Engineered interferon alpha effectively improves clinical outcomes of COVID-19 patients

Chuan Li
Department of Thoracic Surgery, West China Hospital, Sichuan University
Fengming Luo
Department of Pulmonary and Critical Care Medicine, West China Hospital, Sichuan University
Chengwu Liu
Department of Thoracic Surgery, West China Hospital, Sichuan University
Nian Xiong
Department of Neurology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology
Zhihua Xu
Department of Critical Care Medicine, Mianyang Central Hospital
Wei Zhang
Department of Respiratory and Critical Care Medicine, First affiliated hospital, the Second Military Medical University
Ming Yang
Department of Respiratory Medicine, The Public Health Clinical Center of Chengdu
Ye Wang
Department of Pulmonary and Critical Care Medicine, West China Hospital, Sichuan University
Dan Liu
Department of Pulmonary and Critical Care Medicine, West China Hospital, Sichuan University
Chao Yu
Department of Respiratory and Critical Care Medicine, Naval Hospital of Eastern Theater of PLA
Jia Zeng
Department of Aviation Disease, Naval medical center of PLA, the Second Military Medical University
Li Zhang
Department of Respiratory Disease, Wuhan Red Cross Hospital
Duo Li
Department of Respiratory Disease, The Affiliated Hospital of Southwest Medical University
Yanbin Liu
Center of Infectious Diseases, West China Hospital, Sichuan University
Mei Feng
Department of Pulmonary and Critical Care Medicine, West China Hospital, Sichuan University
Ruoyang Liu

Department of Respiratory Disease, Sichuan Second Hospital of T. C. M

Jiandong Mei

Department of Thoracic Surgery, West China Hospital, Sichuan University

Senyi Deng

Department of Thoracic Surgery, West China Hospital, Sichuan University

Zhen Zeng

Department of Thoracic Surgery, West China Hospital, Sichuan University

Yuanhong He

Department of Infectious Disease, The Public Health Clinical Center of Chengdu

Haiyan Liu

Department of Tuberculosis, The Public Health Clinical Center of Chengdu

Zhengyu Shi

Department of Tuberculosis, The Public Health Clinical Center of Chengdu

Meng Duan

Department of Liver Disease, The Public Health Clinical Center of Chengdu

Deying Kang

Department of Evidence based Medicine and Clinical Epidemiology, West China Hospital, Sichuan University

Jiayu Liao

Department of Bioengineering, Bourns College of Engineering, University of California

Weimin Li

Department of Pulmonary and Critical Care Medicine, West China Hospital, Sichuan University

Lunxu Liu (lunxu_liu@aliyun.com)

Department of Thoracic Surgery, West China Hospital, Sichuan University

---

Research Article

Keywords: COVID-19,severe acute respiratory syndrome coronavirus 2,interferon,recombinant super-compound interferon,treatment

DOI: https://doi.org/10.21203/rs.3.rs-65224/v1

Abstract

Interferons are key to the antiviral host defense, yet the therapeutic value of interferon for coronavirus disease 2019 (COVID-19) is unknown. Recombinant super-compound interferon (rSIFN-co) is a new genetically engineered interferon, thus we conducted a multicenter, randomized controlled trial (ChiCTR2000029638) to evaluate the efficacy and safety of recombinant super-compound interferon versus traditional interferon alpha added to baseline antiviral agents (lopinavir–ritonavir or umifenovir) for the treatment of moderate-to-severe COVID-19. Participants received rSIFN-co (12 million international units [IU], twice daily) or interferon alpha (5 million IU, twice daily) nebulization added to baseline antiviral agents for no more than 28 days. The primary outcome was the time to clinical improvement. Secondary outcomes included the overall rate of clinical improvement assessed on day 28，the time to radiological improvement and virus nucleic acid negative conversion, and adverse events. 94 patients hospitalized with moderate-to-severe COVID-19 were included in the safety set (46 patients assigned to rSIFN-co group, 48 to interferon alpha group). Individuals in the rSIFN-co group showed shorter time to clinical improvement (11.5 days vs 14.0 days; $P = 0.019$) as compared to those in the interferon alpha group. The overall rate of clinical improvement on day 28 was much higher in the rSIFN-co group than that in the interferon alpha group (93.5% vs 77.1%; difference, 16.4%; 95% confidence interval 3% to 30%). The time to radiological improvement and the time to virus nucleic acid negative conversion were also much shorter in the rSIFN-co group (8.0 days vs 10.0 days, $P = 0.002$; 7.0 days vs 10.0 days, $P = 0.018$, respectively). Adverse events were reported in 13 (28.3%) patients in the rSIFN-co group and 18 (37.5%) patients in the interferon alpha group. No patients died during the study. Our study showed that rSIFN-co added to antiviral agents was safe and more efficient than interferon alpha plus antiviral agents in the treatment of moderate-to-severe COVID-19. Future clinical study of rSIFN-co therapy alone or combined with other antiviral therapy is warranted.

Introduction

The ongoing coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has affected more than nineteen million people worldwide as of 10 August 2020. Although most of the infections have been self-limited, about 20% of the infected adults have been found to develop severe pneumonia or a critical illness which, in some cases, has led to death[1,2,3]. Thus far, treatment strategies have included standard supportive care, corticosteroids, intravenous immunoglobulin, and empirical or repurposed antiviral therapies (e.g. remdesivir, ribavirin, lopinavir–ritonavir, umifenovir, and interferons, etc.)[4-9].

Interferon alphas, by inducing both innate and adaptive immune responses, have shown antiviral activity against other respiratory coronavirus, such as SARS-Cov and Middle East respiratory syndrome coronavirus (MERS-Cov)[10,11]. During the time period of this study, there were no effective antiviral therapies in treating patients with COVID-19. Interferon alpha nebulization is empirically recommended

for the treatment of SARS-CoV-2 pneumonia by the *Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia* released by the National Health Commission of China[12].

