ORIGINAL ARTICLE

A Trial of Lopinavir–Ritonavir in Adults Hospitalized with Severe Covid-19

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ABSTRACT

BACKGROUND

No therapeutics have yet been proven effective for the treatment of severe illness caused by SARS-CoV-2.

METHODS

We conducted a randomized, controlled, open-label trial involving hospitalized adult patients with confirmed SARS-CoV-2 infection, which causes the respiratory illness Covid-19, and an oxygen saturation (Sao_2) of 94% or less while they were breathing ambient air or a ratio of the partial pressure of oxygen (Pao_2) to the fraction of inspired oxygen (Fio_2) of less than 300 mm Hg. Patients were randomly assigned in a 1:1 ratio to receive either lopinavir–ritonavir (400 mg and 100 mg, respectively) twice a day for 14 days, in addition to standard care, or standard care alone. The primary end point was the time to clinical improvement, defined as the time from randomization to either an improvement of two points on a seven-category ordinal scale or discharge from the hospital, whichever came first.

RESULTS

A total of 199 patients with laboratory-confirmed SARS-CoV-2 infection underwent randomization; 99 were assigned to the lopinavir–ritonavir group, and 100 to the standard-care group. Treatment with lopinavir–ritonavir was not associated with a difference from standard care in the time to clinical improvement (hazard ratio for clinical improvement, 1.24; 95% confidence interval [CI], 0.90 to 1.72). Mortality at 28 days was similar in the lopinavir–ritonavir group and the standard-care group (19.2% vs. 25.0%; difference, –5.8 percentage points; 95% CI, –17.3 to 5.7). The percentages of patients with detectable viral RNA at various time points were similar. In a modified intention-to-treat analysis, lopinavir–ritonavir led to a median time to clinical improvement that was shorter by 1 day than that observed with standard care (hazard ratio, 1.39; 95% CI, 1.00 to 1.91). Gastrointestinal adverse events were more common in the lopinavir–ritonavir group, but serious adverse events were more common in the standard-care group. Lopinavir–ritonavir treatment was stopped early in 13 patients (13.8%) because of adverse events.

CONCLUSIONS

In hospitalized adult patients with severe Covid-19, no benefit was observed with lopinavir–ritonavir treatment beyond standard care. Future trials in patients with severe illness may help to confirm or exclude the possibility of a treatment benefit. (Funded by Major Projects of National Science and Technology on New Drug Creation and Development and others; Chinese Clinical Trial Register number, ChiCTR2000029308.)

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Beginning in December 2019, a novel coronavirus, designated SARS-CoV-2, has caused an international outbreak of respiratory illness termed Covid-19. The full spectrum of Covid-19 ranges from mild, self-limiting respiratory tract illness to severe progressive pneumonia, multiorgan failure, and death.[1-4] Thus far, there are no specific therapeutic agents for coronavirus infections. After the emergence of severe acute respiratory syndrome (SARS) in 2003, screening of approved drugs identified lopinavir, a human immunodeficiency virus (HIV) type 1 aspartate protease inhibitor, as having in vitro inhibitory activity against SARS-CoV, the virus that causes SARS in humans.[5-7] Ritonavir is combined with lopinavir to increase its plasma half-life through the inhibition of cytochrome P450. An open-label study published in 2004 suggested, by comparison with a historical control group that received only ribavirin, that the addition of lopinavir–ritonavir (400 mg and 100 mg, respectively) to ribavirin reduced the risk of adverse clinical outcomes (acute respiratory distress syndrome [ARDS] or death) as well as viral load among patients with SARS.[5] However, the lack of randomization and a contemporary control group and the concomitant use of glucocorticoids and ribavirin in that study made the effect of lopinavir–ritonavir difficult to assess. Similarly, lopinavir has activity, both in vitro[8] and in an animal model,[9] against Middle East respiratory syndrome coronavirus (MERS-CoV), and case reports have suggested that the combination of lopinavir–ritonavir with ribavirin and interferon alfa resulted in virologic clearance and survival.[10-12] However, because convincing data about the efficacy of this approach in humans are lacking,[12] a clinical trial (with recombinant interferon beta-1b) for MERS is currently under way (ClinicalTrials.gov number, NCT02845843).[13-15]

To evaluate the efficacy and safety of oral lopinavir–ritonavir for SARS-CoV-2 infection, we conducted a randomized, controlled, open-label trial, LOTUS China (Lopinavir Trial for Suppression of SARS-Cov-2 in China), in adult patients hospitalized with Covid-19.

METHODS

PATIENTS

Patients were assessed for eligibility on the basis of a positive reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay (Shanghai ZJ Bio-Tec or Sansure Biotech) for SARS-CoV-2 in a respiratory tract sample tested by the local Center for Disease Control (CDC) or by a designated diagnostic laboratory. Male and nonpregnant female patients 18 years of age or older were eligible if they had a diagnostic specimen that was positive on RT-PCR, had pneumonia confirmed by chest imaging, and had an oxygen saturation (Sao_2) of 94% or less while they were breathing ambient air or a ratio of the partial pressure of oxygen (Pao_2) to the fraction of inspired oxygen (Fio_2) (Pao_2:Fio_2) at or below 300 mg Hg. Exclusion criteria included a physician decision that involvement in the trial was not in the patient's best interest, presence of any condition that would not allow the protocol to be followed safely, known allergy or hypersensitivity to lopinavir–ritonavir, known severe liver disease (e.g., cirrhosis, with an alanine aminotransferase level >5× the upper limit of the normal range or an aspartate aminotransferase level >5× the upper limit of the normal range), use of medications that are contraindicated with lopinavir–ritonavir and that could not be replaced or stopped during the trial period (see the Supplementary Appendix, available with the full text of this article at NEJM.org); pregnancy or breast-feeding, or known HIV infection, because of concerns about the development of resistance to lopinavir–ritonavir if used without combining with other antiretrovirals. Patients who were unable to swallow received lopinavir–ritonavir through a nasogastric tube.

