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ORIGINAL RESEARCH

Efficacy and Safety of Reparixin in Patients with Severe COVID-19 Pneumonia: A Phase 3, Randomized, Double-Blind Placebo-Controlled Study

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ABSTRACT

Introduction: Polymorphonuclear cell influx into the interstitial and bronchoalveolar spaces is a cardinal feature of severe coronavirus disease 2019 (COVID-19), principally mediated by interleukin-8 (IL-8). We sought to determine whether reparixin, a novel IL-8 pathway inhibitor, could reduce disease progression in patients hospitalized with severe COVID-19 pneumonia.

Methods: In this Phase 3, randomized, double-blind, placebo-controlled, multicenter study, hospitalized adult patients with severe COVID-19 pneumonia were randomized 2:1 to receive oral reparixin 1200 mg three times daily or placebo for up to 21 days or until hospital discharge. The primary endpoint was the proportion of patients alive and free of respiratory failure at Day 28, with key secondary endpoints

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being the proportion of patients free of respiratory failure at Day 60, incidence of intensive care unit (ICU) admission by Day 28 and time to recovery by Day 28.

Results: Of 279 patients randomized, 182 received at least one dose of reparixin and 88 received placebo. The proportion of patients alive and free of respiratory failure at Day 28 was similar in the two groups {83.5% versus 80.7%; odds ratio 1.63 [95% confidence interval (CI) 0.75, 3.51]; $p = 0.216$}. There were no statistically significant differences in the key secondary endpoints, but a numerically higher proportion of patients in the reparixin group were alive and free of respiratory failure at Day 60 (88.7% versus 84.6%; $p = 0.195$), fewer required ICU admissions by Day 28 (15.8% versus 21.7%; $p = 0.168$), and a higher proportion recovered by Day 28 compared with placebo (81.6% versus 74.9%; $p = 0.167$). Fewer patients experienced adverse events with reparixin than placebo (45.6% versus 54.5%), most mild or moderate intensity and not related to study treatment.

Conclusions: This trial did not meet the primary efficacy endpoints, yet reparixin showed a trend toward limiting disease progression as an add-on therapy in COVID-19 severe pneumonia and was well tolerated.

Trial Registration: ClinicalTrials.gov: NCT04878055, EudraCT: 2020-005919-51.

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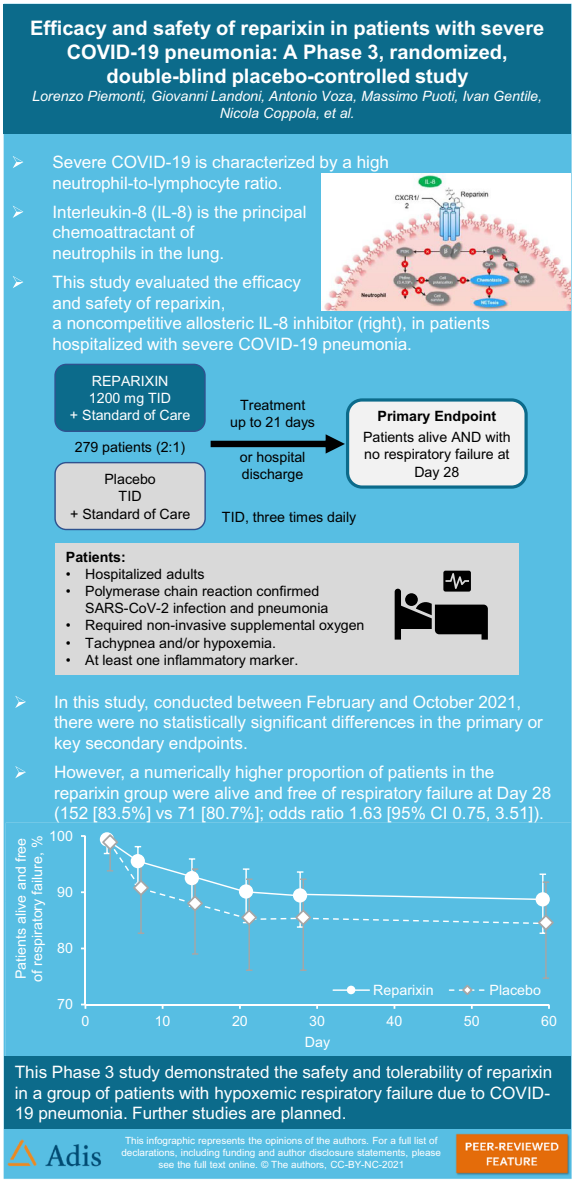
Graphical Abstract:



Keywords: COVID-19; Interleukin-8 (IL-8); Reparixin; SARS-CoV-2

Key Summary Points

Why carry out this study?

High interleukin-8 (IL-8) levels and neutrophil infiltration in the airways are hallmarks of disease severity of several pulmonary conditions, including COVID-19.

Given IL-8 is the principal chemoattractant of neutrophils in the lung, a therapy that targets this pathway is of potential interest.

This study follows a previous Phase 2 study, and was conducted to evaluate the efficacy and safety of reparixin, a noncompetitive allosteric inhibitor of IL-8 receptors, in patients hospitalized with severe COVID-19 pneumonia.

What was learned from the study?

This Phase 3 study demonstrated the safety and tolerability of reparixin in a group of patients with hypoxemic respiratory failure due to COVID-19 pneumonia. Despite no significant effect, it identified a trend in preventing disease deterioration necessitating escalation of care, when combined with standard of care.

Our upcoming studies will build on these encouraging findings by determining the efficacy and safety of reparixin in hospitalized patients with hypoxemic respiratory failure due to pneumonia.

DIGITAL FEATURES

This article is published with digital features, including a graphical abstract, to facilitate understanding of the article. To view digital features for this article, go to https://doi.org/10.6084/m9.figshare.24087894.

