ORIGINAL ARTICLE

Interferon Beta-1b and Lopinavir–Ritonavir for Middle East Respiratory Syndrome

Y.M. Arabi, A.Y. Asiri, A.M. Assiri, H.H. Balkhy, A. Al Bshabshe, M. Al Jeraisy, Y. Mandourah, M.H.A. Azzam, A.M. Bin Eshaq, S. Al Johani, S. Al Harbi, H.A.A. Jokhdar, A.M. Deeb, Z.A. Memish, J. Jose, S. Ghazal, S. Al Faraj, G.A. Al Mekhlafi, N.M. Sherbeeni, F.E. Elzein, F. Al-Hameed, A. Al Saedi, N.K. Alharbi, R.A. Fowler, F.G. Hayden, A. Al-Dawood, M. Abdelzaher, W. Bajhmom, B.M. AlMutairi, M.A. Hussein, and A. Alothman*, for the Saudi Critical Care Trials Group†

ABSTRACT

BACKGROUND

Whether combined treatment with recombinant interferon beta-1b and lopinavir–ritonavir reduces mortality among patients hospitalized with Middle East respiratory syndrome (MERS) is unclear.

METHODS

We conducted a randomized, adaptive, double-blind, placebo-controlled trial that enrolled patients at nine sites in Saudi Arabia. Hospitalized adults with laboratory-confirmed MERS were randomly assigned to receive recombinant interferon beta-1b plus lopinavir–ritonavir (intervention) or placebo for 14 days. The primary outcome was 90-day all-cause mortality, with a one-sided P-value threshold of 0.025. Prespecified subgroup analyses and safety analyses were conducted. Because of the pandemic of coronavirus disease 2019, the data and safety monitoring board requested an unplanned interim analysis and subsequently recommended the termination of enrollment and the reporting of the results.

RESULTS

A total of 95 patients were enrolled; 43 patients were assigned to the intervention group and 52 to the placebo group. A total of 12 patients (28%) in the intervention group and 23 (44%) in the placebo group died by day 90. The analysis of the primary outcome, with accounting for the adaptive design, yielded a risk difference of –19 percentage points (upper boundary of the 97.5% confidence interval [CI], –3; one-sided P=0.024). In a prespecified subgroup analysis, treatment within 7 days after symptom onset led to lower 90-day mortality than use of placebo (relative risk, 0.19; 95% CI, 0.05 to 0.75), whereas later treatment did not. Serious adverse events occurred in 4 patients (9%) in the intervention group and in 10 (19%) in the placebo group.

CONCLUSIONS

A combination of recombinant interferon beta-1b and lopinavir–ritonavir led to lower mortality than placebo among patients who had been hospitalized with laboratory-confirmed MERS. The effect was greatest when treatment was started within 7 days after symptom onset. (Funded by the King Abdullah International Medical Research Center; MIRACLE ClinicalTrials.gov number, NCT02845843.)

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Address reprint requests to Dr. Arabi at P.O. Box 22490, Riyadh 11426, Saudi Arabia, or at arabi@ngha.med.sa.

*Deceased.

†A full list of the members of the Saudi Critical Care Trials Group is provided in the Supplementary Appendix, available at NEJM.org.

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Over the past two decades, emerging coronaviruses (CoVs) have caused three major outbreaks — severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and coronavirus disease 2019 (Covid-19). Since MERS was reported in Saudi Arabia in 2012, at least 2494 cases and 858 associated deaths have been confirmed, with a case fatality rate of 34.4%.[1] Of the patients hospitalized with MERS, many have received prolonged intensive care for respiratory and other organ failure.[2-5] Antiviral therapeutic agents of proven efficacy have been lacking.[6,7]

Various formulations of recombinant interferons and lopinavir–ritonavir have been used for the treatment of MERS on the basis of preclinical and observational data on SARS and MERS.[8-12] Recombinant interferon alfa-2b has shown activity against MERS-CoV in cell culture and, in combination with ribavirin, in a rhesus macaque model of MERS-CoV infection.[9,10,13] However, in some models, recombinant interferon beta has the strongest MERS-CoV inhibitory effects among various preparations of recombinant interferon agents tested in vitro.[13,14] Lopinavir inhibits the replication of SARS-CoV in vitro, and in one cohort of 41 patients with SARS, combination treatment with lopinavir–ritonavir and ribavirin was associated with some improvement in clinical outcomes (acute respiratory distress syndrome [ARDS] or death occurring in fewer patients), as compared with a historical control group of 111 patients who were treated with ribavirin alone.[8] Lopinavir inhibits the replication of MERS-CoV in vitro,[15] and in a marmoset model of MERS-CoV infection, treatment with either recombinant interferon beta-1b or lopinavir–ritonavir was associated with virologic, histologic, and clinical improvement as compared with control.[16] The aim of the MIRACLE (MERS-CoV Infection Treated with a Combination of Lopinavir–Ritonavir and Interferon Beta-1b) trial was to investigate the efficacy of a combination of recombinant interferon beta-1b and lopinavir–ritonavir, as compared with placebo, on 90-day all-cause mortality among hospitalized patients with laboratory-confirmed MERS.