Recombinant super-compound interferon (rSIFN-co) is a new genetically engineered type interferon that was created by changing 65 bases of 60 amino acid genetic codes of interferon alphacon-1 without changing its amino acid composition. The changes altered the protein's spatial conformation, which led to 20 times stronger antiviral activity (including against SARS-CoV), and reduced toxicity and side effects as compared with its prototype [13,14]. rSIFN-co can be safely used in large doses (each dose can be >10 million international units [IU]), making it possible to treat some viral diseases or tumors that require large doses of interferon[13-17]. Therefore, rSIFN-co was considered as a possible therapeutic option for the treatment of COVID-19[17].

We conducted a multicenter, single-blind, randomized controlled trial to evaluate the efficacy and safety of rSIFN-co versus interferon alpha combined with baseline antiviral agents in hospital-admitted adult patients presenting with moderate-to-severe COVID-19.

Results

Patients

Between 10 February and 5 April 2020, a total of 102 patients with COVID-19 from five hospitals in China were recruited and assessed for eligibility. Six patients did not have family consent and the remaining 96 patients were randomly divided into two groups (48 in each group) (**Fig. 1**). In the rSIFN-co group, two patients were excluded (one died within 24 h after randomization, and the other was prescribed interferon alpha instead of rSIFN-co due to the attending physician's misinterpretation of the randomization result). One patient in the interferon alpha group did not receive interferon alpha because of acute exacerbation of the disease and administration of invasive ventilation. Finally, 46 and 48 patients were included in the rSIFN-co group and interferon alpha group, respectively, for evaluation. The study groups were similar at baseline in terms of demographic characteristics, laboratory test results, distribution of ordinal scale scores, chest computed tomography (CT) results, patients' status and therapeutics received after enrollment (**Table 1 and 2**).

The median age of patients was 54.0 years (interquartile range [IQR] 39.8 to 63.3), and 46.8% of the patients were male. In the rSIFN-co group there were 39 moderate cases and 7 severe cases, while in the interferon alpha group, there were 43 moderate cases and 5 severe cases. The median interval between symptom onset and randomization was 14.0 days (IQR 5.0 to 30.0) in the rSIFN-co group and 14.5 days (IQR 7.0 to 31.0) in the interferon alpha group. Some patients (rSIFN-co, n = 26; interferon alpha, n = 27) received symptomatic treatment (such as cough relief and fever reduction) and/or supportive care before randomization. All patients received lopinavir-ritonavir or umifenovir at baseline. During the trial, nine patients received systemic glucocorticoids (four in the rSIFN-co group and five in the interferon alpha group).

One patient in the interferon alpha group suffered disease deterioration and was transferred to another hospital, which was designated for critical ill patients. The other 10 patients (rSIFN-co, *n* = 2; interferon alpha, *n* = 8) were transferred to other hospitals according to the government's unified deployment. The detailed information about the status of these patients when transferred are summarized in **Supplementary Table 1**. The outcomes of these 11 patients were assessed when transferred and included in the final analysis.

Primary outcome

The time to clinical improvement in the rSIFN-co group was statistically shorter than that in the interferon alpha group (median, 11.5 days vs 14.0 days; hazard ratio [HR], 1.76; 95% confidence interval [CI], 1.10 to 2.81; *P* = 0.019) (**Table 3 and Fig. 2**).

Secondary outcomes

The overall rate of clinical improvement on day 28 was much higher in the rSIFN-co group than that in the interferon alpha group (93.5% vs 77.1%; difference, 16.4 percentage points; 95% CI, 3% to 30%) (**Table 3**). The time to radiological improvement in the rSIFN-co group was significantly shorter than that in the interferon alpha group (median, 8.0 days vs 10.0 days; HR, 2.19; 95% CI, 1.32 to 3.62; *P* = 0.002) (**Table 3 and Fig. 2**). The time to virus nucleic acid negative conversion in the rSIFN-co group was also significantly shorter than that in the interferon alpha group (median 7.0 days vs 10.0 days; HR, 1.74; 95% CI 1.10 to 2.74; *P* = 0.018) (**Table 3 and Fig. 2**).The overall rates of radiological improvement on chest CT scans on days 7 and 28 were numerically higher in the rSIFN-co group than those in the interferon alpha group, however a significant difference was only observed between the two groups on day 14 (84.8% vs 66.7%; difference, 18.1 percentage points; 95% CI, 1% to 35%) (**Table 3**). The overall rate of virus nucleic acid negative conversion on day 28 was much higher in the rSIFN-co group than that in the interferon alpha group (97.8% vs 85.4%; difference, 12.4 percentage points; 95% CI, 2% to 23%), while the overall rates on day 7 and 14 in the rSIFN-co group were numerically higher than those in the interferon alpha group (**Table 3**). One patient in the interferon alpha group experienced a secondary bacterial infection and developed respiratory failure on day 6. Tracheal intubation and mechanical ventilation were applied to her. She was transferred to another hospital for further treatment on day 10.

Safety

Adverse events (AEs) were reported by 13 (28.3%) of the 46 patients in the rSIFN-co group and 18 (37.5%) of the 48 patients in the interferon alpha group (**Supplementary Table 2**), most of which were classified as grade 1 or 2 with decreased appetite being the most common in both groups. There were no severe adverse events (SAEs) reported in the rSIFN-co group, while one patient had a secondary bacterial infection followed by respiratory failure in the interferon alpha group. The latter case was deemed to be a non-treatment related SAE, and interferon alpha administration was ceased and invasive mechanical ventilation was applied to this patient. There were no deaths in either group between the initiation of medication and day 28.

Discussion

In this multicenter, head-to-head, randomized controlled trial, the combination of rSIFN-co nebulization and antiviral agents significantly improved the recovery in moderate-to-severe patients with COVID-19 as compared with the combination of interferon alpha and antiviral agents. This benefit was seen in shortening the time to clinical improvement, time to radiological improvement, and time to virus nucleic acid negative conversion. Additionally, the clinical improvement rate on day 28 was also significantly higher in the rSIFN-co group than that in the interferon alpha group. This trial did not enroll any mild or critically ill patients because this was an exploratory study. Instead, we recruited moderate-to-severe patients with COVID-19. Our population mainly consisted of moderate COVID-19 (88.3%) and no patient was given invasive mechanical ventilation or extracorporeal membrane oxygenation at the time of enrollment. During the study period, patients with COVID-19 were admitted to hospitals of different levels according to the severity of the disease following the guidance of health administration department. Our patients were recruited from five hospitals designated for moderate-to-severe patients. The time interval between symptom onset and randomization varied among patients, and some of the participants had received symptomatic treatment and/or supportive care, but without clinical improvement. We distributed the participants evenly between the two groups by randomization. Although some patients were transferred during the middle of the study and failed to complete the whole treatment regimen according to the government's unified deployment, all of them were evaluated before transfer and were included in the final analysis.

