


## ORIGINAL ARTICLE

# Interferon- $\alpha$ -2b aerosol inhalation is associated with improved clinical outcomes in patients with coronavirus disease-2019

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**Aims:** Type 1 interferon (IFN) is used to treat patients with coronavirus disease-2019 (COVID-19) but robust supporting evidence is lacking. We investigated the association between IFN- $\alpha$ -2b and the clinical outcomes of patients with COVID-19.

**Methods:** A total of 1401 patients were enrolled, with 852 (60.8%) patients receiving 5 000 000 U of IFN- $\alpha$ -2b via aerosol inhalation twice daily. The primary outcome was a composite measure consisting of mechanical ventilation, intensive care unit (ICU) admission and death. A subgroup analysis was performed to investigate the impact of the IFN- $\alpha$ -2b initiation schedule on symptom onset.

**Results:** The risk probability for crude endpoints was lower in the IFN- $\alpha$ -2b group (3.8%) than in the non-IFN- $\alpha$ -2b group (9.3%,  $P < .001$ ). After adjusting the confounding factors, IFN- $\alpha$ -2b therapy achieved a reduction of 64% in occurrence of endpoint events (hazard ratio, 0.36; 95% confidence interval [CI], 0.21–0.62). In the subgroup analysis, compared with patients who received IFN- $\alpha$ -2b treatment 0–2 days after symptom onset, the hazard ratio for endpoints was 2.2 (95% CI, 0.43–11.13) in patients who received the therapy 3–5 days after symptom onset, 5.89 (95% CI, 0.99–35.05) in patients who received the therapy 6–8 days after symptom onset, and remained at a high level thereafter.

**Conclusions:** IFN- $\alpha$ -2b aerosol inhalation therapy may be associated with improved clinical outcomes in patients with COVID-19, and delayed IFN- $\alpha$ -2b intervention was associated with increased probabilities of risk events. Further randomized clinical trials are needed to validate the preliminary findings of this study.

## KEYWORDS

COVID-19, interferon, prognosis, SARS-CoV-2

## 1 | INTRODUCTION

At the end of December 2019, an outbreak of pneumonia caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was

reported in China, subsequently giving rise to a global outbreak.<sup>1</sup> As of 28 April 2020, over 3 000 000 cases of coronavirus disease-2019 (COVID-19) have been confirmed worldwide, causing more than 200 000 deaths. The efficacy of broad-spectrum or targeted antivirals on SARS-CoV-2 replication and COVID-19 prognosis is under investigation in randomized clinical trials.<sup>2</sup> Type 1 interferon (IFN) is a non-specific antiviral agent and is widely used to treat emerging viral infections for which no specific drug or vaccine is available.

**Principal investigator:** The authors confirm that the Principal Investigator for this paper is Dr Hongcui Cao. The study uses only anonymous data for analysis and without any individual patient information.

IFN therapy reportedly improves the outcomes of Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV infections.<sup>3–8</sup> *In vitro*, SARS-CoV-2 is substantially more sensitive to IFN-I than MERS-CoV or SARS-CoV.<sup>9</sup> Therefore, IFN is likely to be effective against COVID-19. IFN has been approved by multiple countries for the treatment of COVID-19,<sup>9,10</sup> for which 5 000 000 U of IFN- $\alpha$ -2b by vapor inhalation twice daily is recommended in China. However, the therapeutic effect of IFN- $\alpha$ -2b on COVID-19 is unclear and needs to be evaluated because there is no specific antiviral therapy. Also, the timing of IFN treatment greatly influences the clinical outcomes of coronavirus infection.<sup>3,4,11</sup>

Here we investigated the association between IFN- $\alpha$ -2b aerosol inhalation, the clinical outcomes of patients with COVID-19 and the effect of the IFN initiation schedule on symptom onset.

## 2 | METHODS

### 2.1 | Patients

This multicentre retrospective study was approved by the First Affiliated Hospital, College of Medicine, Zhejiang University, and complied with the ethical guidelines of the Declaration of Helsinki. Patients with COVID-19 who were  $\geq 18$  years of age were enrolled from centres in Zhejiang and Jiangsu Provinces between 17 January and 19 February 2020. The patients were diagnosed according to the diagnostic criteria of the National Health Commission. The requirement for informed consent was waived because the data were anonymized prior to analysis.

### 2.2 | Data collection

The following data were collected from the electronic medical records: epidemiological, demographics, laboratory findings, comorbidities, time from illness onset to hospital admission, time to first dose of antivirals, chest radiological findings on admission, time of the first negative result of a pharyngeal swab, and the duration of hospital stay, with verification by independent physicians. Clinical data were obtained at admission, and laboratory data within 24 hours of admission. COVID-19 cases were confirmed by sequencing or reverse transcriptase-polymerase chain reaction of throat-swab specimens from the upper respiratory tract. The clinical outcomes were followed until 15 March 2020.

### 2.3 | Study definitions

IFN- $\alpha$ -2b treatment comprised 5 000 000 U via aerosol inhalation twice daily. Patients who received only one dose of IFN- $\alpha$ -2b before reaching an endpoint were included in the IFN- $\alpha$ -2b treatment group. Other treatments included supportive and complications treatments and were conducted according to the guidelines for the Diagnosis and

### What is already known about this subject

- Type 1 interferon is a non-specific antiviral and is widely used to treat patients with COVID-19 in China, but robust supporting evidence is lacking.
- We investigated the association between IFN- $\alpha$ -2b and the clinical outcomes of patients with COVID-19.