Methods

Trial Design and Oversight

We conducted this recursive, two-stage, group-sequential, randomized, placebo-controlled, double-blind trial at nine sites in Saudi Arabia. The trial was sponsored by the King Abdullah International Medical Research Center. The trial protocol and statistical analysis plan have been published previously[17,18] and are available with the full text of this article at NEJM.org.

The trial protocol was designed by the management committee (see the Supplementary Appendix, available at NEJM.org) and was approved by the institutional review board at each participating site. Mobile research teams were deployed to initiate trial procedures at some sites. Data monitoring and quality checks were conducted by the management committee and the sponsor. The sponsor supported research coordinators, provided the trial medications, and provided the electronic data platform, trial monitoring, and statistical support. The management committee was responsible for the trial design and management, data analysis, and the interpretation of the results. The members of the management committee vouch for the accuracy and completeness of the data and for the fidelity of the trial to the protocol. The manuscript was written by the writing committee (see the Supplementary Appendix) and was approved for submission for publication by all the authors.

Trial Population and Regimens

The trial included hospitalized patients who had MERS that had been confirmed by real-time reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay, who were 18 years of age or older, and who had evidence of acute organ dysfunction that was judged to be related to MERS. Details of the inclusion and exclusion criteria are provided in Table S1 in the Supplementary Appendix.[17] The methods used for real-time RT-PCR testing are outlined in the Supplementary Appendix. Randomization was stratified according to center and to receipt of invasive or noninvasive mechanical ventilation (yes or no) at the time of enrollment.

Patients in the intervention group received recombinant interferon beta-1b (Betaferon [also called Betaseron], Bayer) as a subcutaneous injection (at a dose of 0.25 mg [8 million IU] in 1 ml of solvent) on alternate days. In addition, patients received oral lopinavir–ritonavir (at a dose of 400 mg of lopinavir and 100 mg of ritonavir; Kaletra, AbbVie) in tablet form every 12 hours. For patients who were unable to take medications

by mouth, lopinavir–ritonavir (at the same dose) was administered as a 5-ml suspension every 12 hours through a nasogastric tube. Patients in the placebo group received a 1-ml subcutaneous injection of normal saline and an oral placebo that consisted of sucrose capsules (or that was administered as 5 ml of normal saline through a nasogastric tube in patients who were unable to take medications by mouth) at the same frequency as the intervention group. The intervention or placebo was administered for 14 days or until hospital discharge, whichever came first. Patients were followed daily until day 28 or hospital discharge and then at day 90. The use of intervention or placebo and the interim analyses were blinded for the research team, treating team, outcome assessors, and patients throughout the trial until the decision to conclude the trial was made according to the recommendation of the data and safety monitoring board (see below).

CLINICAL MONITORING AND OUTCOMES

We collected data on the baseline characteristics of the patients, including demographic characteristics, severity of illness, functional status as assessed with the Karnofsky performance-status score (scores range from 0 [death] to 100 [normal performance status]), coexisting conditions, and laboratory variables. During each patient's hospitalization, we documented the administration of the trial intervention or placebo, adverse events, and laboratory data according to the published schedule.[17] Follow-up respiratory samples (nasopharyngeal swabs or, if available, sputum samples obtained from patients who were not intubated and tracheal aspirates or bronchoalveolar-lavage samples obtained from patients who were intubated) were tested by real-time RT-PCR twice weekly until two consecutive tests were negative. We collected data on cointerventions, including organ support and medications administered during the trial period.[17]

The primary outcome was 90-day all-cause mortality. Secondary outcomes included death in the intensive care unit (ICU), in the hospital, and by day 28; days alive and free from the use of supplemental oxygen, invasive or noninvasive mechanical ventilation, renal-replacement therapy, vasopressors, extracorporeal membrane oxygenation, and organ support; and days out of the ICU in the first 28 days of the study and days of hospital stay among all patients and among patients surviving to day 90 (Table S2). We calculated serial Sequential Organ Failure Assessment (SOFA) scores. Virologic outcomes included time-to-clearance of MERS-CoV RNA and serial cycle-threshold values for the genes *upE* and *ORF1* in respiratory samples. Functional status at day 90 was assessed with the use of the Karnofsky performance-status score. Safety outcomes included reports of serious adverse events and adverse events, graded according to the Common Terminology Criteria for Adverse Events, version 4, of the National Institutes of Health (Table S3).

DATA AND SAFETY MONITORING BOARD AND INTERIM ANALYSES

According to the protocol, the first interim analysis was to be conducted when 34 patients had completed 90 days of follow-up.[17,18] At that time, the data and safety monitoring board advised that the trial be continued, and the total sample size for the trial was reestimated to be 114. Because of the Covid-19 pandemic, the data and safety monitoring board called for an unplanned interim analysis on March 15, 2020. Before performing the second analysis, the statistician on the data and safety monitoring board calculated the futility and efficacy boundaries according to the statistical analysis plan (Table S4).[18] Although the prespecified efficacy boundaries had not been met, the data and safety monitoring board recommended on April 14, 2020, to "terminate subject enrollment and proceed with all haste in analyzing and reporting the results in peer-reviewed format" (see the letter in the Supplementary Appendix).

