptoms (including cough, sore throat, shortness of breath, symptoms of pneumonia and others) onset date + 1.

Table 1: **Characteristics of patient population and interventions at baseline.**

of BIO101, and the consistent beneficial effect of BIO101 was further confirmed by these analyses (Supplementary Figure S1). We performed another subgroup analysis for the primary endpoint before and after the change in the definition of respiratory failure and the related modification of the inclusion criteria to compare the effect of BIO101 vs. Placebo on the primary endpoint in patients recruited under protocol versions 1–11 and under protocol versions 12–13. The results are presented in Supplementary Figure S2 and show consistency for the reduction in the proportion of patients experiencing negative events in favour of BIO101 (protocol versions 1 to 11 adjusted difference –7.08%, 95% CI [–21.1%, 6.91%]) and protocols 12-13 adjusted difference: –16.2%, 95% CI [–33.6%, 1.22%]). We also performed subgroup analyses of the primary endpoints across countries, but since the number of patients recruited in Belgium and USA were low, we merged them with patients recruited in France as we estimated that the populations would likely be comparable. Treatment effects were numerically different between the France, Belgium, and US subgroup (adjusted difference –0.14%, 95% CI [–23.3%, 23.0%]), and the Brazil subgroup (adjusted difference: –12.1%, 95% CI [–25.7%, 1.6%]) (Supplementary Figure S3), but as the confidence intervals are substantially overlapping there is no evidence for a differential treatment effect across regions.

Key secondary outcome

The proportion of subjects discharged from hospital due to improvement (positive events) at day 28 was numerically higher in the BIO101 group (80.1%) than in the placebo group (70.9%) although this difference did not reach statistical significance (adjusted difference: 11.0% [95% CI: –0.4%, 22.4%], p = 0.0568) (Table 2A).

Kaplan Meier analysis: time to mortality and time to respiratory failure

The proportions of subjects with death from any cause at Day 28 (7.9% vs. 14% in the BIO101 vs. placebo arms respectively) using the CMH test indicated that BIO101 had a RR reduction of death of 44.3% compared to placebo but this difference did not reach (nominal) statistical significance (adjusted difference: –6.2%, 95% CI: [–14.5%, 2.2%]) (Table 2). The hazard ratio for time to death through day 90 was 0.554 (95% CI: 0.285, 1.077) a 44.6% reduction in the death rate over 90 days in the BIO101 group that did not reach statistical significance. The difference in the proportions of deaths at 90 days was 0.093 (95% confidence interval (–0.010, 0.195)), representing a 42.9% reduction in the risk of death in the BIO101 group compared to the placebo group, was not statistically significant (Fig. 2B). Finally, Kaplan Meier comparison of the time to respiratory failure at Day 90 between the BIO101 and the placebo

A: Placebo-based multiple imputation for missing data for the first negative and first positive event

	BIO101 350 mg BID (N = 126) n (%)	Placebo (N = 107) n (%)	Comparison of BIO101 350 mg BID vs. placebo		
			Unadjusted difference (%)	Adjusted difference[a] (%) (95% CI)	p-value
Proportion of patients with death or respiratory failure (First negative events) Primary endpoint[d]	19.85 (15.8)	27.86 (26.0)	−10.3	−11.4 (−22.4, −0.4)	**0.0426**
Key secondary endpoint[b] Proportion of patients discharged from hospital due to improvement. (First positive events)	100.94 (80.1)	75.91 (70.9)	9.2	11.0 (−0.4, 22.4)	0.0586
Mortality at day 28[c] Death from any cause	10 (7.9)	15 (14.0)	−6.1	−6.2 (−14.5, 2.2)	0.1476 (nominal)

B

	BIO101 350 mg BID (N = 126) n (%)	Placebo (N = 107) n (%)
Number of subjects with first negative event	17 (13.5)	26 (24.3)
Death from any cause	1 (0.8)	3 (2.8)
Respiratory failure	16 (12.7)	23 (21.5)
Requiring mechanical ventilation	14 (11.1)	15 (14.0)
Requiring ECMO	0	0
Requiring high flow oxygen[d]	2 (1.6)	8 (7.5)
Number of subjects with positive event[e]	93 (73.8)	71 (66.4)