Interferon alphas alone or combined with other antiviral agents have antiviral effects on multiple types of viral infections[10,11,18-21]. Findings from a preliminary, uncontrolled study revealed that interferon alphacon-1 plus corticosteroids was associated with reduced disease-associated impaired oxygen saturation, more rapid resolution of radiographic lung abnormalities in SARS patients, as well as interferon beta combined with ribavirin demonstrating antiviral activity against MERS[10,11]. In addition, SARS-CoV-2 is homologous with MERS-CoV and SARS-CoV and presenting similar properties, combination antiviral therapy with interferon alpha may be effective for COVID-19[22,23]. However, severe COVID-19 patients often develop acute respiratory distress syndrome (ARDS) or secondary haemophagocytic lymphohistiocytosis (sHLH)[24,25]. Both ARDS and sHLH are hallmarks of overwhelmed cytokine productions, so called cytokine storm or cytokine release syndrome (CRS), which is one of main causes of mortality[26,27]. Therefore, it has been controversial in clinic whether interferon alpha alone should be used for treating high pathological viruses, such as SARS-CoV-2, SARS-CoV and MERS-CoV, although interferon alpha were empirically recommended as one of therapeutic option for COVID-19 in clinical practice[12]. In both animal studies and clinic, the early treatment of interferon rescued mice from lethal doses of SARS-CoV and MERS, however, late interferon administration delayed viral clearance and exacerbate immunopathology[28,29]. In supporting the notion of anti-cytokine storm may be beneficial to COVID-19 patients, the administration of anti-inflammation drug, methylprednisolone, slowed down the disease progress and reduced dealth rate[30]. On the other hand, our study suggest that treatment of moderate-to-severe COVID-19 patients with interferon can ameliorate clinical outcomes. This result may

due to the nature of SARS-CoV-2 and related virus infections, such as SARS and MERS, to dysregulation of interferon alpha induction at early stage of infection[31].

rSIFN-co is a new homolog of interferon alpha and not yet commercialized, it has shown stronger antiviral effects and less side effects during preclinical use compared with traditional interferons[13,14,16,17]. Thus, we inferred that rSIFN-co might be a potential superior therapeutics for the treatment of COVID-19, and conducted this exploratory trial. Moreover, given that this study was occurring during a life-threatening pandemic, we chose the traditional interferon alpha as the control rather than having a placebo control. Although there were no antiviral agents confirmed to be effective during the study period, all participants in this study still received baseline antiviral agents (lopinavir–ritonavir or umifenovir) to make sure that all of the patients could benefit from any potential therapeutics.

Recently, a randomized controlled trial (RCT) first confirmed that the combination of interferon beta-1b and antiviral agents accelerated the recovery of patients with mild-to-moderate COVID-19 compared with single antiviral agent alone. They suggested that interferon beta-1b appeared to be a key component of the combination treatment in subgroup analysis[32]. Our study is the first RCT which demonstrated that the combination of interferon alpha and antiviral agents could reach encouraging results, even in moderate-to-severe cases. Meanwhile, our study confirmed the superiority of rSIFN-co versus interferon alpha when used in combination with baseline antiviral agents. The overall rates of clinical improvement were 93.5% and 77.1% on day 28 in the rSIFN-co group and interferon alpha group, respectively. Based on the fact that the baseline antiviral agents (lopinavir–ritonavir or umifenovir) had been shown to be ineffective in treating COVID-19 when used alone[7,8], we argue that the antiviral effects were mainly attributed to the interferon alpha or synergies from the combination. These findings revealed that the combination of interferons with antiviral agents was a potential therapeutic approach for COVID-19. rSIFN-co plus lopinavir–ritonavir or umifenovir might be a potent therapeutics for treating COVID-19. Most recently, remdesivir was proven to be superior to placebo in shortening the time to recovery in adults hospitalized with COVID-19[4]. Combination of rSIFN-co and remdesivir should be strongly expected in the future.

Previous studies on interferon alpha showed that a few patients had influenza-like symptoms, such as pyrexia, myalgia, and rigors, after receiving treatment[18,19,20,21]. The present exploratory study demonstrated the superiority of rSIFN-co over interferon alpha as a therapeutic option for COVID-19 with a low rate of AEs. No patient had influenza-like symptoms in these two groups. However, gastrointestinal AEs, including decreased appetite, nausea, diarrhoea, abdominal discomfort and stomach ache, were relatively common in this study. The incidence of gastrointestinal AEs was similar to previous studies focusing on lopinavir–ritonavir or umifenovir[7,8]. As all of the enrolled patients in this study had received treatment with the antiviral agents, the recorded AEs might be related to those compounds. In addition, one patient's condition in the interferon alpha group deteriorated by what was thought to be the natural progression of SARS-CoV-2 infection, this is a fairly common event in COVID-19 patients. Given these recorded AEs, we conclude that the adding either rSIFN-co or interferon alpha nebulization as a therapeutic option to the current antiviral agents is safe. It should be noted that although rSIFN-co is a

homolog of interferon alpha, it can be used at higher doses with a low rate of AEs. This is one of the reasons that we used a high dose of rSIFN-co in this study as compared to interferon alpha (12 million IU vs 5 million IU). The high doses of rSIFN-co might have contributed to the better outcomes observed in our study.