STATISTICAL ANALYSIS

All the analyses were conducted according to the intention-to-treat principle and in accordance with the published statistical analysis plan.[18,19] Mortality at 90 days in each trial group was a combined estimate of the observed 90-day mortality in the first and second interim analyses, weighted by the inverse variance of each estimate. The absolute difference in risk was calculated as the difference in 90-day mortality between the intervention group and the placebo group. The upper boundary of the 97.5% confidence interval and one-sided P value were calculated to account for the features of the adaptive design of the trial.[18,19] We performed a crude

analysis of the primary outcome that did not account for the current adaptive trial design in order to show the gain in power achieved by the use of this approach in trials of treatment for uncommon conditions, such as MERS.

Prespecified subgroup analyses[17,18] were conducted with the use of multivariable log-binomial regression. We tested for heterogeneity of treatment effect across various subgroups and reported the corresponding P value for interaction. The subgroups were defined on the basis of time since onset of symptoms (≤7 days or >7 days), the score on the Acute Physiology and Chronic Health Evaluation (APACHE) II (>20 points or ≤20 points; scores range from 0 to 71, with higher scores indicating more severe disease and a higher risk of death), use of mechanical ventilation (yes or no), receipt of vasopressor therapy (yes or no), and receipt of renal-replacement therapy (yes or no).

The primary outcome analysis was one-sided with a type I error of 2.5%.[18] All the other analyses were two-sided with a type I error of 5%. To adjust for multiple testing for the secondary analyses, we reported the false discovery rate.[18,20] All the analyses were conducted with the use of SAS software, version 9.4 (SAS Institute).

RESULTS

PATIENTS

From November 2016 through April 2020, we assessed 182 patients, of whom 96 were enrolled and underwent randomization. One patient in the intervention group withdrew informed consent and did not give permission for data use. Of the 95 remaining patients, 43 were assigned to receive recombinant interferon beta-1b and lopinavir–ritonavir (intervention) and 52 to receive placebo (Fig. S1 and Table S5). Among the screened patients, 58 did not meet the eligibility criteria, most commonly because of an absence of new organ dysfunction that was judged to be related to MERS (in 41 patients). Another 28 patients were eligible for the trial but were not enrolled, mainly because of a lack of informed consent (in 26 patients).

The patients' baseline demographic characteristics, Karnofsky performance-status scores, coexisting conditions, severity-of-illness measures, and organ support and laboratory variables were similar in the two trial groups (Table 1 and Table S6). At the time of enrollment, 18 patients (42%) in the intervention group and 21 patients (40%) in the placebo group were receiving invasive or noninvasive mechanical ventilation.

The median time from illness onset to enrollment was 7 days in the intervention group and 7.5 days in the placebo group (Table 2). Patients received a median of 7 doses of recombinant interferon beta-1b or 7 doses of corresponding placebo; they also received a median of 27 doses of lopinavir–ritonavir or 26 doses of corresponding placebo. Courses that were shorter than 14 days were related to early deaths or to interruption of the regimen, with elevated liver-enzyme levels being the most frequent reason for dose interruption (Table S7). During the trial period, the frequency of various cointerventions did not differ significantly between the two groups (Table 2 and Table S8).

PRIMARY OUTCOME

Death from any cause at 90 days occurred in 12 patients (28%) in the intervention group and in 23 patients (44%) in the placebo group. The analysis of the primary outcome (90-day mortality) that accounted for the adaptive design yielded a risk difference of –19 percentage points (upper boundary of the 97.5% confidence interval [CI], –3; one-sided P=0.024) (Fig. 1A, Table 3, and Table S9).

The prespecified subgroup analysis showed that patients who had been treated within 7 days after symptom onset had lower 90-day mortality with the intervention than with placebo (relative risk, 0.19; 95% CI, 0.05 to 0.75), whereas patients who had been treated after 7 days did not (relative risk, 1.18; 95% CI, 0.63 to 2.21; P=0.006 for interaction; false discovery rate for multiple comparisons, 0.03). Across the other prespecified subgroups, there was no evidence of heterogeneity of treatment effect on the primary outcome (Fig. 1B).

SECONDARY OUTCOMES

The median number of days that patients were free from invasive or noninvasive mechanical ventilation was 16 days (interquartile range, 0 to 28) in the intervention group, as compared with 5.5 days (interquartile range, 0 to 28) in the placebo group; the median number of days that patients were alive outside the ICU was 9 days (interquartile range, 0 to 28) and 0 days (inter-