TRIAL DESIGN AND OVERSIGHT

This was an open-label, individually randomized, controlled trial conducted from January 18, 2020, through February 3, 2020 (the date of enrollment of the last patient), at Jin Yin-Tan Hospital, Wuhan, Hubei Province, China. Because of the emergency nature of the trial, placebos of lopinavir–ritonavir were not prepared. Eligible patients were randomly assigned in a 1:1 ratio to receive either lopinavir–ritonavir (400 mg and 100 mg, orally; freely provided by the national health authority) twice daily, plus standard care, or standard care alone, for 14 days. Standard care comprised, as necessary, supplemental oxygen, noninvasive and invasive ventilation, antibiotic agents, vasopressor support, renal-replacement therapy, and extracorporeal membrane oxygenation (ECMO). To balance the distribution of oxy-

gen support between the two groups as an indicator of severity of respiratory failure, randomization was stratified on the basis of respiratory support methods at the time of enrollment: no oxygen support or oxygen support with nasal duct or mask, or high-flow oxygen, noninvasive ventilation, or invasive ventilation including ECMO. The permuted block (four patients per block) randomization sequence, including stratification, was prepared by a statistician not involved in the trial, using SAS software, version 9.4 (SAS Institute). To minimize allocation bias, we performed allocation concealment with an interactive Web-based response system until randomization was finished on the system through a computer or phone.

The trial was approved by the institutional review board of Jin Yin-Tan Hospital. Written informed consent was obtained from all patients or from the patient's legal representative if the patient was too unwell to provide consent. The trial was conducted in accordance with the principles of the Declaration of Helsinki and the Good Clinical Practice guidelines of the International Conference on Harmonisation. The authors were responsible for designing the trial and for compiling and analyzing the data. The authors vouch for the completeness and accuracy of the data and for the adherence of the trial to the protocol. Full details about the trial design are provided in the protocol, available at NEJM.org.

CLINICAL AND LABORATORY MONITORING

Patients were assessed once daily by trained nurses using diary cards that captured data on a seven-category ordinal scale and on safety from day 0 to day 28, hospital discharge, or death. Safety was monitored by the Good Clinical Practice office from Jin Yin-tan Hospital. Other clinical data were recorded using the WHO-ISARIC (World Health Organization–International Severe Acute Respiratory and Emerging Infections Consortium) case record form (https://isaric.tghn.org).[16] Serial oropharyngeal swab samples were obtained on day 1 (before lopinavir–ritonavir was administered) and on days 5, 10, 14, 21, and 28 until discharge or death had occurred and were tested at Teddy Clinical Research Laboratory (Tigermed–DiAn Joint Venture), using quantitative real-time RT-PCR (see the Supplementary Appendix). RNA was extracted from clinical samples with the MagNA Pure 96 system, detected and quantified by Cobas z480 qPCR (Roche), with the use of LightMix Modular Wuhan CoV assays (TIB MOBIOL). These samples were obtained for all 199 patients who were still alive at every time point. Sampling did not stop when a swab at a given time point was negative. Baseline throat swabs were tested for detection of E gene, RdRp gene, and N gene, and samples on the subsequent visits were quantitatively and qualitatively detected for E gene. Clinical data were recorded on paper case record forms and then double-entered into an electronic database and validated by trial staff.

OUTCOME MEASURES

The primary end point was the time to clinical improvement, defined as the time from randomization to an improvement of two points (from the status at randomization) on a seven-category ordinal scale or live discharge from the hospital, whichever came first. The end point of clinical improvement was used in our previous influenza study[17] and was also recommended by the WHO R&D Blueprint expert group.[18] Ordinal scales have been used as end points in clinical trials in patients hospitalized with severe influenza.[16-19] The seven-category ordinal scale consisted of the following categories: 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 nasal high-flow oxygen therapy, noninvasive mechanical ventilation, or both; 6, hospitalized, requiring ECMO, invasive mechanical ventilation, or both; and 7, death.

Other clinical outcomes included clinical status as assessed with the seven-category ordinal scale on days 7 and 14, mortality at day 28, the duration of mechanical ventilation, the duration of hospitalization in survivors, and the time (in days) from treatment initiation to death. Virologic measures included the proportions with viral RNA detection over time and viral RNA titer area-under-the-curve (AUC) measurements.

Safety outcomes included adverse events that occurred during treatment, serious adverse events, and premature discontinuation of treatment. Adverse events were classified according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

STATISTICAL ANALYSIS

The trial was initiated in rapid response to the Covid-19 public health emergency, at which time there was very limited information about clinical outcomes in hospitalized patients with Covid-19. The original total sample size was set at 160, since it would provide the trial with 80% power to detect a difference, at a two-sided significance level of $\alpha = 0.05$, of 8 days in the median time to clinical improvement between the two groups, assuming that the median time in the standard-care group was 20 days and that 75% of the patients would reach clinical improvement. The planned enrollment of 160 patients in the trial occurred quickly, and the assessment at that point was that the trial was underpowered; thus, a decision was made to continue enrollment by investigators. Subsequently, when another agent (remdesivir) became available for clinical trials, we decided to suspend enrollment in this trial.