### What this study adds

- IFN- $\alpha$ -2b aerosol inhalation was associated with improved clinical outcomes of patients with COVID-19.
- Earlier IFN- $\alpha$ -2b intervention may be associated with a lower risk of worse outcomes.

Treatment of COVID-19 of the National Health Commission (seventh edition).

The patients were classified as mild, moderate, severe or critical according to the guidelines on the diagnosis and treatment of COVID-19. Based on the clinical information, we classified patients with COVID-19 as moderate (mild or moderate), severe or critical. The onset of COVID-19 was defined as the time when symptoms were first noticed. Patients who tested negative for SARS-CoV-2 in respiratory samples on two consecutive days were considered not to be shedding virus, and the negative time was defined as the day of the first negative test.

### 2.4 | Outcome

The composite endpoint was defined as at least one of the following: (1) respiratory failure requiring mechanical ventilation, (2) other organ failure and need for intensive care unit (ICU) monitoring and treatment, and (3) death.<sup>12</sup> If the patient met several criteria for an event, the calculation was based on the time of the first criterion.

The duration of hospitalization and the interval from symptom onset or admission to a negative nucleic acid test were also examined. Furthermore, the association between days from onset to IFN- $\alpha$ -2b therapy and clinical outcomes was assessed.

### 2.5 | Statistical analysis

Continuous variables are expressed as means and standard deviation or medians and interquartile range (IQR) and were compared by *t*-test or Mann–Whitney U-test, as appropriate. Categorical variables are expressed as percentages and were evaluated by chi-squared test or

Fisher exact test. The log-rank test was used to evaluate differences in event-free survival between the two groups. A Cox regression analysis adjusted for the benchmark covariate was conducted to assess the robustness of the results if the proportional risk assumption held. Results are expressed as hazard ratios (HRs) and 95% confidence intervals (CIs). For non-linear relations, we used restricted cubic spline Cox regression analyses to assess the relationship between the initiation time of IFN- $\alpha$ -2b treatment and the clinical outcome after adjusting for sex, age, smoking status, CRP, NLR, other antivirals, clinical type at admission and comorbidities. Statistical analysis was performed using SPSS version 19.0 (IBM, Armonk, NY) and R version 3.4 (R Foundation, Vienna, Austria) software. All tests were two-tailed and  $P < .05$  was considered to indicate statistical significance.

## 2.6 | Other sensitivity analyses

We performed further sensitivity analyses to assess the robustness of the results. First, mechanical ventilation or ICU monitoring without death were considered component endpoints and were analysed separately. Second, the outcome was examined in 12 prespecified subgroups defined according to the following baseline characteristics: (1) age ( $< 60$  vs.  $\geq 60$  years), (2) sex (male vs. female), (3) duration from onset to admission (median value) ( $< 4$  vs.  $\geq 4$  days), (4) C-reactive protein (CRP) level ( $< 8$  vs.  $\geq 8$  mg/L), (5) neutrophil-to-lymphocyte ratio (NLR; median value) ( $< 2.41$  vs.  $\geq 2.41$ ), (6) presence of hypertension (yes vs. no), (7) presence of diabetes (yes vs. no), (8) smoking status (yes vs. no), (9) clinical type on admission (moderate vs. severe), (10) treatment with lopinavir/ritonavir (yes vs. no), (11) treatment with arbidol (yes vs. no), and (12) treatment with glucocorticoids (yes vs.

no). Third, among the 1401 patients eligible for analysis, five received only one dose of IFN- $\alpha$ -2b before the endpoint event, and 549 did not receive IFN- $\alpha$ -2b. After the main analysis, we compared the 847 participants who received more than one dose of IFN- $\alpha$ -2b with the 549 who did not receive the drug.

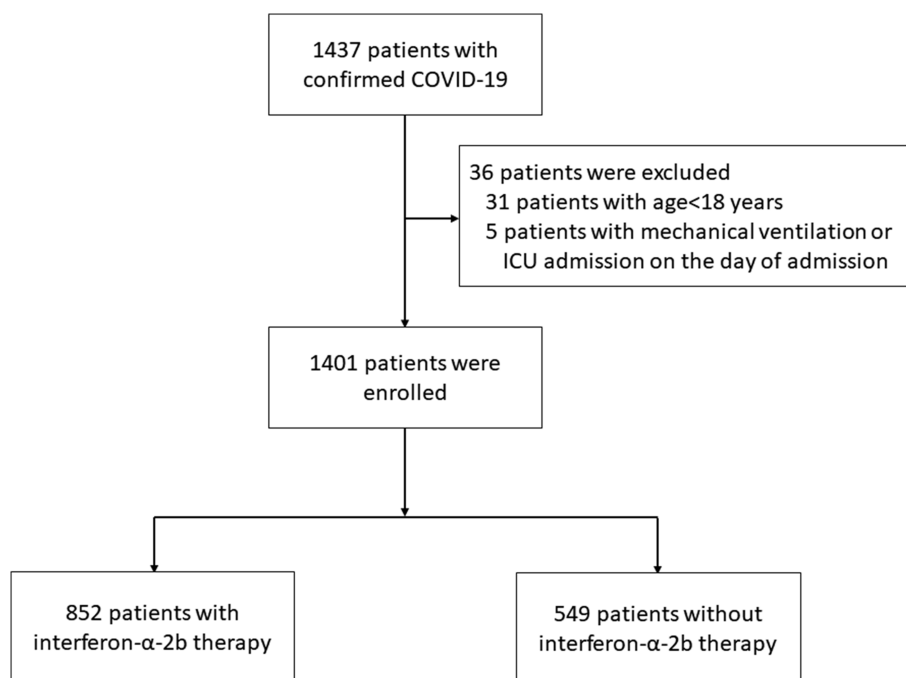
Finally, following the primary analyses, we performed a propensity score analysis to minimize the effect of IFN- $\alpha$ -2b treatment selection bias and to control for potential confounding factors.<sup>13</sup>