Table 1. Characteristics of the Patients at Baseline.*

Characteristic	Intervention Group (N=43)	Placebo Group (N=52)
Median age (IQR) — yr	56 (43–67)	56 (43.5–67)
Male sex — no. (%)	31 (72)	44 (85)
Body-mass index	28.3±5.5	27.2±5.7
Acquisition of MERS — no. (%)		
Community-acquired acquisition	28 (65)	38 (73)
Nosocomial acquisition	15 (35)	14 (27)
Coinfection with any other virus or bacterium — no. (%)	6 (14)	7 (13)
APACHE II score†	19.1±10.4	20.4±11.1
Median SOFA score (IQR)‡	6 (3–9)	6 (3–9.5)
Median Karnofsky performance-status score (IQR)§	90 (70–100)	100 (80–100)
Coexisting conditions — no. (%)		
Any chronic coexisting condition	38 (88)	45 (87)
Chronic cardiac disease	9 (21)	13 (25)
Chronic pulmonary disease	3 (7)	2 (4)
Chronic renal disease	9 (21)	15 (29)
Diabetes with chronic complications	18 (42)	17 (33)
Location at time of randomization — no. (%)		
Ward	14 (33)	13 (25)
ICU	29 (67)	39 (75)
Randomization stratum — no. (%)		
Mechanical ventilation	18 (42)	21 (40)
No mechanical ventilation	25 (58)	31 (60)
Interventions before randomization — no. (%)		
Renal-replacement therapy	10 (23)	14 (27)
Vasopressor therapy	8 (19)	12 (23)
Neuromuscular blockade	11 (26)	15 (29)
Glucocorticoids	15 (35)	15 (29)
Laboratory results before randomization — median (IQR)		
Platelet count per mm^3	181,000 (145,000–254,000)	182,500 (154,000–235,000)
White-cell count per mm^3	6300 (5000–9000)	6900 (4100–8900)
Lymphocyte count per mm^3	1000 (500–1300)	800 (600–1200)
Aspartate aminotransferase — U/liter	55 (33–79)	78 (45–114)
Alanine aminotransferase — U/liter	42 (29–62)	46 (22–76)
Bilirubin — μmol/liter	9 (6–12)	9 (6–16)
Creatinine — μmol/liter	79 (64–202)	94 (68–315)

* Plus–minus values are means ±SD. Patients in the intervention group received recombinant interferon beta-1b and lopinavir–ritonavir. Continuous variables were compared between the two trial groups with the use of an independent Student's t-test or Mann–Whitney test, and categorical variables were compared with the use of a chi-square test or Fisher's exact test. None of the baseline characteristics differed significantly between the two trial groups. Data on the following characteristics were missing as follows: on the body-mass index (the weight in kilograms divided by the square of the height in meters), for one patient in the placebo group; on the lymphocyte count, for five patients in the intervention group and three in the placebo group; on the aspartate aminotransferase level, for two and one, respectively; on the alanine aminotransferase level for one and one, respectively; and on the bilirubin level, for one in the placebo group. To convert the values for bilirubin to milligrams per deciliter, divide by 17.1. To convert the values for creatinine to milligrams per deciliter, divide by 88.4. Additional details on the baseline characteristics are provided in Table S6. ICU denotes intensive care unit, IQR interquartile range, and MERS Middle East respiratory syndrome.

† Scores on the Acute Physiology and Chronic Health Evaluation (APACHE) II range from 0 to 71, with higher scores indicating more severe disease and a higher risk of death.

‡ Scores on the Sequential Organ Failure Assessment (SOFA) range from 0 to 24, with higher scores indicating a greater degree of organ dysfunction.

§ Karnofsky performance-status scores range from 0 (death) to 100 (normal performance status).

Table 2. Interventions and Cointerventions during the Trial Period.*

Variable	Intervention Group (N=43)	Placebo Group (N=52)
Interventions — median (IQR)		
No. of interferon beta-1b or placebo injection doses	7 (5–7)	7 (4–7)
No. of lopinavir–ritonavir or placebo doses	27 (14–28)	26 (12.5–28)
Time from onset of symptoms to randomization — days	7 (5–11)	7.5 (5–10)
Time from admission to randomization — days	2 (1–4)	1 (1–3)
Time from randomization to receipt of the first dose — days	0 (0–0)	0 (0–0)
Time from the first positive RT-PCR assay to receipt of the first dose — days	1 (0–3)	1 (1–2.5)
Cointerventions		
Vasopressor therapy — no. (%)	21 (49)	30 (58)
Renal-replacement therapy — no. (%)	19 (44)	24 (46)
Neuromuscular blockade — no. (%)	22 (51)	28 (54)
Invasive mechanical ventilation — no. (%)	26 (60)	35 (67)
Noninvasive mechanical ventilation — no. (%)	4 (9)	9 (17)
Extracorporeal membrane oxygenation — no. (%)	6 (14)	5 (10)
Glucocorticoids — no. (%)	27 (63)	31 (60)
Median duration of glucocorticoid therapy (IQR) — days†	8 (6–13)	9 (4–18)

* Continuous variables were compared between the two trial groups with the use of an independent Student's t-test or Mann–Whitney test, and categorical variables were compared with the use of a chi-square test or Fisher's exact test. None of the variables differed significantly between the two trial groups. Additional details on interventions and cointerventions are provided in Table S8. RT-PCR denotes reverse transcriptase–polymerase chain reaction.

† The analysis of duration of glucocorticoid therapy included only patients who received a glucocorticoid.

quartile range, 0 to 18), respectively. Results of other secondary outcomes are shown in Table 4 and Figure S2. The time to clearance of MERS-CoV RNA and the serial cycle-threshold values for the genes *upE* and *ORF1* in respiratory samples did not differ significantly between the two groups (Table 4 and Fig. S3). At day 90, the median Karnofsky performance-status score was 70 points (interquartile range, 0 to 100) in the intervention group and 50 points (interquartile range, 0 to 100) in the placebo group.