Our study has several limitations. Firstly, the total number of trial patients was small, although it is not uncommon for an exploratory study, further studies are encouraged to confirm these results with more patients. Secondly, the median interval between symptom onset and randomization was longer in our study than that in other reports[1,2] and some patients received symptomatic treatment (such as cough relief and fever reduction) and/or supportive care before randomization. Thirdly, we were unable to mask research staff to the treatment allocation, which might introduce potential performance bias when they were doing ordinal scale measurements. However, this was mitigated because they were trained. In addition, the secondary endpoints included some objective parameters like the time to virus nucleic acid negative conversion which also supported the clinical findings. Fourthly, dissimilar baseline concurrent therapeutics, such as antiviral agents, antibiotics, corticosteroids or immunoglobulins, might be other possible confounders, but we endeavoured to minimize these effects by randomization.

In conclusion, our study showed that rSIFN-co added to antiviral agents was safe and more efficient than interferon alpha plus antiviral agents in the treatment of moderate-to-severe COVID-19. Future clinical study of rSIFN-co therapy alone or combined with other antiviral therapy is warranted.

Methods

Trial design and patients

This is a multicenter, prospective, randomized controlled, single-blind, clinical trial (Chinese Clinical Trial Registry, ChiCTR2000029638) conducted between 10 February and 5 April 2020. We recruited patients from five hospitals in Wuhan city, Hubei province, and in Chengdu city, Sichuan province, China.

Eligible patients were males and non-pregnant females aged 18 years or older, diagnosed with moderate-to-severe COVID-19 pneumonia according to the *Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia* released by the National Health Commission of China (**Supplementary Table 3**)[12]. Moderate COVID-19 patients were featured by fever, respiratory symptoms, and radiographic pneumonia, while severe COVID-19 patients featured by any of the following signs: dyspnea, respiratory frequency ≥30/minute, oxygen saturation ≤94%, and PaO2/FiO2 ratio <300mmHg. The diagnosis of COVID-19 pneumonia was confirmed with quantitative reverse transcription–polymerase chain reaction (qRT-PCR) of SARS-CoV-2 nucleic acid by nasopharyngeal swab test and chest CT scans. Patients who received symptomatic treatment and/or supportive care before enrollment but had no clinical improvement were also involved. We excluded patients if they presented with any condition that would not allow the protocol to be followed safely; had a history of allergy or hypersensitivity to interferons or any of the ingredients

used in this trial; had a history of myocardial infarction and other serious cardiovascular diseases; were unable to receive nebulized compound; and/or voluntarily requested to withdraw from the trial.

The patients who met all the following criteria were considered cured and could be discharged from the hospital if their body temperature remained normal for at least three days, their respiratory symptoms relieved, and they obtained two consecutive negative tests for SARS-CoV-2 (interval between tests was more than 24 h).

The study protocol was approved by the institutional review board in West China Hospital, Sichuan University, Chengdu, China (2020 Review, No. 10). Each patient or the patient's legal representative received oral and written information about the trial and signed an informed consent form before enrollment. The study was undertaken in full accordance with the Declaration of Helsinki and Good Clinical Practice guidelines and reported according to CONSORT (Consolidated Standards of Reporting Trials) guidelines. The study protocol is available in the supplementary materials.

Randomization and procedures

Eligible patients were randomized in a 1:1 ratio using a computer-generated random number table to the rSIFN-co group or the interferon alpha group. The study medications were prepared by the medical ward nurses and then dispensed to the participants. Patients were blinded to treatment allocation, whereas treating physicians were aware of group allocations.

Patients received nebulized rSIFN-co (12 million IU, twice daily) or nebulized interferon alpha (interferon alpha-2a or interferon alpha-2b, 5 million IU, twice daily) immediately after randomization for no more than 28 days. The baseline antiviral agents were lopinavir-ritonavir (400 mg and 100 mg, orally, twice daily) or umifenovir (200 mg, orally, three times a day), which were freely provided by the national health authority. All patients received the standard care as well as the interferon treatment, and were subjected to the laboratory, and radiographic examinations. Clinical, laboratory, and radiographic assessments were conducted at baseline. Patients were assessed once daily by trained researchers from day 0 to day 28. A complete blood count, serum biochemical tests (renal function, liver function), and a nasopharyngeal swab test for SARS-CoV-2 using the qRT-PCR assay (approved by the National Medical Products Administration) were conducted every 3 days, while chest CT scans were conducted every 5 or 7 days. The virus nucleic acid negative conversion was defined as two consecutive negative tests for SARS-CoV-2 (interval of more than 24 h). Moreover, chest CT scans was graded by the changed areas of ground-glass opacity and consolidation compared with the baseline by two independent radiologists. Data were collected and recorded on paper case report forms and then entered into an electronic database and validated by trial staffs.

Primary outcome

The primary outcome was the time to clinical improvement, defined as the time from enrollment to an improvement of two points on a seven-category ordinal scale[33] (**Supplementary Table 4**) or live discharge

from the hospital, whichever came first.

Secondary outcomes

Secondary outcomes included the time to radiological improvement defined as the time from enrollment to radiological improvement on chest CT scans, the time to virus nucleic acid negative conversion defined as the time from enrollment to two consecutive negative tests for SARS-CoV-2 via qRT-PCR on nasopharyngeal swabs samples, the overall rate of clinical improvement assessed on day 28. Safety outcomes included treatment-emergent AEs and SAEs, classified according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. AEs and SAEs were assessed and recorded once daily by trained researchers from day 0 to day 28. Other secondary outcomes included overall rates of radiological improvement on days 7, 14, and 28 on chest CT scans, overall rates of virus nucleic acid negative conversion via qRT-PCR on nasopharyngeal swabs samples on days 7, 14, and 28, and the rates of deterioration or death on day 28.

Statistical analysis

This trial was designed as an exploratory one and was not powered statistically to measure a specific outcome, thus sample size estimates were not based on statistical power assessments. All participants who received study medications at least once were included in the safety analysis. The time to clinical improvement was assessed after all patients had reached day 28. Patients that failed to reach clinical improvement or died before day 28 were considered as right-censored. We used the Kaplan-Meier method to analyze the time to events in the safety population with a log-rank test. We used a Cox model to estimate the HRs with 95% CIs. We used the rate difference between groups to compare the event rates of secondary or other outcomes. A two-sided α of less than 0.05 was considered statistically significant. We used the StataSE software, version 14.0 for statistical analysis.