Primary efficacy analysis was on an intention-to-treat basis and included all the patients who had undergone randomization. The time to clinical improvement was assessed after all patients had reached day 28, with failure to reach clinical improvement or death before day 28 considered as right-censored at day 28 (right-censoring occurs when an event may have occurred after the last time a person was under observation, but the specific timing of the event is unknown). The time to clinical improvement was portrayed by Kaplan–Meier plot and compared with a log-rank test. Hazard ratios with 95% confidence intervals were calculated by means of the Cox proportional-hazards model. Five patients who had been assigned to the lopinavir–ritonavir group did not receive any doses (three of them died within 24 hours) but were included in the intention-to-treat analysis, since no reciprocal removals occurred in the standard-care group. A modified intention-to-treat analysis that excluded three early deaths was also performed. Post hoc analyses include subgroup analysis for National Early Warning Score 2 (NEWS2)[19] of 5 or below or greater than 5 and those who underwent randomization up to 12 days or more than 12 days after the onset of illness.

Because the statistical analysis plan did not include a provision for correcting for multiplicity in tests for secondary or other outcomes, results are reported as point estimates and 95% confidence intervals. The widths of the confidence intervals have not been adjusted for multiplicity, so the intervals should not be used to infer definitive treatment effects for secondary outcomes. Safety analyses were based on the patients' actual treatment exposure. Statistical analyses were conducted with SAS software, version 9.4 (SAS Institute).

RESULTS

PATIENTS

Of the 199 patients who underwent randomization, 99 patients were assigned to receive lopinavir–ritonavir and 100 patients to standard care alone. Of the 99 patients assigned to receive lopinavir–ritonavir, 94 (94.9%) received treatment as assigned (Fig. 1). In the lopinavir–ritonavir group, 5 patients did not receive any doses of lopinavir–ritonavir: 3 because of early death within 24 hours after randomization and 2 others because the attending physician refused to prescribe lopinavir–ritonavir after randomization.

The median age of patients was 58 years (interquartile range [IQR], 49 to 68 years), and 60.3% of the patients were men (Table 1). The median interval time between symptom onset and randomization was 13 days (IQR, 11 to 16 days) (Table 2). There were no important between-group differences in demographic characteristics, baseline laboratory test results, distribution of ordinal scale scores, or NEWS2 scores at enrollment. During the trial, systemic glucocorticoids were administered in 33.0% of the patients in the lopinavir–ritonavir group and in 35.7% of those in the standard-care group.

PRIMARY OUTCOME

Patients assigned to lopinavir–ritonavir did not have a time to clinical improvement different from that of patients assigned to standard care alone in the intention-to-treat population (median, 16 day vs. 16 days; hazard ratio for clinical improvement, 1.31; 95% confidence interval [CI], 0.95 to 1.85; P = 0.09) (Fig. 2). In the modified intention-to-treat population, the median time to clinical improvement was 15 days in the lopinavir–ritonavir group, as compared with 16 days in the standard-care group (hazard ratio, 1.39; 95% CI, 1.00 to 1.91) (Table S1 and Fig. S1 in the Supplementary Appendix). In the intention-to-treat population, lopinavir–ritonavir treatment within 12 days after the onset of symptoms was associated

with shorter time to clinical improvement (hazard ratio, 1.25; 95% CI, 1.77 to 2.05), but later treatment with lopinavir–ritonavir was not (hazard ratio, 1.30; 95% CI, 0.84 to 1.99) (Fig. S2A and S2B). No significant differences were observed when the time to clinical improvement was assessed by NEWS2 score at entry in the intention-to-treat population (Fig. S3A and S3B). In addition, when the time to clinical deterioration (defined as a one-category increase on the seven-category scale) was compared between the two groups, no difference was observed (hazard ratio for clinical deterioration, 1.01; 95% CI, 0.76 to 1.34) (Fig. S4).

SECONDARY OUTCOMES

The 28-day mortality was numerically lower in the lopinavir–ritonavir group than in the standard-care group for either the intention-to-treat population (19.2% vs. 25.0%; difference, –5.8 percentage points; 95% CI, –17.3 to 5.7) or the modified intention-to treat population (16.7% vs. 25.0%; difference, –8.3 percentage points; 95% CI, –19.6 to 3.0) (Table 3).

Patients in the lopinavir–ritonavir group had a shorter stay in the intensive care unit (ICU) than those in the standard-care group (median, 6 days vs. 11 days; difference, –5 days; 95% CI, –9 to 0), and the duration from randomization to hospital discharge was numerically shorter (median, 12 days vs. 14 days; difference, 1 day; 95% CI, 0 to 3]). In addition, the percentage of patients with clinical improvement at day 14 was higher in the lopinavir–ritonavir group than in the standard-care group (45.5% vs. 30.0%; difference, 15.5 percentage points; 95% CI, 2.2 to 28.8) (Fig. S5). There were no significant differences for other outcomes such as duration of oxygen therapy, duration of hospitalization, and time from randomization to death.