INTRODUCTION

Although most patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) experience mild disease [1], a proportion develops pneumonia and hypoxemic respiratory failure that can lead to fatal outcomes. The immunological phenotype of this severe "coronavirus disease 2019" (COVID-19) is characterized by a high neutrophil-to-lymphocyte ratio that correlates with disease severity [2, 3], predominantly due to a pronounced increase in neutrophil count [4]. Neutrophils mount anti-pathogen responses including the generation of neutrophil extracellular traps (NETosis), degranulation, and de novo production of cytokines and chemokines. These are vital innate immune responses against SARS-CoV-2, as they appear to be the most activated cellular immune responses in COVID-19 [5, 6]. However, these same processes may contribute to lung tissue damage and systemic complications such as thrombosis, acute respiratory distress syndrome, and multisystem inflammatory disease in children [7, 8]. Interleukin-8 [IL-8, also known as chemokine ligand 8 (CXCL8)] is the principal chemoattractant of neutrophils in the lung, with elevated systemic levels associated with poor outcomes in COVID-19 [9, 10].

Reparixin is a noncompetitive allosteric inhibitor of the IL-8 receptors 1 and 2 (CXCR1/CXCR2), which inhibits IL-8 mediated chemotaxis of human neutrophils [11], and their downstream biological effects such as NETosis [12]. In animal models of acute lung injury, reparixin significantly reduced capillary permeability and interstitial/alveolar neutrophil migration, improving gas exchange in both prophylactic and therapeutic approaches [13]. Similar biological and clinical outcomes, in terms of neutrophil infiltration and lung damage, were obtained using reparixin in lung injury due to influenza A virus and *Streptococcus pneumoniae* [14]. Reparixin also reduced pulmonary fibrosis and improved lung function in a particulate matter mice model [15]. In a mice sepsis model, reparixin decreased neutrophil extracellular traps (NET) formation without impairment of bacterial clearance, improving organ function and decreasing mortality [12].

In the pulmonary clinical setting, the role of reparixin in the prevention of primary graft dysfunction was investigated in a randomized controlled trial that included 114 patients; reparixin, although well tolerated with a good overall safety profile, was unable to show a statistically significant effect of reparixin on functional and clinical outcomes after lung transplantation [16]. In a previous Phase 2 study, completed during the first stages of the pandemic, reparixin in addition to standard of care (SoC) reduced disease progression in patients hospitalized with COVID-19 pneumonia, as determined by the proportion of patients who required supplemental oxygen, mechanical ventilation, intensive care unit (ICU) admission, and/or rescue medication for any reason (16.7% in the reparixin group versus 42.1% in the SoC group; $p = 0.02$) [17].

To further explore these initial findings, we conducted a larger Phase 3 study to evaluate the efficacy and safety of reparixin in patients hospitalized with severe COVID-19 pneumonia.

METHODS

Study Design

This was a randomized, double-blind, placebo-controlled, multicenter study to evaluate the efficacy and safety of reparixin in hospitalized adult patients with severe COVID-19 pneumonia, conducted between February and October 2021. The definition of severe COVID-19 followed the National Institutes of Health (NIH) recommendation, and required the presence of hypoxemia in combination with lung infiltrates and/or tachypnea (Table S1 in the electronic supplementary material) [18]. The protocol and all required clinical trial documentation were approved by the independent ethics committee of each investigational study site before the study was initiated. The central ethics committee for this study was *EC IRCSS Istituto Nazionale Per Le Malattie Infettive* (approval number 254). The study complied with the tenets of the Declaration

of Helsinki and the International Conference of Harmonization Tripartite Guidelines for Good Clinical Practice (ICH/CPMP/135/95), and was registered at EudraCT (2020-005919-51, 19 January 2021) and ClinicalTrials.gov (NCT04878055, 7 May 2021). All patients provided informed consent to participate in the study.

Participants

Eligible patients were hospitalized adults (aged 18–90 years) with polymerase chain reaction confirmed SARS-CoV-2 infection within 10 days of randomization, and radiologically verified pneumonia that required non-invasive supplemental oxygen. Patients had tachypnea (respiratory rate $\geq$ 24 breaths/min without oxygen) and/or hypoxemia [partial pressure of oxygen (PaO_2) to fraction of inspiration O_2 (FiO_2) ratio 100–300 mmHg or peripheral arterial oxygen saturation (SpO_2) $\leq$ 94% while breathing ambient air]. In addition, patients had at least one of the following inflammatory markers: lactate dehydrogenase above normal range, C-reactive protein $\geq$ 100 mg/L, IL-6 $\geq$ 40 pg/mL, serum ferritin $\geq$ 900 ng/mL, or serum cross-linked fibrin $>$ 20 μg/mL. Patients were excluded if they had moderate/severe hepatic dysfunction (Child–Pugh score B–C, or aspartate aminotransferase $>$ 5 times the upper limit of normal), moderate/severe renal dysfunction (estimated glomerular filtration rate $\leq$ 50 mL/min/1.73 m^2, or were on continuous renal replacement therapy, hemodialysis, or peritoneal dialysis), history of hypersensitivity to ibuprofen (metabolite of reparixin) or to more than one nonsteroidal anti-inflammatory drug or to more than one sulfonamide medication. Patients with severe active bleeding, or a recent history of such, were also excluded. All patients provided written informed consent prior to any study-related procedure. A complete list of the inclusion and exclusion criteria is in Table S1 in the electronic supplementary material.