Declarations

Data availability

All requests for raw and analyzed data will be reviewed by the West China Hospital to verify whether the request is subject to any intellectual property or confidentiality obligations. Patient-related data not included in the paper were generated as part of clinical trials and may be subject to patient confidentiality. Any data and materials that can be shared will be released via a material transfer agreement. All other data that support the findings of this study will be provided by the corresponding author upon reasonable request when possible. All data shared will be de-identified.

Acknowledgements

This clinical trial was supported by the New Coronavirus (2019-nCoV) Emergency Project of Sichuan University (grant no. 2020scunCoVEmergency10001) and the Novel Coronavirus Pneumonia Epidemic Research Project in West China Hospital, Sichuan University (grant no. HX-2019-nCoV016). The study

was designed, conducted, analyzed, and interpreted by the authors entirely independently of the funding sources. We thank all the study participants and their families, the participating researchers at all study centers that were provided throughout this study.

We thank Sichuan Huiyang Life Science & Technology Corporation for providing rSIFN-co as a kind gift.

Author contributions

L. Liu, C. Li, and F. Luo were responsible for the initial study design and had roles in planning and coordination of the study. F. Luo and X. Nian were responsible for study implementation and patient enrollment. L. Liu, C. Li, F. Luo, C. Liu, W. Li and J. Liao had roles in writing, conceiving and revising the article. C. Li and C. Liu had roles in data acquisition, data analysis, and graph generations. D. Kang, J. Mei, S. Deng and Z. Zeng had roles in data interpretation, and the literature search. Z. Xu, W. Zhang, M. Yang, Y. Wang, D. Liu, C. Yu, J. Zeng, L. Zhang, D. Li, Y. Liu, M. Feng, R. Liu, Y. He, H. Liu, Z. Shi, and D. Meng were responsible for recruitment and clinical care of the participants. All authors reviewed and approved the final version of the manuscript.

Competing interests:

The authors declare no competing interests.

References

1. Zhou, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* **395**, 1054-1062 (2020).
2. Wu, & McGoogan, J.M. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. *JAMA* **323**, 1239-1242 (2020).
3. Chen, N. et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive *Lancet* **395**, 507-513 (2020).
4. Beigel, J.H. et al. Remdesivir for the Treatment of COVID-19 - Preliminary Report. *Engl. J. Med.* https://www.doi.org/10.1056/NEJMoa2007764 (2020).
5. Horby, et al. Dexamethasone in Hospitalized Patients with Covid-19 - Preliminary Report. *N. Engl. J. Med*. https://www.doi.org/10.1056/NEJMoa2021436 (2020).
6. Sanders, J.M., Monogue, M.L., Jodlowski, Z. & Cutrell, J.B. Pharmacologic Treatments for Coronavirus Disease 2019 (COVID-19): A Review. *JAMA* **323**, 1824-1836 (2020).
7. Lian, N. et al. Umifenovir treatment is not associated with improved outcomes in patients with coronavirus disease 2019: A retrospective *Clin. Microbiol. Infect.* **26**, 917-921 (2020).
8. Cao, B. et al. A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe COVID-19. *Engl. J. Med* **382**, 1787-1799 (2020).

9. Hu, K. et al. Efficacy and Safety of Lianhuaqingwen Capsules, a repurposed Chinese Herb, in Patients with Coronavirus disease 2019: A multicenter, prospective, randomized controlled Phytomedicine. https://www.doi.org/10.1016/j.phymed.2020.153242 (2020).
10. Loutfy, R. et al. Interferon alphacon-1 plus corticosteroids in severe acute respiratory syndrome: a preliminary study. *JAMA* **290**, 3222-3228 (2003).
11. Omrani, A.S. et al. Ribavirin and interferon alpha-2a for severe Middle East respiratory syndrome coronavirus infection: a retrospective cohort *Lancet Infect. Dis.* **14**, 1090-1095 (2014).
12. Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7). *Med. J.* **133**, 1087-1095 (2020).
13. Liu, Y. et al. 21-Possibility to Partly Win the War Against Cancer. In *Recent Advances in Cancer Research and Therapy* (Elsevier, Oxford, 2012).
14. Wei, Treatment of viral diseases with recombinant interferon α. United States patent US 8287852 B2 (2012).
15. Zhang, K.J. et al. A Potent In Vivo Antitumor Efficacy of Novel Recombinant Type I Interferon. *Cancer Res.* **23**, 2038-2049 (2017).
16. Zhang, et al. Activity of superior interferon alpha against HIV-1 in severe combined immunodeficient mice reconstituted with human peripheral blood leukocytes. *Chin. Med. J.* **124**, 396–400 (2011).
17. Liu, C. et al. Research and Development on Therapeutic Agents and Vaccines for COVID-19 and Related Human Coronavirus Diseases. *ACS Cent. Sci.* **6**, 315-331 (2020).
18. Janssen, H.L. et al. Pegylated interferon alpha-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomized trial. *Lancet* **365**, 123-129 (2005).
19. Fried, W. et al. Peginterferon alpha-2a plus ribavirin for chronic hepatitis C virus infection. *N. Engl. J. Med.* **347**, 975-982 (2002).
20. Torriani, J. et al. Peginterferon Alpha-2a plus ribavirin for chronic hepatitis C virus infection in HIV-infected patients. *N. Engl. J. Med.* **351**, 438-450 (2004).
21. Wedemeyer, et al. Peginterferon alpha-2a plus tenofovir disoproxil fumarate for hepatitis D (HIDIT-II): a randomized, placebo controlled, phase 2 trial. *Lancet Infect. Dis.* **19**, 275-286 (2019).
22. Lu, R. et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* **395**, 565-74 (2020).
23. Lai, C.C., Shih, P., Ko, W.C., Tang, H.J. & Hsueh, P.R. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges. *Int. J. Antimicrob Agents* **55**:105924 (2020).
24. Qin, C. et al. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. *Infect. Dis.* https://doi.org/10.1093/cid/ciaa248 (2020).
25. Huang, C. et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* **395**, 497-506 (2020).

