Efficacy and safety of zapnometinib in hospitalised adult patients with COVID-19 (RESPIRE): a randomised, double-blind, placebo-controlled, multicentre, proof-of-concept, phase 2 trial

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Summary

Background Zapnometinib is an oral, non-ATP-competitive, small-molecule inhibitor of MEK1/MEK2 with immunomodulatory and antiviral properties. We aimed to investigate the safety and efficacy of zapnometinib in patients with COVID-19.

Methods In this randomised, double-blind, placebo-controlled, multicentre, proof-of-concept, phase 2 trial, we recruited hospitalised adults with moderate or severe COVID-19 from 18 hospitals in Germany, India, Romania, South Africa, and Spain. Those requiring ICU admission or ventilator support at screening or randomisation were excluded. Patients were randomly assigned (1:1) to receive oral zapnometinib (900 mg on Day 1; 600 mg on Days 2–6) or matching placebo, on top of standard of care. Randomisation, stratified by baseline clinical severity status (CSS 3 or 4, measured on a 7-point ordinal scale), was done using Interactive Response Technology. Patients, investigators, and the sponsor were masked to treatment allocation. The primary endpoint was CSS at Day 15 and was conducted on the full analysis set (FAS: all patients who were randomised to the study, received at least one dose of study medication and had at least one post-dose assessment of CSS, as randomised). Safety analyses were conducted on the safety analysis set (all study participants who received at least one dose of study medication, as treated). This study is registered at ClinicalTrials.gov (NCT04776044) and EudraCT (2020-004206-59).

Findings The trial was terminated early as the emergence of the Omicron variant impacted recruitment. Between 12th April 2021 and 9th August 2022, 104 of the planned 220 patients were enrolled and randomly assigned, 103 were treated, and 101 were included in the FAS (zapnometinib: n = 50; placebo: n = 51). The primary outcome was not significantly different between the two groups, but patients on zapnometinib had higher odds of improved CSS versus placebo (odds ratio [OR] 1.54 [95% CI 0.72–3.33]; p = 0.26). Predefined subgroup analyses identified trends for improved CSS in patients with severe disease at baseline (OR 2.57 [0.76–8.88]; p = 0.13) and non-Omicron variants (OR 2.36 [0.85–6.71]; p = 0.10); the p value of the CSS subgroup by Treatment interaction term in the model was p = 0.28. The frequency and intensity of adverse events was low and similar between arms. Twenty (39.2%) patients treated with zapnometinib experienced adverse events compared with eighteen (34.6%)

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patients treated with placebo. One patient receiving zapnometinib and two patients receiving placebo died during the study. None of the deaths were considered related to study medication.

Interpretation These results provide proof-of-concept for the innovative approach of targeting the Raf/MEK/ERK pathway in patients with hospitalised moderate/severe COVID-19. Further clinical studies will be required to evaluate the clinical benefit of zapnometinib in this and other indications.

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Keywords: COVID-19; Anti-viral; Immunomodulator

Research in context

Evidence before this study

We searched PubMed up to March 2022, for English-language articles on phase 2 randomised clinical trials and meta-analyses regarding the use of MEK inhibitors in the treatment of moderate/severe COVID-19. We searched using the terms "COVID-19", "SARS-CoV-2 infection", "MEK inhibitors", "trametinib", "cobimetinib", "selumetinib", "phase 2", "randomised". This search revealed no evidence for the use of zapnometinib or other MEK inhibitors in the treatment of moderate/severe COVID-19.

Added value of this study

Data obtained from the RESPIRE study indicate the potential of the oral MEK-inhibitor zapnometinib as a safe short-term treatment option for hospitalised patients with moderate/severe COVID-19. Data indicate that patients treated with zapnometinib have a higher probability (odds ratio) of improved clinical severity status (CSS) than patients treated with placebo. The safety profile of zapnometinib was found to be similar to placebo. These data must be put in the context of the study being terminated early and therefore underpowered due to the emergence of the omicron variant of COVID-19 leading to a significant reduction in the number of COVID-19 patients being hospitalized. While several agents have demonstrated clinical value in patients with moderate/severe COVID-19, and are now included in treatment guidelines, therapeutic options are still limited. The principle of inhibiting the Raf/MEK/ERK pathway with non-ATP-competitive drugs like zapnometinib offers the potential to both inhibit viral replication and prevent a hyperinflammatory immune response disease pathway. The dual effect of zapnometinib is a promising and innovative therapeutic principle to be evaluated in future clinical trials in severe respiratory disease, including seasonal influenza.

Implications of all the available evidence

With the availability of effective vaccines for COVID-19 and the increasing prevalence of variants of lower pathogenicity such as Omicron, the numbers of patients with COVID-19 requiring hospitalisation are currently considerably lower than was seen at the height of the pandemic. With this positive outlook, it is important not to lose sight of the fact that a considerable number of COVID-19 patients still end up requiring hospital treatment. It also remains possible that SARS-CoV-2 variants of concern with increased pathogenicity and immune signatures conferring resistance to vaccines may yet emerge. Zapnometinib with its mechanism of action agnostic to variant type therefore remains relevant in the development of the armamentarium for the treatment of COVID-19 and is therefore a strong candidate for further clinical development.

Since MEK inhibition has the potential to target multiple RNA virus types beyond SARS-CoV-2 e.g., influenza and respiratory syncytial virus (RSV) this further underlines zapnometinib as being a potential candidate for development as a broad-spectrum therapeutic to treat patients with moderate/severe viral respiratory infections.

Introduction

The rapid emergence of SARS-CoV-2 and global spread of COVID-19 highlighted the need for broad-acting therapeutics that are effective against severe viral diseases. At the onset of the COVID-19 pandemic there were no effective treatments available. Initial therapeutic attempts involved repurposing existing agents such as antivirals (e.g., remdesivir) and immunomodulators (e.g., corticosteroids).[1–3] Over time, specific therapies targeting SARS-CoV-2 were developed and effective vaccination programmes introduced. The high levels of morbidity and mortality in the early days of the COVID-19 pandemic, coupled with the lack of treatment options, highlight the pressing need for broad-acting agents to treat moderate/severe viral respiratory infections.

