Supplemental Online Content

Cheng L-l, Guan W-j, Duan C-y, et al. Effect of recombinant human granulocyte colony–stimulating factor for patients with coronavirus disease 2019 (COVID-19) and lymphopenia: a randomized clinical trial. *JAMA Intern Med*. Published online September 10, 2020. doi:10.1001/jamainternmed.2020.5503

This supplemental material has been provided by the authors to give readers additional information about their work.

eMethods

Participating sites

Four participating sites were invited. Of these, no eligible patient was screened in one of the sites (The First Affiliated Hospital of Guangzhou Medical University). Therefore, all patients were from three participating sites located in Guangzhou (Guangzhou No. 8 People's Hospital) and Wuhan city (Wuhan Union Hospital, Wuhan Hankou Hospital).

Inclusion criteria

1. Patients aged 15-80 years who tested positive to reverse transcription polymerase-chain-reaction (RT-PCR) assay for SARS-CoV-2 in nasopharyngeal sample (including mild disease, common disease and severe disease as defined by the Protocol for the Diagnosis and Treatment of Coronavirus disease 2019 (version 5), drafted by the Chinese National Health Commission.

2. Having peripheral blood leukocyte count of 150 $\times 10^9$/L or less, and peripheral blood lymphocyte (PBL) count of 0.8 $\times 10^9$/L or lower

Exclusion criteria

1. Critical Covid-19 (developed respiratory failure needing mechanical ventilation, shock or other organ failure requiring admission to intensive care unit)
2. Any comorbidity (e.g., hypertension, diabetes, coronary heart disease)
3. Malignancy
4. Breastfeeding and pregnant women
5. Severe mental disorders
6. Unstable angina pectoris or ischemic infarction or cardiac angiography within 6 months, severe stenosis of the main streams of coronary artery;
7. Cerebral infarction or hemorrhage within 6 months
8. Allergic or intolerant to rhG-CSF
9. Other conditions determined by the investigators.

Definitions of the outcomes

Primary outcome

The primary outcome used for evaluating the efficacy is the time to clinical improvement which defined as the duration from randomization to the improvement of at least one point on a seven-category ordinal scale.

Secondary outcomes

Secondary outcomes included the following:

post-treatment lymphocyte count;

mortality;

proportions of patients critical conditions;

viral loads;

Hospital stay days;

Oxygen support days.

Safety outcomes

Safety outcomes included the following:

adverse events;

serious adverse events;

premature discontinuation of treatment.

eRESULTS

eTable 1. Treatments received after enrollment

Characteristic	rhG-CSF (n = 100)	Usual care (n = 100)	Total (N = 200)
Treatments during the study period, No. (%)	-	-	-
Non-invasive mechanical ventilation	16 (16)	43 (43)	59 (29.5)
Invasive mechanical ventilation	2 (2)	14 (14)	16 (8)
Extracorporeal membrane oxygenation	1 (1)	3 (3)	4 (2)
Glucocorticoid therapy	25 (25)	32 (32)	57 (28.5)
Duration of corticosteroids therapy, median (IQR), d	4 (3-5)	4 (3-6)	4 (3-5)

rhG-CSF: recombinant human granulocyte colony stimulating factor; none of the study participants had documented comorbidities according to our exclusion criteria

eTable 2. Sensitivity analysis results for the primary outcome

Characteristic*	rhG-CSF	Usual care	Hazard ratio (95%CI)†		
				Model 1	Model 2
Primary analysis					
Sample size	100	100	Unadjusted	1.29 (0.98 to 1.71)	1.22 (0.91 to 1.65)
Time to clinical improvement, median (IQR), d	12 (10 to 16)	13 (11 to 17)	Adjusted	1.28 (0.95 to 1.71)	1.23 (0.91 to 1.65)
Sensitivity analysis based on actual treatment exposure					
Sample size	98	102	Unadjusted	1.34 (1.02 to 1.77)	1.23 (0.91 to 1.65)
Time to clinical improvement, median (IQR), d	12 (10 to 16)	13 (11 to 17)	Adjusted	1.34 (1.00 to 1.79)	1.23 (0.91 to 1.66)