## 3 | RESULTS

### 3.1 | Clinical characteristics and symptoms at admission

From 17 January to 19 February, 2020, a total of 1401 patients were enrolled in this study from among 1437 patients with confirmed COVID-19 from 47 centres in Zhejiang and Jiangsu Provinces. Of these, 31 patients  $< 18$  years of age and five patients who experienced mechanical ventilation or ICU admission on the day of admission were excluded (Figure 1).

Among the patients, 852 (60.8%) received at least one dose of IFN- $\alpha$ -2b. The patients who received IFN- $\alpha$ -2b therapy were younger (means 48.06 vs. 49.73 years,  $P = .039$ ), had a shorter interval from symptom onset to admission (median 4 vs. 5 days,  $P < .001$ ), a higher CRP level (median 8.75 vs. 7.95 mg/L,  $P = .044$ ), and a higher NLR (median 2.46 vs. 2.29,  $P = .042$ ). Fever and cough were the main symptoms, and occurred at similar frequencies, in both groups. The characteristics of the patients with COVID-19 are summarized in Table 1, and the patients with missing CRP and/or NLR data are shown in Table S1 in the Supporting Information.



**FIGURE 1** Flowchart of patient selection

**TABLE 1** Characteristics of COVID-19 patients with and without interferon treatment

Variables	Unmatched			Matched		
	Control n = 549	Interferon n = 852	SD	Control n = 355	Interferon n = 355	SD
Age (years)	49.73 (15.42)	48.06 (14.33)	0.11	49.15 (15.56)	51.06 (14.96)	0.12
BMI (kg/m <sup>2</sup> ) <sup>a</sup>	23.69 (3.34)	23.77 (3.43)	0.02	23.65 (3.36)	23.84 (3.34)	0.06
Duration from onset to admission (days)	5 (2–7)	4 (2–7)	0.19	5 (2–7)	4 (2–7)	0.23
Temperature (°C)	37.82 (0.86)	37.93 (0.84)	0.14	37.86 (0.88)	37.87 (0.82)	0.01
Female	289 (52.6%)	422 (49.5%)	0.06	184 (51.8%)	162 (45.6%)	0.12
Severe type on admission	31 (5.7%)	44 (5.2%)	0.02	23 (6.5%)	22 (6.2%)	0.01
Current smoker	41 (7.5%)	57 (6.7%)	0.03	28 (7.9%)	19 (5.4%)	0.10
<b>Laboratory examination</b>						
Leucocytes (10 <sup>9</sup> /L) <sup>a</sup>	5.10 (4.09–6.55)	4.84 (3.85–6.04)	0.19	4.98 (3.95–6.48)	4.89 (3.97–6.15)	0.10
Neutrophil lymphocyte ratio <sup>a</sup>	2.29 (1.61–3.87)	2.46 (1.69–3.80)	0.11	2.49 (1.60–4.06)	2.38 (1.66–3.72)	0.10
International normalized ratio <sup>a</sup>	1.07 (0.12)	1.05 (0.10)	0.18	1.06 (0.12)	1.05 (0.10)	0.11
Serum creatinine (μmol/L) <sup>a</sup>	63.01 (52.48–75.50)	66.00 (55.00–78.00)	0.07	64.00 (53.35–77.50)	66.95 (55.15–78.89)	0.07
C-reactive protein (mg/L) <sup>a</sup>	7.95 (2.50–24.30)	8.75 (3.00–23.24)	0.11	8.10 (2.64–23.59)	9.10 (2.96–24.20)	0.02
<b>Symptoms</b>						
Fever	417 (76.0%)	692 (81.2%)	0.13	273 (76.9%)	277 (78.0%)	0.03
Cough	288 (52.5%)	509 (59.7%)	0.15	193 (54.4%)	209 (58.9%)	0.09
Sore throat	37 (6.7%)	105 (12.3%)	0.19	25 (7.0%)	38 (10.7%)	0.13
Muscle ache	54 (9.8%)	86 (10.1%)	0.01	31 (8.7%)	34 (9.6%)	0.03
Fatigue	134 (24.4%)	187 (22.0%)	0.06	79 (22.3%)	80 (22.5%)	0.01
Shortness of breath	49 (8.9%)	37 (4.3%)	0.18	26 (7.3%)	20 (5.6%)	0.07
Diarrhoea	39 (7.1%)	64 (7.5%)	0.02	27 (7.6%)	26 (7.3%)	0.01
Vomiting	14 (2.6%)	38 (4.5%)	0.1	8 (2.3%)	13 (3.7%)	0.08
Headache	24 (4.4%)	63 (7.4%)	0.13	17 (4.8%)	22 (6.2%)	0.06
<b>Coexisting comorbidity</b>						
Hypertension	110 (20.0%)	176 (20.7%)	0.02	73 (20.6%)	91 (25.6%)	0.12
Cardiovascular diseases	23 (4.2%)	24 (2.8%)	0.07	16 (4.5%)	14 (3.9%)	0.03
Diabetes	47 (8.6%)	58 (6.8%)	0.07	31 (8.7%)	24 (6.8%)	0.07
COPD	7 (1.3%)	7 (0.8%)	0.04	2 (0.6%)	3 (0.8%)	0.03
Chronic liver disease	24 (4.4%)	62 (7.3%)	0.12	20 (5.6%)	45 (12.7%)	0.25
Chronic renal disease	2 (0.4%)	7 (0.8%)	0.06	2 (0.6%)	4 (1.1%)	0.06
Pregnant	1 (0.2%)	5 (0.6%)	0.07	0 (0%)	3 (0.6%)	0.13
Asthma	5 (0.9%)	5 (0.6%)	0.04	3 (0.8%)	3 (0.8%)	0.00
Cancer	7 (1.3%)	12 (1.4%)	0.01	5 (1.4%)	9 (2.5%)	0.08
Immunosuppression	1 (0.2%)	2 (0.2%)	0.01	1 (0.3%)	0 (0.0%)	0.08
<b>Treatment</b>						
Other antivirals	82 (14.9%)	47 (5.5%)	0.31	43 (12.1%)	37 (10.4%)	0.05
Arbidol	325 (59.2%)	645 (75.7%)	0.36	224 (63.1%)	278 (78.3%)	0.34
Lopinavir/ritonavir	169 (30.8%)	656 (77.0%)	1.05	165 (46.5%)	169 (47.6%)	0.02
Glucocorticoids	123 (22.4%)	188 (22.1%)	0.01	91 (25.6%)	79 (22.3%)	0.08
IVIgT	54 (9.9%)	140 (16.5%)	0.2	43 (12.1%)	59 (16.6%)	0.13
Antibiotics	227 (41.5%)	337 (39.6%)	0.04	166 (46.9%)	140 (39.5%)	0.15