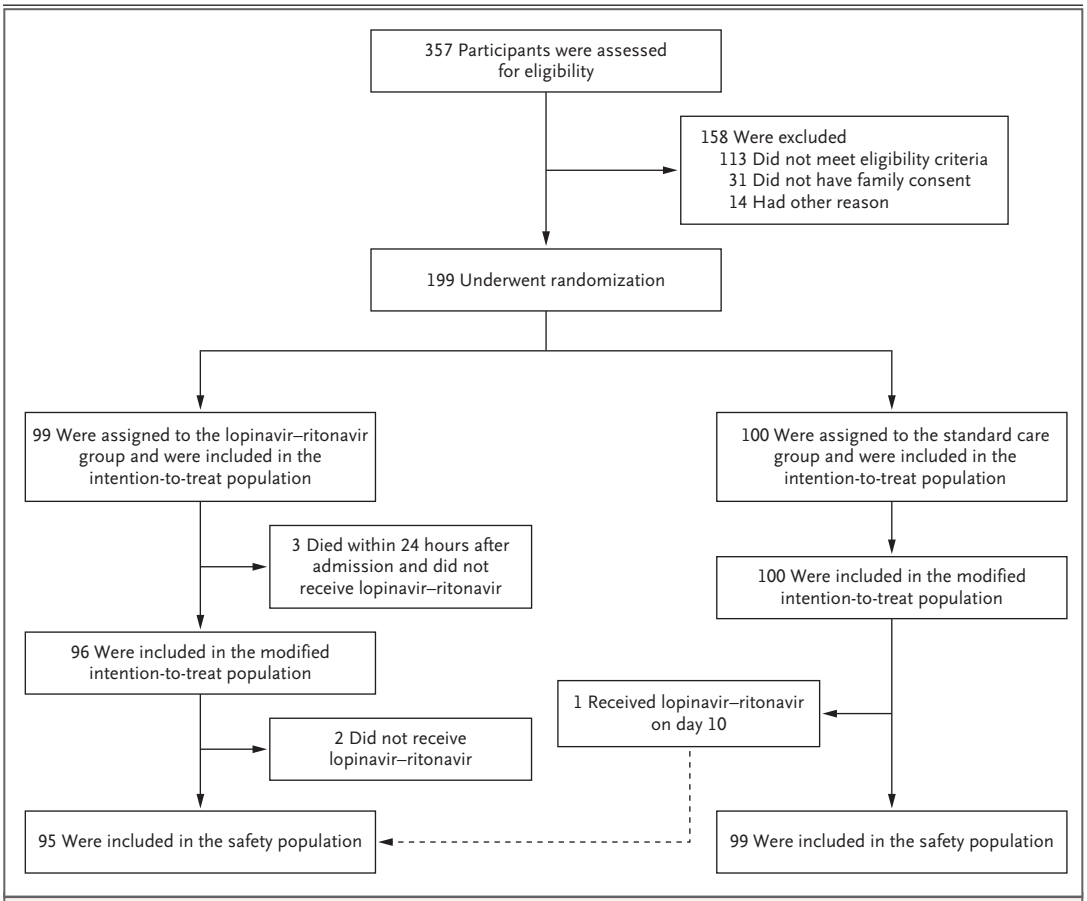

Figure 1. Randomization and Treatment Assignment.

Table 1. Demographic and Clinical Characteristics of the Patients at Baseline.*

Characteristic	Total (N=199)	Lopinavir–Ritonavir (N=99)	Standard Care (N=100)
Age, median (IQR) — yr	58.0 (49.0–68.0)	58.0 (50.0–68.0)	58.0 (48.0–68.0)
Male sex — no. (%)	120 (60.3)	61 (61.6)	59 (59.0)
Coexisting conditions — no. (%)			
Diabetes	23 (11.6)	10 (10.1)	13 (13.0)
Cerebrovascular disease	13 (6.5)	5 (5.1)	8 (8.0)
Cancer	6 (3.0)	5 (5.1)	1 (1.0)
Body temperature, median (IQR) — °C	36.5 (36.4–36.8)	36.5 (36.4–37.0)	36.5 (36.5–36.8)
Fever — no. (%)	182 (91.5)	89 (89.9)	93 (93.0)
Respiratory rate >24/min — no. (%)	37 (18.8)	21 (21.6)	16 (16.0)
Systolic blood pressure <90 mm Hg — no. (%)	2 (1.0)	2 (2.0)	0
White-cell count ($\times 10^{-9}$/liter) — median (IQR)	7.0 (5.1–9.4)	7.3 (5.3–9.6)	6.9 (4.9–9.1)
4–10 $\times 10^{-9}$/liter — no. (%)	137 (70.3)	64 (67.4)	73 (73.0)
<4 $\times 10^{-9}$/liter — no. (%)	20 (10.3)	12 (12.6)	8 (8.0)
>10 $\times 10^{-9}$/liter — no. (%)	38 (19.5)	19 (20.0)	19 (19.0)
Lymphocyte count ($\times 10^{-9}$/liter) — median (IQR)	0.9 (0.6–1.2)	0.8 (0.6–1.4)	0.9 (0.5–1.2)
≥1.0 $\times 10^{-9}$/liter — no. (%)	73 (37.4)	37 (38.9)	36 (36.0)
<1.0 $\times 10^{-9}$/liter — no. (%)	122 (62.6)	58 (61.1)	64 (64.0)
Platelet count ($\times 10^{-9}$/liter) — median (IQR)	207.0 (158.0–284.0)	201.0 (155.0–287.0)	210.0 (163.0–269.5)
≥100 $\times 10^{-9}$/liter — no. (%)	186 (95.4)	91 (95.8)	95 (95.0)
<100 $\times 10^{-9}$/liter — no. (%)	9 (4.6)	4 (4.2)	5 (5.0)
Serum creatinine (μmol/liter) — median (IQR)	69.5 (57.2–82.5)	70.7 (56.4–82.7)	67.4 (58.4–82.5)
≤133 μmol/liter — no. (%)	189 (96.9)	93 (96.9)	96 (97.0)
>133 μmol/liter — no. (%)	6 (3.1)	3 (3.1)	3 (3.0)
Aspartate aminotransferase (U/liter) — median (IQR)	34.0 (26.0–45.0)	33.0 (25.0–42.0)	34.0 (27.0–45.0)
≤40 U/liter — no. (%)	155 (79.5)	78 (81.3)	77 (77.8)
>40 U/liter — no. (%)	40 (20.5)	18 (18.8)	22 (22.2)
Alanine aminotransferase (U/liter) — median (IQR)	33.0 (22.0–55.0)	33.0 (22.0–53.5)	34.0 (22.0–59.0)
≤50 U/liter — no. (%)	115 (59.0)	61 (63.5)	54 (54.5)
>50 U/liter — no. (%)	80 (41.0)	35 (36.5)	45 (45.5)
Lactate dehydrogenase (U/liter) — median (IQR)	325.0 (245.0–433.0)	322.0 (243.0–409.0)	327.0 (245.0–470.0)
≤245 U/liter — no. (%)	50 (25.8)	24 (25.3)	26 (26.3)
>245 U/liter — no. (%)	144 (74.2)	71 (74.7)	73 (73.7)
Creatine kinase (U/liter) — median (IQR)	69.0 (44.0–115.0)	57.0 (42.0–126.0)	72.0 (45.0–110.0)
≤185 U/liter — no. (%)	168 (86.6)	81 (85.3)	87 (87.9)
> 185 U/liter — no. (%)	26 (13.4)	14 (14.7)	12 (12.1)