Note: Only the first occurrence of any event up to Day 28 was considered for the primary endpoint. Bold is used to draw attention on the p value of the primary analysis of the primary endpoint indicating that the study "was positive" with a p value below 0.05. [a]Adjusted difference in proportions is calculated using a Cochran-Mantel-Haenszel test, stratified by RAS pathway modulator use (Yes/No), gender, co-morbidities and receiving CPAP/BiPAP/HFO2 at study entry (Yes/No/Missing). [b]The first negative event rates and the first positive event rates are the averages of treatment arm estimates from the imputed complete data sets. The SAS procedure PROC MIANALYZE is used to derive unadjusted difference, adjusted difference with 95% CI and 2-sided p-value for treatment comparison combining the imputed complete data sets. [c]Patients with a missing outcome for death are considered as having survived. [d]This is considered respiratory failure for subjects who were enrolled under protocol version 1–11. Subjects who were recruited under protocol version 12 or later are not counted in this category. [e]The positive event is official discharge from hospital care by the department due to improvement in participant condition (self-discharge by participant is not considered a positive event). Requiring high-flow oxygen for subjects who were enrolled under protocol version 1–11 is considered respiratory failure and negative event. Only the first occurrence of any event up to day 28 considered for negative and positive event.

Table 2: **Primary and secondary endpoints.**

groups did not reach statistical significance either (HR: 0.530 (95% CI: 0.279, 1.006) (Fig. 2C).

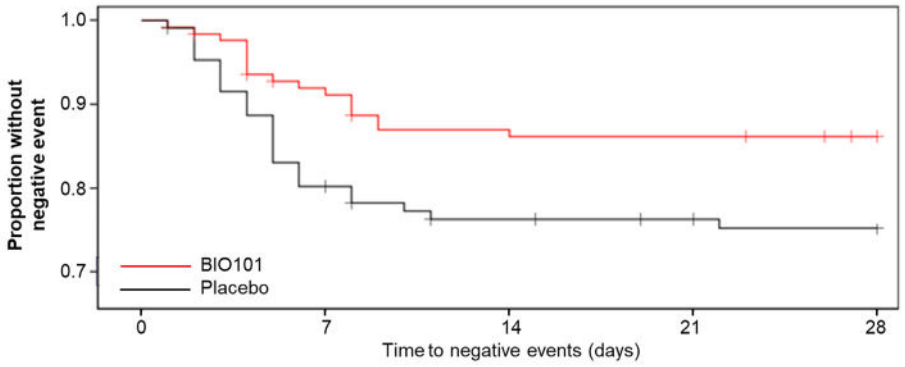
Safety outcomes and treatment emergent adverse events

A lower proportion of patients treated with BIO101 experienced any type of TEAEs than placebo subjects (73 [57.0%] vs. 67 [64.4%], respectively). Overall, 26 (11.2%) subjects had treatment-related TEAEs, 12 (9.4%) in the BIO101 group and 14 (13.5%) in the placebo group (Table 3). A lower proportion and number of serious TEAEs were reported in the BIO101 group (32 subjects [25.0%]), 59 serious events), vs. the placebo group (32 subjects, [30.8%], 64 serious events). During the on-intervention period, the most frequent serious (≥5% of subjects in any group) TEAEs were respiratory failure, acute respiratory distress syndrome, acute respiratory failure, and hypoxia with a lower frequency (19 subjects [14.8%]) in BIO101 than in placebo (29 subjects [27.8%]), supporting the efficacy results (Table 3). Forty-four subjects (19.0%) had 51 TEAEs leading to discontinuation from the study or of the study drug (18 BIO101 treated subjects [14.1%], 21 events and 26 placebo subjects [25.0%], 30 events). Orthostatic hypotension, an event of special interest, occurred as frequently on BIO101 (9 participants (7.0%), 11 events) as on placebo (8 participants [7.7%], 10 events). Other common TEAEs were constipation (10 [7.8%] and 16 [15.4%] subjects in the BIO101 and placebo groups, respectively, hyperkalaemia (7 [5.5%] and 9 [8.7%] subjects in the BIO101 and placebo groups, respectively), and hypokalaemia (7 [5.5%] subjects and 1 [1.0%] subject in the BIO101 and placebo groups, respectively). An association between BIO101 and increase in gamma-glutamyltransferase cannot be excluded but seems unlikely (Supplementary Table S2). Mean differences in vital signs including blood pressure, laboratory parameters and ECG parameters between the BIO101 and placebo groups were mostly not clinically significant and similar in both treatment arms. Physical examination findings were also aligned to the underlying COVID-19 condition in most subjects indicating a very good safety profile of BIO101.