26. Ruan, Q., Yang, , Wang, W., Jiang, L. & Song, J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Med.* **46**, 846-848 (2020).
27. Ramos-Casals, M., Brito-Zerón, , López -Guillermo, A., Khamashta, M.A. & Bosch, X. Adult haemophagocytic syndrome. *Lancet* **383**, 1503-1516 (2014).
28. Channappanavar, R. et al. Dysregulated Type I Interferon and Inflammatory Monocyte-Macrophage Responses Cause Lethal Pneumonia in SARS-CoV-Infected Mice. *Cell Host Microbe* **19**, 181-193 (2016).
29. Channappanavar, R. et al. IFN-I response timing relative to virus replication determines MERS coronavirus infection outcomes. *Clin. Invest.* **129**, 3625-3639 (2019).
30. Wu, et al. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. *JAMA Intern. Med.* 180, 1-11 (2020).
31. Blanco-Melo, D. et al. Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. *Cell* **181**, 1036-1045 (2020).
32. Hung, F. et al. Triple combination of interferon beta-1b, lopinavir-ritonavir, and ribavirin in the treatment of patients admitted to hospital with COVID-19: an open-label, randomized, phase 2 trial. *Lancet* **395**, 1695-1704 (2020).
33. Wang, et al. Comparative Effectiveness of Combined Favipiravir and Oseltamivir Therapy Versus Oseltamivir Monotherapy in Critically Ill Patients With Influenza Virus Infection. *J. Infect. Dis.* **221**, 1688-1698 (2020).

Tables

Table 1. Baseline demographic, clinical, laboratory, and radiographic characteristics of the study population

Characteristics	Total (*n* = 94)	rSIFN-co (*n* = 46)	Interferon alpha (*n* = 48)
Age (years)[a]	54.0 (39.8-63.3)	51.0 (33.5-59.3)	56.0 (49.3-69.0)
Male (*n* (%))	44 (46.8)	21 (45.7)	23 (47.9)
Component (*n* (%))			
Moderate	83 (88.3)	39 (84.8)	44 (91.7)
Severe	11 (11.7)	7 (15.2)	5 (10.4)
Comorbidities (*n* (%))			
Hypertension	18 (19.1)	7 (15.2)	11 (22.9)
Diabetes	9 (9.6)	3 (6.5)	6 (12.5)
Heart disease	7 (7.4)	3 (6.5)	4 (8.3)
Cerebrovascular disease	5 (5.3)	3 (6.5)	2 (4.2)
Tuberculosis	3 (3.2)	3 (6.5)	0
Liver diseases	6 (6.4)	3 (6.5)	3 (6.3)
COPD	1 (1.1)	1 (2.2)	0
Body temperature (°C)[a]	36.7 (36.4-36.9)	36.8 (36.5-37.0)	36.7 (36.4-36.9)
Fever (*n* (%))[b]	15 (16.0)	8 (17.4)	7 (14.6)
Cough (*n* (%))	51 (54.3)	26 (56.5)	25 (52.1)
Expectoration (*n* (%))	21 (22.3)	10 (21.7)	11 (22.9)
Fatigue (*n* (%))	21 (22.3)	11 (23.9)	10 (20.8)
Myalgia (*n* (%))	12 (12.8)	7 (15.2)	5 (10.4)
Anhelation (*n* (%))	15 (16.0)	6 (13.0)	9 (18.8)
Dyspnea (*n* (%))	8 (8.5)	5 (10.9)	3 (6.3)
Pharyngalgia (*n* (%))	7 (7.4)	4 (8.7)	3 (6.3)

Poor appetite (*n* (%))	7 (7.4)	3 (6.5)	4 (8.3)
Diarrhoea (*n* (%))	5 (5.3)	2 (4.3)	3 (6.3)
Other (*n* (%))	7 (7.4)	4 (8.7)	3 (6.3)
Respiratory rate (breaths per min)[a]	20 (18.8-20.0)	20 (18.0-20.0)	20 (19.0-20.0)
Respiratory rate >24 breaths per min (*n* (%))	6 (6.4)	3 (6.5)	3 (6.3)
Heart rate (beats per min)[a]	82.5 (77.8-93.0)	82.0 (77.6-90.0)	83.0 (77.3-93.0)
Oxygen saturation[a]	98.0 (97.0-99.0)	98.0 (97.0-99.0)	98.0 (96.3-98.0)
Oxygen saturation <94% (*n* (%))	3 (3.2)	2 (4.3)	1 (2.1)
White blood cell count ($\times 10^{-9}$ /L)[a]	5.4 (4.4-6.8)	5.4 (4.6-6.8)	5.5 (4.2-6.8)
4–10	76 (80.9)	39 (84.8)	39 (81.2)
<4	16 (17.0)	7 (15.2)	9 (18.8)
>10	2 (2.1)	0	2 (4.2)
Lymphocyte count ($\times 10^{-9}$/L)[a]	1.5 (1.1-1.8)	1.5 (1.1-1.8)	1.37 (0.9-1.8)
≥1.0	75 (79.8)	39 (84.8)	36 (75.0)
<1.0	19 (20.2)	7 (15.2)	12 (25.0)
Platelet count (10^{-9} /L)[a]	206 (167.5-251.3)	208 (176.5-247.8)	198.5 (155.0-260.8)
≥100	89 (94.7)	44 (95.7)	45 (93.7)
<100	5 (5.3)	2 (4.3)	3 (6.3)
Serum creatinine (μmol/L)[a]	69.0 (54.0-81.5)	65.5 (51.8-76.7)	71.9 (59.5-94.5)
≤133	90 (95.7)	46 (100.0)	44 (91.7)
>133	4 (4.3)	0	4 (8.3)
Alanine aminotransferase (U/L)[a]	21.0 (13.9-34.8)	23.7 (17.9-50.8)	16.7 (11.4-28.3)
≤50	79 (84.0)	35 (76.1)	44 (91.7)
>50	15 (16.0)	11 (23.9)	4 (8.3)
Aspartate aminotransferase (U/L)[a]	20.8 (15.0-28.3)	23.5 (15.5-34.4)	18.4 (13.9-24.5)
≤40	80 (85.1)	37 (80.4)	43 (89.6)