*rhG-CSF: recombinant human granulocyte colony stimulating factor; 95%CI: 95% confidence interval

†Unadjusted: Only included treatment group into model. Adjusted: Included treatment group, oxygen therapy and center into model. Model 1: Fine and Gray proportional sub-distribution hazards model ; Model 2: Cause-specific proportional hazards model

eTable 3. A list of the details pertaining to the patients who progressed to death during the study

rhG-CSF	Usual care
Patient No. 37 was rated as having grade 4 at enrollment (having critically ill Covid-19 on day 4 and died on day 10)	Patient No. 2 was rated as having grade 5 at enrollment (having critically ill Covid-19 on day 7 and died on day 14)
Patient No. 70 was rated as having grade 4 at enrollment (having critically ill Covid-19 on day 2 and died on day 5)	Patient No. 12 was rated as having grade 5 at enrollment (having critically ill Covid-19 on day 27 and died on day 7)
	Patient No. 14 was rated as having grade 3 at enrollment (having critically ill Covid-19 on day 6 and died on day 13)
	Patient No. 15 was rated as having grade 3 at enrollment (having critically ill Covid-19 on day 5 and died on day 14)
	Patient No. 22 was rated as having grade 5 at enrollment (having critically ill Covid-19 on day 8 and died on day 13)
	Patient No. 28 was rated as having grade 4 at enrollment (having critically ill Covid-19 on day 7 and died on day 15)
	Patient No. 33 was rated as having grade 5 at enrollment (having critically ill Covid-19 on day 8 and died on day 15)
	Patient No. 44 was rated as having grade 4 at enrollment (having critically ill Covid-19 on day 67 and died on day 16)
	Patient No. 46 was rated as having grade 3 at enrollment (having critically ill Covid-19 on day 8 and died on day 19)
	Patient No. 86 was rated as having grade 4 at enrollment (having critically ill Covid-19 on day 2 and died on day 3)

eTable 4. Fatality rate in the intention-to-treat population

Characteristic	rhG-CSF (n = 100)	Usual care (n = 100)	Risk difference (95%CI)
Day 21 fatality, No. (%)	2 (2.0)	10 (10.0)	-8.0 (-15.6 to -1.3)
Day 28 fatality, No. (%)	3 (3.0)	12 (12.0)	-9.0 (-17.1 to -1.6)
Day 60 fatality, No. (%)	3 (3.0)	13 (13.0)	-10.0 (-18.2 to -2.4)

The 28- and 60-day fatality was calculated based on the follow-up information

eTable 5. Association between oxygen therapy and PBL count

Oxygen therapy	PBL count, $\times 10^9$/L	
	⩽0.4	**>0.4**
Not requiring supplemental oxygen	0 (0.0%)	26 (24.8%)
Requiring supplemental oxygen	41 (43.2%)	79 (75.2%)
Requiring HFNC or non-invasive mechanical ventilation	54 (56.8%)	0 (0.0%)
Total	95 (100.0%)	105 (100.0%)

