Multicenter randomized controlled trial for rhG-GSF in the treatment of coronavirus disease 2019 (COVID-19)

PROTOCOL

(COVID-19 study)

Version 2.0 **(**Feb. 01, 2020)

Authors: COVID-19 Study Group

Contents

Abbreviations

Abbreviation	Full name
COVID-19	Coronavirus disease 2019
GM-CSF	Granulocyte macrophage colony stimulating factor
IL-6	Interleukin-6
SARS	Severe acute respiratory syndrome
rhG-CSF	recombinant human granulocyte colony stimulating factor
ICU	Intensive care unit
RCT	Randomized controlled trial
PD-1	Programmed death-1
CRP	C reactive protein
SARS-CoV-2	Severe acute respiratory syndrome coronavirus-2
RT-PCR	Reverse transcription polymerase chain reaction
TBNK	T and B lymphocyte and NK cells
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-10	Interleukin-10
TNF-α	Tumor necrosis factor-α
IFN-γ	Γ-interferon
LOCF	Last observation carry forward
ITT	Intention-to-treat population
SS	Safety set
SAE	Serious adverse event
GCP	Good clinical practice
HCT	hematocrit
PaO2	Partial pressure of oxygen
ADR	Adverse drug reaction
AE	Adverse event
ALP	Alkaline phosphatase

AST	Aspartate aminotransferrase
LDH	Lactate dehydrogenase
ARDS	Acute respiratory distress syndrome
QD	Once daily
BUN	Blood urea nitrogen
CDC	Center for disease control and prevention
CFDA	Chinese food and drug administration
ECMO	Extracorporeal membrane oxygenation
IEC	Independent ethics committee
IP	Investigational product
IRB	Institutional review board
PCR	Polymerase chain reaction
SAP	Statistical analysis plan

88

89

Abstract

Title	**Effects of Recombinant human Granulocyte Colony Stimulating Factor for Covid-19 with lymphopenia: A multicenter, randomized controlled trial**
PI	Prof Nan-shan Zhong
Lead site	The First Affiliated Hospital of Guangzhou Medical University
Participating sites	The First Affiliated Hospital of Guangzhou Medical University, Guangzhou No. 8 People's Hospital, Wuhan Union Hospital, Wuhan Hankou Hospital
Objective	To determine the effectiveness and safety of recombinant human Granulocyte Colony Stimulating Factor for Covid-19 with lymphopenia
Study design	Multicenter, randomized, standard-of-care controlled, superiority trial
Study population	**Inclusion criteria** 1. Patients aged 18 years or greater who tested positive to reverse transcription polymerase-chain-reaction (RT-PCR) assay for SARS-CoV-2 in oropharyngeal sample, had pneumonia as confirmed by chest imaging; meeting the diagnostic criteria established by the *Protocol for the Diagnosis and Treatment of Coronavirus Disease (Trial version 5)* by the National Health Commission 2. Peripheral blood leukocyte count being 1500 per cubic milliliters or less and peripheral blood lymphocyte (PBL) count of 800 per cubic milliliters or lower **Exclusion criteria** 1. Critical illness requiring invasive ventilation, developed shock or other organ failure that requires admission to intensive care unit 2. Any comorbidity (e.g., chronic obstructive pulmonary disease, hypertension, diabetes, coronary heart disease) 3. Malignancy 4. Breastfeeding and pregnant 5. Severe mental disorders 6. Unstable coronary heart diseases (angina pectoris, myocardial infarction, or severe stenosis of coronary arteries as indicated by angiography) within 6 months 7. Cerebral infarction or hemorrhage within 6 months 8. Intolerant or allergic to rhG-CSF 9. Deemed ineligible by the investigators

<table>
<tr><td>Diagnostic criteria</td><td>Meeting the diagnostic criteria established by the Protocol for the Diagnosis and Treatment of Coronavirus Disease (Trial version 5) by the National Health Commission</td></tr>
<tr><td>Sample size</td><td>Totally 200 cases (100 cases in each group)</td></tr>
<tr><td>Interventions</td><td>Treatment group: Standard-of-care (SOC) alone or plus rhG-CSF (5 μg/kg subcutaneously once daily from days 0 to 2)

Control group: SOC

Standard-of-care treatment: Based on the Protocol for the Diagnosis and Treatment of Coronavirus Disease (Trial version 5) by the National Health Commission, SOC comprised resting, supportive treatment (supplemental oxygen, non-invasive ventilation, or intravenous antibiotics), supplementation of nutrition and energy, water and electrolyte equilibrium, and maintenance of homeostasis</td></tr>
<tr><td>Endpoints</td><td>Primary endpoint
The time to clinical improvement (endorsed by the World Health Organization R&D Blueprint expert group) within 21 days - the duration from randomization to the improvement of at least one point on a seven-category ordinal scale or discharge from hospital, whichever occurred first
The 7-category scale
<table><tr><th>Score</th><th>Criteria</th></tr><tr><td>1</td><td>Non-hospitalized with normal activities</td></tr><tr><td>2</td><td>Non-hospitalized but unable to resume normal activities</td></tr><tr><td>3</td><td>Hospitalized but not requiring supplemental oxygen</td></tr><tr><td>4</td><td>Hospitalized and requiring supplemental oxygen</td></tr><tr><td>5</td><td>Hospitalized and requiring nasal high-flow oxygen therapy, noninvasive mechanical ventilation, or both</td></tr><tr><td>6</td><td>Hospitalized and requiring extracorporeal membrane oxygenation, invasive ventilation, or both</td></tr><tr><td>7</td><td>Death</td></tr></table>
Secondary endpoints
Lymphocyte count at each visit
Mortality at day 21</td></tr>
</table>