Procedures

In addition to SoC, patients were randomly assigned 2:1 to reparixin 1200 mg tablets three times daily by mouth or identical placebo (reparixin and placebo tablet composition are in Table S2 in the electronic supplementary material), using an interactive response system, based on a randomization list created by an independent statistician using a computer-generated stratified permuted block scheme. Randomization was stratified by site, sex, and age ($<$ 65 versus $\geq$ 65 years). After randomization, patients unwilling or unable to swallow could receive tablets crushed and dispersed in water via naso-gastric tube, if already positioned. The duration of treatment was up to 21 days or until hospital discharge if occurring earlier than 21 days. Given the evolving nature of the disease, SoC was not strictly defined, and included any medication used during the study to treat COVID-19 pneumonia. Demographic data, medical history, previous and concomitant medications were collected at screening and/or at baseline. Clinical, laboratory, and hematology assessments were performed at screening, baseline, on Days 3, 7, 14, and 21 and on the day of discharge from hospital (or maximum at Day 28). Follow-up visits, in person or via telephone call, were conducted on Days 60 and 90, unless consent was withdrawn.

Clinical assessments included the 7-point ordinal scale recommended by the World Health Organization (WHO-OS) [19], oxygenation status (SpO_2, PaO_2, FiO_2, and PaO_2/FiO_2), severity of dyspnea [assessed using a visual analogue scale (VAS) and a 100 mm Likert scale], and use of supplemental oxygen. The seven WHO-OS categories were: (1) not hospitalized, with resumption of normal activities; (2) not hospitalized, but unable to resume normal activities; (3) hospitalized, not requiring supplemental oxygen; (4) hospitalized, requiring supplemental oxygen; (5) hospitalized, requiring high-flow oxygen therapy, non-invasive mechanical ventilation, or both; (6) hospitalized, requiring extracorporeal membrane oxygenation (ECMO), invasive mechanical ventilation, or both; and (7) death. Supplemental oxygen was defined as any oxygen administered through nasal cannula or non-invasive ventilation (NIV), including high-flow nasal cannula, bilevel-positive airway pressure, continuous positive airway pressure, or non-

rebreather mask. On Days 60 and 90 information was collected on patients' general condition, the occurrence of clinically important adverse events, WHO-OS, need for supplemental oxygen or NIV, and new hospitalization and/or ICU admission since hospital discharge.

Outcomes

The primary endpoint was the proportion of patients alive and free of respiratory failure at Day 28, defined as no need for invasive mechanical ventilation or ECMO, or admission to ICU due to worsening respiratory function. The four key secondary endpoints were the proportion of patients alive and free of respiratory failure at Day 60, mortality up to Day 28, the incidence of ICU admission or death up to Day 28, and the time to recovery as defined by reversion to WHO-OS category 1, 2, or 3 up to Day 28. Additional secondary endpoints, which included the proportion of patients alive and free of respiratory failure at fixed time points and other measures of clinical improvement and level of care, are listed in Table S3 in the electronic supplementary material. Safety was assessed throughout the study in terms of the occurrence of adverse events (AEs), and hematology and laboratory evaluations.

Statistical Analysis

With a 2:1 (reparixin:placebo) randomization ratio and a one-sided alpha of 0.025, a total of 264 evaluable patients would have allowed an overall power of 90% to detect a group difference $\geq$ 20% in the proportion of patients alive and free of respiratory failure at Day 28 in favor of reparixin, assuming that the proportion of patients alive and free of respiratory failure in the placebo group was approximately 60%. The primary and key secondary endpoints were analyzed by a logistic regression model, adjusted by treatment, sex, age group, and presence of concomitant disease as fixed effects, and site as a random effect, with a one-sided test used to evaluate the difference between treatment groups. Time to recovery was analyzed using the cumulative incidence function, with the treatment groups compared by means of a Gray's test.

The full analysis set (FAS), which comprised all randomized patients who received at least one dose of study drug, was used for all efficacy analyses, with patients analyzed according to treatment allocation. The per-protocol (PP) population, consisting of all patients in the FAS who did not have any major protocol deviations, was used for the sensitivity analysis of the primary endpoint. Safety analyses were performed using the safety population, which was the FAS with patients analyzed according to treatment received. All statistical analyses and data processing were performed using the Statistical Analysis Systems (SAS) Software (release 9.4).

RESULTS

A total of 279 patients were randomized at 14 Italian sites; 270 received at least one dose of study treatment (182 and 88 in the reparixin and placebo groups, respectively), with 221 patients (148 and 73, respectively) completing treatment (Fig. 1). The patients' baseline characteristics and clinical parameters were similar in the two groups (Table 1). The distribution of WHO-OS scores was similar in the two groups, with all patients having a score of 4 or 5, i.e., requiring supplemental oxygen, with half receiving high-flow supplemental oxygen and/or NIV. All patients had radiological imaging confirming lung involvement, and approximately one-third had ground-glass opacifications.

A total of 229 patients took at least one medication for COVID-19, most commonly glucocorticoids [219 (84.8%)] and remdesevir [74 (27.4%)]. Few patients received tocilizumab [two (1.1%) in the reparixin group versus none in the placebo group] or anakinra [nine (4.9%) versus two (2.3%)]. Prophylactic anticoagulants were used by 142 patients (78.0%) in the reparixin group and 65 (73.9%) in the placebo group.

The mean ($\pm$ SD) duration of treatment was 9.5 $\pm$ 4.9 days [median 9.0 (range 1, 21 days)] in the reparixin group and 9.6 $\pm$ 4.8 days [8.0 (1,

21) days] in the placebo group. Compliance to study medication was similar in the two arms: a median of 97.2% [interquartile range (IQR) 93.3%–100%] in the reparixin group and 97.9% (IQR 94.2%–100%) in the placebo group. Compliance ≥ 80% was reported in 163 patients (89.6%) in the reparixin group and 81 (92.0%) in the placebo group.