eTable 6. Subgroup analysis results

Characteristic*	rhG-CSF	Usual care	Difference†
Patients with PBL count of equal or less than 0.4 ×10^9/L at baseline	n=46	n=49	
Primary outcome			
Time to clinical improvement, median (IQR), d	12 (9 to 15)	14 (11 to 18)	1.86 (1.23 to 2.83) ‡
Secondary outcomes			
Patients progressing to critical condition, No. (%)	0 (0.0)	11 (22.4)	-22.5 (-35.9 to -10.3)
Day 21 fatality, No. (%)	0 (0.0)	8 (16.3)	-16.3(-29.0 to -5.4)
Oxygen support duration, median (IQR), d	10 (8 to 12)	10 (8 to 14)	-1 (-2 to 1)
Hospital stay, median (IQR), d	13 (10 to 16)	14 (11 to 17)	-1 (-3 to 1)
Lymphocyte cell count on Day 5, median (IQR), ×10^9/L	0.96 (0.86 to 1.05)	0.53 (0.42 to 0.62)	0.43 (0.37 to 0.50)
Patients with PBL count of greater than 0.4 ×10^9/L at baseline	n=54	n=51	
Primary outcome			
Time to clinical improvement, median (IQR), d	12 (11 to 17)	12 (10 to 17)	0.92 (0.64 to 1.33) ‡
Secondary outcomes			
Patients progressing to critical condition, No. (%)	2 (3.7)	4 (7.8)	-4.1 (-15.1 to -5.9)
Day 21 fatality, No. (%)	2 (3.7)	2 (3.9)	-0.2(-9.9 to 9.1)
Oxygen support duration, median (IQR), d	10 (9 to 12)	9 (8 to 13)	1.0 (-1 to 2)
Hospital stay, median (IQR), d	14 (12 to 17)	14 (11 to 18)	0.0 (-2 to 2)
Lymphocyte cell count on Day 5, median (IQR), ×10^9/L	1.16 (1.05 to 1.22)	0.72 (0.66 to 0.83)	0.41 (0.35 to 0.47)

*IQR, interquartile range; rhG-CSF: recombinant human granulocyte colony-stimulating factor; PBL: peripheral blood lymphocyte; critical conditions included acute respiratory distress syndrome, sepsis or septic shock; 95%CI: 95% confidence interval

† The difference in the primary or secondary endpoints was expressed as the difference of the rate or median levels by using the Hodges–Lehmann estimate and the 95% CIs.

‡The hazard ratio for clinical improvement was estimated by the Fine and Gray proportional sub-distribution hazards model.

§The change from baseline in the viral load was compared, and the mean difference of least-squares means was estimated with the mixed-effect model, with the baseline level being the covariate.

eTable 7. Sensitivity *post hoc* subgroup analysis for the primary outcome

Subgroup*	No. of event/patients		Hazard ratio (95%CI)†
	rhG-CSF	Usual care	
PBL count, ×10^9/L			
<=0.3	10/13	3/13	3.10 (1.22 to 7.87)
0.3 to 0.4	33/33	29/36	1.67 (1.08 to 2.59)
0.4 to 0.6	23/26	27/27	0.86 (0.55 to 1.36)
> 0.6	25/28	21/24	1.16 (0.69 to 1.95)

*rhG-CSF: recombinant human granulocyte colony stimulating factor; PBL: peripheral blood lymphocyte; 95%CI: 95% confidence interval

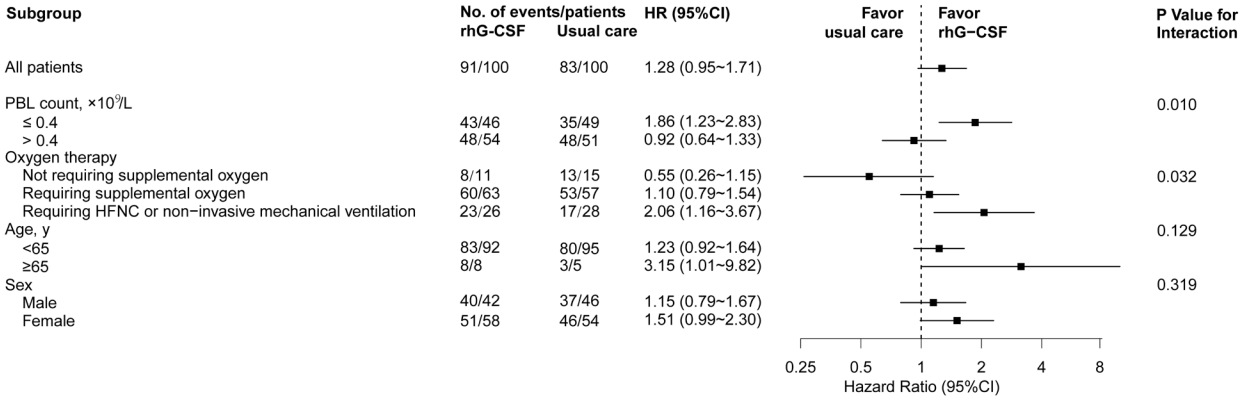
†For each subgroup, the hazard ratio for clinical improvement was estimated by the Fine and Gray proportional sub-distribution hazards model. Only treatment group was included in the model.