	The proportion of patients progressing to critical conditions (acute respiratory distress syndrome, sepsis or septic shock) at day 21 Viral loads and detection rate at each visit The duration of oxygen therapy Duration of hospitalization
Safety	Adverse events
Follow-up	From enrollment to day 21 or discharge from hospital after randomization
Superiority	The improvement of at least one point on a seven-category ordinal scale or discharge from hospital would be shorter in the treatment group
Statistical analysis	**Hypothesis** H0: No difference in the duration from enrollment to clinical improvement between the two groups H1: Shorter duration from enrollment to clinical improvement in treatment group **Main outcomes** Kaplan-Meier plot will be applied, with Gray's test for comparison of the between-group differences; Fine and Gray proportion sub-distribution hazards model will be applied, along with the HR and the 95%CI. Right-censoring of data at day 21 will be conducted in case of a failure to reach to the clinical improvement. Death events before day 21 will be treated as the competing risk event.
Anticipated progress	2020.02.03~2020.04.10

91

92

93

94 # Amendment records

Date of revision	Version	Chapter	Main contents
February 15	V2.0	2.1; 2.4	The First Affiliated Hospital of Guangzhou Medical University was not in the list because no patient was included. We have further adjusted the sample size to 40 per group for the patients to be recruited from Guangzhou No. 8 People's Hospital

95

Signature for the principal investigator (lead site)

I have reviewed and confirmed the study protocol, and will fulfill my responsibilities based on the Chinese laws, Declaration of Helsinki, the Good Clinical Practice and the study protocol. I consent to dispatch the data audit managers to ensure the reliability, accuracy and completeness of the data and the timely entry to warrant high quality of the clinical trial findings. I declare that the investigators will maintain the confidentiality of the personal information and other relevant data. I will implement necessary measures to protect the safety, rights and responsibility of the study participants. I will implement the updated protocol after notifying the participating sites to amend the study protocol and obtaining ethics committee approval.

Lead site：The First Affiliated Hospital of Guangzhou Medical University

PI：Nan-shan Zhong (Print) _____(Signature)

Date：2020-2-18 **Phone**：_____

Address：151 Yanjiang Road, Guangzhou **Postal code**：510120

Signature page of the study protocol

I will take the responsibility of the study investigator by directly guiding the conduct of the clinical trial based on the Good Clinical Practice. Having received the investigator's protocol, I have been aware of and reviewed the research background before initiating the clinical. I consent to take my responsibilities based on the Chinese laws, Declaration of Helsinki, the Good Clinical Practice and the study protocol. I will not implement the updated protocol after notifying the participating sites to amend the study protocol and obtaining ethics committee approval unless the circumstance when the safety, rights and responsibility of the study participants should be protected. I will medical decisions related to the clinical practice to ensure that all study participants who have developed adverse events during the trial to receive timely management, and document and report all these adverse events based on the national regulations.

I will ensure the reliability, accuracy and completeness of the data and the timely entry to warrant high quality of the clinical trial findings. I will cooperate with the monitoring or audit which is executed by the data monitor or audit managers dispatched by the sponsor. I declare that I will maintain the confidentiality of the personal information and other relevant data. I consent to disclose my names, occupation, and the expenditure associated with the clinical trial, and prohibit any commercial or economic behaviors related to the clinical trial to the sponsor. I consent to submit the research findings for drug registry and publication.

Lead site: The First Affiliated Hospital of Guangzhou Medical University

PI: Nan-shan Zhong (Print) _____(Signature)

Date: 2020-2-18 **Phone**: _____

Address: 151 Yanjiang Road, Guangzhou **Postal code**: 510120

Clinical trial protocol

1 Background and objective

1.1 Study background

Coronavirus disease 2019 (COVID-19) has masqueraded globally. The incubation period of Covid-19 might be longer than SARS and is highly contagious. Some of the patients might readily progress to critical illnesses or death. In a study of 138 patients with Covid-19 recently published in JAMA [1], the dynamic changes in peripheral blood leukocyte and lymphocyte count correlated significantly with the prognosis of patients with Covid-19. The leukocyte count did not exceed the upper limit of normal from onset of disease to day 19 among the survivors, whereas the leukocyte count rapidly increased since day 7 and exceeded the upper limit of normal since day 9 among the non-survivors. It was also reported that lymphopenia existed in a considerable proportion of survivors and non-survivors with Covid-19. However, there was a more dramatic decrease in the lymphocyte count among the non-survivors who had both a lower level at baseline but also sustained decrease throughout the course of the disease. Moreover, the peripheral blood lymphocyte count fell to an extremely low level since day 5 after the onset of disease (remarkable reductions) in non-survivors whereas the decrease in lymphocyte count was relatively minor among survivors (only reaching to the nadir at day 9).