SAFETY OUTCOMES

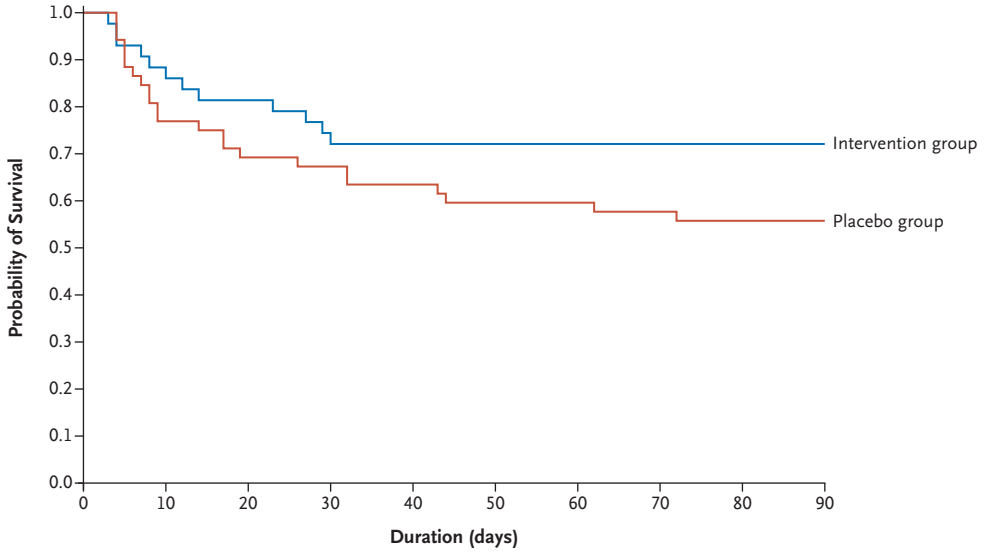
Serious adverse events were reported in 4 patients (9%) in the intervention group and in 10 (19%) in the placebo group. The majority of serious adverse events were related to elevated liver-enzyme levels. The incidence of adverse events did not differ significantly between the two trial groups (Tables S10 and S11).

DISCUSSION

We found that, among hospitalized patients with laboratory-confirmed MERS, treatment with recombinant interferon beta-1b and lopinavir–ritonavir resulted in lower 90-day mortality than use of placebo. The treatment effect was observed in patients who were treated within 7 days after symptom onset, among whom mortality was lower with the intervention than with placebo. In contrast, a similar treatment effect was not observed with later initiation of therapy. Our findings of an important time-to-treatment effect on mortality are consistent with earlier observations in patients with severe influenza who were given oseltamivir[21-24] and with the results of a recent trial of remdesivir in patients with severe Covid-19.[25]

A majority of patients in the intervention group received the 14-day planned course of treatment

A



No. at Risk

Intervention group	43	38	35	32	31	31	31	31	31	31
Placebo group	52	40	36	35	33	31	31	30	29	29

B

Subgroup	Intervention Group	Placebo Group	Relative Risk (95% CI)	P Value for Interaction	FDR
	no. of patients with event/ no. of patients (%)				
Time from onset of symptoms to enrollment				0.006	0.03
≤7 days	2/23 (9)	12/26 (46)	0.19 (0.05–0.75)		
>7 days	10/20 (50)	11/26 (42)	1.18 (0.63–2.21)		
APACHE II score				0.30	0.75
>20	11/21 (52)	18/24 (75)	0.70 (0.44–1.12)		
≤20	1/22 (5)	5/28 (18)	0.25 (0.03–2.02)		
Mechanical ventilation				0.54	0.82
Yes	8/18 (44)	13/21 (62)	0.72 (0.39–1.33)		
No	4/25 (16)	10/31 (32)	0.50 (0.18–1.39)		
Vasopressor therapy				0.70	0.82
Yes	5/8 (62)	10/12 (83)	0.75 (0.41–1.36)		
No	7/35 (20)	13/40 (32)	0.62 (0.28–1.37)		
Renal-replacement therapy				0.82	0.82
Yes	5/10 (50)	10/14 (71)	0.70 (0.35–1.41)		
No	7/33 (21)	13/38 (34)	0.62 (0.28–1.37)		

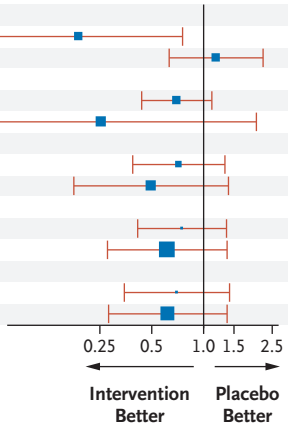


Figure 1. Probability of Survival and Subgroup Analyses.