Discussion

Since it was discovered that SARS-CoV-2 uses ACE2 as a receptor,[11–13] we and others have posed the hypothesis that binding of SARS-CoV-2's spike protein to ACE2 may lead to a dysregulation of the RAS.[14] An imbalance between levels of Ang-II and of Ang-1-7 could provoke multiple pathophysiological features of COVID-19,

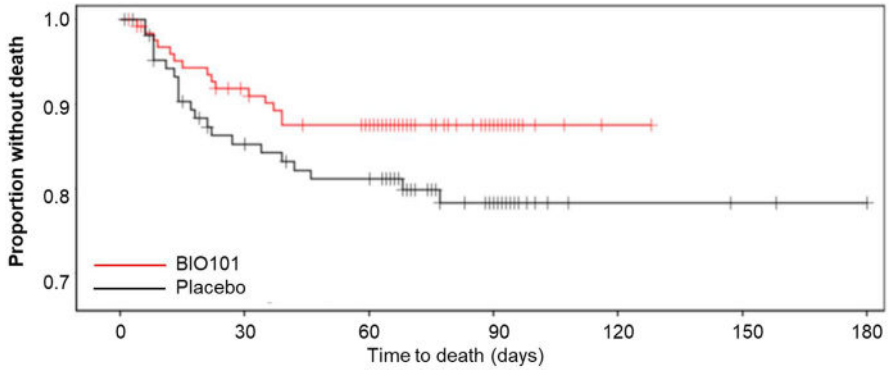
a Time to primary outcome (negative events: respiratory failure or early death).



Number of subjects at risk

BIO101	126	112	105	104	101
Placebo	107	85	76	74	72

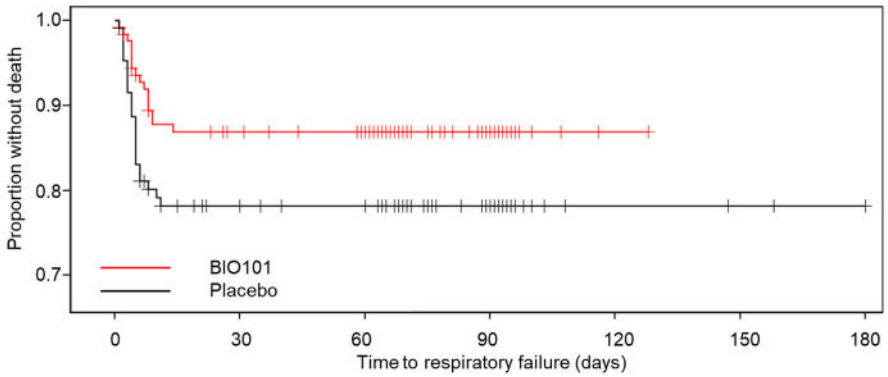
b Time to death.



Number of subjects at risk

BIO101	126	109	100	52	1	0
Placebo	107	84	78	44	3	2	1

c Time to respiratory failure



Number of subjects at risk

BIO101	126	101	96	48	1	0
Placebo	107	72	69	41	3	2	1

including vasoconstriction, thrombosis, inflammatory reaction, which is potentially ultimately responsible for the respiratory symptoms observed during COVID-19.[18–21] Therefore, it has been suggested that restoring RAS balance either by inhibition of the classical arm of the RAS (ACE/Ang-II/AT1R) with classical ACE inhibitors or AT1R blockers (ARBs) or alternatively by stimulation of the protective arm of the RAS (ACE2/angiotensin 1-7/MasR axis) with AT2R or MasR activators would benefit patients with severe respiratory symptoms associated with COVID-19.[18]