The mechanisms underlying these changes in the leukocyte and lymphocyte count among patients with Covid-19 remain unclear. A study suggested that the inflammatory cytokine storm was the main contributor of the progression to critical illness among patients with Covid-19 [2]. After the infection with SARS-CoV-2, the activated T cells and mononuclear cells will precipitate to the inflammatory cytokine storm via the signaling pathways of granulocyte macrophage colony stimulating factor and interleukin-6 that led to lung injury [3]. Therefore, proactive treatment should be initiated once the cytokine storm and SIRS occur.

There have been a number of treatments for critical illness of Covid-19 [4]: 1. Treatments against shock (for maintenance of the vital signs): infusion to increase the blood volume, use of vasopressors, mechanical ventilation if indicated to protect the organ function; 2. Supportive and symptomatic treatment (revitalization): infusion, and maintenance of the water and electrolyte equilibrium, energy and nutritional support; 3. Suppressing excessive

activation of the immune cells and production of the cytokines (for promoting rehabilitation): Adequate use of corticosteroids, non-steroidal anti-inflammatory drugs or free radical scavenger (high-dose vitamin C and E); 4. Antibodies to neutralize the cytokine storm (precision treatment): Monoclonal antibodies that target specially at the corresponding cytokines to prevent from further progression or death.

Based on these understandings, there have been multiple randomized controlled trials that are on the way. Interleukin-6 receptor blocker tocilizumab (recombinant humanized interleukin-6 monoclonal antibody) has been proposed to antagonize the inflammatory cytokine storm. There has also been a phase III trial with tocilizumab in patients with Covid-19. Anti-PD-1 medications have also been proposed to activate the immune pathways for the treatment of Covid-19 (ChiCTR2000029806). Regardless, a precise evaluation should be needed before initiating the therapies. Interleukin-6 inhibitors should be applied in case of the excessive immune responses and anti-PD-1 agents should be applied in case of the suppressed immune function. However, the clinical application of these drugs would be challenging because there could be overlap in the states.

We think that the progression of disease cannot be totally explained by the cytokine storm when analyzed based on the dynamic changes in peripheral blood leukocyte count. Leukopenia along with lymphopenia also existed in a number of patients with mild Covid-19. However, no remarkable signs of cytokine storms were identified upon discharge from the hospital. We therefore hypothesized that the SARS-CoV-2 might have predisposed to the marked reduction in the lymphocyte count.

Upon literature review [5.6], along with the mechanistic investigations published in 2003, we think that the viral infections in the lymph tissues resulted in suppressed immunity that led to aberrant immune responses (lymphopenia, lung injury). The cytokine storm would subsequently predispose to further aggravation of the lung injury, resulting in organ dysfunction or even death. Therefore, some pilot studies have attempted to manage SARS with the recombinant human granulocyte colony stimulating factor (rhG-CSF), with remarkable therapeutic outcomes -- markedly increased lymphocyte count after treatment. Notably, no major adverse impacts (aggravation of pneumonia) or aggravation of inflammatory responses were identified. The possible mechanisms might be that rhG-CSF led to decreased responsiveness to the viral antigen and up-regulated immunity and ameliorated

inflammatory responses.

Based on the homology of SARS-CoV and SARS-CoV-2 as well as the successful treatment experience during the pilot investigations, we have planned for the investigation of the effects of rhG-CSF in patients with Covid-19. For instance, we have administered rhG-CSF in two patients with peripheral blood leukocyte count being $<2.5\times10^9$/L and lymphocyte count $< 0.8\times10^9$/L. Injection of rhG-CSF (5ug/kg) for a single dose led to a marked increase in the peripheral blood leukocyte and lymphocyte count. Intriguingly, the lymphocyte count sustained despite progressively declined leukocyte count thereafter. Meanwhile, we also noted progressively decrease temperature at days 2-3 and clinical improvement. The patients were finally discharged home after achieving viral assay conversion. We have also attempted to manage another six patients with normal leukocyte count but decreased lymphocyte count, and achieved similar clinical and laboratory test findings. None of the eight patients had documented aggravation of pneumonia.

Therefore, we think that it would be justified to conduct an open-label, multicenter clinical trial of rhG-CSF in patients with Covid-19 who had lymphopenia. Our findings will provide a solid basis for further exploration of the mode of action of rhG-CSF in patients with Covid-19.

1.2 Objectives

Primary objective: To determine the effectiveness of recombinant human Granulocyte Colony Stimulating Factor for Covid-19 with lymphopenia

Secondary objective: To determine the safety of recombinant human Granulocyte Colony Stimulating Factor for Covid-19 with lymphopenia

1.3 Hypothesis

H0: No difference in the duration from enrollment to clinical improvement between the two groups

H1: Shorter duration from enrollment to clinical improvement in treatment group

2 Trial design

Multicenter, randomized, standard-of-care controlled, superiority trial

2.1 Multicenter

The First Affiliated Hospital of Guangzhou Medical University, Guangzhou No. 8 People's Hospital, Wuhan Union Hospital, Wuhan Hankou Hospital

2.2 Allocation

Treatment group: Standard-of-care (SOC) alone or plus rhG-CSF (5 μg/kg subcutaneously once daily from days 0 to 2)