Zapnometinib (ATR-002) is a highly specific small-molecule inhibitor of MEK1 and MEK2 (both protein kinases and members of the Ras/Raf/MEK/ERK signalling cascade). MEK inhibition by zapnometinib

offers a dual effect: immunomodulation and antiviral activity. Zapnometinib has been shown to abate the pro-inflammatory cytokine response to viral infection in vitro and in vivo, and thus may prevent a "cytokine storm", the hyperinflammatory immune response that can be triggered by particular viral infections.[4,5] In patients with COVID-19, the cytokine storm is associated with COVID-19 severity and has been described as a significant cause of COVID-19-related death.[6,7] This host-targeting effect may mitigate overactive inflammatory responses, such as those leading to acute respiratory distress syndrome in patients who are severely ill with COVID-19 or influenza.[4,6]

In addition to immunomodulation of the host response, zapnometinib has antiviral activity. Activation of the MEK pathway is essential for replication of many RNA viruses including influenza viruses, hantaviruses, respiratory syncytial virus (RSV), and coronaviruses.[8,9] Inhibition of the MEK pathway has been shown to reduce virus propagation in vitro and in vivo.[4,8,10,11] The antiviral activity of zapnometinib was primarily established in influenza virus infection models, where it demonstrated broad antiviral activity against different influenza virus strains, including strains resistant to other antivirals such as baloxavir marboxil.[11,12] Inhibition of MEK1/2 by zapnometinib or other MEK-inhibitors blocks the formation of functional influenza virus particles in the host cell, ultimately reducing the viral load in mice.[10,11] Zapnometinib has also been shown to inhibit MEK1/2 in SARS-CoV-2 infected cells and significantly reduce virus production.[4] To date, the antiviral activity of zapnometinib has been demonstrated in influenza virus, hantavirus, RSV, SARS-CoV-2 and other coronaviruses, Borna disease virus, dengue virus, and human metapneumovirus[4,10,11] (and Atriva Therapeutics unpublished data).

At the time of the onset of the COVID-19 pandemic, zapnometinib was under investigation as a novel treatment option for severe influenza. The immunomodulatory properties of zapnometinib and its broad-acting antiviral activity, combined with preclinical data showing that zapnometinib effectively inhibits SARS-CoV-2, suggested that zapnometinib may be an effective treatment for patients with COVID-19. Consequently, the RESPIRE trial was initiated to investigate the safety and efficacy of zapnometinib as a treatment for hospitalised patients with COVID-19. Here we report the findings of this proof-of-concept trial.

Materials and methods

Study design

RESPIRE was a randomised, double-blind, placebo-controlled, proof-of-concept, multi-centre, phase 2 clinical trial that assessed the safety and efficacy of zapnometinib in addition to standard of care for the treatment of adult patients hospitalised with COVID-19. It was conducted at 18 hospitals in Germany, India, Romania, South Africa, and Spain (17 hospitals provided randomised patients). The protocol was approved by the respective regulatory authorities and by the Ethics Committees concerned and is available online in the Supplementary Material. The trial was registered (ClinicalTrials.gov: NCT04776044/EudraCT: 2020-004206-59), was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice and adhered to the CONSORT reporting guidelines.

Patients

Patients aged ≥18 years with a laboratory-confirmed diagnosis of SARS-CoV-2 infection presenting as COVID-19 requiring hospitalisation were eligible. All patients had a clinical severity status (CSS) of either 3 (hospitalised, not requiring supplemental oxygen) or 4 (hospitalised, requiring supplemental oxygen). Patients with a rapidly worsening clinical condition, or those requiring ICU admission or ventilator support at screening or at randomisation, were excluded. Other exclusion criteria included suspected infection other than SARS-CoV-2; history of malignant disease, autoimmune disease, or severe liver, kidney, blood, cardiac, pulmonary, neurological, or endocrine disease; or uncontrolled hypertension. Full eligibility criteria are included in the protocol (see Supplementary Materials). All patients provided written informed consent prior to enrolment.

Randomisation and masking

Eligible patients were randomly assigned (1:1) to receive zapnometinib or placebo using interactive response technology (IRT). The randomisation list and allocation list were provided by a statistician not involved in the analyses. Patients, investigators, the study team, and the Sponsor remained blinded to treatment allocation throughout the study. Only the statistician and laboratory conducting pharmacokinetic analyses had access to allocation information. Randomisation was stratified by trial site and by CSS at baseline (3 or 4) within trial sites.

Procedures

Patients received 900 mg (6 tablets) of zapnometinib orally on Day 1 followed by 600 mg (4 tablets) once daily on Days 2–6, or matching placebo tablets (matched for size, colour, and general appearance). No dose modifications were permitted. All other care and medication of the patients was at the investigator's discretion as per local standards of care, and could include remdesivir, dexamethasone, and other therapies as deemed appropriate (including approved treatments and those used off-label). Certain medications known to be metabolised by CYP2C8 and CYP2C9 were prohibited, based on in vitro data showing that zapnometinib inhibits these enzymes at clinically relevant levels of exposure (Atriva Therapeutics, unpublished data on file).

Patients were assessed up to Day 15 and again on Days 21, 30, and 90 for CSS, adverse events (AEs), and concomitant medication use. Patient-reported degree of dyspnoea and vital signs (including Glasgow Coma Scale for level of consciousness) were evaluated daily on Days 1–15, 21, and 30, and laboratory values on Days 1, 3, 5, 8, 11, 15, and 30. Vital signs were evaluated three times daily on Days 1–7. Assessments on Days 9, 10, and 12–14 were only required if the patient remained hospitalised.

CSS was determined using a 7-point ordinal scale (based on the 8-point ordinal scale recommended by the World Health Organization [WHO] for measuring illness severity over time in patients with COVID-19).[13] Severity scores were determined at each time point and ranged from [1] (not hospitalised, no limitations of activities) to [7] (death). Patient-reported degree of dyspnoea was measured on a 7-point Likert scale (grading change in current breathing from baseline ranging from markedly worse to markedly better) and a visual analogue scale (from 0 = no shortness of breath at all to 100 = maximum shortness of breath).