In agreement with this scientific rationale the COVA trial met its primary endpoint, showing that BIO101, a MasR activator, significantly reduces the proportion of patients with death or respiratory failure at day 28 (p = 0.0426). This represents a 43.8% relative reduction in risk of death or respiratory failure, an effect slightly larger than originally expected in the sample size estimation (37.5%). Benefits of BIO101 were confirmed by logistic regression (OR: 0.47), by subgroup analysis, and by Kaplan–Meier analysis of time to negative event over 28 days. By contrast, the nominally increased proportion of patients treated with BIO101 to experience positive events at day 28 (key secondary endpoint: 80.1% vs. 70.9% in the placebo group) did not reach statistical significance. In secondary analysis, the 44.3% relative reduction in risk of death at 28 days in the BIO101 group also did not reach statistical significance. Both the time to death and time to respiratory failure over 90 days showed numerical but not statistically significant differences in favor of BIO101. The safety profile of BIO101 in COVID-19 patients was favorable with similar proportions of patients with any TEAEs in the BIO101 group compared to placebo (73 [57.0%] vs. 67 [64.4%]) and lower frequencies of respiratory failure events.

Our results, and those of other clinical studies, suggests that the role of the RAS in COVID-19 pathophysiology may be more complex than previously thought.[9,28] Indeed, early in the pandemic it has been reported that C21 (an activator of the AT2R) improved respiratory function in patients with severe COVID-19,[22] but this was not confirmed by the results of a subsequent phase 3 study (ATTRACT-3, NCT04880642), communicated through a press release. More recently, two clinical trials testing TRV-027 (an AT1R blocker), and TXA-127 (an analogue of Ang-1-7), did not show clinical improvement in the same patient population.[23] These contrasting results between the effect of BIO101 and of these other compounds may be explained by the complexity of the RAS composed of many proteases, receptors and peptidic ligands with often opposing effects.[28] Because BIO101 does not interfere with AT1R like TRV-027, our positive results in COVID-19 perhaps suggest a particular importance of MasR activation in the regulation of respiratory function.[28]

When comparing the effect of BIO101 with those of TXA-127 which shares the same molecular target (MasR),[24] it has been shown that peptidic ligands such as TXA-127/Ang-1-7 and non peptidic ligands such as BIO101 (20-hydroxyecdysone) bind to an overlapping but not identical pocket.[29] Consequently, MasR activation by Ang-1-7 and BIO101 probably does not induce the same signaling cascade.[24,29,30] In addition, Ang-1-7's half-life in humans is approximately half an hour[31] and is shorter compared to BIO101's half-life (2.4 h–4.9 h).[32] Moreover, in contrast with BIO101, Ang-1-7 is cleaved into several peptides (Ang-1-5, Ang-1-4, and others) susceptible to evoke different biological effects. All these elements could explain the differences in efficacy of BIO101 and TXA-127 on COVID-19 respiratory symptoms and mortality.[23] Future analysis of the concentration of RAS biomarkers in plasma of patients with COVID-19 are needed and will help unravel the multiple roles and effects of RAS modulation during this disease.

In the COVA study (as in previous studies[4,5]), approximately half of the patients included in the mITT analysis were hypertensive, and this proportion was similar in the BIO101 and the placebo groups (Table 1). Based on the MasR agonist activity of BIO101, future post-hoc analysis to determine if the effect of BIO101 is different in hypertensive and normotensive patients or in ACE inhibitors or ARBs users vs. nonusers needs to be performed to better understand the pathophysiology of the disease and the role of RAS in COVID-19.