Panel A shows a Kaplan–Meier survival analysis involving all the patients enrolled in the trial. Panel B shows the results of prespecified subgroup analyses of the primary outcome (90-day all-cause mortality). Two-sided P values for interaction are reported. The false discovery rate (FDR) accounts for multiplicity by calculating the expected proportion of tests with false positives at a specified rank of a set of tests. The size of each square is proportional to the subgroup sample size. Scores on the Acute Physiology and Chronic Health Evaluation (APACHE) II range from 0 to 71, with higher scores indicating more severe disease and a higher risk of death.

Table 3. 90-Day All-Cause Mortality (Primary Outcome).

Variable	Intervention Group (N=43)	Placebo Group (N=52)	Risk Difference (Upper Boundary of 97.5% CI)	One-Sided P Value
			percentage points	
Death by day 90 — no. (%)	12 (28)	23 (44)		
Primary analysis: 90-day mortality — %*	29	48	–19 (–3)	0.024
Crude analysis: 90-day mortality — %†	28	44	–16 (3)	0.05

* The primary analysis accounted for the adaptive design of the trial according to the published statistical analysis plan. Mortality at 90 days in each trial group was a combined estimate of the observed 90-day mortality from the first and second interim analyses, weighted by the inverse variance of each estimate. The risk difference was calculated as the difference in 90-day mortality between the intervention group and the placebo group. The upper boundary of the 97.5% confidence interval was calculated. A one-sided P value was calculated with the use of the Z test for difference in proportion. A one-sided P value of 0.025 or less was considered to indicate statistical significance.

† A crude analysis was conducted, for illustration purposes, with the use of a Z test. The reported one-sided P value does not account for the planned stage-wise approach or for the error spending and conditional error principle used in the design of the trial, which provide more gain in power than the traditional crude approach.[19] Results of multivariate analyses are reported in Table S12.

with recombinant interferon beta-1b and lopinavir–ritonavir. The incidences of interruptions in the trial regimen and of adverse events were similar in the intervention group and the placebo group, although serious adverse events were numerically more common in the placebo group than in the intervention group. Our findings suggest that these events were related to the disease rather than to the treatment with recombinant interferon beta-1b and lopinavir–ritonavir. An open-label, randomized trial of lopinavir–ritonavir in 199 patients with Covid-19 also showed that lopinavir–ritonavir monotherapy was associated with a lower incidence of serious adverse events than was observed in control patients receiving standard care, although the treatment was not associated with a significantly shorter time to clinical improvement.[26]

One practical consideration related to our trial is the administration of lopinavir–ritonavir through a nasogastric tube in critically ill patients. The crushing of lopinavir–ritonavir tablets is associated with reduced and unpredictable bioavailability (reduction by approximately 50%; range, 5 to 75).[27] Consequently, patients treated in the MIRACLE trial who were unable to swallow tablets received lopinavir–ritonavir as a suspension.[17] Preliminary data from the RECOVERY (Randomised Evaluation of Covid-19 Therapy) and Solidarity trials of lopinavir–ritonavir monotherapy in hospitalized patients with Covid-19 showed no significant difference in mortality, but data about the timing of the intervention are not available at this time.[28,29]

MERS-CoV and SARS-CoV-1, the virus that causes SARS, was found to suppress the release of interferon in preclinical studies.[30-35] SARS-CoV-2, the virus that causes Covid-19, causes similar effects in diminishing type I and type III interferon signatures in infected primary human bronchial cells and in a ferret model.[36] Patients with severe Covid-19 have impaired type 1 interferon signatures, as compared with patients who have mild or moderate illness.[37] Recently, inborn errors of type 1 interferon immunity or autoantibodies against interferon alfa subtypes and, less commonly, interferon beta have been described in patients with life-threatening Covid-19.[38,39] These lines of evidence support studies of treatment with interferon beta in patients with Covid-19.

In a recent randomized, controlled trial, a combination of lopinavir–ritonavir, ribavirin, and recombinant interferon beta-1b alleviated symptoms and shortened the duration of viral RNA detection and hospital stay in patients with mild Covid-19, as compared with patients who received treatment with lopinavir–ritonavir alone.[40] In that trial, recombinant interferon beta-1b was omitted in patients who were recruited after day 7 after symptom onset because of concerns about its proinflammatory effects.[40-42] Our trial showed that benefit was likely with early treatment but unlikely with later therapy. Small, randomized, controlled trials of interferon beta-1a or inter-