For the primary endpoint, 152 patients (83.5%) in the reparixin group and 71 (80.7%) in the placebo group were alive and free of respiratory failure at Day 28 [odds ratio (OR) 1.63; $p = 0.216$; Table 2 and Fig. 2]. This was confirmed in the various sensitivity analyses, including in the PP population {1.92 [95% confidence interval (CI) 0.77, 4.79]; $p = 0.162$}, when patients who were in the ICU at baseline were excluded [1.84 (0.81, 4.19); $p = 0.148$], when only complete cases were considered [1.32 (0.41, 4.24); $p = 0.638$], or when analyzed by means of multiple imputation under missing at random assumptions [1.70 (0.74, 3.92); $p = 0.215$].

For the key secondary outcomes (Table 2), 141 patients (88.7%) in the reparixin group and 66 (84.6%) in the placebo group were alive and free of respiratory failure at Day 60 (OR 1.77; $p = 0.195$), with 10 (6.0%) and 7 (8.6%), respectively dying up to Day 28 (0.47; $p = 0.170$). A total of 141 patients in the reparixin group and 63 in the placebo group recovered by Day 28, i.e., reverted to WHO-OS scores

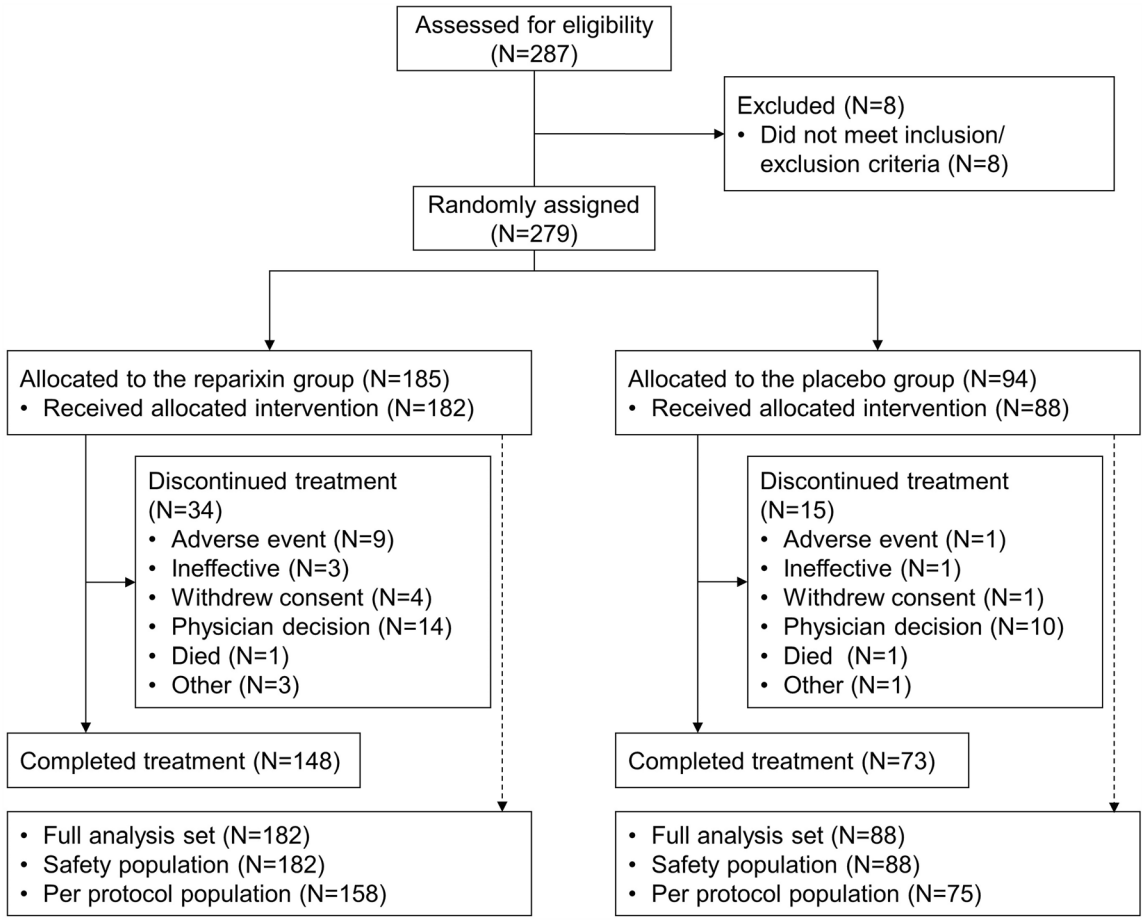


Fig. 1 Patient disposition. The full analysis set and the safety population consisted of all randomized patients who received at least one dose of study medication. The full analysis set was performed according to the intent-to-treat principle and was used for the efficacy analyses. The safety set was used for the safety analyses

Table 1 Demographic and clinical characteristics of patients at baseline

	Reparixin (*N* = 182)	**Placebo (*N* = 88)**
Age, years	61.3 ± 11.8	60.0 ± 12.0
Age ≥ 65 years	70 (38.5)	30 (34.1)
Male gender	132 (72.5)	63 (71.6)
Body-mass index (kg/m^2)	28.8 ± 4.8	28.9 ± 4.8
> 30 kg/m^2	51 (28.0)	29 (33.0)
Race		
White/Caucasian	170 (93.4)	84 (95.5)
Black	2 (1.1)	1 (1.1)
Other	10 (5.5)	3 (3.4)
Smoking history		
Never smoker	122 (67.0)	65 (73.9)
Former smoker	43 (23.6)	20 (22.7)
Current smoker	9 (4.9)	1 (1.1)
WHO clinical severity score[a]		
Score = 4	90 (49.5)	44 (50.0)
Score = 5	92 (50.5)	44 (50.0)
Dyspnea VAS scale	54.5 ± 25.2	55.2 ± 23.6
SpO_2, %	96.2 ± 2.4	96.5 ± 2.7
PaO_2, mmHg	90.3 ± 27.9	94.2 ± 34.6
FiO_2, %	0.5 ± 0.2	0.5 ± 0.2
PaO_2/FiO_2 ratio	200.1 ± 63.7	199.3 ± 67.2
Concomitant medications for COVID-19		
Glucocorticoids	149 (81.9)	70 (79.5)
Remdesivir	46 (25.3)	28 (31.8)
Interleukin inhibitors (anti-ILRa and anti-IL6)	11 (6.0)	2 (2.3)
COVID-19 immunization	2 (1.1)	0
At least one concomitant disease	153 (84.1)	73 (83.0)
Vascular disorders	91 (50.0)	48 (54.5)
Hypertension	88 (48.4)	47 (53.4)