(Continues)

TABLE 1 (Continued)

Variables	Unmatched		SD	Matched		SD
	Control n = 549	Interferon n = 852		Control n = 355	Interferon n = 355	
Chest x-ray/CT findings			0.43			0.29
Normal	68 (12.4%)	50 (5.9%)		43 (12.1%)	23 (6.5%)	
Unilateral pneumonia	70 (12.8%)	143 (16.8%)		50 (14.1%)	59 (16.6%)	
Bilateral pneumonia	353 (64.3%)	457 (53.6%)		215 (60.6%)	196 (55.2%)	
Multiple mottling and ground-glass opacity	58 (10.6%)	202 (23.7%)		47 (13.2%)	77 (21.7%)	

Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; SD, standardized difference.

<sup>a</sup>Missing patient data: 447 BMI, 27 serum creatinine, 2 leucocytes 14 neutrophil lymphocyte ratio, 125 international normalized ratio, and 51 C-reactive protein.

TABLE 2 In-hospital outcome with and without interferon treatment

Variables	Unmatched		P-value	Matched		P-value
	Control n = 549	Interferon n = 852		Control n = 355	Interferon n = 355	
Negative days from onset	20(14–28)	17(13–24)	<0.001	20(14–27)	18(13–25)	0.052
Hospitalization time of discharged patients	18(12–26)	17(13–23)	0.003	18(12–25)	17(13–25)	0.668
Negative days from admission	14(9–23)	13(9–19)	<0.001	14(9–21)	13(9–21)	0.530
ICU admission	40 (7.3%)	23 (2.7%)	<0.001	28 (7.9%)	14 (3.9%)	0.039
Mechanical ventilation	35 (6.4%)	25 (3.1%)	0.002	25 (7.0%)	10 (2.8%)	0.015
ECMO	7 (1.3%)	8 (0.9%)	0.551	4 (1.1%)	3 (0.8%)	1
Venovenous haemofiltration	3 (0.6%)	2 (0.2%)	0.34	2 (0.6%)	2 (0.6%)	1
Undischarged	7 (1.3%)	4 (0.5%)	0.095	4 (1.1%)	3 (0.8%)	1
Lung transplantation	1 (0.2%)	1 (0.1%)	1	0 (0.0%)	1 (0.3%)	1
Died	0 (0%)	1 (0.1%)	1	0 (0.0%)	0 (0.0%)	1
Shock	1 (0.2%)	5 (0.6%)	0.413	0 (0.0%)	2 (0.6%)	0.479
Composite endpoint	51 (9.3%)	32 (3.8%)	<0.001	38 (10.7%)	16 (4.5%)	0.002

Abbreviations: ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit.