In addition to the complexity of the RAS, differences in protocol designs between the COVA trial and the

Fig. 2: A) Kaplan–Meier curve of time to negative event in the mITT analysis set (adjusted difference 10.9%, 95% CI [0.6%, 21.2%]). Hazard Ratio (95% CI): 0.489 (0.265, 0.904)/Two-sided p-value Log-rank p = 0.0223. Note: negative events: death or respiratory failure including mechanical ventilation, ECMO and high-flow oxygen (HFO2). HFO2 was considered respiratory failure and negative event only for subjects who were enrolled under protocol version 1–11. Only the first occurrence of any event up to Day 28 was considered for negative event. B) Kaplan–Meier curve of time to death through day 90. Hazard ratio was 0.554 (95% CI: 0.285, 1.077), a 44.6% reduction in the death rate over 90 days in the BIO101 group. The difference in the proportions of deaths at 90 days was 0.093 (95% confidence interval (−0.010, 0.195)) representing a 42.9% reduction in the risk of death in the BIO101 group compared to the placebo but this difference was not statistically significant. C) Kaplan–Meier curve of time to respiratory failure. Hazard ratio for time to respiratory failure through day 90 was (HR: 0.530 (95% CI: 0.279, 1.006) a non-statistically significant difference. Note: respiratory failure including mechanical ventilation, ECMO and high-flow oxygen (HFO2). HFO2 for subjects who were enrolled under protocol version 1–11. Only the first occurrence of any event up to Day 28 was considered.

Number Of Subjects With	BIO101 350 mg BID (N = 128) n (%) [E]	Placebo (N = 104) n (%) [E]	Total (N = 232) n (%) [E]
Intervention period: on-intervention			
Any TEAE	73 (57.0) [216]	67 (64.4) [193]	140 (60.3) [409]
Any serious TEAE	32 (25.0) [59]	32 (30.8) [64]	64 (27.6) [123]
Any non-serious TEAE	57 (44.5) [157]	53 (51.0) [129]	110 (47.4) [286]
Any Grade 1 TEAE	44 (34.4) [86]	32 (30.8) [54]	76 (32.8) [140]
Any Grade 2 TEAE	31 (24.2) [63]	36 (34.6) [63]	67 (28.9) [126]
Any Grade 3 TEAE	26 (20.3) [41]	26 (25.0) [41]	52 (22.4) [82]
Any Grade 4 TEAE	8 (6.3) [12]	11 (10.6) [20]	19 (8.2) [32]
Any Grade 3 or more TEAE	33 (25.8) [67]	33 (31.7) [88]	66 (28.4) [160]
Any fatal TEAE	14 (10.9) [14]	15 (14.4) [17]	29 (12.5) [31]
Any treatment related TEAE[a]	12 (9.4) [20]	14 (13.5) [17]	26 (11.2) [37]
Any serious treatment related TEAE[a]	0	3 (2.9) [3]	3 (1.3) [3]
Any TEAE for which the study or study drug was discontinued	18 (14.1) [21]	26 (25.0) [30]	44 (19.0) [51]
Any TEAE for which the study drug was temporarily interrupted	4 (3.1) [4]	3 (2.9) [6]	7 (3.0) [10]
Any TEAE of special interest[b]	9 (7.0) [11]	8 (7.7) [10]	17 (7.3) [21]
Respiratory, thoracic, and mediastinal disorders	29 (22.7) [48]	33 (31.7) [52]	62 (26.7) [100]
Respiratory failure	12 (9.4) [14]	19 (18.3) [21]	31 (13.4) [35]
Metabolism and nutrition disorders	20 (15.6) [3]	19 (18.3) [25]	39 (16.8) [62]
Hyperkalaemia	7 (5.5) [8]	9 (8.7) [9]	16 (6.9) [17]
Hypokalaemia	7 (5.5) [10]	1 (1.0) [1]	8 (3.4) [11]
Vascular disorders	18 (14.1) [23]	12 (11.5) [15]	30 (12.9) [38]
Orthostatic hypotension	9 (7.0) [11]	8 (7.7) [10]	17 (7.3) [21]
Gastrointestinal disorders	16 (12.5) [20]	21 (20.2) [24]	37 (15.9) [44]
Constipation	10 (7.8) [10]	16 (15.4) [16]	26 (11.2) [26]

Note: At each level of subject summarization, a subject was counted once if the subject reported one or more events. n represents the number of subjects at each level of summarization. [E] represents the number of events at each level of summarization. AEs were coded using MedDRA, Version 25.0. Grades are NCI-CTCAE toxicity grades. TEAEs were defined as AEs starting on or after first administration of any study drug. [a]Treatment-related AEs included AEs with probable/likely, certain, conditional/unclassified, un-assessable/unclassifiable, or missing relationship to study drug. [b]Adverse events of special interest included orthostatic hypotension.