Figure legends

eFigure 1. Subgroup analysis of the primary outcome

The events denote the number of observed clinical improvement during the follow-up.

rhG-CSF: recombinant human granulocyte colony stimulating factor; PBL: peripheral blood lymphocyte; HR: hazards ratio; HFNC: high-flow nasal cannula



eFigure 2. The dynamic changes in peripheral blood white blood cell count

WBC: White blood cell

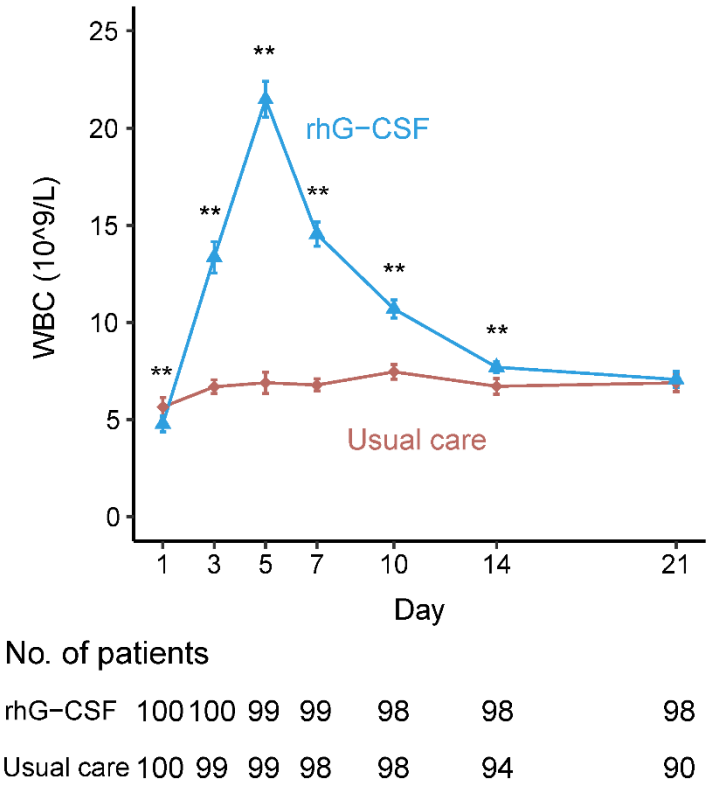
The bars indicate 95% confidence intervals.

The blue curve indicates the rhG-CSF group whereas the red curve denotes the control group (usual care).

*: P<0.05; **: P<0.01 for the comparison at individual time points between the two groups;

There was no statistical significance for the comparison between the two groups should no asterisk be demonstrated for any time point.

rhG-CSF: recombinant human granulocyte colony stimulating factor



eFigure 3: Dynamic changes in the mean T lymphocyte, CD8+ T cell and natural killer cell count

Panel A: peripheral blood lymphocyte cell count in all study participants;

Panel B: peripheral blood CD8+ T lymphocyte cell count in all study participants;