* The values shown are based on available data. Laboratory values for white-cell count, lymphocyte count, platelet count, lactate dehydrogenase, and creatine kinase were available for 95 patients in the lopinavir–ritonavir group; and values for serum creatinine, aspartate aminotransferase, and alanine aminotransferase were available for 96 patients in that group. Laboratory values for serum creatinine, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and creatine kinase were available for 99 patients in the standard-care group. To convert the values for creatinine to milligrams per deciliter, divide by 88.4. IQR denotes interquartile range.

Table 2. Patients' Status and Treatments Received at or after Enrollment.*

Characteristic	Total (N=199)	Lopinavir–Ritonavir (N=99)	Standard Care (N=100)
NEWS2 score at day 1 — median (IQR)	5.0 (4.0–6.0)	5.0 (4.0–6.0)	5.0 (4.0–7.0)
Seven-category scale at day 1			
3: Hospitalization, not requiring supplemental oxygen — no. (%)	28 (14.1)	11 (11.1)	17 (17.0)
4: Hospitalization, requiring supplemental oxygen — no. (%)	139 (69.8)	72 (72.7)	67 (67.0)
5: Hospitalization, requiring HFNC or noninvasive mechanical ventilation — no. (%)	31 (15.6)	15 (15.2)	16 (16.0)
6: Hospitalization, requiring ECMO, invasive mechanical ventilation, or both — no. (%)	1 (0.5)	1 (1.0)	0
Days from illness onset to randomization — median (IQR)	13 (11–16)	13 (11–17)	13 (10–16)
Earlier (≤12 days of symptom onset) — no. (%)	90 (45.2)	42 (42.4)	48 (48.0)
Later (>12 days of symptom onset) — no. (%)	109 (54.8)	57 (57.6)	52 (52.0)
Mean viral load — $\log_{10}$ copies per ml at day 1	4.0±2.1	4.4±2.0	3.7±2.1
Using interferon at enrollment — no. (%)	22 (11.1)	9 (9.1)	13 (13.0)
Treatments during study period — no. (%)			
Vasopressors	44 (22.1)	17 (17.2)	27 (27.0)
Renal-replacement therapy	9 (4.5)	3 (3.0)	6 (6.0)
Noninvasive mechanical ventilation	29 (14.6)	10 (10.1)	19 (19.0)
Invasive mechanical ventilation	32 (16.1)	14 (14.1)	18 (18.0)
ECMO	4 (2.0)	2 (2.0)	2 (2.0)
Antibiotic agent	189 (95.0)	94 (94.9)	95 (95.0)
Glucocorticoid therapy	67 (33.7)	32 (32.3)	35 (35.0)
Days from illness onset to glucocorticoid therapy — median (IQR)	13 (11–17)	13 (12–19)	13 (9–17)
Days of glucocorticoid therapy — median (IQR)	6 (3–11)	7 (3–11)	6 (2–12)

* Plus–minus values are means ±SD. ECMO denotes extracorporeal membrane oxygenation, HFNC high-flow nasal cannula for oxygen therapy, and NEWS2 National Early Warning Score 2.

VIROLOGY

A total of 69 patients (35%) who had a diagnostic respiratory tract sample that was positive on RT-PCR had a negative RT-PCR result on the throat swab taken after consent. The mean (±SD) baseline viral RNA loads in the throat swabs taken after consent were slightly higher in the lopinavir–ritonavir group than in the standard-care group at randomization (4.4±2.0 $\log_{10}$ copies per milliliter vs. 3.7±2.1) (Table 2). The viral RNA loads over time did not differ between the lopinavir–ritonavir recipients and those receiving standard care (Fig. 3), including analysis according to duration of illness (Fig. S6).

The percentage of patients with detectable viral RNA for SARS-CoV-2 was similar in the lopinavir–ritonavir group and the standard-care group on any sampling day (day 5, 34.5% vs. 32.9%; day 10, 50.0% vs. 48.6%; day 14, 55.2% vs. 57.1%; day 21, 58.6% vs. 58.6%; and day 28, 60.3% vs. 58.6%) (Table S2).

SAFETY

A total of 46 patients (48.4%) in the lopinavir–ritonavir group and 49 (49.5%) in the standard-care group reported adverse events between randomization and day 28 (Table 4). Gastrointestinal adverse events including nausea, vomiting, and diarrhea were more common in lopinavir–ritonavir group than in the standard-care group (Table 4).