### 3.2 | Factors for IFN- $\alpha$ -2b use in patients with COVID-19

To determine factors associated with IFN- $\alpha$ -2b use, a stepwise logistic regression model was performed. Neutrophil lymphocyte ratio (NLR), international normalized ratio (INR) body temperatures, fever, sore throat, vomiting, evidence of pneumonia, treatment with arbidol, and treatment with lopinavir/ritonavir were the independent variables included in the model. As shown in Table S2 in the Supporting Information, NLR (OR = 0.96), fever (OR = 0.53), evidence of pneumonia (OR = 2.19), treatment with arbidol (OR = 1.72) and treatment with lopinavir/ritonavir (OR = 6.45) were independently associated with IFN- $\alpha$ -2b use.

### 3.3 | Association of IFN- $\alpha$ -2b therapy with clinical outcomes

Disease progression or death occurred in 83 patients at a median of 5 days after admission in the entire cohort. In detail, one patient died, six had septic shock and received vasoactive medications, two had lung transplantation, 63 (4.5%) were admitted to the ICU, 60 (4.3%) received mechanical ventilation, and 15 (1.1%) received extracorporeal membrane oxygenation (Table 2). The composite endpoints were documented in 32 (3.8%) patients in the IFN- $\alpha$ -2b treatment group, compared with 51 (9.3%) in the non-IFN- $\alpha$ -2b treatment group. Up to 15 March 2020, 11 patients had not been discharged, four of whom were in the IFN- $\alpha$ -2b treatment group.

Compared to the non-IFN- $\alpha$ -2b treatment group, the Kaplan–Meier curve for event-free survival of the IFN- $\alpha$ -2b treatment group showed an HR of 0.39 (95% CI, 0.25–0.61,  $P < .001$ ) in the unadjusted model, and 0.36 (95% CI, 0.21–0.62,  $P < .001$ ) after adjusting for sex, age, smoking status, interval from onset to admission, CRP, NLR, other antivirals, glucocorticoids, clinical type at admission and comorbidities (Figure 2). The adjusted HR was 0.43 (95% CI, 0.23–0.78,  $P = .006$ ) for mechanical ventilation and 0.41 (95% CI, 0.22–0.75,  $P = .004$ ) for ICU admission (Table 3).

Compared to the non-IFN- $\alpha$ -2b treatment group, the IFN- $\alpha$ -2b treatment group had a shorter median interval from admission to a negative nucleic acid test (13 vs. 14 days,  $P < .001$ ) and from symptom onset to first negative nucleic acid test (17 vs. 20 days,  $P < .001$ , Table 2). The duration of hospitalization of discharged patients differed significantly between the two groups (17 vs. 18 days,  $P = .003$ ) (Table 2).

### 3.4 | Subgroup analysis and sensitivity analysis

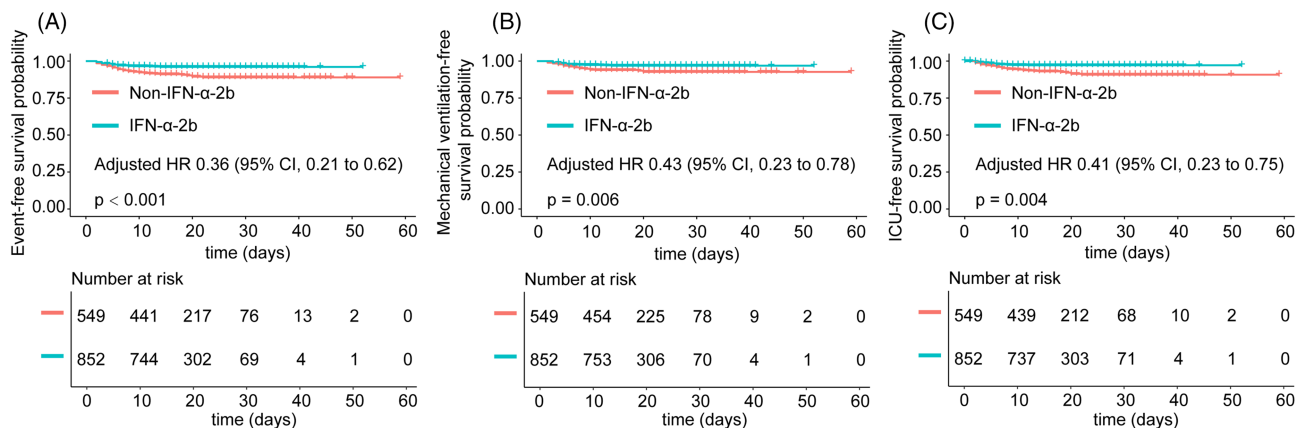
To determine whether the findings were robust to potential confounders, we performed analyses stratified by prespecified

subgroups; the analyses were adjusted for all variables other than the stratification variable. In the IFN- $\alpha$ -2b treatment group, the risk of composite endpoint events was lower than that of the non-IFN- $\alpha$ -2b treatment group irrespective of subgroup (HR 0.17–0.55) (Figure 3). The adjusted HRs for mechanical ventilation and ICU admission in the IFN- $\alpha$ -2b treatment group compared to the non-IFN- $\alpha$ -2b treatment group were 0.20–0.91 and 0.03–0.99, respectively (Figures S1 and S2 in the Supporting Information).