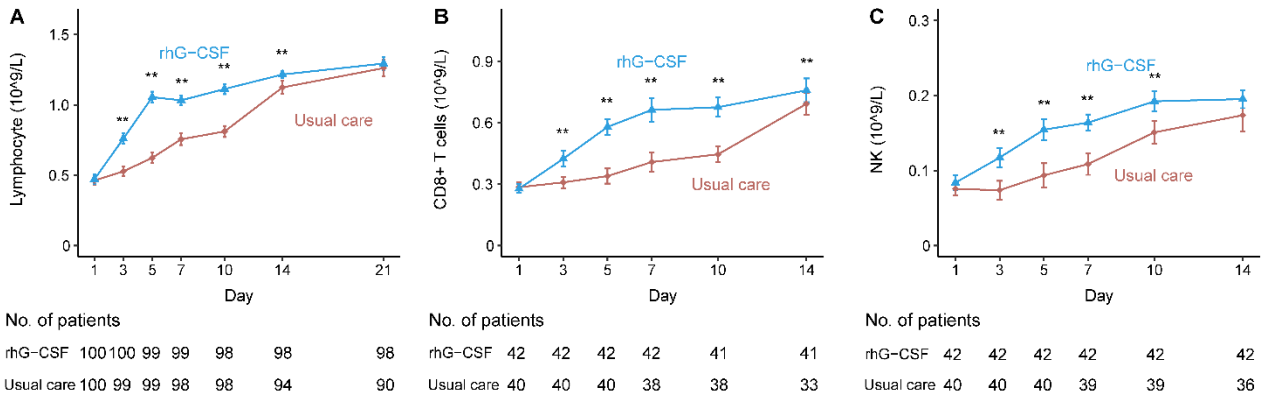
Panel C: peripheral blood natural killer cell count in all study participants;

The blue curve indicates the rhG-CSF group whereas the red curve denotes the control group (usual care).

*: P<0.05; **: P<0.01 for the comparison at individual time points between the two groups;

There was no statistical significance for the comparison between the two groups should no asterisk be demonstrated for any time point.

rhG-CSF: recombinant human granulocyte colony stimulating factor



eFigure 4. The dynamic changes in peripheral blood CD4+ T cell and B cell count

Panel A: CD4+ T cell count in the overall analysis;

Panel B: B cell count in the overall analysis;

The bars indicate 95% confidence intervals.

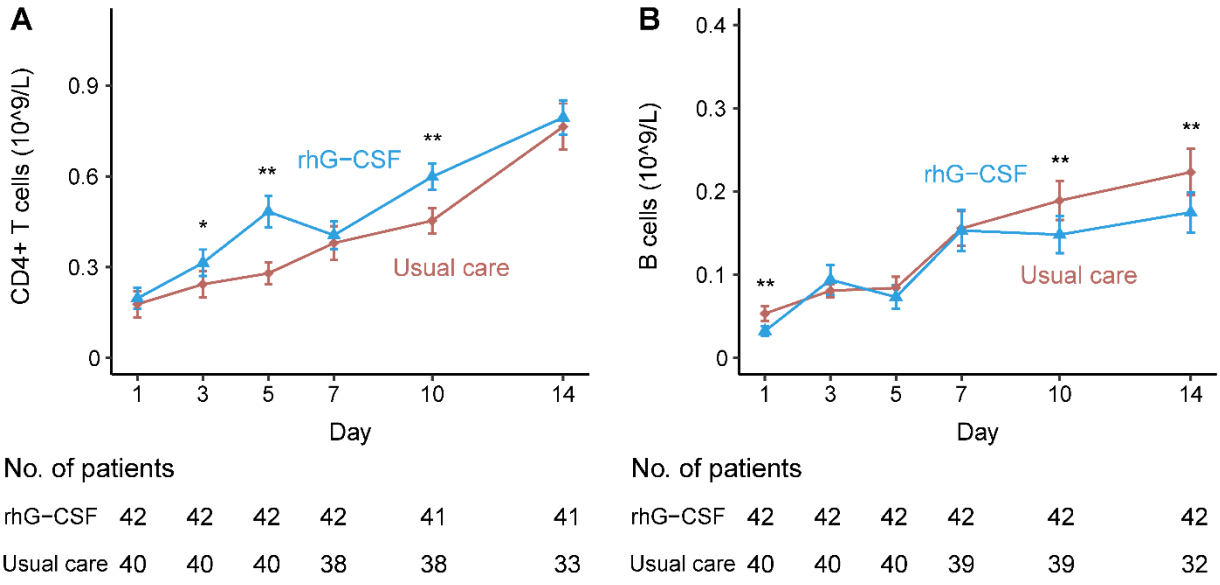
The blue curve indicates the rhG-CSF group whereas the red curve denotes the control group (usual care).