Control group: SOC

2.3 Randomization

Because the decision whether or not to initiate oxygen therapy (nasal cannula or mask, or high-flow oxygen, non-invasive ventilation) will be based on patient's disease severity, we will stratify the patients to balance the distribution of oxygen therapy between the two groups (non-invasive, invasive ventilation or ECMO). A statistician blinded to trial allocation will be responsible for generating the permuted block (four patients per block) randomization sequence by using SAS software (version 9.4, SAS Institute, USA)

2.4 Sample size

No report was available to provide the basis for sample size estimation. Assuming a two-sided significance level of α=0.05, the median duration of 14 days in SOC group and 70% of patients would achieve clinical improvement, 192 patients would be needed to provide a power of 90% to detect a 6-day difference in the median time to clinical improvement. In light of the relatively low drop-out rate, we consider that a total of 200 cases (100 per group) would be sufficient to power the analysis.

Table 1. Study participant number assignment

Participating sites	Number	Treatment group	Control group
The First Affiliated Hospital of Guangzhou Medical University	1	0	0
Guangzhou No. 8 People's Hospital	2	40	40
Wuhan Union Hospital	3	10	10
Wuhan Hankou Hospital	4	50	50

2.5 Blinding

We have adopted an open-label design.

2.6 Superiority

The improvement of at least one point on a seven-category ordinal scale or discharge from hospital would be shorter in the treatment group

2.7 Follow-up and timing

We will recruit hospitalized patients for continuous monitoring until discharge from hospital or the end-of-treatment.

2.8 Flow chart

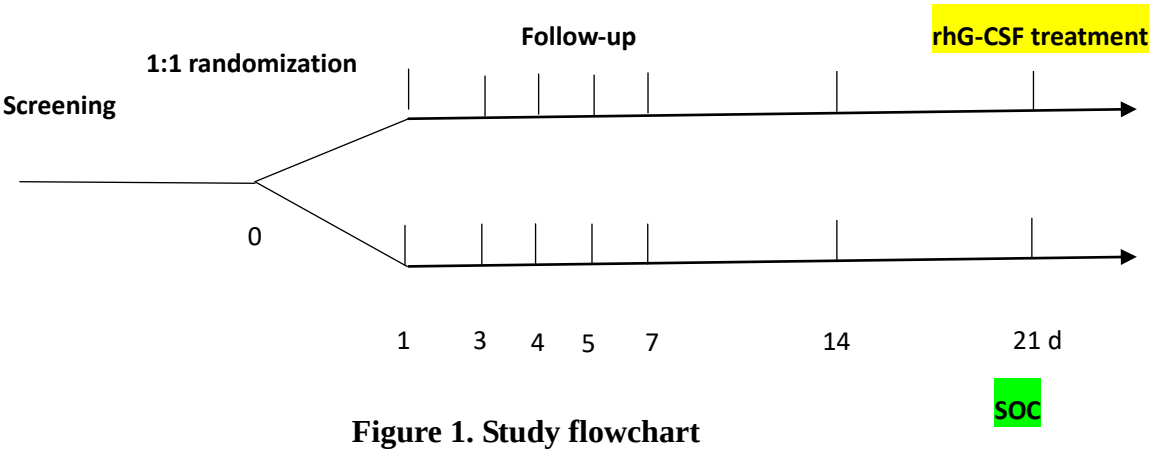

Figure 1. Study flowchart

1. Baseline and enrollment

Day 0

History: name, sex, time of onset, onset of fever, previous use of medications and the duration

Vital signs: temperature, heart rate, respiratory rate, blood pressure, oxygen saturation

Symptoms and treatment: fever, cough, dyspnea, oxygen therapy (nasal cannula, transnasal humidified high-flow oxygenation, non-invasive, invasive ventilation, ECMO)

Laboratory assessment: blood routine test, liver enzymes (AST, ALT and LDH); cardiac enzymes (CKMB, etc.), renal function (including blood creatinine), blood gas analysis, PCT, CRP, lymphocyte subset (CD4+T, CD8+T and NK cells), oropharyngeal swab SARS-CoV-2 assay for Orf1ab segment, chest imaging

7-category scale assessed daily

2. Follow-up

Patients will be followed-up until death or 21 days, whichever occurs first. The survival status, duration of hospitalization, date of death and cause of death (if applicable) will be recorded.

Contents

- 7-category scale daily assessment
- Current symptoms or overall well-being
- Vital signs
- Supportive treatment (nasal cannula, transnasal high-flow humidification, non-invasive, invasive ventilation, and ECMO)
- Study medication
- Laboratory assessment (if indicated), blood routine test, liver enzymes (AST, ALT and LDH); cardiac enzymes (CKMB, etc.), renal function (including blood creatinine), blood gas analysis, PCT, CRP, lymphocyte subset (CD4+T, CD8+T and NK cells), oropharyngeal swab SARS-CoV-2 assay for Orf1ab segment
- Adverse events or serious adverse events
- Discharge/death/any outcomes

3. Final visits

In our study, the primary endpoint is the time to clinical improvement (endorsed by the World Health Organization R&D Blueprint expert group) within 21 days - the duration from randomization to the improvement of at least one point on a seven-category ordinal scale or discharge from hospital, whichever occurs first. Therefore, follow-up visit at day 21 would be crucial to data collection. Patients will be discharged if meeting the criteria defined by the *Protocol for the Diagnosis and Treatment of Coronavirus Disease (Trial version 5)*. Telephone visits will be adopted for patients discharged home.