Imaging data results, laboratory data, and use of standard of care treatment (including the need for supplemental oxygen, ventilator support [invasive, non-invasive, high flow], renal replacement therapy [RRT], vasopressor treatment, and use of extracorporeal membrane oxygenation [ECMO]) were also recorded throughout the study period. Nasopharyngeal swabs and sputum, and blood and plasma samples, were collected for exploratory analysis of SARS-CoV-2 viral load and biomarkers of immune response, respectively. These data will be reported separately.

Outcomes

The primary objective of the trial was to demonstrate the efficacy of zapnometinib versus placebo, on top of standard of care, based on the CSS at Day 15 (primary endpoint). The key secondary endpoint was the time from randomisation to discharge from hospital. Other secondary endpoints comprised the time to discharge from hospital or to score of ≤2 maintained for 24 h in National Early Warning Score 2 (NEWS2), whichever occurred first; time to resolution of fever [defined as ≤36.6 °C (axilla), ≤37.2 °C (oral) or ≤37.8 °C (rectal or tympanic) for at least 24 h without antipyretics for 24 h]; time to SpO2 >94% on room air maintained for 24 h; CSS over the hospital period calculated as the area under the curve from the 7-point ordinal scale at Days 3, 5, 8, 11, 15, and 30, survival time up to Day 30, and time to event/AUC analyses of the various endpoints (see Supplementary Materials).

Statistical analyses

Sample size estimation

The sample size estimation was based on the primary endpoint (CSS at Day 15). The distribution of CSS across the 7 categories in the placebo arm at Day 15 was derived from publications available at the time of study planning. Day 15 was chosen because it was assumed that the distinction between less and more severe courses of COVID-19 would have occurred for most patients at this day. To discover a treatment effect on the 7-point CSS scale quantified by an odds ratio (OR) of 2.18 with 85% statistical power using ordinal logistic regression analysis, 99 evaluable patients were required for each treatment arm (alpha = 0.05, two-sided). The assumed OR of 2.18 for sample size estimation was accepted as a clinically relevant effect size in pre-trial discussions with regulatory bodies and ethics committees.

Analysis sets

The statistical analyses were conducted according to a modified intent-to-treat principle. The Safety Analysis Set (SAS) was used for all safety analyses and comprised all randomised patients who received at least one dose of investigational medicinal product (IMP); all safety analyses were conducted as treated. The Full Analysis Set (FAS) was used for efficacy analyses and included all patients from the SAS who had at least one post-baseline assessment of CSS (the primary endpoint). The Per Protocol Set (PPS) comprised all patients of the FAS who followed the study protocol without major protocol violations. All efficacy analyses were conducted as randomised; some analyses of the primary endpoint were additionally conducted as treated.

The study used stratified randomisation by baseline CSS and site. According to EMA Guidelines on investigation of subgroups in confirmatory clinical trials (Final Version 2019)[14] subgroup analyses by baseline CSS 3 and 4 for the primary and key secondary endpoints were conducted.

Statistical analysis of the primary endpoint

The null hypothesis of no shift across the seven ordered categories of the CSS scale at Day 15 when comparing the two treatment groups was tested using logistic regression analysis for ordered categorical data. A proportional odds model with cumulative logit link was used. The CSS at baseline (3 or 4, categorical) and the 4C Mortality Score for COVID-19[15] were included into the model as covariates. For subgroup analyses, this model was extended with the subgroup terms (if not already included) and a subgroup by treatment interaction term. The treatment effect was estimated as the (proportional) OR of zapnometinib versus placebo with a two-sided 95% profile likelihood confidence interval (CI). The validity of the proportional odds assumption was investigated and confirmed for all primary and key-secondary FAS based and subgroup analyses at study Day 15; violations of the proportional odds assumption were found for the Omicron subgroup, CSS analysis and for a worst CSS subgroup analysis. Baseline CSS 3

and CSS 4 as well as virus variant were pre-planned for subgroup analyses; baseline CSS was considered as mandatory because it was implemented in stratified randomisation. Results of overall and subgroup analyses were presented in addition as forest plots.

Statistical analysis of the key-secondary endpoint

The time to discharge from hospital was analysed using the Cox proportional hazards model including treatment and clinical severity status at baseline as covariates and 4C Mortality Score as stratum instead of covariate because of questionable proportional hazard assumption. The model provided hazard ratios with 95% profile likelihood CIs. As a hazard ratio of >1 is beneficial for time to discharge, the term rate ratio was used instead of hazard ratio. Subgroup analyses were planned and conducted as for the primary endpoint.

Handling of missing values

Missing CSS values were primarily imputed by logical rules such as last observation carried forward for visits missed as result of premature discontinuation if the last known CSS value of the patient was 1 (i.e., not hospitalised, no limitations of activities). If a missing CSS value could not be replaced by one of these logical rules, imputation was done using an ordinal generalised linear mixed model with treatment group, visit, the treatment-by-visit interaction, baseline clinical severity status, and 4C Mortality Score for COVID-19 as fixed effects, and with study participant as random effect (longitudinal modelling).

Deaths and other competing events in Cox Proportional hazards modelling were taken into account by censoring at the end of the observation period (i.e., study Day 30).

Missing covariate values were imputed based on prediction from correlated variables using linear regression.

General

Statistical tests of the endpoints were planned to be done two-sided on an alpha level of 0.05; as the planned sample size was not reached, p values were interpreted in the sense of exploratory data analyses. All statistical analyses were conducted using SAS version 9.4 (SAS Institute Inc).

Role of the funding source

The Sponsor/funders (Atriva Therapeutics) were involved in the trial design, trial management, data collection, data analysis, data interpretation, and report writing. The Federal Ministry of Education and Research, Germany (Bundesministerium für Bildung und Forschung; BMBF) had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data and all authors approved the final manuscript for submission.