The number of patients was less than the total number in the overall analysis because not all findings were available.

*: P<0.05; **: P<0.01 for the comparison at individual time points between the two groups;

There was no statistical significance for the comparison between the two groups should no asterisk be demonstrated for any time point.

rhG-CSF: recombinant human granulocyte colony stimulating factor



eFigure 5. SARS-CoV-2 viral load by reverse transcription polymerase chain reaction on throat swabs

The cycle threshold (Ct) values of Orf1b gene of SARS-CoV-2 on RT-PCR assay that were detected in throat swabs.

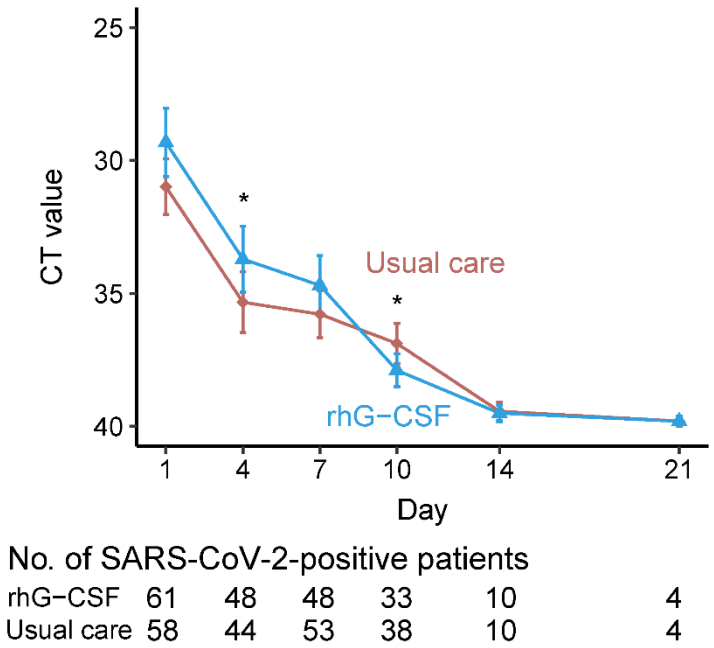
The bars indicate 95% confidence intervals. The Ct value correlates inversely with viral RNA copy numbers. Negative samples denoted a Ct value of 40, which was the lower limit of detection.

The blue curve indicates the rhG-CSF group whereas the red curve denotes the control group (usual care).

*: P<0.05; **: P<0.01 for the comparison at individual time points between the two groups

There was no statistical significance for the comparison between the two groups should no asterisk be demonstrated for any time point.

rhG-CSF: recombinant human granulocyte colony stimulating factor; RT-PCR: reverse transcription polymerase chain reaction