Table 1 continued

	Reparixin (N = 182)	Placebo (N = 88)
Metabolism and nutrition disorders	69 (37.9)	37 (42.0)
Diabetes mellitus	38 (20.9)	18 (20.5)
Dyslipidemia	22 (12.1)	9 (10.2)
Reproductive system and breast disorders	27 (14.8)	7 (8.0)
Benign prostatic hyperplasia	24 (13.2)	6 (6.8)
Cardiac disorders	16 (8.8)	9 (10.2)
Respiratory, thoracic, and mediastinal disorders	16 (8.8)	6 (6.8)

Data are mean ± standard deviation, or number (percent)
VAS visual analog scale, SpO_2 peripheral arterial oxygen saturation, PaO_2 partial pressure of oxygen, FiO_2 fraction of inspired oxygen, *COVID-19* coronavirus disease 2019
[a]World Health Organization (WHO) clinical severity score of 4 indicates hospitalized, requiring supplemental oxygen; score of 5 indicates hospitalized, requiring high-flow oxygen therapy, non-invasive ventilation, or both

Table 2 Primary and key secondary outcomes

	Reparixin (N = 182)	Placebo (N = 88)	Reparixin versus placebo, odds ratio (95% CI)	p-Value
Primary outcome				
Patients alive and free or respiratory failure at Day 28	152/182 (83.5)[a]	71/88 (80.7)[a]	1.63 (0.75, 3.51)	0.216
Key secondary outcomes				
Patients alive and free of respiratory failure at Day 60	141/159 (88.7)	66/78 (84.6)	1.77 (0.75, 4.22)	0.195
28 days mortality rate	10/168 (6.0)	7/81 (8.6)	0.47 (0.16, 1.39)	0.170
28 days incidence of ICU admission (including those who died)	27/171 (15.8)	18/83 (21.7)	0.56 (0.25, 1.28)	0.168
Cumulative incidence of recovery up to Day 28 (95% CI)	81.6% (74.8%, 86.7%)	74.9% (64.0%, 83.0%)		0.167

Data are the number of patients with the event/number of patients considered in the model (%), except for ICU admission, which is the number of events, and the cumulative incidence of recovery, which represents the cumulative incidence function of recovery events (i.e., patients who reverted to categories 1, 2, or 3 of the 7-point World Health Organization ordinal scale (WHO-OS) over the population of patients with WHO-OS disease severity above 3 during the 28 days of observation and 95% confidence interval)
ICU intensive care unit
[a]The primary outcome was analyzed using a logistic regression model with multiple imputation under missing not at random; at Day 28, data were available from 170 and 83 patients in the reparixin and placebo groups, respectively

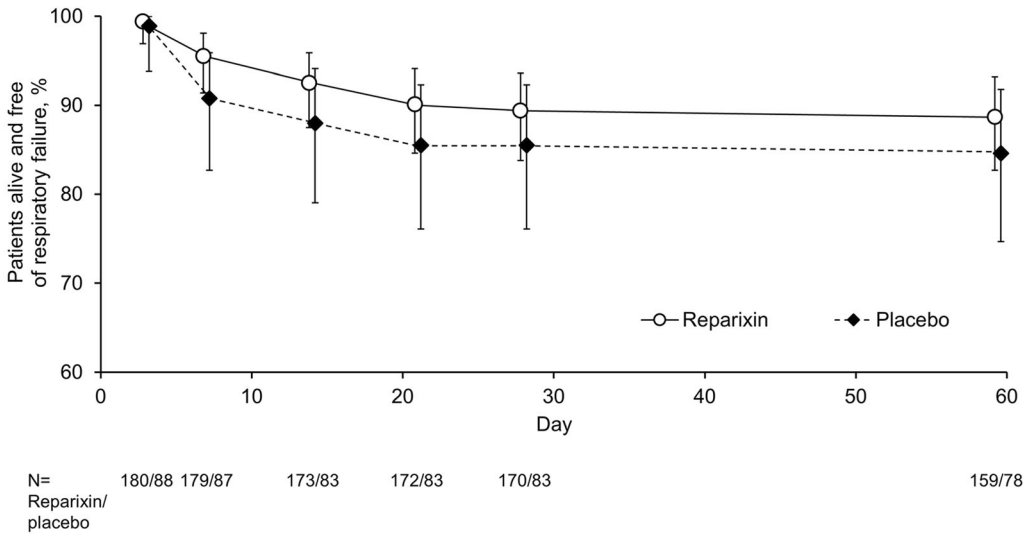


Fig. 2 Proportion of patients alive and free of respiratory failure at each visit throughout the study (full analysis set)