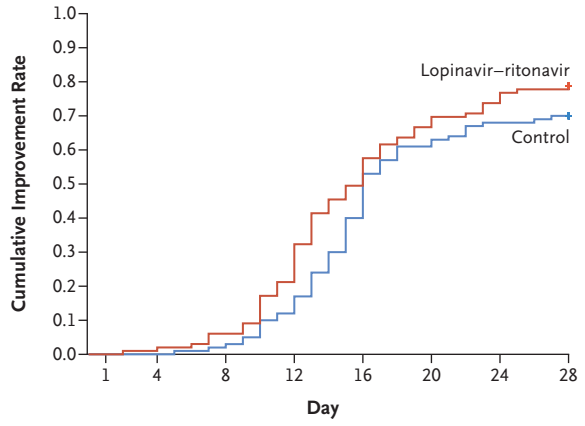

Figure 2. Time to Clinical Improvement in the Intention-to-Treat Population.

The percentages of patients with laboratory abnormalities were similar in the two groups (Table 4). Serious adverse events occurred in 51 patients: 19 events in the lopinavir–ritonavir group and 32 events in the standard-care group (Table 4). There were 4 serious gastrointestinal adverse events in the lopinavir–ritonavir group but none in the standard-care group; all 4 events were judged by the investigators to be related to the trial medication. Respiratory failure, acute kidney injury, and secondary infection were more common in patients receiving standard care. All deaths during the observation period were judged by the site investigators to be unrelated to the intervention.

DISCUSSION

This randomized trial found that lopinavir–ritonavir treatment added to standard supportive care was not associated with clinical improvement or mortality in seriously ill patients with Covid-19 different from that associated with standard care alone. However, in the modified intention-to-treat analysis, which excluded three patients with early death, the between-group difference in the median time to clinical improvement (median, 15 days vs. 16 days) was significant, albeit modest. Of note, the overall mortality in this trial (22.1%) was substantially higher than the 11% to 14.5% mortality reported in initial descriptive studies of hospitalized patients with Covid-19,[1,2] which indicates that we enrolled a severely ill population.

Our patient population was heterogeneous with regard to duration and severity of illness at enrollment; accelerated clinical recovery (16.0 days vs. 17.0 days) and reduced mortality (19.0% vs. 27.1%) were observed in a post hoc subgroup of those treated within 12 days after the onset of symptoms, but not in those treated later (Fig. S2A and S2B). The question of whether earlier lopinavir–ritonavir treatment in Covid-19 could have clinical benefit is an important one that requires further study. The finding is consistent with studies showing that patients with SARS-CoV-2 viral pneumonia have progression in the second week of illness[1] and with the time-to-treatment effects observed in previous antiviral studies in SARS[20] and severe influenza.[21-23] In addition, we found that the numbers of lopinavir–ritonavir recipients who had serious complications (acute kidney injury and secondary infections) or requiring noninvasive or invasive mechanical ventilation for respiratory failure were fewer than in those not receiving treatment. These observations are hypothesis-generating and require additional studies to determine whether lopinavir–ritonavir treatment given at a certain stage of illness can reduce some complications in Covid-19.

We did not find that adding lopinavir–ritonavir treatment reduced viral RNA loads or duration of viral RNA detectability as compared with standard supportive care alone. SARS-CoV-2 RNA was still detected in 40.7% of the patients in the lopinavir–ritonavir group at end of the trial (day 28). A recent report showed that the median duration of viral shedding in Covid-19 was 20 days in patients with severe illness and could be as long as 37 days.[24] Neither that study nor the current one found evidence that lopinavir–ritonavir exerted a significant antiviral effect. The reasons for the apparent lack of antiviral effect are uncertain, but the sampling methods used in the current trial were most likely suboptimal. Samples were taken only intermittently (on days 1, 5, 10, 14, 21, and 28), and more frequent sampling in the first 5 days could have provided more detailed characterization of viral load kinetics in the two groups over this critical period.

