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

(COVID-19 study)

STATISTICAL ANALYSIS PLAN

Version 1.0 **(**Apr. 01, 2020)

Authors: COVID-19 Study Group

Author for correspondence:

Chongyang Duan and Pingyan Chen

Department of Biostatistics, Southern Medical University,

510515, Guangzhou, China

Email: chenpy99@126.com

Contents

Primary Rationale for Amendment: 4

List of abbreviations and definition of terms 5

Abstract 6

1. Introduction 7

2. Study design 7

2.1 Overview 7

2.2 Sample size estimation 7

2.3 Definitions of the outcomes 7

2.3.1 Primary outcome *7*

2.3.2 Secondary outcomes *8*

2.3.3 Safety outcomes *8*

3. Statistical analysis 8

3.1 Analysis principles 8

3.2 Data quality control 9

3.3 Trial profile 9

3.4 Patients characteristics and baseline comparisons 9

3.5 Primary outcome 9

3.6 Secondary outcomes 10

3.7 Adverse events 10

3.8 Other variables 10

3.9 Patients drop out or switch between groups 10

3.10 Subgroup analysis 10

3.11 Sensitivity analysis 11

3.12 Tables and figures for the main paper 11

4. References **12**

Appendix 1: Proposed format of data tables in the publication of the main results **13**

Table 1: Baseline characteristics of the study participants **13**

Table 2: Results primary and secondary outcomes **16**

Table 3: Comparison of adverse reactions **17**

Appendix 2: Figures for the main results paper **18**

Figure 1: Flowchart of inclusion and follow-up **18**

Figure 2: KM plots of time to clinical improvement **18**

Figure 3: Changes in lymphocyte and viral loads during the follow-up period **18**

Figure 4: Forest plot of subgroups (sample plot) **18**

Appendix 3: Statement of contribution of the authors **19**

Primary Rationale for Amendment:

There was no amendment for the statistical analysis plan in this study.

List of abbreviations and definition of terms

CI	Confidence interval
HR	Hazard ratio
IQR	Interquartile range
rhG-CSF	recombinant human granulocyte colony stimulating factor
SD	Standard deviation
SOC	Standard of care

Abstract

Background: Several latest reports have documented lymphopenia in more than 80% of patients with Covid-19 upon hospital admission. The recombinant human granulocyte colony stimulating factor (rhG-CSF) is a biologic capable of increasing peripheral blood leukocyte and lymphocyte count in both mice models and human. Our pilot observation showed rhG-CSF could be indicated for those Covid-19 patients with lymphopenia.

Objective: We aimed to examine the efficacy and safety of rhG-CSF in patients with Covid-19 who had lymphopenia.

Methods: All data collected by participating researchers will be reviewed and formally assessed. Information pertaining to the baseline characteristics of patients will be selected and for each item statistically relevant descriptive elements are described. Information relevant to the rhG-CSF and standard-of-care (SOC) group is classified and, for each item, descriptive statistical analyses are planned for comparison between the rhG-CSF and SOC groups. Finally, for the outcomes which are classified as primary and secondary, the most appropriate statistical comparison to be made between groups are described.

Results: A statistical analysis plan (SAP) has been developed for the results of our study. This plan will allow a comprehensive description of baseline characteristics, features of the treatments, along with pre-determined statistical assessment of relevant outcome in a way that is transparent, available to the public, verifiable and pre-determined before completion of data collection.

Conclusions: We have developed a pre-determined SAP for the study which is to be followed to avoid analysis bias arising from prior knowledge of the study findings.

Trial registration: Chinese Clinical Trial Registry (No.: ChiCTR2000030007)

1. Introduction

Since December 2019, coronavirus disease 2019 (Covid-19) has resulted in a global outbreak. Several latest reports have documented lymphopenia in more than 80% of patients with Covid-19 upon hospital admission. The recombinant human granulocyte colony stimulating factor (rhG-CSF) is a biologic capable of increasing peripheral blood leukocyte and lymphocyte count in both mice models and human. Our pilot observation showed that rhG-CSF could be indicated for those Covid-19 patients with lymphopenia.

2. Study design

2.1 Overview

The study is a prospective, multicenter, randomized, open-label, standard-of-care control clinical trial.

The primary aim of this study is to evaluate the efficacy of rhG-CSF for Covid-19 patients with lymphopenia compared with standard-of-care (SOC).

The null hypothesis is that there is no difference in the time to clinical improvement.