4. Premature withdrawal

- Drug-related serious adverse events
- Discharge

- Death
- Patients requesting withdrawal
- Cessation of study medications for safety reasons

319

Study flow

	Screening	Baseline	Treatment						
Visit	V0	V1	V2	V3	V4	V5	V6	V7	V8
Time point	Day 0	Day 1	Day 3	Day 4	Day 5	Day 7	Day 10	Day 14	Day 21
History taking									
Informed consent	●								
History acquisition	●								
Demographics	●								
Ascertainment of inclusion	●	●							
Efficacy and safety assessment									
Symptoms and treatment: fever, cough and dyspnea, oxygen therapy	●	●	●	●	●	●	●	●	●
Vital signs (temperature, heart rate, blood pressure, oxygen saturation)	●	●	●	●	●	●	●	●	●
Complications (ARDS, septic shock, bacteremia)	●	●	●	●	●	●	●	●	●
7-category scale*	●	●	●	●	●	●	●	●	●
Chest imaging	●								●
Laboratory assessment									
Whole blood cell count	●	●	●		●	●	●	●	●
Liver function, renal function and cardiac enzymology#	●					●			●
Arterial blood gas analysis	●								
PCT	●								
CRP	●								

T lymphocyte subset&	●	●	●		●	●	●	●	●
Common pathogens	●								
Oropharyngeal swab	●	●		●		●	●	●	●
Lower respiratory tract specimens (sputum, tracheal aspirates, and BALF)	●	●		●		●	●	●	●
Trial medications									
Study medications		●	●	●					
Concomitant medications		●	●	●	●	●	●	●	●
Concomitant treatment		●	●	●	●	●	●	●	●
Adverse events		●	●	●	●	●	●	●	●

21-day follow-up consists of daily assessment during hospitalization; telephone visits will be scheduled if discharged home

*7-category scale to be evaluated at 8-9 am each day

liver function assessment: AST, ALT and LDH; cardiac enzymes (CKMB, etc.), renal function (including blood creatinine)

& Lymphocyte subsets and enumeration: CD4+T, CD8+T lymphocytes; NK cells and percentages

3 Study participants

3.1 Diagnostic criteria

Based on the *Protocol for the Diagnosis and Treatment of Coronavirus Disease (Trial version 5)* by the National Health Commission

3.2 Inclusion criteria

1. Patients aged 18 years or greater who tested positive to reverse transcription polymerase-chain-reaction (RT-PCR) assay for SARS-CoV-2 in oropharyngeal sample, had pneumonia as confirmed by chest imaging; meeting the diagnostic criteria established by the *Protocol for the Diagnosis and Treatment of Coronavirus Disease (Trial version 5)* by the National Health Commission

2. Peripheral blood leukocyte count being 1500 per cubic milliliters or less and peripheral blood lymphocyte (PBL) count of 800 per cubic milliliters or lower

3.3 Exclusion criteria

1. Critical illness requiring invasive ventilation, developed shock or other organ failure that requires admission to intensive care unit

2. Any comorbidity (e.g., chronic obstructive pulmonary disease, hypertension, diabetes, coronary heart disease)

3. Malignancy

4. Breastfeeding and pregnant

5. Severe mental disorders

6. Unstable coronary heart diseases (angina pectoris, myocardial infarction, or severe stenosis of coronary arteries as indicated by angiography) within 6 months

7. Cerebral infarction or hemorrhage within 6 months

8. Intolerant or allergic to rhG-CSF

9. Deemed ineligible by the investigators

Safety outcomes

Safety outcomes will include the following:

adverse events;

serious adverse events;

premature discontinuation of treatment.

3.3 Cessation/withdrawal criteria

1. Having serious and intolerable adverse events, and deemed directly by the study investigators (i.e. serious allergic reactions needing cessation of the study medications)
2. Loss of data affecting the assessment of the primary endpoints
3. Pregnant during the study
4. Patients requesting withdrawal。

Patients who had adverse events should be follow-up until the complete resolution of the adverse events and deemed stabilized by the investigators. Recordings should also be made on the CRF.

3.4 Management of withdrawal and cessation

Patients will have the rights to withdraw from the study at any time and will not subject to discrimination. The reasons leading to premature withdrawal will be recorded on the CRF. Follow-up visits will be scheduled for all possible means (including the day 21 visits).