Table 3. Outcomes in the Intention-to-Treat Population.*

Characteristic	Total (N=199)	Lopinavir–Ritonavir (N=99)	Standard Care (N=100)	Difference†
Time to clinical improvement — median no. of days (IQR)	16.0 (15.0 to 17.0)	16.0 (13.0 to 17.0)	16.0 (15.0 to 18.0)	1.31 (0.95 to 1.80)‡
Day 28 mortality — no. (%)	44 (22.1)	19 (19.2)§	25 (25.0)	−5.8 (−17.3 to 5.7)
Earlier (≤12 days after onset of symptoms)	21 (23.3)	8 (19.0)	13 (27.1)	−8.0 (−25.3 to 9.3)
Later (>12 days after onset of symptoms)	23 (21.1)	11 (19.3)	12 (23.1)	−3.8 (−19.1 to 11.6)
Clinical improvement — no. (%)				
Day 7	8 (4.0)	6 (6.1)	2 (2.0)	4.1 (−1.4 to 9.5)
Day 14	75 (37.7)	45 (45.5)	30 (30.0)	15.5 (2.2 to 28.8)
Day 28	148 (74.4)	78 (78.8)	70 (70.0)	8.8 (−3.3 to 20.9)
ICU length of stay — median no. of days (IQR)	10 (5 to 14)	6 (2 to 11)	11 (7 to 17)	−5 (−9 to 0)
Of survivors	10 (8 to 17)	9 (5 to 44)	11 (9 to 14)	−1 (−16 to 38)
Of nonsurvivors	10 (4 to 14)	6 (2 to 11)	12 (7 to 17)	−6 (−11 to 0)
Duration of invasive mechanical ventilation — median no. of days (IQR)	5 (3 to 9)	4 (3 to 7)	5 (3 to 9)	−1 (−4 to 2)
Oxygen support — days (IQR)	13 (8 to 16)	12 (9 to 16)	13 (6 to 16)	0 (−2 to 2)
Hospital stay — median no. of days (IQR)	15 (12 to 17)	14 (12 to 17)	16 (13 to 18)	1 (0 to 2)
Time from randomization to discharge — median no. of days (IQR)	13 (10 to 16)	12 (10 to 16)	14 (11 to 16)	1 (0 to 3)
Time from randomization to death — median no. of days (IQR)	10 (6 to 15)	9 (6 to 13)	12 (6 to 15)	−3 (−6 to 2)
Score on seven-category scale at day 7 — no. of patients (%)				
2: Not hospitalized, but unable to resume normal activities	4 (2.0)	4 (4.0)	0	
3: Hospitalization, not requiring supplemental oxygen	29 (14.6)	12 (12.1)	17 (17.0)	
4: Hospitalization, requiring supplemental oxygen	109 (54.8)	58 (58.6)	51 (51.0)	
5: Hospitalization, requiring HFNC or noninvasive mechanical ventilation	35 (17.6)	14 (14.1)	21 (21.0)	
6: Hospitalization, requiring ECMO, invasive mechanical ventilation, or both	10 (5.0)	6 (6.1)	4 (4.0)	
7: Death	12 (6.0)	5 (5.1)	7 (7.0)	
Seven-category scale at day 14 — no. of patients (%)				
2: Not hospitalized, but unable to resume normal activities	71 (35.7)	43 (43.4)	28 (28.0)	
3: Hospitalization, not requiring supplemental oxygen	32 (16.1)	8 (8.1)	24 (24.0)	
4: Hospitalization, requiring supplemental oxygen	45 (22.6)	25 (25.3)	20 (20.0)	
5: Hospitalization, requiring HFNC or noninvasive mechanical ventilation	11 (5.5)	5 (5.1)	6 (6.0)	
6: Hospitalization, requiring ECMO, invasive mechanical ventilation, or both	8 (4.0)	3 (3.0)	5 (5.0)	
7: Death	32 (16.1)	15 (15.2)	17 (17.0)	

* Clinical improvement was defined as a decline of two categories on the modified seven-category ordinal scale of clinical status, or hospital discharge. ICU denotes intensive care unit.
† Differences were expressed as rate differences or median differences (Hodges–Lehmann estimate) and 95% confidence intervals.
‡ The hazard ratio for clinical improvement was estimated by Cox proportional-risk model.
§ This total includes 3 patients who died within 24 hours after randomization and did not receive lopinavir–ritonavir.

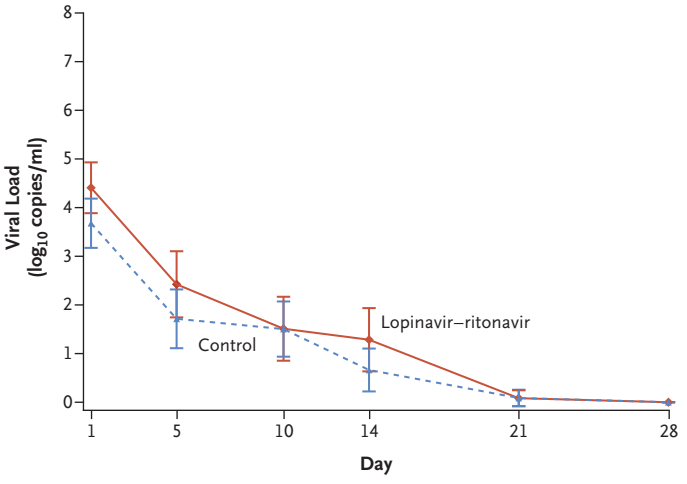

Figure 3. Mean Change from Baseline in SARS-CoV-2 Viral RNA Load by qPCR on Throat Swabs.

I bars indicate 95% confidence intervals. Results less than the lower limit of quantification of polymerase-chain-reaction (PCR) assay and greater than the limit of qualitative detection are imputed with 1 $\log_{10}$ copies per milliliter; results for patients with viral-negative RNA are imputed with 0 $\log_{10}$ copies per milliliter. Among the 199 patients, 130 (59 patients in the lopinavir–ritonavir group and 71 in the standard-care group) had virologic data that were used for viral load calculation, whereas the rest of the patients had undetectable viral RNA on throat swabs over the time.

In addition, previous studies have shown that throat-swab specimens have lower viral loads than nasopharyngeal samples,[25] and importantly, we were unable to do sampling of lower respiratory tract secretions. Of note, depending on cell type used, the 50% effective concentrations (EC_{50}) of lopinavir in vitro for SARS-CoV has ranged from 4.0 to 10.7 μg per milliliter,[5,6,8] although other studies reported that lopinavir was inactive[26] or that higher concentrations (25 μg per milliliter) were required for inhibition.[7] For MERS-CoV, the EC_{50} values have ranged from 5 to approximately 7 μg per milliliter).[1,8,13] Both the mean peak (9.6 μg per milliliter) and trough (5.5 μg per milliliter) serum concentrations of lopinavir in adults just approach these concentrations. Whether the EC_{50} value is an adequate threshold and whether unbound lopinavir concentrations in human plasma are sufficient for inhibition of SARS-CoV-2 are questionable.[1]

Nearly 14% of lopinavir–ritonavir recipients were unable to complete the full 14-day course of administration. This was due primarily to gastrointestinal adverse events, including anorexia, nausea, abdominal discomfort, or diarrhea, as well as two serious adverse events, both acute gastritis. Two recipients had self-limited skin eruptions. Such side effects, including the risks of hepatic injury, pancreatitis, more severe cutaneous eruptions, and QT prolongation, and the potential for multiple drug interactions due to CYP3A inhibition, are well documented with this drug combination. The side-effect profile observed in the current trial arouses concern about the use of higher or more prolonged lopinavir–ritonavir dose regimens in efforts to improve outcomes.