The results did not change after exclusion of the five patients who received only one dose of IFN- $\alpha$ -2b before the endpoint event from the treatment group (HR 0.30 [95% CI, 0.17–0.53,  $P < .001$ ] for the primary outcome, 0.39 [95% CI, 0.21–0.74] for mechanical ventilation, and 0.34 [95% CI, 0.18–0.65] for ICU admission) (Table S3 in the Supporting Information).

### 3.5 | Association of IFN- $\alpha$ -2b therapy with clinical outcomes after PS matching

PS matching was applied; 355 non-IFN- $\alpha$ -2b and 355 IFN- $\alpha$ -2b treated patients were matched. The summaries of balance for matched data are shown in Table 1. The PS model included variables such as clinical



**FIGURE 2** Event-free survival probability: (A) composite endpoint-free survival probability; (B) mechanical ventilation-free survival probability; and (C) ICU-free survival probability

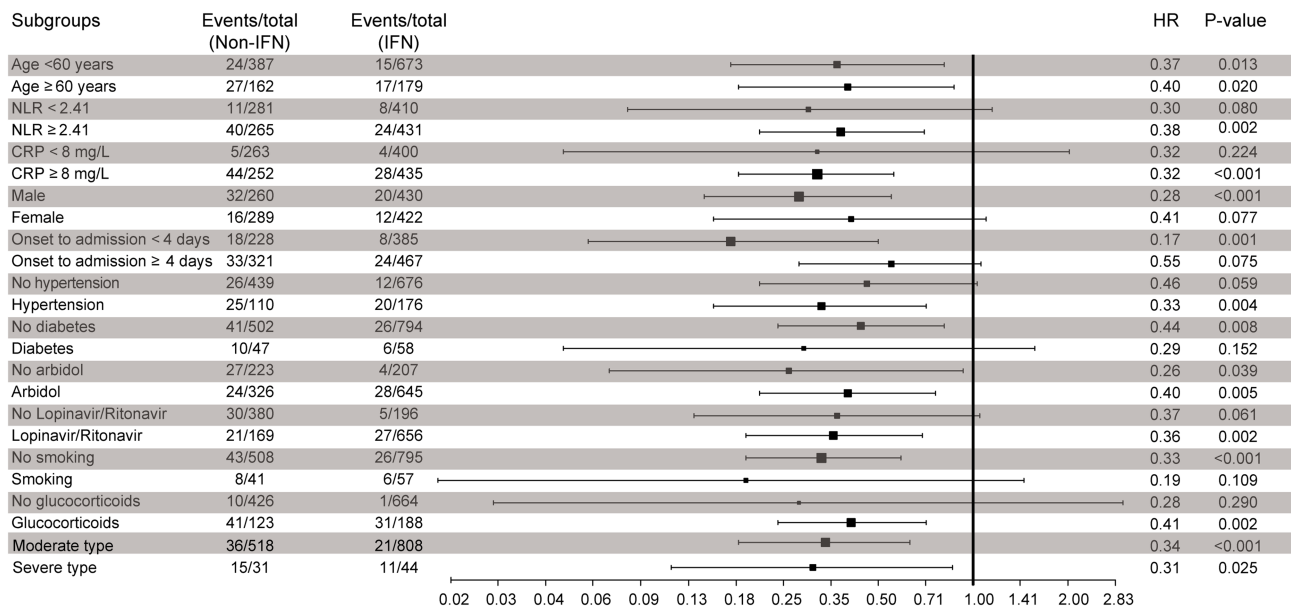
**TABLE 3** Hazard ratios for outcomes with and without interferon treatment

	Unmatched				Matched	
	Crude		Model I <sup>a</sup>		Model I <sup>a</sup>	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Composite endpoints	0.39 (0.25–0.61)	<0.0001	0.36 (0.21–0.62)	0.0002	0.43 (0.23–0.80)	0.0079
Mechanical ventilation	0.45 (0.27–0.75)	0.0023	0.43 (0.23–0.78)	0.0057	0.46 (0.21–1.00)	0.051
ICU admission	0.36 (0.22–0.60)	0.0001	0.41 (0.22–0.75)	0.0039	0.61 (0.30–1.22)	0.163

Abbreviations: CI, confidence interval; HR, hazard ratio; ICU, intensive care unit.

<sup>a</sup>Model I: adjusted for sex, age, smoking status, neutrophil lymphocyte ratio, C-reactive protein, duration from onset to admission, other antivirus drug treatments including lopinavir/ritonavir and arbidol, clinical type on admission, use of glucocorticoids and comorbidities (hypertension, cardiovascular diseases, diabetes, chronic liver disease).





**FIGURE 3** Subgroup analysis of composite endpoints according to IFN- $\alpha$ -2b treatment

symptom, sex, age, smoking status, CRP, NLR, duration from symptom onset to admission, other antiviral drug treatments, clinical type on admission and comorbidity. Finally, regarding the effect of IFN- $\alpha$ -2b on clinical outcomes, the survival plot (Figure S3 in the Supporting Information) showed an HR of 0.43 (95% CI, 0.23–0.80;  $P = .008$ ) for event-free survival, 0.46 (95% CI, 0.21–1.00;  $P = .051$ ) for mechanical ventilation-free survival, and 0.61 (95% CI, 0.30–1.22;  $P = .163$ ) for ICU admission in the IFN- $\alpha$ -2b treatment group compared to the non-IFN- $\alpha$ -2b treatment group. PS matching for confounding factors resulted in a similar effect of IFN- $\alpha$ -2b on COVID-19 (Table 3).