Results

Recruitment to RESPIRE began in April 2021 and it was planned to randomise 220 patients. In February 2022 it was observed that enrolment into the trial had decelerated. This was due to recruitment challenges arising from the lower frequency of hospitalisations for COVID-19 as a result of the propagation of vaccination programmes and the increasing prevalence of the less severe Omicron variant.[16,17] The Sponsor decided to conduct an unplanned interim analysis which occurred in May 2022. At this analysis, the independent data monitoring committee (iDMC) reviewed unblinded data from 104 recruited patients. Following the meeting, the iDMC recommended that the trial should continue as designed; however, the Sponsor decided to terminate the trial early, while blinded to the study findings, due to the unfavourable recruitment outlook. Early termination of the trial reduced the planned statistical power considerably; therefore, statistically significant results could not be expected, however clinical relevance of estimated trends in favour of zapnometinib was nevertheless observed.

As of the date of the database lock following study termination (12 July 2022), 104 patients had been enrolled and randomised. Of these, 103 patients had been treated and comprised the SAS (zapnometinib [n = 51]; placebo [n = 52]; Fig. 1). The FAS included 101 patients (zapnometinib [n = 50]; placebo [n = 51]). Two treated patients were excluded from the FAS: one patient from the zapnometinib arm had no post-baseline value of CSS recorded, and one patient from the placebo arm was excluded due to major protocol deviation. Based on pharmacokinetic data, it was detected that four patients received the wrong treatment due to a labelling error (two patients planned to receive placebo were treated with zapnometinib and two patients planned to receive zapnometinib were treated with placebo). To investigate the impact of this error on the primary endpoint an "as treated" analysis was conducted with the full analysis set.

The median age of the patients was 56.0 years (IQR 43.0–69.0 years; Table 1). There was a slight difference in the distribution of sex between arms; a higher proportion of patients in the zapnometinib arm were male, whereas the placebo arm contained an equal ratio of men to women. Baseline CSS was well balanced between arms: 40.0% of patients had a CSS score of 4 (hospitalised, requiring supplemental oxygen) in the zapnometinib arm and 41.2% of patients in the placebo arm. The distribution of Omicron versus non-Omicron variants was also balanced between arms. None of the patients were vaccinated against SARS-CoV-2 before or during their participation in the study. There were no

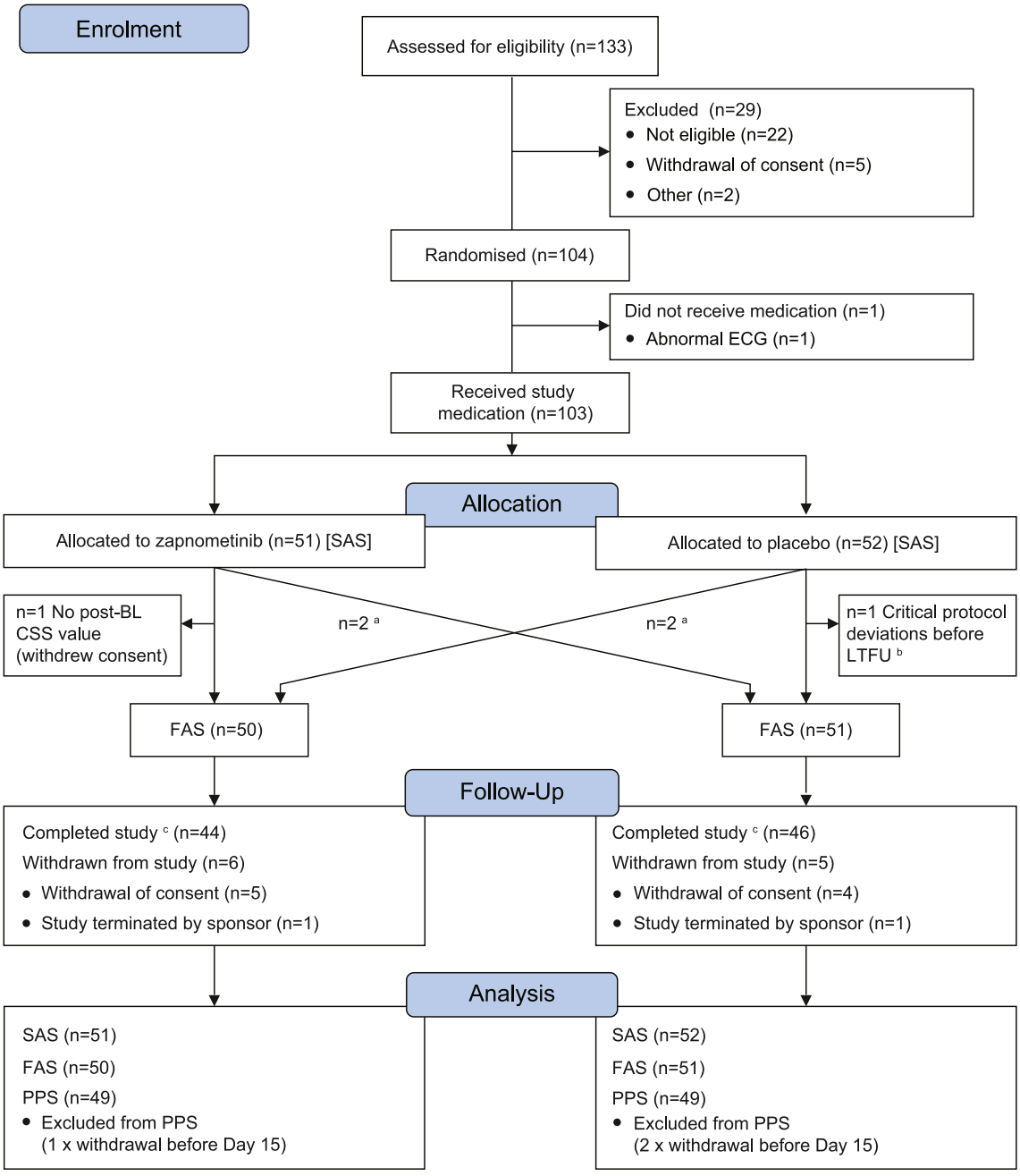

Fig. 1: CONSORT flow diagram. a Two patients randomised to the zapnometinib arm received placebo, two patients randomised to the placebo arm received zapnometinib. The assignment of these patients to treatment groups depends on the analysis sets, namely, as treated for the SAS but as randomised for the FAS. b Patient excluded from FAS as the PCR test for confirmation of COVID-19 infection was not done. c Includes patients who died during the trial (zapnometinib arm: n = 1; placebo arm: n = 2). BL, baseline; FAS, full analysis set; LTFU, lost to follow-up; PK, pharmacokinetic; PPS, per protocol set; SAS, safety analysis set.

notable differences between treatment arms in medical history, concomitant diseases, or prior medication use (Table 1).