Table 4. Secondary Outcomes.*

Outcome	Intervention Group (N=43)	Placebo Group (N=52)	Relative Risk (95% CI)
Death from any cause — no. (%)			
By day 28	10 (23)	17 (33)	0.71 (0.36–1.39)
During ICU stay	12 (28)	22 (42)	0.66 (0.37–1.17)
During hospital stay	13 (30)†	23 (44)	0.68 (0.40–1.18)
Alive at day 90 — no. (%)			
Receiving renal-replacement therapy	6 (14)	3 (6)	2.42 (0.64–9.11)
Receiving supplemental oxygen	3 (7)	1 (2)	3.63 (0.39–33.63)
Receiving invasive mechanical ventilation	2 (5)	1 (2)	2.42 (0.23–25.78)
Median duration (IQR) — days‡			
Free from supplemental oxygen therapy	4 (0–19)	0 (0–17)	
Free from invasive or noninvasive mechanical ventilation	16 (0–28)	5.5 (0–28)	
Free from renal-replacement therapy	28 (7–28)	22 (0–28)	
Free from vasopressor therapy	27 (3–28)	24.5 (0–28)	
Free from extracorporeal membrane oxygenation	28 (0–28)	28 (0–28)	
Free from organ support	15 (0–28)	5 (0–27.5)	
Outside the ICU	9 (0–28)	0 (0–18)	
Of hospital stay			
Among all patients	22 (11–40)	17.5 (8–34)	
Among patients surviving to 90 days	26 (14–43)	20 (10–37)	
Virologic outcomes			
Median no. of days to MERS-CoV RNA clearance (IQR)§			
Among all patients	17 (9–25)	20 (10–33)	
Among patients surviving to 90 days	13 (8–21)	12 (9–22)	
Functional outcome			
Median Karnofsky performance-status score at day 90 (IQR)¶	70 (0–100)	50 (0–100)	
Safety outcomes			
Serious adverse event — no. (%)‖	4 (9)	10 (19)	0.48 (0.16–1.43)
Acute pancreatitis	0	1 (2)	—
Elevation of alanine aminotransferase level to >5× ULN	4 (9)	9 (17)	0.54 (0.18–1.62)
Other serious adverse event	0	1 (2)	—

* The 95% confidence intervals have not been adjusted for multiplicity, and therefore inferences drawn from these intervals may not be reproducible. MERS-CoV denotes MERS coronavirus, and ULN upper limit of the normal range.
† One patient in the intervention group died during the hospital stay after day 90.
‡ The calculations of days free from supplemental oxygen therapy, days free from mechanical ventilation, days free from renal-replacement therapy, days free from vasopressor therapy, days free from extracorporeal membrane oxygenation, days free from organ support, and days outside the ICU were based on 28 days of observation.
§ Days to clearance of MERS-CoV RNA were censored by death or hospital discharge.
¶ Data on the Karnofsky performance-status score at day 90 were not available for one patient in the placebo group.
‖ P values for serious adverse events are provided in Table S10.

feron beta-1b have suggested clinical benefit in hospitalized patients with Covid-19,[43,44] and preliminary results of a phase 2 study involving patients with mild-to-moderate Covid-19 have suggested that treatment with inhaled recombinant interferon beta-1a results in a lower risk of severe disease (involving the use of mechanical ventilation or resulting in death) than placebo.[45] Recombinant interferon beta-1a and interferon beta-1b are currently under study in patients

with Covid-19 in multiple randomized, controlled trials (ClinicalTrials.gov numbers, NCT04492475 and NCT02735707; ISRCTN Registry number, ISRCTN83971151).

The immunomodulatory effects of recombinant interferon beta-1b have been examined in unselected patients with moderate-to-severe ARDS.[46-48] An early-phase, nonrandomized study showed that treatment with recombinant interferon beta-1a was associated with lower 28-day mortality than use of placebo.[46] A phase 3, randomized, controlled trial showed no difference between interferon beta-1a and placebo in the primary composite outcome (death and the number of ventilator-free days at day 28).[47] A post hoc analysis showed that use of recombinant interferon beta-1a with glucocorticoids was associated with higher mortality, whereas the administration of recombinant interferon beta-1a without glucocorticoids was not.[47,48] Hydrocortisone inhibits interferon beta signaling and the up-regulation of CD73 in human lung tissues ex vivo.[49] In our trial, approximately 30% of the patients were receiving glucocorticoids at the time of enrollment and another 30% did so during the course of the trial. More data are needed about the biologic interaction of glucocorticoids with recombinant interferon beta therapy in patients with Covid-19. This is especially important given the common use of glucocorticoids in patients with Covid-19 and the recent findings of a survival benefit with dexamethasone in hospitalized patients with Covid-19 who were receiving supplemental oxygen or greater levels of ventilatory support.[50]

We did not observe accelerated viral RNA clearance with the intervention or differences between the two groups in the cycle-threshold values over time. Our virologic findings are limited by a lack of data on quantitative viral RNA detection or infectious virus isolation from lower respiratory tract samples. In one study of SARS-CoV-2 in nonhuman primates, the effect of early antiviral therapy with remdesivir appeared to be greater on infectious virus recovery in bronchoalveolar-lavage samples than on viral RNA detection in bronchoalveolar-lavage or upper respiratory tract samples.[51] It may be that prolonged viral RNA detectability in patients with MERS makes this an insensitive marker of antiviral efficacy.

Some of the features of the results of our trial are direct consequences of the challenges of conducting a trial involving patients with an uncommon disease that is sporadic in geographic and temporal distribution. Variation in the number of enrolled patients according to site, owing to regional distribution of cases and to some sites being assigned to be MERS referral hospitals, led to low recruitment at many sites and to a slight sample-size imbalance between the two trial groups.