After PS matching, the median intervals from admission to a negative nucleic acid test (13 vs. 14 days,  $P = .530$ ), hospitalization duration (17 vs. 18 days,  $P = .668$ ), and the interval from symptom onset to a negative nucleic acid test (18 vs. 20 days,  $P = .052$ ) were not significantly different between the two groups (Table 2).

### 3.6 | Association of IFN- $\alpha$ -2b initiation with clinical outcomes

The median time from symptom onset to IFN- $\alpha$ -2b treatment was 5 days (interquartile range [IQR], 3–7 days). Restricted cubic spline Cox regression showed a spline at 7 days, which enabled the time–HR relationships before and after to be modelled as the following two linear relations: (1) the HR for composite endpoints increased up to around 7 days (from onset to treatment) and (2) was thereafter maintained at a high but constant level, resulting in an approximately flat slope at  $> 7$  days (Figure S4 in the Supporting Information). Specifically, compared with patients who received IFN- $\alpha$ -2b treatment 0–2 days after symptom onset, the hazard ratio for composite endpoints was 2.2 (95% CI, 0.43–11.13) in patients who received the therapy 3–5 days after symptom onset and 5.89 (95% CI, 0.99–35.05)

in patients who received the therapy 6–8 days after symptom onset, and remained at a high level thereafter. The association between the onset-to-treatment interval and component endpoints (i.e., mechanical ventilation or ICU admission) exhibited a similar tendency (Table 4, Figure S4 in the Supporting Information). The characteristics of patients with COVID-19 who received IFN- $\alpha$ -2b treatment stratified by onset-to-treatment interval are summarized in Table S4 in the Supporting Information.

## 4 | DISCUSSION

Our results suggested that IFN- $\alpha$ -2b in combination with standard treatment improves the clinical outcomes of patients with COVID-19. This clinical benefit remained evident in all subgroups after adjusting for confounding factors. Also, IFN- $\alpha$ -2b therapy reduced the frequency of composite endpoint events by 61%. Moreover, the effect was enhanced by 64% after adjusting for confounding factors. In a subgroup analysis of patients who received IFN- $\alpha$ -2b, we explored the association between the IFN- $\alpha$ -2b initiation schedule and clinical outcomes; delayed interferon intervention was associated with increased probabilities of risk events.

The efficacy of broad-spectrum or targeted antivirals on SARS-CoV-2 replication and COVID-19 prognosis is under investigation in randomized clinical trials. The innate immune response, particularly the production of IFN-I (IFN- $\alpha$  and IFN- $\beta$ ), is the first line of defence against viral infection. After detection of viral invasion of host cells, IFN activates interferon-stimulated genes (ISGs), which encode downstream proteins that interfere with viral metabolism, replication and spread; it also promotes cytokine secretion by activating adaptive immunity.<sup>14</sup> Blocking of IFN-I signalling enhances monocyte, macrophage and neutrophil infiltration of the lung, promotes inflammatory

**TABLE 4** The associations between interferon starting treatment time and the outcomes

Onset-to-treatment	Composite endpoints		Mechanical ventilation		ICU admission	
	Events/Total (n)	Adjusted <sup>a</sup> HR (95% CI)	Events/Total (n)	Adjusted <sup>a</sup> HR (95% CI)	Events/Total (n)	Adjusted <sup>a</sup> HR (95% CI)
0–2 days	2/209	Reference	2/209	Reference	2/209	Reference
3–5 days	9/279	2.20 (0.43–11.13) 0.342	8/279	2.44 (0.46–12.81) 0.292	6/279	1.22 (0.21–7.01) 0.824
6–8 days	12/206	5.89 (0.99–35.05) 0.052	10/206	4.40 (0.64–30.32) 0.133	7/206	4.43 (0.66–29.77) 0.126
9–11 days	6/109	7.59 (0.76–76.07) <i>P</i> = .085	3/109	2.41 (0.15–37.90) 0.532	5/109	17.62 (1.41–220.10) 0.026
≥ 12 days	3/50	5.44 (0.25–119.77) 0.283	2/50	3.32 (0.09–127.23) 0.519	3/50	19.00 (0.79–458.93) 0.700

Abbreviations: CI, confidence interval; HR, hazard ratio; ICU, intensive care unit.