Treatment adherence was high in both arms: The mean proportion of tablets taken was 97.8% in the zapnometinib arm and 97.9% in the placebo arm. Mean duration of treatment was 5.8 (±0.7) and 5.7 (±1.0) days, respectively. Fourteen patients discontinued the trial early (seven in each arm), mainly due to withdrawal of consent (Fig. 1).

	Zapnometinib arm (n = 50)	Placebo arm (n = 51)	Total (n = 101)
Sex, n (%)			
Female	17 (34.0)	26 (51.0)	43 (42.6)
Male	33 (66.0)	25 (49.0)	58 (57.4)
Age, years			
Mean (SD)	54.1 (18.6)	56.8 (15.6)	55.4 (17.1)
Median (IQR)	54.5 (38.0–70.0)	57.0 (44.0–68.0)	56.0 (43.0–69.0)
Race, n (%)			
Asian	21 (42.0)	22 (43.1)	43 (42.6)
Black/African American	3 (6.0)	2 (3.9)	5 (5.0)
White	26 (52.0)	27 (52.9)	53 (52.5)
CSS, n (%)			
3 (hospitalised, not requiring supplemental oxygen)	30 (60.0)	30 (58.8)	60 (59.4)
4 (hospitalised, requiring supplemental oxygen)	20 (40.0)	21 (41.2)	41 (40.6)
SARS-CoV-2 variant			
Non-Omicron	27 (54.0)	29 (56.9)	56 (55.4)
Omicron	23 (46.0)	22 (43.1)	45 (44.6)
Median time since hospitalisation, days (IQR)	2.0 (2.0–3.0)	2.0 (2.0–3.0)	2.0 (2.0–3.0)
Median time since onset of symptoms, days (IQR)	7.0 (4.0–10.0)	7.0 (5.0–10.0)	7.0 (5.0–10.0)
COVID-19 symptoms, n (%)			
Cough	46 (92.0)	48 (94.1)	94 (93.1)
Dyspnoea	28 (56.0)	33 (64.7)	61 (60.4)
Fever	41 (82.0)	36 (70.6)	77 (76.2)
4C Mortality score			
Median (IQR)	5.0 (2.0–8.0)	5.0 (2.0–8.0)	5.0 (2.0–8.0)
<9 points, n (%)	41 (82.0)	40 (78.4)	81 (80.2)
≥9 points, n (%)	9 (18.0)	11 (21.6)	20 (19.8)

Note: the majority of patients received systemic steroids as local SOC and per investigator discretion (not prespecified in study protocol). CSS, clinical severity status; FAS, full analysis set; IQR, interquartile range; SD, standard deviation.

Table 1: **Baseline demographics and disease characteristics (FAS).**

On Day 15, the OR for an improved CSS score with zapnometinib versus placebo (primary endpoint) was 1.54 (95% CI: 0.72–3.33; p = 0.26; Fig. 2) in the pre-planned analysis, as randomised. In the FAS-based as-treated analysis, the estimated OR was 1.76 (95% CI 0.82–3.81, p = 0.15). Data were similar in the PPS as randomised (OR 1.45 [95% CI 0.67–3.17]; p = 0.35). In a pre-defined subgroup analysis, a trend for improvement in CSS with zapnometinib versus placebo was observed for patients with more severe disease (CSS 4) at baseline (OR 2.57 [95% CI 0.76–8.88]; p = 0.13). The p value of the CSS subgroup by Treatment interaction term in the model was p = 0.28. For the as-treated analysis, ORs of 1.37 (95% CI 0.51–3.70; p = 0.53) and 2.56 (95% CI 0.76–8.87; p = 0.13) were estimated for the patients with CSS 3 or CSS 4 at baseline, respectively.

There was also a trend for higher efficacy of zapnometinib in patients with non-Omicron variants compared to Omicron SARS-CoV-2 (OR 2.36 [95% CI 0.85–6.71]; p = 0.10). The p value of the interaction term was p = 0.25.

For the key secondary endpoint (the time from randomisation to hospital discharge), a trend to greater reduction was observed with zapnometinib versus placebo among patients with a CSS of 4 at baseline. The median time to discharge from hospital was 8.5 days (95% CI: 7.0–12.0) for patients treated with zapnometinib compared with 10.0 days (95% CI: 8.0–15.0) for patients treated with placebo (rate ratio 1.59 [95% CI 0.73–3.57]; p = 0.25; Fig. 3), equivalent to ~1.5 days shorter. In the overall population, the rate ratio was only moderately different between arms (1.31 [95% CI: 0.81–2.13]; p = 0.27; Fig. 3). The p value of the CSS subgroup by Treatment interaction term was p = 0.53.

The results of other secondary endpoints evaluated in this study generally demonstrated benefits of zapnometinib over placebo. These findings also supported the observation of a greater clinical benefit being observed in patients with more severe disease (CSS 4), consistent with the subgroup analyses conducted on the primary endpoint discussed above (Supplementary Tables S1–S13).