Our trial has several limitations. In particular, the trial was not blinded, so it is possible that knowledge of the treatment assignment might have influenced clinical decision-making that could have affected the ordinal scale measurements we used. We will continue to follow these patients to evaluate their long-term prognosis. The characteristics of the patients at baseline were generally balanced across the two groups, but the somewhat higher throat viral loads in the lopinavir–ritonavir group raise the possibility that this group had more viral replication. Although we did not observe differences between groups in the frequency of use of concurrent pharmacologic interventions, such as glucocorticoids, this might have been another confounder. In addition, approximately 45% and 40% of the patients in lopinavir–ritonavir group had positive RNA detection by throat swabs on day 14 and day 28, respectively, but we do not know if infectious virus was still present, since we did not attempt virus isolation or assess the possible emergence of SARS-CoV-2 variants with reduced susceptibility to lopinavir. Finally, we do not have data on the lopinavir exposure levels in these seriously and often critically ill patients.

In conclusion, we found that lopinavir–ritonavir treatment did not significantly accelerate clinical improvement, reduce mortality, or diminish throat viral RNA detectability in patients with serious Covid-19. These early data should inform future studies to assess this and other medication in the treatment of infection with SARS-CoV-2. Whether combining lopinavir–ritonavir with other antiviral agents, as has been done in SARS[5,20] and is being studied in MERS-CoV,[15] might en-

Table 4. Summary of Adverse Events in the Safety Population.*

Event	Lopinavir–Ritonavir (N=95)		Standard Care (N=99)	
	Any Grade	Grade 3 or 4	Any Grade	Grade 3 or 4
	number (percent)			
Any adverse event	46 (48.4)	20 (21.1)	49 (49.5)	11 (11.1)
Lymphopenia	16 (16.8)	12 (12.6)	12 (12.1)	5 (5.1)
Nausea	9 (9.5)	1 (1.1)	0	0
Thrombocytopenia	6 (6.3)	1 (1.1)	10 (10.1)	2 (2.0)
Leukopenia	7 (7.4)	1 (1.1)	13 (13.1)	0
Vomiting	6 (6.3)	0	0	0
Increased aspartate aminotransferase	2 (2.1)	2 (2.1)	5 (5.1)	4 (4.0)
Abdominal discomfort	4 (4.2)	0	2 (2.1)	0
Diarrhea	4 (4.2)	0	0	0
Stomach ache	4 (4.2)	1 (1.1)	1 (1.0)	0
Neutropenia	4 (4.2)	1 (1.1)	8 (7.6)	0
Increased total bilirubin	3 (3.2)	3 (3.2)	3 (3.0)	2 (2.0)
Increased creatinine	2 (2.1)	2 (2.1)	7 (7.1)	6 (6.1)
Anemia	2 (2.1)	2 (2.1)	5 (5.0)	4 (4.0)
Rash	2 (2.1)	0	0	0
Hypoalbuminemia	1 (1.1)	1 (1.1)	4 (4.0)	1 (1.0)
Increased alanine aminotransferase	1 (1.1)	1 (1.1)	4 (4.0)	1 (1.0)
Increased creatine kinase	0	0	1 (1.0)	0
Decreased appetite	2 (2.1)	0	0	0
Prolonged QT interval	1 (1.1)	0	0	0
Sleep disorders and disturbances	1 (1.1)	0	0	0
Facial flushing	1 (1.1)	0	0	0
Serious adverse event	19 (20.0)	17 (17.9)	32 (32.3)	31 (31.3)
Respiratory failure or ARDS	12 (12.6)	12 (12.6)	27 (27.3)	27 (27.3)
Acute kidney injury	3 (3.2)	2 (2.1)	6 (6.1)	5 (5.1)
Secondary infection	1 (1.1)	1 (1.1)	6 (6.1)	6 (6.1)
Shock	2 (2.1)	2 (2.1)	2 (2.0)	2 (2.0)
Severe anemia	3 (3.2)	3 (3.2)	0	0
Acute gastritis	2 (2.1)	0	0	0
Hemorrhage of lower digestive tract	2 (2.1)	1 (1.1)	0	0
Pneumothorax	0	0	2 (2.0)	2 (2.0)
Unconsciousness	1 (1.1)	0	0	0
Disseminated intravascular coagulation	1 (1.1)	0	1 (1.0)	1 (1.0)
Sepsis	0	0	1 (1.0)	1 (1.0)
Acute heart failure	0	0	1 (1.0)	1 (1.0)

* Adverse events that occurred in more than 1 patient after randomization through day 28 are shown. Some patients had more than one adverse event. Since there are no adverse event grades criteria for serum levels of hypersensitivity troponin (cardiac biomarker) and serum lipid, the proportions of patients with values worse than baseline values are listed here. The proportion of increased hypersensitivity troponin was higher in the standard-care group than in the lopinavir–ritonavir group (14.1% vs. 9.5%). A total of 55 patients (52.4%) in the standard-care group and 65 (68.4%) in the lopinavir–ritonavir group had lipid levels that were normal at enrollment but abnormal after enrollment. All deaths were due to respiratory failure. ARDS indicates acute respiratory distress syndrome.

hance antiviral effects and improve clinical outcomes remains to be determined.

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APPENDIX

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