Table 3 Secondary and exploratory outcomes of interest

Endpoint	Reparixin (N = 182)	Placebo (N = 88)	Reparixin versus placebo, p-value
Clinical severity score, change from baseline on Day 3, mean ± SD	−0.1 ± 0.4	0.0 ± 0.5	0.047
PaO_2, change from baseline on Day 3, mean ± SD	13.377 ± 36.568	−5.274 ± 41.103	0.002
PaO_2/FiO_2 ratio, change from baseline on Day 3, mean ± SD	30.329 ± 81.291	0.398 ± 87.607	0.005
Incidence of invasive mechanical ventilation or ECMO followed by death up to Day 28, % (95% CI)	1.9 (0.4, 5.3)	8.6 (3.5, 17.0)	0.018
Incidence of invasive mechanical ventilation or ECMO followed by death up to Day 60, % (95% CI)	2.0 (0.4, 5.8)	9.2 (3.8, 18.1)	0.034
ICU admissions up to Day 28, number [adjusted mean rate per 4 weeks (Poisson regression)]	12 (0.0014)	12 (0.0034)	0.002 [RR 0.43 (0.25, 0.73)]
Invasive mechanical ventilation use or ECMO up to Day 28, number [adjusted mean rate per 4 weeks (Poisson regression)]	10 (0.05)	9 (0.10)	0.016 [RR 0.49 (0.27, 0.88)]
ICU admission, invasive mechanical ventilation use or ECMO, or death up to Day 28, number [adjusted mean rate per 4 weeks (Poisson regression)]	32 (0.13)	28 (0.27)	0.005 [RR 0.47 (0.28, 0.80)]

PaO_2 partial pressure of oxygen, *FiO_2* fraction of inspired oxygen, *ECMO* extracorporeal membrane oxygenation, *ICU* intensive care unit

Table 4 Treatment-emergent adverse events (safety population)

	Reparixin (*N* = 182)	**Placebo (*N* = 88)**
At least one adverse event	83 (45.6)	48 (54.5)
Infections and infestations	22 (12.1)	16 (18.2)
Sepsis	3 (1.6)	5 (5.7)
Urinary tract infection	3 (1.6)	6 (6.8)
Gastrointestinal disorders	22 (12.1)	13 (14.8)
Constipation	13 (7.1)	10 (11.4)
Respiratory, thoracic, and mediastinal disorders	17 (9.3%)	13 (14.8)
Respiratory failure	12 (6.6)	9 (10.2)
At least one treatment-related adverse event	10 (5.5)	8 (9.1)
Alanine aminotransferase increased	0	2 (2.3)
Respiratory failure	1 (0.5)	2 (2.3)
At least one serious adverse event	20 (11.0)	13 (14.8)
Respiratory failure	11 (6.0)	7 (8.0)
Pulmonary embolism	0	2 (2.3)
At least one treatment-related serious adverse event	0	0
At least one severe adverse event	16 (8.8)	12 (13.6)
At least one treatment-related severe adverse event	0	0
At least one adverse event leading to treatment discontinuation	19 (10.4)	11 (12.5)
Respiratory failure	8 (4.4)	5 (5.7)
At least one adverse event leading to death	10 (5.5)	7 (8.0)
Respiratory failure	8 (4.4)	4 (4.5)
Acute respiratory failure	0	1 (1.1)
Circulatory collapse	1 (0.5)	1 (1.1)
Multiple organ dysfunction syndrome	0	1 (1.1)
Sepsis	1 (0.5)	0

Data are number of patients (%). The listed terms are the most common system organ classes and preferred terms within system organ class for adverse events, and the most common preferred terms elsewhere

of 1, 2, or 3, corresponding to cumulative incidence functions of 81.6% and 74.9%, respectively ($p = 0.167$). The proportions of patients requiring ICU admission up to Day 28 when deaths were also considered as events were 15.8% and 21.7% (0.56; $p = 0.168$). In a post-hoc analysis based on the logistic regression model that excluded patients who died, 28-day ICU admission occurred in 20 (12.1%) patients in the reparixin group versus 18 (21.7%) with placebo [0.40 (0.17, 0.95); $p = 0.038$].

In a post-hoc analysis based on a Poisson regression model, over follow-up durations of 168.46 days in the reparixin group and

Table 5 Hematology and laboratory values, baseline and change from baseline at end of treatment (safety population)

Parameter	Reparixin (*N* = 182)	Placebo (*N* = 88)
Platelet count, $10^3/\mu L$		
Baseline	249.0 ± 92.4 (*N* = 175)	254.5 ± 92.3 (*N* = 87)
Change from baseline at end of treatment	40.5 ± 121.3 (*N* = 106)	18.4 ± 129.3 (*N* = 59)
Leukocytes, $10^3/\mu L$		
Baseline	8.475 ± 4.096 (*N* = 175)	8.502 ± 3.661 (*N* = 87)
Change from baseline at end of treatment	1.531 ± 4.596 (*N* = 106)	0.917 ± 3.709 (*N* = 59)
Neutrophils, $10^3/\mu L$		
Baseline	8.243 ± 10.536 (*N* = 174)	8.981 ± 13.091 (*N* = 86)
Change from baseline at end of treatment	0.974 ± 8.596 (*N* = 103)	−0.078 ± 15.413 (*N* = 59)
Lymphocytes, $10^3/\mu L$		
Baseline	1.255 ± 2.380) (*N* = 174)	1.253 ± 1.621 (*N* = 86)
Change from baseline at end of treatment	0.946 ± 1.091 (*N* = 103)	0.722 ± 1.479 (*N* = 59)
Albumin, g/dL		
Baseline	3.3980 ± 0.4350 (*N* = 117)	3.3848 ± 0.4456 (*N* = 63)
Change from baseline at end of treatment	−0.1258 ± 0.4892 (*N* = 62)	0.0197 ± 0.4283 (*N* = 30)
Aspartate aminotransferase (U/L)		
Baseline	49.0 ± 68.5 (*N* = 179)	41.6 ± 20.5 (*N* = 88)
Change from baseline at end of treatment	−17.7 ± 35.6 (*N* = 101)	93.5 ± 753.8 (*N* = 55)
Alanine aminotransferase (U/L)		
Baseline	52.7 ± 59.9 (*N* = 176)	52.9 ± 40.7 (*N* = 88)