There was no apparent difference in the use of concomitant medications between study arms. Systemic corticosteroids were used by 29 (56.9%) and 32 (61.5%) patients in the zapnometinib arm and placebo arm,

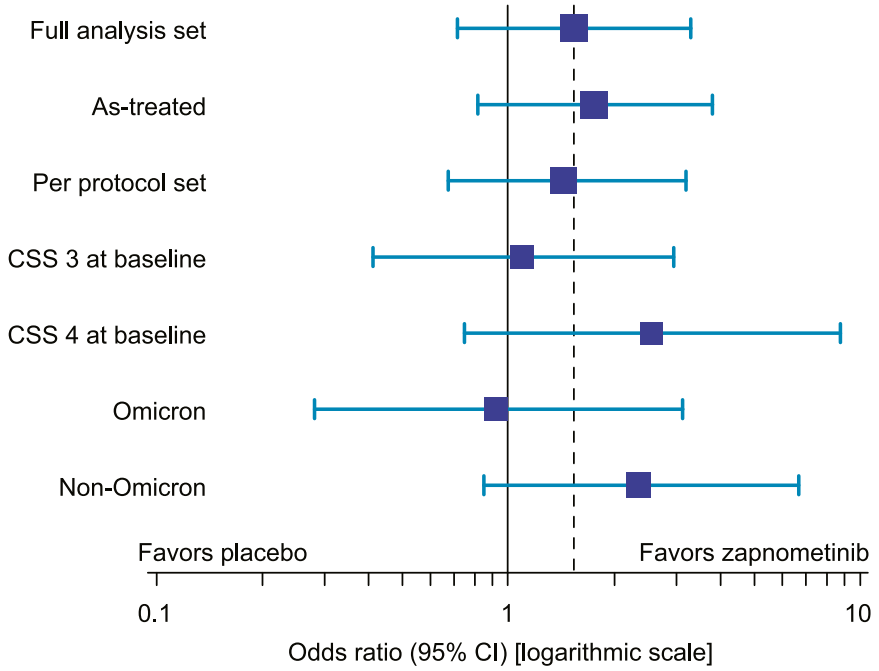

Fig. 2: Forest plot of odds ratios for clinical severity status at Day 15, overall and in subgroups (FAS). CI, confidence interval; CSS, clinical severity status; PPS, per protocol set.

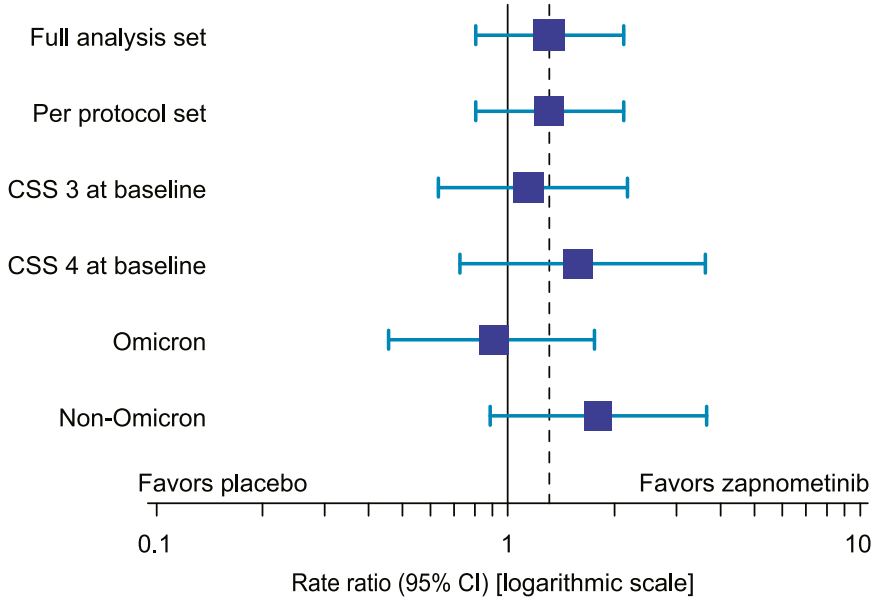

Fig. 3: Forest plot of rate ratios for time to hospital discharge, overall and in subgroups (FAS). CI, confidence interval; CSS, clinical severity status; PPS, per protocol set.

respectively, and direct acting antivirals by 26 (51.0%) and 23 (44.2%), respectively. Overall, 26/49 (53.1%) evaluable patients in the zapnometinib arm and 25/51 (49.0%) patients in the placebo arm were treated with dexamethasone, remdesivir, baricitinib or tocilizumab during the study.

Protocol deviations

Major protocol deviations were identified in three (5.9%) of patients treated with zapnometinib and six (11.3%) of patients treated with placebo. The majority of these deviations reflected enrolment of patients who did not satisfy the study inclusion/exclusion criteria. Patients with major protocol deviations were excluded from the per protocol population.

Safety and tolerability

The frequency of AEs was low and similar between arms (Table 2). Increased alanine aminotransferase and diarrhoea were the most common treatment-emergent AEs (TEAEs), albeit at low frequencies in both arms. Overall, no apparent effect on parameters of liver function was seen in either arm. Most TEAEs were mild or moderate in intensity; 7/103 (6.8%) patients experienced a severe event, more frequently in the placebo arm (5/52 [9.6%] versus 2/51 [3.9%] in the zapnometinib arm). The only severe TEAE to occur in more than 5% of patients in either arm was severe dyspnoea (reported only in the placebo arm [3/52 [5.8%]). No serious TEAEs were reported in more than 5% of patients in either arm. Three patients died during the trial (two in the placebo arm [one due to cardiorespiratory arrest and one due to acute respiratory failure] and one in the zapnometinib arm [due to respiratory arrest]). All deaths occurred before Day 30, and all were judged by the investigators to be unrelated to study drug.