>40	14 (14.9)	9 (19.6)	5 (10.4)
Creatine kinase (U/L)[a]	56.0 (42.8-82.7)	56.0 (40.3-73.7)	57.0 (43.7-93.0)
≤185	88 (93.6)	44 (95.7)	44 (91.7)
> 185	6 (6.4)	2 (4.3)	4 (8.3)
C-reactive protein (mg/DI)[a]	4.2 (1.1-4.2)	3.1 (1.0-15.8)	6.1 (1.3-29.1)
Chest CT scans (*n* (%))			
Ground-glass opacity infiltration	66 (70.2)	34 (73.9)	32 (66.7)
Unilateral	11 (11.7)	8 (17.4)	3 (6.3)
Bilateral	55 (58.5)	26 (56.5)	29 (60.4)
Consolidation	34 (36.2)	21 (45.7)	12 (25.0)
Unilateral	4 (4.3)	2 (4.3)	2 (4.2)
Bilateral	30 (31.9)	20 (43.5)	10 (20.8)
Pleural effusion	7 (7.4)	2 (4.3)	5 (10.4)

[a]Data are shown as the median (IQR). None of the differences between the two study groups was significant ($P \geq 0.05$). [b]Fever was defined as body temperature ≥

37.3 ℃.

Table 2. Patients' status and treatments received after enrollment

	rSIFN-co (*n* = 46)	Interferon alpha (*n* = 48)
Days from illness onset to randomization (days)[a]	14.0 (5.0-30.0)	14.5 (7.0-31.0)
Seven-category scale on day 1 (*n* (%))		
3: Hospitalization, not requiring supplemental oxygen	11 (23.9)	11 (22.9)
4: Hospitalization, requiring supplemental oxygen	20 (43.5)	28 (58.3)
5: Hospitalization, requiring high-flow nasal cannula or noninvasive mechanical ventilation	15 (32.6)	9 (18.8)
Receiving lopinavir–ritonavir (*n* (%))	22 (47.8)	20 (41.7)
Receiving umifenovir (*n* (%))	24 (52.2)	28 (58.3)
Oxygen therapy support (*n* (%))	35 (76.1)	42 (87.5)
Nasal cannula	32 (69.6)	39 (81.3)
Mask	2 (4.3)	2 (4.2)
Non-invasive mechanical ventilation	1 (2.2)	0
Invasive mechanical ventilation	0	1 (2.1)
Duration of oxygen support[a]	12.0 (10.0-16.0)	14.0 (10.0-17.5)
Antibiotic (*n* (%))	13 (28.3)	8 (16.7)
Glucocorticoid therapy (*n* (%))	4 (6.5)	5 (10.4)
Duration of glucocorticoid therapy[a]	5.5 (4.0-7.8)	4.0 (3.0-6.5)
Immunoglobulins (*n* (%))	2 (4.3)	1 (2.1)

[a]Data are shown as the median (IQR). None of the differences between the two study groups was significant ($P \geq 0.05$).

Table 3. Outcomes in the study population

	rSIFN-co (*n* = 46)	Interferon alpha (*n* = 48)	Difference[b]
Seven-category scale on day 7 (*n* (%))			
2: Not hospitalized, but unable to resume normal activities	6 (13.0)	6 (12.5)	
3: Hospitalization, not requiring supplemental oxygen	9 (19.6)	12 (25.0)	
4: Hospitalization, requiring supplemental oxygen	23 (50.0)	21 (43.8)	
5: Hospitalization, requiring high-flow nasal cannula or noninvasive mechanical ventilation	8 (17.4)	8 (16.7)	
6: Hospitalization, requiring extracorporeal membrane oxygenation, invasive mechanical ventilation, or both	0	1 (2.0)	
Seven-category scale on day 14 (*n* (%))			
2: Not hospitalized, but unable to resume normal activities	27 (58.7)	21 (43.8)	
3: Hospitalization, not requiring supplemental oxygen	10 (21.7)	15 (31.2)	
4: Hospitalization, requiring supplemental oxygen	8 (17.4)	8 (16.7)	
5: Hospitalization, requiring high-flow nasal cannula or noninvasive mechanical ventilation	1 (2.2)	3 (6.3)	
6: Hospitalization, requiring extracorporeal membrane oxygenation, invasive mechanical ventilation, or both	0	1 (2.0)	
Seven-category scale on day 28 (*n* (%))			
2: Not hospitalized, but unable to resume normal activities	44 (95.6)	44 (91.6)	
3: Hospitalization, not requiring supplemental oxygen	1 (2.2)	1 (2.1)	
4: Hospitalization, requiring supplemental oxygen	1 (2.2)	2 (4.2)	
5: Hospitalization, requiring high-flow nasal cannula or noninvasive mechanical ventilation	0	0	
6: Hospitalization, requiring extracorporeal membrane	0	1 (2.1)	

oxygenation, invasive mechanical ventilation, or both			
Time to clinical improvement (days)[a]	11.5 (9.3-16.0)	14.0 (10.0-18.0)	1.76 (1.10-2.81)
Clinical improvement rates (*n* (%))			
Day 7	5 (10.9)	3 (6.3)	4.6 (-0.07-0.16)
Day 14	30 (65.2)	19 (39.6)	25.6 (0.06-0.45)
Day 28	43 (93.5)	37 (77.1)	16.4 (0.03-0.30)
Time to radiological improvement (days) [a]	8.0 (6.0-8.3)	10.0 (7.0-13.0)	2.19 (1.32-3.62)
Radiological improvement rates (*n* (%))			
Day 7	20 (43.5)	13 (25.0)	18.5 (-0.03-0.35)
Day 14	39 (84.8)	32 (66.7)	18.1 (0.01-0.35)
Day 28	42 (91.3)	38 (79.2)	12.1 (-0.02-0.26)
Time to virus nucleic acid negative conversion (days) [a]	7.0 (5.0-13.0)	10.0 (6.3-16.8)	1.74 (1.10-2.74)
Virus nucleic acid negative conversion rates (*n* (%))			
Day 7	23 (50.0)	15 (31.3)	18.7 (-0.01-0.38)
Day 14	35 (76.1)	31 (64.6)	11.5 (-0.07-0.30)
Day 28	45 (97.8)	41 (85.4)	12.4 (0.02-0.23)
Day 28 mortality (*n* (%))	0	0	-
Deterioration rates (*n* (%))	0	1 (2.1)	-2.1 (-0.08-0.04)

[a]Data are shown as the median (IQR). [b]The hazard ratio was estimated by Cox model for time to events. Differences were expressed as rate differences and 95% confidence intervals for the overall rates of

clinical improvement, radiological improvement on chest CT scans and virus nucleic acid negative conversion on days 7, 14, and 28 and deterioration rate.