eFigure 6. Subgroup analysis of the time to clinical improvement at day 21

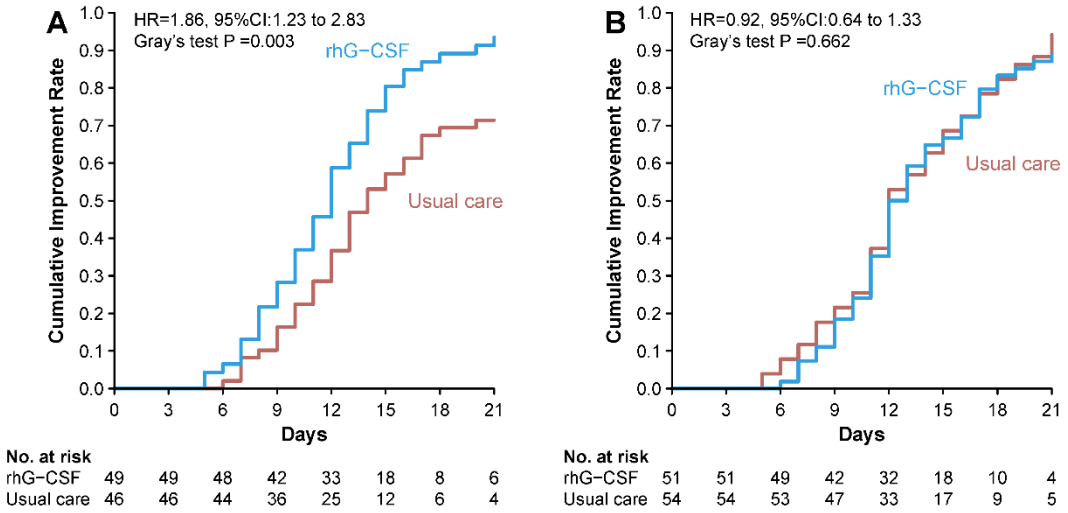
Figure E6-A. Time to Clinical Improvement in population with peripheral blood lymphocyte cell count equal to or less than 0.4 $\times 10^9$/L

Figure E6-B. Time to Clinical Improvement in population with peripheral blood lymphocyte cell count greater than 0.4 $\times 10^9$/L

The blue curve indicates the rhG-CSF group whereas the red curve denotes the control group (usual care).

The hazards ratio of achieving clinical improvement, along with the 95% confidence interval and the P value, is also displayed.

rhG-CSF: recombinant human granulocyte colony stimulating factor



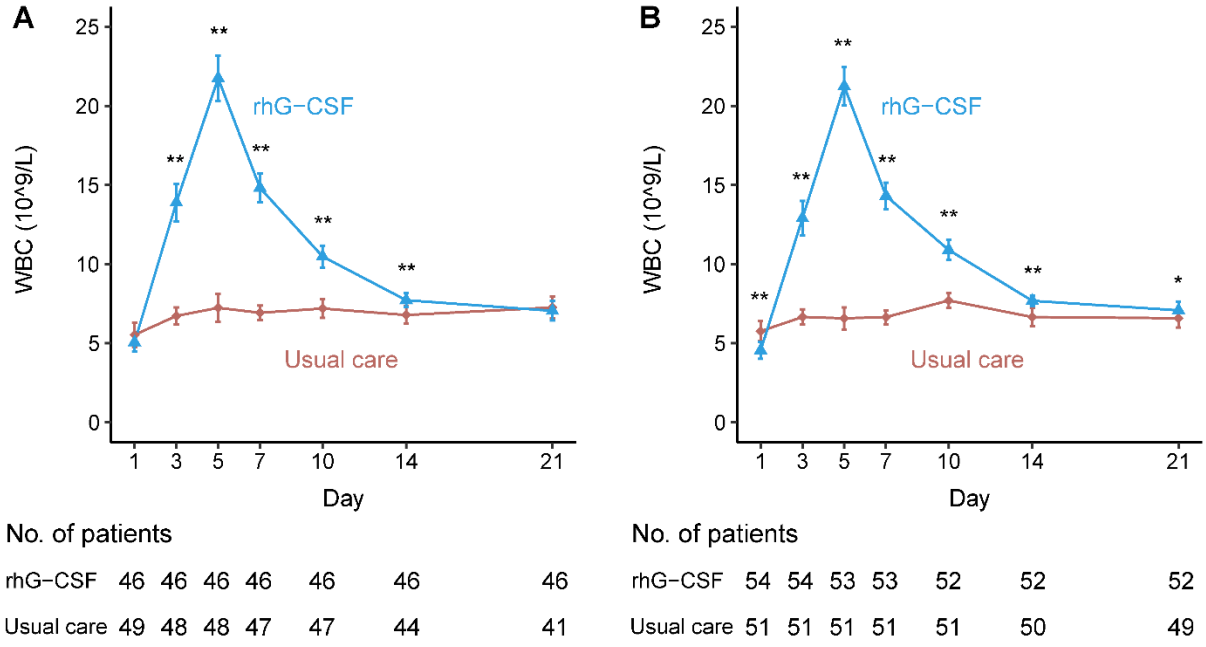
eFigure 7. Subgroup analysis of the dynamic changes in peripheral blood leukocyte count