3.5 Participant recruitment

Within Guangzhou No. 8 People's Hospital, Wuhan Union Hospital, Wuhan Hankou Hospital

4 Treatment interventions

Treatment group: Standard-of-care (SOC) alone or plus rhG-CSF (5 μg/kg subcutaneously once daily from days 0 to 2)

Control group: SOC

Standard-of-care treatment: Based on the *Protocol for the Diagnosis and Treatment of Coronavirus Disease (Trial version 5)* by the National Health Commission, SOC comprised resting, supportive treatment (supplemental oxygen, non-invasive ventilation, or intravenous antibiotics), supplementation of nutrition and energy, water and electrolyte equilibrium, and maintenance of homeostasis

4.1 SOC

Based on the *Protocol for the Diagnosis and Treatment of Coronavirus Disease (Trial*

version 5) by the National Health Commission

1) Lying on bed, strengthening supportive therapy, energy supplementation; maintaining water and electrolyte equilibrium, maintaining homeostasis; closely monitoring vital signs and digital oxygen saturation
2) Monitor blood routine, urine routine test, CRP, biochemical parameters (liver, cardiac and renal function), coagulation, arterial blood gas analysis
3) Effective oxygen therapy via nasal cannula, facial masks or high-flow oxygen therapy
4) Antiviral treatment if indicated
5) Antibiotics if indicated

4.2 G-CSF

rhG-CSF injections

1) Main components: rhG-CSF, which is derived from the human cystic carcinoma cells and contains 175 amino acid residues (C845H1339N223O243S9; molecular weight 18,748.88)
2) Adjuvant: polysorbate 80, mannitol, acetic acid, sodium hydroxide, water for injection
3) Size: 0.3 ml: 75 μg; 0.6 ml: 150 μg; 1.2 ml: 300μg
4) Storage: 2℃-10℃, avoid frozen
5) Manufacturer: Kyowa Hakko Kirin China Pharmaceutical Co. Ltd.

4.3 Concomitant medications or treatment

4.3.1 Medications with indication

Antiviral drugs, antibiotics, corticosteroids, paracetamol, albumin, anti-hypertensive drugs, glucose-lowering drugs, inotropic drugs or vasopressors (if indicated)

4.3.2 Medications with caution

Di-yu-sheng-bai tablets, berberin hydrochloride tablets, Sheng-bai granules, Sheng-bai oral solution, Sheng-bai Rehabilitation oral solutions

4.3.3 Rescue medications

Adrenaline, atropine, antihistamines, corticosteroids, bronchodilators

4.3.4 Medications with contraindications

Drugs with antiviral effects (i.e., arbidol, lopinavir/ritonavir, chloroquine, hydrochloroquine)

4.3.5 Concomitant medications and management of treatment

1) Medications that should be maintained must be recorded in the CRF. The name, dose, and timing should be documented for all concomitant medications.

2) Concomitant medications should be maintained based on the instructions of the investigations during the trial.

3) Other necessary medications would be allowed if clinically indicated despite the symptoms are not indicated. However, this should also be documented in the CRF.

5 Potential risks and benefits

5.1 Known potential risks

Serious adverse events such as shock, interstitial pneumonia, acute respiratory distress syndrome, an increase in the naive blood cells; other adverse events might include skin rashes, flushes (incidence <1%), neutrophil infiltrating macular, Sweet syndrome (incidence unknown), osteodynia (incidence 1%-5%), back pain, chest pain, arthralgia (incidence <1%), nausea and vomiting (incidence <1%), abnormal liver function, increased GOT and GPT (incidence <1%), elevated LDH and ALP (incidence 1%-5%), fever, headache, malaise, palpitation, increased uric acid, and elevated CRP (incidence ＜1%)

5.2 Known potential benefits

The role of rhG-CSF in the treatment of Covid-19 remains largely unclear. However, based on pilot observations, patients might benefit from the treatment because rhG-CSF may increase the leukocyte and lymphocyte count. The conduct of this trial will derive updated knowledge that will benefit more patients and better design of future clinical trials to explore the mode of action of rhG-CSF.

6 Management of the study medications

6.1 Storage

Storage: 2℃-10℃, avoid frozen. Designated personnel will be responsible for the acquisition and dispense of the study medications. The study medications will be dispensed to the study participants who fulfilled the eligibility criteria.

6.2 Preparation

The research nurse will be responsible for the subcutaneous injection of rhG-CSF (5μg/kg) at day 1, 2 and 3 based on the body weight of the patients

6.3 Dose and usage

Treatment group: Standard-of-care (SOC) alone or plus rhG-CSF (5 μg/kg subcutaneously once daily from days 0 to 2)

Control group: SOC

6.4 Duration and timing

Treatment group: Standard-of-care (SOC) alone or plus rhG-CSF (5 μg/kg subcutaneously once daily for 3 consecutive days)

Control group: SOC

Duration: 14 days

6.5 Counting of the study medications

The investigators designated the staffs to store a sufficient amount of records, including the date of receipt, batch number, date of expiry, the amount of drug delivery, the date of dispensation and retrieval. Study medications will be destroyed based on the standardized procedures. Records of drug destroy will also be made accordingly.

7 Endpoints and assessment

7.1 Efficacy

Primary endpoint

The time to clinical improvement (endorsed by the World Health Organization R&D Blueprint expert group) within 21 days - the duration from randomization to the improvement of at least one point on a seven-category ordinal scale or discharge from hospital, whichever occurs first

The 7-category scale

Score	Criteria
1	Non-hospitalized with normal activities
2	Non-hospitalized but unable to resume normal activities
3	Hospitalized but not requiring supplemental oxygen
4	Hospitalized and requiring supplemental oxygen
5	Hospitalized and requiring nasal high-flow oxygen therapy, noninvasive mechanical ventilation, or both
6	Hospitalized and requiring extracorporeal membrane oxygenation, invasive ventilation, or both