Figures

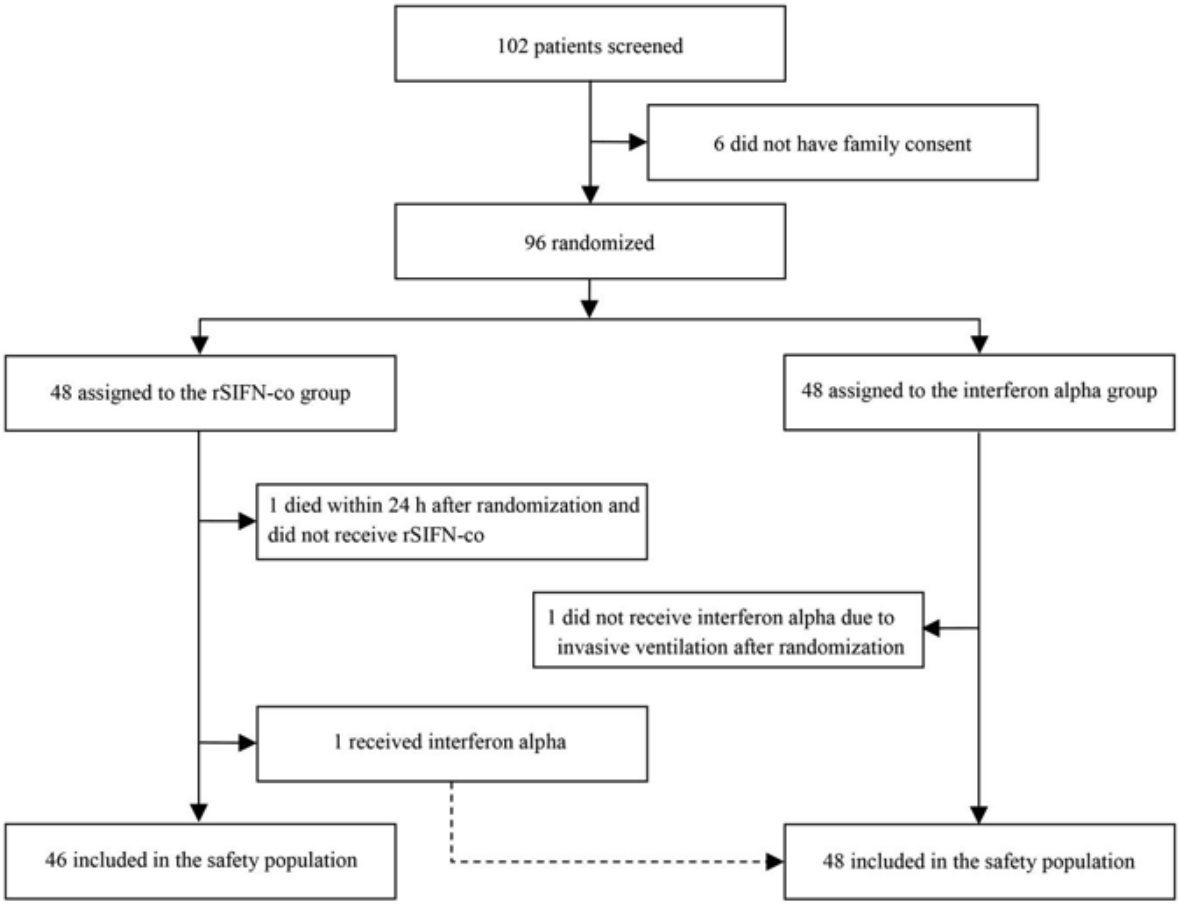

Figure 1

Trial profile.

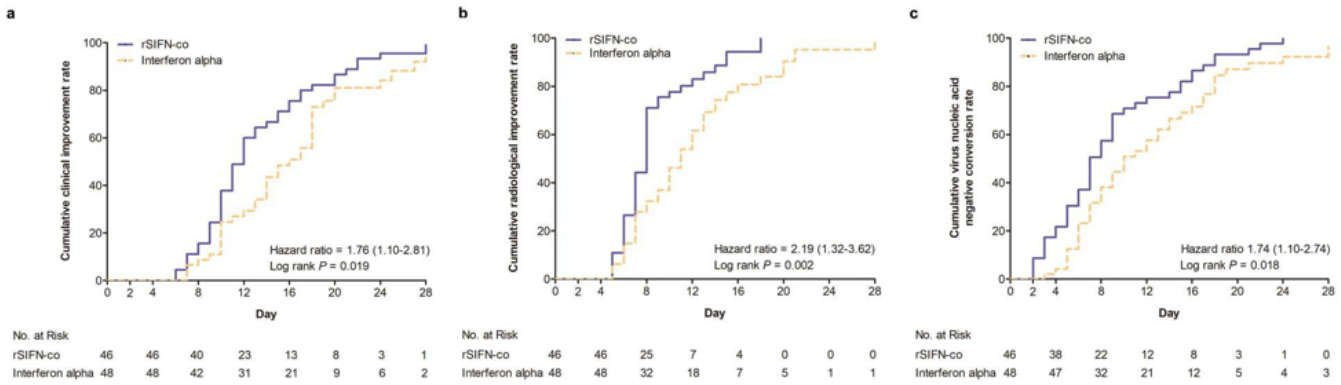

Figure 2

Outcomes over time. a, Time to clinical improvement; b, Time to radiological improvement on chest CT scans; c, Time to virus negative conversion. Analysis was performed by log-rank (Mantel-Cox) test.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarytables.doc