Discussion

The data presented here suggest the potential clinical benefit of zapnometinib as a treatment for COVID-19, particularly in hospitalised patients requiring supplemental oxygen. A clinically relevant trend for improvement was observed for the primary endpoint of CSS at Day 15. In patients with more severe disease activity, an OR exceeding 2 was observed. The effect on the key secondary endpoint was also stronger in patients with more severe disease at baseline. Consistent with this, zapnometinib was shown to be more efficacious in patients infected with non-Omicron variants of SARS-CoV-2, which typically lead to more severe disease compared to the Omicron variant.[18] While these treatment differences were not statistically significant, most likely due to the small sample size caused by the early termination of the trial, they do indicate a possible clinical benefit of zapnometinib. Also, the p values of the interaction terms of the three models discussed above were all $p > 0.2$ which was expected because of the small sample sizes of the subgroups: baseline CSS 3 and CSS 4. Our conclusion of a better zapnometinib treatment effect in more severe patients is mainly based on the size of the effect estimates in the CSS 4 subgroup of

n, %	Zapnometinib arm (n = 51)	Placebo arm (n = 52)	Total (n = 103)
Any TEAEs	20 (39.2)	18 (34.6)	38 (36.9)
TEAEs occurring in >5% of either arm			
ALT increased	3 (5.9)	1 (1.9)	4 (3.9)
Diarrhoea	4 (7.8)	3 (5.8)	7 (6.8)
Dyspnoea	1 (2.0)	3 (5.8)	4 (3.9)
Cough	0	3 (5.8)	3 (2.9)
Headache	0	3 (5.8)	3 (2.9)
Any severe TEAE	2 (3.9)	5 (9.6)	7 (6.8)
Severe TEAEs occurring in >5% of either arm			
Dyspnoea	0	3 (5.8)	3 (2.9)
Any serious TEAE	3 (5.9)	4 (7.7)	7 (6.8)
Serious TEAEs occurring in >5% of either arm			
None	0	0	0
Any TEAE leading to discontinuation of IMP	1 (2.0)	0	1 (1.0)
Any TEAE leading to withdrawal from trial	1 (2.0)	2 (3.8)	3 (2.9)
Any ADR	11 (21.6)	8 (15.4)	19 (18.4)
ADRs occurring in >5% of either am			
ALT increased	3 (5.9)	1 (1.9)	4 (3.9)
Diarrhoea	3 (5.9)	1 (1.9)	4 (3.9)
Any severe ADR	1 (2.0)	0	1 (1.0)
Any serious ADR	2 (3.9)	0	2 (1.9)
Death	1 (2.0)	2 (3.8)	3 (2.9)

ADR, adverse drug reaction (an AE judged to be at least possibly related to zapnometinib or placebo); ALT, alanine aminotransferase; SAS, safety analysis set; (TE)AE, (treatment-emergent) adverse event.

Table 2: **Summary of safety data (SAS).**

the primary and key secondary analyses and their increase compared to the CSS 3 subgroup. These are the first clinical data for zapnometinib as treatment for patients with any type of viral infection.

The RESPIRE study was well designed to demonstrate proof-of-concept of zapnometinib treatment. The study primary endpoint and key secondary endpoint are consistent with those used in other trials of treatments for severe COVID-19.[3,19] The primary endpoint was based on the 8-point ordinal scale recommended by the WHO and widely used in COVID-19 trials.[13] In the RESPIRE trial, two stages on this ordinal scale were combined ("Intubation and mechanical ventilation" [6 points] and "Ventilation + additional organ support – pressors, RRT, ECMO" [7 points]) since patients with rapidly worsening clinical condition, or those requiring ICU admission or ventilator support at screening or at randomisation, were excluded and these stages were only necessary to document deterioration.

Zapnometinib had a favourable safety profile that was like that seen in the placebo arm. The overall incidence of TEAEs was low and the events that were reported were predominantly mild/moderate and non-serious in nature. We did not observe any significant levels of class effects associated with MEK inhibition (such as rash, inflammatory effects cardiovascular complications, and ocular effects[20–23]). This likely reflects the short duration of treatment in the RESPIRE trial, as these MEK class effects are typically observed with long-term use in oncology indications.

Zapnometinib acts by modulating the host immune response, in addition to its antiviral properties. Therefore, the study protocol aimed to enrol patients that were pathogenetically at the transitional stage from declining viral replication to beginning immune derailment. The trial protocol did not mandate an exact day of symptom onset for patient enrolment; the population happened to be randomised in the second week post infection (approximately between days 8–14). The goal was to prevent further decline into a hyperinflammatory state of the immune system, translating into prevention of a worsening of clinical symptoms. The overall mortality in the study was low compared to other trials in hospitalised patients with COVID-19. This reflected the intent of the sponsor to focus on the above patient population, not on more severely affected patients, where the specific immunomodulatory intervention may have come too late to prevent the sepsis-like terminal disease pathway. In addition, to comply with expectations from regulatory authorities during the study protocol approval process, the Sponsor was required to exclude individuals with certain severe conditions from study participation, given pre-existing concerns on the safety profile of MEK-inhibitors used in other indications (e.g., oncology).

Analyses of biomarker data from RESPIRE are ongoing and include analyses of pharmacodynamic data collected throughout the trial. The assessment of immunological markers and viral load may shed light on the relative contribution of the dual benefit of MEK inhibition (immunomodulation and inhibition of viral replication) on the clinical efficacy of zapnometinib.

A limitation of the RESPIRE trial was the small sample size due to the early termination of the study: only 51 patients were treated with zapnometinib. Furthermore, the population of patients in which the largest signs of efficacy were observed (those with more severe disease [CSS 4] at baseline) comprised approximately 40% of the study population. Thus, it is tempting to speculate that the trial may have been successful in showing a statistically significant treatment effect with zapnometinib if more patients with severe disease had been recruited. The RESPIRE trial was designed at the time the Alpha variant of SARS-CoV-2 was dominant, but by the time the trial was terminated, Omicron was prevalent and effective global vaccination programs had reduced the proportion of patients hospitalised with more severe COVID-19.

Background COVID-19 therapy was not specified by the study protocol and was at the investigator's discretion as per local standards of care applicable at the time the study was running. This design decision was necessary to make the study acceptable from an ethical perspective, but did introduce some risk that differences in therapeutic approaches between sites and countries could have impacted on study results; the small sample size did not permit a detailed evaluation of this study design limitation.