Table 5 continued

Parameter	Reparixin(N = 182)	Placebo(N = 88)
Change from baseline at end of treatment	11.3 ± 53.9	112.5 ± 674.0
	(N = 98)	(N = 54)
Bilirubin (mg/dL)		
Baseline	0.6015 ± 0.2665	0.5615 ± 0.2593
	(N = 175)	(N = 85)
Change from baseline at end of treatment	0.1360 ± 0.5102	0.0683 ± 0.4215
	(N = 96)	(N = 53)
Direct bilirubin (mg/dL)		
Baseline	0.257 ± 0.128	0.266 ± 0.169
	(N = 147)	(N = 79)
Change from baseline at end of treatment	0.050 ± 0.256	0.063 ± 0.385
	(N = 75)	(N = 45)
Serum creatinine (mg/dL)		
Baseline	0.863 ± 0.201	0.850 ± 0.181
	(N = 181)	(N = 88)
Change from baseline at end of treatment	−0.034 ± 0.191	0.025 ± 0.223
	(N = 101)	(N = 59)
Estimated glomerular filtration rate (mL/min/1.73 m^2)		
Baseline	91.051 ± 26.353	93.294 ± 24.224
	(N = 126)	(N = 62)
Change from baseline at end of treatment	6.491 ± 21.817	1.953 ± 27.675
	(N = 71)	(N = 45)
Ferritin (ng/mL)		
Baseline	1136.178 ± 849.540	1129.519 ± 1033.543
	(N = 142)	(N = 73)
Change from baseline at end of treatment	−346.038 ± 807.985	741.083 ± 4716.879
	(N = 60)	(N = 40)

Data are mean ± standard deviation

80.07 days in the placebo group, the adjusted mean rates of ICU admission days over 4 weeks were 0.0014 in the reparixin group and 0.0034 in the placebo group, equating to a rate ratio of 0.43 (95% CI 0.25, 0.73; p = 0.002). Additional secondary and exploratory endpoints are listed in Table 3.

Safety

A total of 205 treatment-emergent AEs (TEAEs) were reported in 83 patients (45.6%) in the reparixin group compared with 116 TEAEs in 48 patients (54.5%) in the placebo group (Table 4). Serious TEAEs were reported in 20 patients (11.0%) in the reparixin group (23 TEAEs) and 13 patients (14.8%) in the placebo group (16 TEAEs), none of which was related to treatment. The most common TEAEs by system organ class were: gastrointestinal disorders [21 patients (11.5%) in the reparixin group and 12 (13.6%) in the placebo group] and infections and infestations [13 patients (7.1%) in the reparixin group and 14 (15.9%) in the placebo group].

TEAEs leading to treatment discontinuation were reported in 10.4% of patients receiving reparixin versus 12.5% receiving placebo, whereas TEAEs leading to death were reported in 10 patients (5.5%) in the reparixin group and 7 (8.0%) in the placebo group, again none treatment related. Respiratory failure was the most common TEAE leading to death, in 8 patients (4.4%) in the reparixin group and 4 (4.5%) in the placebo group (Table 4). There were no substantial changes from baseline in hematology or laboratory parameters in either group (Table 5), and no significant vital sign or ECG changes.

DISCUSSION

This Phase 3 study did not demonstrate efficacy of reparixin as compared with placebo in adults for severe COVID-19 pneumonia. Nonetheless, there was a positive trend in favor of reparixin in the primary and the secondary efficacy endpoints. More patients in the reparixin group were alive and free of respiratory failure at Days 28 and 60, fewer patients died by Day 28, fewer needed to be transferred to ICU for deterioration of respiratory status, and more had a meaningful recovery. This was consistently supported by the other endpoints; for example, reparixin was associated with statistically significant improvements in the exploratory analyses of serious events, i.e., invasive mechanical ventilation use or ECMO followed by death up to Days 28 and 60, indicating that reparixin may prevent the most invasive rescue treatments that are frequently linked to death. Furthermore, patients in the reparixin group started improving earlier, with significant differences in clinical status and oxygenation status on Day 3 (the first post-dose assessment).

The COVID-19 pandemic has led to close to 7 million deaths worldwide, with 1 million deaths being reported in the USA alone [20], and millions more affected directly and indirectly through impacts on their livelihood, employment opportunities, education, and long-term health-related quality of life. However, the impact of COVID-19 on respiratory pathology, along with the efforts taken to manage the disease, have not only reshaped our awareness of viral pneumonias, but has helped to advance the way we manage patients with infection-related acute hypoxemic respiratory failure.

Pharmacologic management of COVID-19, other than anti-virals, has focused on therapies that modulate the hyperinflammatory state effected by the excessive production of cytokines by a deregulated immune system, which is increasingly recognized as a key feature of severe COVID-19 [21]. Such immunomodulators include steroids, IL-6 inhibitors, and Janus kinase (JAK) inhibitors. The large RECOVERY study demonstrated a significant drop in mortality following early low-dose dexamethasone in patients with COVID-19, with the greatest benefit seen in mechanically ventilated patients whereas there was no benefit for those not requiring supplemental oxygen [22]. Unfortunately, this benefit is associated with significant drawbacks, especially when the use of steroids is protracted, in particular beyond the currently advised 10 days. Indeed, a meta-analysis of 21,350 patients with COVID-19 concluded that the overall effect of steroids on COVID-19-related mortality is less certain than anticipated, with the caveat that there was great heterogeneity among the studies reviewed [23]. When added to steroids, tocilizumab, the most widely used IL-6 receptor inhibitor, led to an improvement of in-hospital mortality—although when optimal SoC was applied this benefit was observed only among the sickest

patients [24]. Furthermore, the risk of late-onset infectious complications due to tocilizumab exposure remains a concern [25]. This becomes even more of an issue with inhibition of the JAK–STAT pathway through the use of JAK inhibitors, since the involvement of JAKs in the immune response, especially via interferon-gamma, means that their potential adverse events include immunosuppression with reactivation of latent infection or development of new secondary infections, compounded by thrombotic events, cardiotoxicity, and hepatotoxicity [26].