7	Death

Secondary endpoints

Lymphocyte count at each visit

Mortality at day 21

The proportion of patients progressing to critical conditions (acute respiratory distress syndrome, sepsis or septic shock) at day 21

Viral loads and detection rate at each visit

The duration of oxygen therapy

7.2 Efficacy/safety

(1) Vital signs (temperature, heart rate, respiratory rate) and important signs

(2) Symptoms and treatment: fever, cough, dyspnea and oxygen saturation

(3) Complications (ARDS, septic shock, bacteremia)

(4) 7-category scale

(5) Chest imaging

(6) Laboratory testing

① blood routine test: leukocyte, lymphocyte count

② T lymphocyte subsets and enumeration (CD4+T, CD8+T and NK cell count and percentage)

③ Oropharyngeal swab SARS-CoV-2 assay for Orf1ab segment (Ct value), with internal control for RT-PCR

④ Blood gas analysis, PCT, CRP, liver enzymes (AST, ALT and LDH); cardiac enzymes (CKMB, etc.), renal function (including blood creatinine), other pathogens

(7) Adverse events and serious adverse events

8 Adverse events

8.1 Definition

Adverse events are defined as any abrupt medical events after receiving the medical products or clinical trials, regardless of the causality associated with the treatment.

Adverse events could be any adverse or accidental abnormal signs (including laboratory test abnormalities), symptoms or diseases regardless of the causality associated with the interventions.

8.2 Range

All suspected adverse drug events include: the adverse reactions associated with drug overdose, abuse, suspension, allergies, toxicity or beyond all expected reactions; any irrelevant disease, including exacerbation of the preexisting diseases; accidents or injuries; laboratory or physical examination abnormalities.

8.3 Observation and documentation of adverse events

The investigators should be responsible for informing the study participants the actual changes in the clinical conditions before and after the therapeutic interventions. Inductive queries should be avoided. While monitoring the efficacy, the investigators should closely monitor the incidence of adverse events and determine the cause and severity and implement further management, follow up for the clinical prognosis and document the details in the CRF.

8.4 Severity grading

Mild: Acceptable or causing only slight discomforts, not affecting daily life, not needing clinical interventions

Moderate: Affecting daily life, tolerable, but needing general clinical management

Severe: Having objective manifestations, significantly affecting daily life, intolerable, needing rest at bed, and needing active clinical management

8.5 Causality inference

Based on the five-category criteria, the causality could be classified as ① Definitely casual; ② Probably causal; ③ Possibly causal; ④ Probably not causal; ⑤ Definitely not causal. Drug-related adverse events consist of those definitely causal, probably causal and possibly causal.

Causality inference algorithms

Causality	Results

	1	2	3	4	5
Definitely causal	+	+	−	+	+
Probably causal	+	+	−	+	?
Possibly causal	+	+	±	±	?
Probably not causal	+	−	±	±	?
Definitely not causal	−	−	−	−	−

+ Present − Absent ± not readily determined ? unknown

8.6 Time frame

The duration of adverse events is defined as the time between signing the informed consent to the completion of the clinical trial. All adverse events should be documented in the CRF regardless of the causality with the therapeutic interventions.

8.7 Serious adverse events

Events that meeting any of the following criteria

a. Resulting in death;

b. life-threatening;

c. Persisting or severe disabilities, or permanent loss of capacity;

d. Needing hospitalization nor prolonged hospitalization;

e. Resulting in congenital malformation

OR

Any other conditions possibly threatening the life or needing immediate surgeries based on the medical judgement.

All serious adverse events should be reported through telephone or fax to the study investigators as well as the sponsor, and report to the ethics committee of the participating site and the lead site, and the provincial and national Food and Drug Administration as well as the National Health Commission, within 24 hours upon identification.

8.8 Follow-up

All adverse evens should be closely monitored until finally being resolved, or completion of the final report as of the completion of the whole clinical trial. Follow-up should not be ceased for serious adverse events despite the completion of the clinical trial.

9 Data management

9.1 Entry

(1) Data manager will notify the investigators and request for reply in case issues or concerns have been raised before data entry. Data response query will be applied for all queries and responses.

(2) The data manager will have to fully understand the contents and coding before data entry. The process of coding and all contents in the table will be recorded in a designated coding document. The nomenclature of the database should be standardized, readily reviewed and readily searched. Accuracy, safety and confidentiality should be ensured.

(3) Database: The Epidata software will be adopted.

(4) Data entry and management will be processed by designated data managers.

(5) The data manager should work with the principal investigators, and determine the data evaluation and logical audit based on the CRF. The computerized programs will be drafted. Erroneous data entry will be minimized and the reasons be spotted. All records will be appropriately documented.

9.2 Data audit and cleaning

(1) The data manager should work with the principal investigators, and determine the data evaluation and logical audit based on the CRF.

(2) The CRF will be stored in order after the data entry and audit is completed. Catalogue numbers will be filled for further retrieval of the records.

(3) The database will be locked after audit and verification of the data. No further amendment will be allowed once locking has been completed. Issues identified after database locking will be further amended with the statistical model after further confirmation and recorded in relevant documents.

9.3 Data audit meeting

The investigators will schedule for data audit meetings based on the actual progress, to determine the unresolved data query and catalogue the data sets.