<sup>a</sup>Adjusted for sex, age, smoking status, neutrophil lymphocyte ratio, C-reactive protein, other antiviral drug treatments including lopinavir/ritonavir and arbidol, clinical type on admission and comorbidities (hypertension, cardiovascular diseases, diabetes, chronic liver disease).

responses and impairs specific T-cell responses in an animal model of MERS-CoV infection.<sup>3</sup> Chu et al. reported that the levels of type I, II and III IFN were low during SARS-CoV-2 infection.<sup>15</sup> MERS-CoV and SARS-CoV are closely related to SARS-CoV-2 and have similar characteristics,<sup>16</sup> and their nucleocapsid proteins suppress expression of the gene encoding IFN.<sup>6,17</sup> In animal models of MERS-CoV, IFN- $\alpha$ -2b increased the serum IFN- $\alpha$  level up to 37-fold compared to untreated animals 2 days after infection. Although exogenous supply of IFN exerted little effect on the serum levels of inflammatory factors and chemokines, it reduced the levels of interleukin-6, IFN- $\gamma$  and monocyte chemoattractant protein-1 in the lung, moderated the cytokine storm and improved the clinical outcomes.<sup>8</sup> Consistently, the outcomes of MERS-CoV infection were improved by IFN in preclinical studies.<sup>3–5</sup> Furthermore, IFN-I has been employed in the management of patients with SARS-CoV and accelerated resolution of radiographic lung abnormalities and restoration of oxygen saturation level.<sup>7</sup> Therefore, exogenous supply of IFN is likely to promote antiviral immunity and may be useful as an alternative treatment for COVID-19. Indeed, this is consistent with a recent report of a key role for IFN in the treatment of COVID-19.<sup>18</sup>

Intriguingly, IFN upregulates angiotensin-converting enzyme 2 (ACE2) expression in addition to its immunomodulatory effect. In brief, ACE2 is expressed in specific cell subsets, such as airway epithelial cells, and is implicated in SARS-CoV-2 invasion and COVID-19 progression. Taking ACE2 expression into consideration, the overall effect of IFN therapy needs further investigation. On the one hand, ACE2 serves as a crucial SARS-CoV receptor; acute lung failure in mice was worsened by injection of SARS-CoV spike proteins but attenuated by blocking the renin-angiotensin pathway.<sup>19</sup> Because IFN supplementation upregulates ACE2 expression, patients may be more vulnerable to viral invasion and more likely to experience disease aggravation.<sup>20</sup> On the other hand, ACE2 expression is reduced by SARS-CoV infection, resulting in extensive vasoconstriction, endothelial dysfunction and acute lung

injury.<sup>19,21</sup> Also, ARDS animal models suggest that exogenous ACE2 supplementation can improve clinical outcomes by modulating aberrant inflammation and increasing oxygenation.<sup>22</sup> IFN is effective in vitro and in certain animal models,<sup>3,4,8,23</sup> but does not improve the clinical outcomes of coronavirus-infected patients.<sup>10</sup> Also, IFN therapy showed limited effect on the high mortality rate of MERS-CoV.<sup>11,24</sup> However, two preclinical studies indicated the importance of timing: starting IFN treatment before peak viral load can reduce disease severity and mortality; conversely, delayed IFN treatment does not inhibit viral replication, and leads to increased infiltration and activation of inflammatory cells in the lungs and enhanced expression of proinflammatory cytokines, leading to fatal pneumonia.<sup>3,4</sup>

Based on the above rationale, we investigated the therapeutic effect of the onset-to-treatment interval on the clinical outcomes of COVID-19. Delayed IFN intervention was associated with increased probabilities of risk events, with a peak risk at about 7 days, coinciding with the peak SARS-CoV-2 load.<sup>25</sup> Furthermore, animal models have shown that the timing of IFN-I responses and maximal viral replication were key determinants of the results.<sup>3,4</sup> Our results indicated that earlier IFN intervention reduces the risk of events, possibly by immune modulation and ACE2 upregulation but has limited efficacy when the viral load peaked. Therefore, the timing of IFN- $\alpha$ -2b therapy is important and further investigation is needed to verify our results.

This is the largest report of an association between IFN- $\alpha$ -2b aerosol inhalation and improved clinical outcomes in patients with COVID-19 in China. We detected a curvilinear relationship between the interval from symptom onset to IFN- $\alpha$ -2b treatment and clinical outcomes. Delayed IFN intervention was associated with increased probabilities of risk events. However, this study had several limitations. First, there are limitations inherent to any observational study. That said, after adjusting for confounders including coexisting comorbidities, baseline disease severity and antivirals, we obtained



consistent results from different statistical models. However, IFN- $\alpha$ -2b treatment was not randomly assigned, and potential unmeasurable confounding factors may have biased the results. Second, there is no effective indicator of whether an inhaled dose is sufficient, but it may be possible to use the serum interferon levels for this purpose. Third, the data were collected retrospectively through electronic medical record system, and information like side effects data were not obtained. Thus the other side effects of IFN- $\alpha$ -2b treatment could not be assessed.

## 5 | CONCLUSION

From a large cohort of hospitalized patients with COVID-19, PS matching and a subgroup analysis revealed that IFN- $\alpha$ -2b aerosol inhalation in combination with standard treatment may be associated with improved clinical outcomes of patients with COVID-19. Delayed IFN intervention was associated with increased probabilities of risk events, and so the timing of IFN therapy is an important clinical consideration. Further randomized clinical trials are needed to validate our findings.

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### COMPETING INTERESTS

The authors have no conflicts of interest to declare.

### CONTRIBUTORS

H.C. and L.L. were responsible for the concept and study design. J.Y., X.L., L.T., X.S., J.M., F.L., J.W., Q.P. and J.Y. acquired the data and prepared the manuscript. J.Y., X.L. and L.T. analysed the data and J.Y. and L.T. were responsible for the Statistical analysis. All the authors reviewed and approved the final manuscript.

### DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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