Strengths of our trial include the randomized, double-blind, placebo-controlled design. We followed patients up to 90 days for mortality. The survival curves in our trial continued to diverge beyond day 28, which may be related to additional deaths in the placebo group as a consequence of organ failure — a result that suggests the importance of longer-term follow-up in trials of therapeutic agents for patients with severe or critical Covid-19. We followed a pragmatic approach in relation to standard of care, in order to reflect current practice and to facilitate the enrollment of patients. Because of the uncommon, episodic nature of MERS, we used a recursive two-stage design that provided flexibility to introduce sample-size adjustment while allowing for continual learning from the observed data without compromise of the overall trial type I error. Finally, a trial design with early futility boundaries provided a gain in power over the traditional approach.[18,19] Limitations of the trial include its early termination, which reduced the power. With a sample size of 95, the trial had insufficient power to detect differences in secondary analyses. An inherent problem of adaptive designs is that early stopping might lead to overestimation of the treatment effect.

In this trial involving hospitalized patients with laboratory-confirmed MERS, we found that combination therapy with recombinant interferon beta-1b and lopinavir–ritonavir led to lower mortality at 90 days than placebo. The effect was greatest when treatment was started within 7 days after symptom onset.

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APPENDIX

The authors' full names and academic degrees are as follows: Yaseen M. Arabi, M.D., Ayed Y. Asiri, M.D., Abdullah M. Assiri, M.D., Hanan H. Balkhy, M.D., Ali Al Bshabshe, M.D., Majed Al Jeraisy, Pharm.D., Yasser Mandourah, M.D., Mohamed H.A. Azzam, M.D., Abdulhadi M. Bin Eshaq, M.D., Sameera Al Johani, M.B., B.S., Shmeylan Al Harbi, Pharm.D., B.C.P.S, Hani A.A. Jokhdar, M.B., Ch.B., Ph.D., Ahmad M. Deeb, R.N., M.S.N., Ziad A. Memish, M.D., Jesna Jose, M.Sc., Sameeh Ghazal, M.D., Sarah Al Faraj, M.D., Ghaleb A. Al Mekhlafi, M.D., Nisreen M. Sherbeeni, M.D., Fatehi E. Elzein, M.D., Fahad Al-Hameed, M.D., Asim Al Saedi, M.D., Naif K. Alharbi, Ph.D., Robert A. Fowler, M.D.C.M., Frederick G. Hayden, M.D., Abdulaziz Al-Dawood, M.D., Mohamed Abdelzaher, M.D., Wail Bajhmom, M.B., B.S., Ph.D., Badriah M. AlMutairi, R.N., Mohamed A. Hussein, M.S.C.S., M.S.P.H., Ph.D., and Adel Alothman, M.D.

The authors' affiliations are as follows: the Intensive Care Department (Y.M.A., A.A.-D.) and the Departments of Infection Prevention and Control (H.H.B.), Pathology and Laboratory Medicine (S.A.J.), Pharmaceutical Care (S.A.H.), and Medicine (A.A.), King Abdulaziz Medical City, Ministry of National Guard Health Affairs, the College of Medicine (Y.M.A., S.A.J., A.A.-D., A.A.) and the College of Pharmacy (M.A.J., S.A.H.), King Saud bin Abdulaziz University for Health Sciences, Prince Mohammed bin Abdulaziz Hospital (A.Y.A., Z.A.M., S.G., S.A.F.), Infection Prevention and Control, Preventive Health (A.M.A.), and Deputyship for Public Health (H.A.A.J.), Ministry of Health, Clinical Trials Services (M.A.J., A.M.D., B.M.A.) and the Departments of Biostatistics and Informatics (J.J., M.A.H.) and Infectious Disease Research (N.K.A.), King Abdullah International Medical Research Center, the Military Medical Services, Ministry of Defense (Y.M.), the Department of Intensive Care Services (G.A.A.M.), and the Infectious Diseases Division (N.M.S., F.E.E.), Prince Sultan Military Medical City, and the College of Medicine, Alfaisal University (Z.A.M.), Riyadh, the Department of Critical Care Medicine, King Khalid University, Aseer Central Hospital, Abha (A.A.B.), Medical Services (M.H.A.A.) and the Department of Critical Care Medicine (M.A.), King Abdullah Medical Complex, the Health Directorate, Ministry of Health (M.H.A.A.), and the Internal Medicine Department, King Fahad General Hospital, Ministry of Health (W.B.), the Intensive Care Department (F.A.-H.) and the Department of Infection Prevention and Control (A.A.S.), Ministry of National Guard Health Affairs, and the College of Medicine and King Saud bin Abdulaziz University for Health Sciences, King Abdullah International Medical Research Center (F.A.-H., A.A.S.), Jeddah, and the Intensive Care Department, King Khalid Hospital, Najran (A.M.B.E.) — all in Saudi Arabia; the World Health Organization, Geneva (H.H.B.); the Hubert Department of Global Health, Rollins School of Public Health, Emory University, Atlanta (Z.A.M.); the Departments of Critical Care Medicine and Medicine, Sunnybrook Hospital, and the Institute of Health Policy Management and Evaluation, University of Toronto, Toronto (R.A.F.); and the Division of Infectious Diseases and International Health, Department of Medicine, University of Virginia School of Medicine, Charlottesville (F.G.H.).

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