WBC: White blood cell

Panel A: WBC count in patients with lymphocyte cell count being equal to or less than 0.4 $\times 10^9$/L;

Panel B: WBC count in patients with lymphocyte cell count being greater than 0.4 $\times 10^9$/L.

There was no statistical significance for the comparison between the two groups should no asterisk be demonstrated for any time point.



eFigure 8. Subgroup analysis of the dynamic changes in the mean T lymphocyte subset count

Panel A: peripheral blood lymphocyte cell count in patients with lymphocyte cell count being equal to or less than 0.4 $\times 10^9$/L;

Panel B: peripheral blood lymphocyte cell count in patients with lymphocyte cell count being greater than 0.4 but lower than 0.8 $\times 10^9$/L;

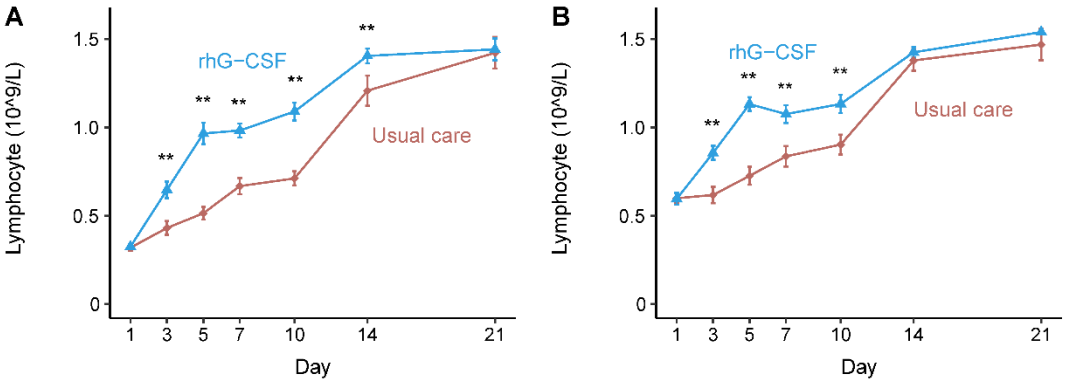
Panel C: peripheral blood CD8+ T lymphocyte cell count in patients with lymphocyte cell count being equal to or less than 0.4 $\times 10^9$/L;

Panel D: peripheral blood CD8+ T lymphocyte cell count in patients with lymphocyte cell count being greater than 0.4 but lower than 0.8 $\times 10^9$/L;

Panel E: peripheral blood natural killer cell count in patients with lymphocyte cell count being equal to or less than 0.4 $\times 10^9$/L;

Panel F: peripheral blood natural killer cell count in patients with lymphocyte cell count being equal to or less than 0.4 but lower than 0.8 $\times 10^9$/L.

There was no statistical significance for the comparison between the two groups should no asterisk be demonstrated for any time point.



eFigure 9. Subgroup analysis of the dynamic changes in peripheral blood lymphocyte subset count

Panel A: CD4+ T cell count in patients with lymphocyte cell count being equal to or less than 0.4 $\times 10^9$/L;

Panel B: CD4+ T cell count in patients with lymphocyte cell count being greater than 0.4 but lower than 0.8 $\times 10^9$/L;

Panel C: B cell count in patients with lymphocyte cell count being equal to or less than 0.4 $\times 10^9$/L;

Panel D: B cell count in patients with lymphocyte cell count being greater than 0.4 but lower than 0.8 $\times 10^9$/L;

There was no statistical significance for the comparison between the two groups should no asterisk be demonstrated for any time point.