A further limitation may be found in the statistical approach adopted in the time-to-event analyses; for such analyses, including the key-secondary endpoint time to hospital discharge, patients who died were censored at the end of the observation period. This is however considered to be a minor methodological limitation in view of only three deaths occurring in the study.

It is important to note that none of the patients in the RESPIRE study had received vaccination against COVID-19. At the time the RESPIRE study was in development multiple COVID-19 vaccines have either been approved or were under Regulatory review. Despite the effectiveness of vaccination and the increasing prevalence of variants of SARS-CoV-2 with lower pathogenicity, a substantial number of patients with COVID-19 continue to require hospital treatment. While the RESPIRE study did not evaluate the effectiveness of zapnometinib in patients who developed severe COVID-19 despite vaccination, its indirect mechanism of action would lead to the expectation that it would continue to be effective in such patients.

These results obtained support the further development of zapnometinib as an innovative approach for targeting the intracellular Raf/MEK/ERK signalling pathway in patients with hospitalised moderate/severe viral respiratory infections where the disease progress is driven by an inflammatory process. A phase 2 trial

(SURVIVE) is currently under development which will include patients with severe disease caused by influenza. Zapnometinib has several characteristics that favour its use as an agent of first-line defence in the face of future viral threats. The oral formulation is easy to administer. The production processes of the drug substance as well as of the oral dosage formulation (tablets) are straightforward and scalable. With its excellent stability properties for both the drug substance and the drug product, zapnometinib is particularly suitable for stockpiling. Importantly, as zapnometinib targets the host Raf/MEK/ERK pathway, there is a low risk of inducing viral resistance, thus overcoming a limitation of many direct-acting antiviral agents used in a variety of viral infections.[24–26] The activity of zapnometinib is unlikely to be affected by variant changes, which have resulted in regulatory authorities reversing their recommendations for most previously developed direct acting monoclonal antibodies against SARS-CoV-2.[27–29]

In conclusion, the results of the RESPIRE trial provide proof-of-concept for the innovative approach of targeting the intracellular Raf/MEK/ERK signalling pathway in patients with COVID-19. These results also suggest that zapnometinib may have a role in the treatment of future variants of COVID-19 with the potential to cause severe illness in humans and possibly other severe respiratory infections caused by RNA viruses. Further clinical studies will be required to confirm these results in COVID-19 and other serious viral infections.

Contributors

Authors G.R., S.S., M.B., W.K., D.N., and M.W. conceived the study and designed the study protocol. Authors G.R., H.P., G.M., O.S., A.T., and M.W. recruited and managed patients in the study and collected data. All authors analysed and interpreted the data. Authors G.R., S.S., M.B., T.O., W.K., and M.W. prepared the first draft of the manuscript (based on an outline drafted and agreed by all authors). O.P. and W.K. had full access to and verified all study data. All authors were given the opportunity to comment on the draft report and reviewed and approved the final version.

Data sharing statement

Requests for access to anonymised patient data from participants in this study can be submitted to the corresponding author. Reasonable requests for data will be considered from researchers conducting approved clinical studies providing that the provision of such data does not contravene any relevant patient confidentiality or data protection laws, or pose a commercial or legal conflict of interest.

Declaration of interests

G.R. reports personal fees from Astra Zeneca, Atriva Therapeutics, Boehringer Ingelheim, GSK, Insmed, MSD, Sanofi, Novartis, and Pfizer for consultancy during advisory board meetings, and personal fees from Astra Zeneca, Berlin Chemie, BMS, Boehringer Ingelheim, Chiesi, Essex Pharma, Grifols, GSK, Insmed, MSD, Roche, Sanofi, Solvay, Takeda, Novartis, Pfizer and Vertex for lectures including service on speakers' bureaux. S.S., M.B., and T.O. are employees of Atriva Therapeutics. H.P. and G.M. have nothing to declare. O.S. reports investigator fees from Atriva Therapeutics, Algernon Pharmaceuticals, Atea Pharmaceuticals, Diffusion Pharmaceuticals, Regeneron Pharmaceuticals, PureTech, Celltrion Inc., and Adagio Therapeutics. W.K. reports payment from Atriva Therapeutics to provide statistical support during the RESPIRE study. D.N. reports payment, funded by Atriva Therapeutics, as an employee of the contract research organisation that supported the RESPIRE study. O.P. reports grants to his institution from the Federal Ministry of Education and Research, Germany (Bundesministerium für Bildung und Forschung; BMBF), personal fees for consulting from Atriva Therapeutics, receipt of equipment and materials to his institution from Atriva Therapeutics and is a shareholder of Atriva Therapeutics. A.T. reports consulting fees from Pfizer and Janssen, and payment/honoraria for lectures, presentations, speakers' bureaus, manuscript writing or educational events, and participation in a Data Safety Monitoring Board or Advisory Board, from Pfizer, Janssen, and Biomerieux. M.W. reports grants from the Deutsche Forschungsgemeinschaft, BMBF, Deutsche Gesellschaft für Pneumologie, European Respiratory Society, Marie Curie Foundation, Else Kröner Fresenius Stiftung, Capnetz Stiftung, Bayer Health Care, Biotest, and Pantherna, consulting fees from Insmed, Pantherna, Pherecydes, Aptarion, Glaxo Smith Kline, Inflarx, and Biotest, and payment or honoraria for lectures, presentations, speakers' bureaus, manuscript writing or educational events from Astra Zeneca, Insmed, Chiesi, Novartis, Teva, Actelion, Boehringer Ingelheim, Glaxo Smith Kline, Biotest, Bayer Health Care. M.W. also holds relevant patents: EPO 12181535.1: IL-27 for modulation of immune response in acute lung injury (2012), WO/2010/094491: Means for inhibiting the expression of Ang-2 (2010), DE 102020116249.9: Camostat/Niclosamide cotreatment in SARS-CoV-2 infected human lung cells (2020/21), and PCT/EP2021/075627: New medical use of cystic fibrosis transmembrane conductance regulator (CFTR) modulators (2021).

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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.2023.102237.

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