9.4 Database locking

The database will be locked once all data queries have been resolved and the database catalogue has been completed. In principle, no further amendment will be made after

locking the database. Caution should be exercised should the database be unlocked. Repeated unlocking should be avoided.

9.5 Statistical analysis

Analysis principles

- All tests are two-sided, the nominal level of type I error will be 5% and the confidence level for all confidence intervals (CI) will be 95%.
- There will be no imputation of the missing values. The number of observations used in the analysis will be reported.
- Intention-to-treat principle will be used to deal with the non-compliance.
- All analysis will be exploratory analysis except for the one for the primary outcome.
- Subgroup analyses will be carried out irrespective of whether there is a significant treatment effect on the primary outcome.
- For the repeatedly measured outcomes, the difference with the baseline data (follow-up minus the baseline) will be analysed and the baseline data will be included in the model as covariates.
- Analyses will be conducted primarily using SAS software (version 9.4, SAS Institute, Cary, North Carolina, USA). Figures will be plotted using R packages.

Patients characteristics and baseline comparisons

Description and statistical inference of the following baseline characteristics will be presented by treatment group. No statistical inference will be performed for the baseline variables. Baseline measures for all patients will be tabulated.

Primary outcome

For the time to clinical improvement, death events before day 21 will be treated as competing risk event, and analyses will be performed by using the Gray's test and Fine and Gray proportion sub-distribution hazards model. The rates of clinical improvement in different treatment groups will be presented as Kaplan-Meier curves. Gray's test P-value and HR with a 95% confidence interval estimated by Fine and Gray proportion sub-distribution hazards model will be reported.

Secondary outcomes

The post-treatment lymphocyte and viral loads will be presented by using profile plots. Descriptive analysis and exploratory analysis by using the linear mixed-effect model will be performed. The post-treatment lymphocyte will be treated as nun-normally distributed, and the median with IQR will be used to present the data at each time point.

For the mortality and proportion of patients progressing to critical conditions, the rate difference with 95% Newcombe-Score CI will be reported. Log-rank test and Cox regression will be also performed for the time to death and time to progressing to critical conditions.

The median hospital stay (days) and oxygen support days with IQR will be reported.

Adverse events

Rates of adverse reactions in different treatment groups will be presented.

Subgroup analysis

Subgroup analyses will be carried out for the primary outcomes.

Planned subgroup analysis:

- Lymphocyte category: <=400, >400 cell/μl
- Oxygen therapy
- Age category: <65, ≥65 years
- Gender: male, female

More details see attached SAP.

10 Ethics considerations

The Declaration of Helsinki and Good Clinical Practice regulations should be strictly enforced during the conduct of the trial. Study participants could enter the trial after formally signing the informed consent forms. Confidentiality should be protected.

10.1 Ethics committee

The investigators should ensure that the clinical trial has been reviewed and approved by the ethics committee which is qualified for the Chinese Good Clinical Practice. Before initiation of the clinical trial, the investigators should submit the study protocol, informed consent form and other necessary files to the ethics committee for review. The sponsor

should inform the ethics committee all serious adverse events that might have affected the safety of the study participants, took place during further amendment of the study protocols and during the study. The investigators are responsible for reporting to the ethics committee the progress related to the clinical trial. The investigators should timely submit all copies to the ethics committee. The ethics committee should confirm the titles and the number of the trial protocols upon review and approval for the study protocol, and document the files and the dates of review and approval.

10.2 Informed consent forms

- The investigators should inform the study participants both verbally and literally the information related to the clinical trial. The study participants and their guardians or the legitimate representatives (if necessary) have the rights to be informed of further details of the clinical trial.

- The informed consent form (along with the study protocol) should be submitted to the ethics committee for review and approval. The informed consent form should be printed in Chinese. If necessary, the investigators should explain to the study participants in an understandable fashion. The study participants should have a sufficient amount of time for review before formally signing the informed consent form.

- The informed consent form should be signed by the study participants or their legal guardians, along with the date of signature. The signing of informed consent form could be taking place with another individual who witnesses the whole procedures. Each signed informed consent form should be stored and archived by the investigator for monitoring, audit and inspection by the sponsor or the national Food and Drug Administration.

10.3 Confidentiality

The investigators are responsible for maintaining the confidentiality of the study participants. Capital letter, numbers and codes could be adopted for identification of the study participants. No names could be appearing directly on the CRF. The source document should be stored, along with the code, names and family address. Strict confidentiality should be maintained.

11 Quality control

11.1 Standardized operating procedures

All the conduct of the trial should abide by the standardized operating procedures. The investigators and the sponsors should adhere to the clinical trial protocol to ensure appropriate control of the quality based on the standardized operating procedures.

11.2 Laboratory quality control

All central laboratories should establish the standardized procedures and quality control program for the tests.

11.3 Clinical audit

The principal investigators could authorize the audit managers to perform data audit based on the requirement of the Good Clinical Practice and the study protocol.

11.4 Investigation

The principal investigators will be responsible for the investigation of the conduct of the study.

12 Organization and implementation

It is planned that the first patient will be enrolled on 2019.2.3 and the last patient will complete the trial at day 21 after the treatment.

13 Participating investigators

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Wuhan Union Hospital	Nuo-fu Zhang, Yu Hu
Wuhan Hankou Hospital	Ai-lan Chen

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