IL-8 is one of the main neutrophil chemoattractors in the lung. Neutrophils abound in the lungs of patients with fatal COVID-19 [2, 3] and their activation-related functions such as the production of reactive oxygen species, proteases, inflammatory mediators, and release of NETs can result in local tissue injury. NETs are a constant histopathologic feature in the lungs of patients with fatal COVID-19, where their spatial distribution correlates closely with local IL-8 levels [27]. In fact, IL-8 together with other $ELR^{+}CXCL$ (i.e., those with the amino acid sequence Glu-Leu-Arg present) chemokines acting on CXCR1 and CXCR2, are among the most effective NET promoters [28, 29], both persistent and out of proportion to tissue viral load [27]. IL-8 pathway activation is a marker of disease status with serum levels increasing in correlation with progression of disease severity in patients with COVID-19 [30]. Our previous experience with reparixin supported further exploration of the role of IL-8 inhibition in severe COVID-19.

The current study was designed on assumptions informed by the first wave of COVID-19 that approximately 60% of patients in the placebo group would be alive and free of respiratory failure at Day 28, with a difference $\geq$ 20% in favor of reparixin. These assumptions were not confirmed, given the rapidly evolving natural history and treatment options for COVID-19. In our previous Phase 2 study in a similar patient population, 42.1% of patients in the placebo group experienced the composite endpoint of need for supplemental oxygen or mechanical ventilation use, ICU admission, or requirement of rescue medication by Day 28 (compared with 16.7% in the reparixin group) [17]. In addition, 15.8% of patients in this group died, whereas in the current study mortality by Day 28 was 8.6% in the placebo group and 6.0% in the reparixin group. Given that the patients enrolled in the two studies had comparable COVID-19 severity (as assessed using WHO-OS), the difference in outcomes is most likely attributable to the adaption of new therapies for COVID-19 that became SoC in the meantime, as well as changes in the proportion of vaccinated patients and changes in disease epidemiology and natural history. As disease-related mortality decreases, therapies that have demonstrated a positive effect on survival during the first wave of the disease are likely to lose this effect in subsequent studies. As an example, in the latest tocilizumab study, REMDACTA [24], which failed to demonstrate a survival benefit, mortality in both groups (18% versus 20% with tocilizumab versus placebo, respectively) was much lower than that in the RECOVERY and REMAP-CAP studies that established a mortality benefit (REMAP-CAP, 28% versus 36%; RECOVERY 31% versus 35% [31, 32]).

Lack of mortality benefit, however, does not equate to lack of efficacy, and our results demonstrate a beneficial effect of reparixin on important patient-centered outcomes such as transfer to ICU for deterioration of respiratory status. Even 1 day away from the ICU or not intubated translates to considerable gains in psychological strain for patients and their families and financial cost [33–35]. Reparixin appears to assist in achieving this goal, thus adding to the COVID-19 armamentarium in a meaningful way. Furthermore, and in contrast to other COVID-19 therapies [23, 36–38], reparixin was not associated with any safety signals, and was very well tolerated. Compared with those receiving placebo, fewer patients in the reparixin group had TEAEs, overall, serious, treatment-related, or severe TEAEs, or TEAEs leading to treatment discontinuation or death. Given that the most commonly reported TEAEs were related to the underlying disease (e.g., respiratory failure or respiratory distress), the fact that patients receiving reparixin experienced fewer AEs is a further indication of efficacy. Consistently with the previous trials

[17, 39], there was a very low incidence of secondary infection despite the widespread use of glucocorticoids.

The main limitation of the study is that, because it was conducted during a surge of the pandemic in Italy, study conduct was at times difficult, leading to a high amount of missing clinical and pharmacokinetic data, especially at later time points. Furthermore, the identity and prevalence of SARS-CoV-2 variants in patients participating in the study are unknown, as the majority of the participating centers did not have access to variant screening methods. However, during the study period, the SARS-CoV-2 alpha variant was predominant, corresponding to an increase in infections during the second wave of the epidemic, whereas the omicron variant and its sublineages, which emerged in South Africa in November 2021, were not included in the study [40]. Moreover, there was a rapid change in the prevailing SARS-CoV-2 variant in Italy during the conduction of the study, with prevalence of the alpha variant increasing from 3.5% in December 2020 to 86.7% by March 2021 [40]. Given that evolving viral strains differ in their ability to evade host immunity, it is unclear whether an immune modulator that acts primarily through regulation of neutrophil activation would have the same efficacy across variants. In addition, the attempt to collect inflammatory markers to investigate the link between inflammatory status and drug efficacy was not successful. Finally, although the proportion of patients with concomitant glucocorticoid use was collected, the length of administration was not captured in the study database.

CONCLUSIONS

This Phase 3 study demonstrated the safety and tolerability of reparixin in a group of patients with hypoxemic respiratory failure due to COVID-19 pneumonia and indicated that when reparixin is used in addition to optimal therapy, it may still offer an additional advantage in preventing disease deterioration leading to ICU admission and mechanical ventilation or death. Reparixin is free of significant side effects and is not associated with increased risk for secondary infections, which is a significant advantage over currently used immunomodulators. Given the decrease in hospitalizations for COVID-19, our upcoming studies will build on these encouraging findings by expanding the study population to hospitalized patients with acute respiratory failure in pneumonia including COVID-19.

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Data Availability. The datasets generated during and/or analyzed during the current

study are available from the corresponding author on reasonable request.

Declarations

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