Table 3: **Adverse events overview.**

study by Self and colleagues[23] could also explain these contradictory results. For instance, the primary outcomes evaluated by Self and colleagues: oxygen-free days differed from the proportion of patients with respiratory failure or death which was evaluated in the present study. Moreover, they didn't evaluate mortality further than 28 days. In addition, the limited drug exposure (5 days maximum of treatment) in their study compared to the longer treatment period with BIO101 (mean number of days on treatment of 8.9 days in the BIO101 group) spanning from 1 to 28 days maximum, appear as a further plausible cause for the different clinical results reported.

It is important to note that some limitations of the present COVA study are due to the fact the trial was designed and launched very early on during the COVID-19 pandemic. Indeed, at the time in March 2020, no strain other than the initial SARS-CoV-2 variant was known and it was difficult to anticipate the evolutions of the variants in relationship with severity of the disease and this explain why this information was not collected. However, due to BIO101's mode of action through activation of the MasR, it is anticipated that viral strains would not influence BIO101's effects. Similarly, early in 2020 the commercialization of a vaccine was highly hypothetical, therefore the vaccine status of the patients included in the COVA study was not collected either. Moreover, during the inclusion period the standard of care evolved as well with the use of hydroxychloroquine being replaced mostly with remdesivir and the use of corticosteroids (particularly dexamethasone). These improvements in patients' management might explain why we observed a substantially lower rate of respiratory failure or death in the placebo group (26.0%) than expected (40%). Other factors may also have had an impact on the results of our study, for instance the early termination of the COVA trial after recruitment of 238 instead of the initially planned 310 subjects: but also the higher proportion of patients using immunosuppressants in the placebo group, and the higher proportion of males in the BIO101 group (male gender being a risk factor for severe COVID-19[2,33]). All those factors may also explain the absence of statistically significant effects of BIO101 on some secondary endpoints such as the proportion of patients discharged from hospital or death at Day 28, despite numerical difference in favour of BIO101 treatment.

In conclusion, the COVA trial results demonstrated statistically significant efficacy of BIO101 on the primary endpoint (43.8% RR reduction of proportion of death or respiratory failure, p = 0.0426), with number needed to treat of 9, and confirm the good safety profile of BIO101. These results support the clinical relevance of RAS modulation through activation of the MASR for treating COVID-19 and support further investigation on the use of BIO101 as treatment for patients hospitalized with severe pneumonia due to COVID-19.[30]

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Serge Camelo, Rob van Maanen, and Richard Kay wrote the initial manuscript. Serge Camelo, Rob van Maanen, Waly Dioh, Capucine Morelot, Suzanna Lobo, Gaetand Plantefeve, Richard Kay and Stanislas Veillet, were responsible for the decision to submit the manuscript for publication.

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All authors reviewed the manuscript and approved the decision to submit for publication.

Data sharing statement

Data are the property of Biophytis. However, the Company is fully committed to providing qualified scientific researchers access to anonymized patient data for product and indication that have been approved by regulators in the US and EU. Requests for access to the data can be submitted to Biophytis via email to rob.vanmaanen@biophytis.com. Data requestors will need to sign a data access agreement.

Declaration of interests

Biophytis declares that potential commercial interests had no impact on the scientific conduct of the study nor on the analysis/interpretation of data. Cendrine Tourette, Luis Esmeraldino, Pierre Jean Dilda, René Lafont, Serge Camelo, Sandrine Rabut, Waly Dioh, Rob van Maanen and Stanislas veillet are Biophytis company employees, Anait Azbekyan Samuel Agus and Mounia Chabane are former Biophytis company employees. Jean Mariani is emeritus professor at Sorbonne University and consultant for Biophytis, Richard Kay is a consultant for Biophytis.

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Fabiano Ramos, MD declares that its institution: Hospital São Lucas da Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Porto Alegre, Rio Grande do Sul, Brazil received financial resources to conduct the study from Biophytis.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2023.102383.

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