2.2 Sample size estimation

The sample size estimation is based on the time to clinical improvement within 21 days. There is no prior literature report which would provide the basis for sample size estimation. Assuming the median duration of the SOC group of 14 days, 192 patients (96 per group) with 134 events would be needed to provide a power of 90% to detect a 6-day difference in the median time to clinical improvement under a two-sided significance level of α=0.05. Considering a low rate of drop-out, 200 patients (100 per group) were planned to be enrolled.

2.3 Definitions of the outcomes

2.3.1 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.

2.3.2 Secondary outcomes

Secondary outcomes will include the following:

post-treatment lymphocyte count;

mortality;

proportions of patients critical conditions;

viral loads;

Hospital stay days;

Oxygen support days.

2.3.3 Safety outcomes

Safety outcomes will include the following:

adverse events;

serious adverse events;

premature discontinuation of treatment.

3. Statistical analysis

3.1 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 analyzed and the baseline data will be included in the model as covariables.

- Analyses will be conducted primarily using SAS software (version 9.4, SAS Institute, Cary, North Carolina, USA). Figures will be plotted using R packages.

3.2 Data quality control

All responsible data collectors will be trained at the beginning of this study. Double entry is required for all variables and double validation is required for all other variables. The hospital coordinator at each collaborating site ensures that all data needed are collected.

3.3 Trial profile

The flow chart of inclusion and follow-up is displayed in a diagram (Appendix 2; Figure 1). The report will include the number of patients who met the inclusion criteria and the number included and reasons for exclusion of the non-included patients.

3.4 Patients characteristics and baseline comparisons

Description and statistical inference of the following baseline characteristics will be presented by treatment group.

Discrete variables will be summarised by frequencies and percentages. Percentages will be calculated according to the number of patients for whom the data are available. The number of missing values will be added in a footnote in the corresponding summary table.

Continuous variables will be summarised by the use of standard measures of central tendency and dispersion, either mean and standard deviation [Mean ± SD] for variables identified with #, or median and 25%, 75% quartiles[Median(IQR)] with †.

No statistical inference will be performed for the baseline variables.

Baseline measures for all patients will be tabulated.

3.5 Primary outcome

For the time to clinical improvement, death events before day 21 will be treated as the 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.

3.6 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 data will be summarised by frequencies and percentages, and 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.

The Hodges-Lehmann estimate of the location shift with a 95% asymptotic confidence interval will be reported for the comparison of medians.

3.7 Adverse events

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

3.8 Other variables

In our trial, we will also collect the data of physical examination, clinical symptoms, PSI scores, and other laboratory tests. Statistical analysis methods will be used appropriately for those variables.

3.9 Patients drop out or switch between groups

During the study, patients may switch between the two groups. The intention-to-treat principle will be used to deal with patients who had switched to the other group. Sensitivity analyses will be used to assess the robustness of the results among the patients who had switched to the other group.

The intention-to-treat principle will be also used to deal with the patients who dropped out.

3.10 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
- Baseline clinical grade

Forest plots will be constructed to illustrate subgroup analyses.

3.11 Sensitivity analysis

Planned sensitivity analysis:

- sensitivity for the patients who had switched to the other group

3.12 Tables and figures for the main paper

The proposed tables and figures for the main results are presented in Appendix 1 and 2.

Table 1 will report the key baseline characteristics of the participants by treatment group.

Table 2 will report the primary and secondary outcomes.

Table 3 will report the incidence of adverse reactions.

In addition, the following figures will be prepared:

- A diagram illustrating the flow chart of inclusion and follow-up (Appendix 2; Figure 1).
- Kaplan-Meier plot of time to clinical improvement (Appendix 2, Figure 2).
- A Forest plot of the treatment effect among different subgroups (Appendix 2, Figure 3).
- A profile plot of the changes in lymphocyte and viral loads during the 21 days follow-up period among different groups (Appendix 2, Figure 4).

A more extensive list of tables and figures used to report additional information is available upon request.

4. References

1. World Health Organization. https://covid19.who.int/

2. Guan WJ, Ni ZY, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med*. 2020 Feb 28. doi: 10.1056/NEJMoa2002032.

3. Zhao H, Guo M, Sun X, et al. Effects of Recombinant Human Granulocyte Colony-Stimulating Factor on Central and Peripheral T Lymphocyte Reconstitution After Sublethal Irradiation in Mice. *J Radiat Res*. 2013;54:83-91

4. Ang CC, Tay YK. Hematological Abnormalities and the Use of Granulocyte-Colony-Stimulating Factor in Patients With Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis. *Int J Dermatol*. 2011;50:1570-8

Appendix 1: Proposed format of data tables in the publication of the main results

Table 1: Baseline characteristics of the study participants

	rhG-CSF	**Standard-of-care**
	(n = XXX)	**(*n* = XXX)**
Age, y[#]	XX.XX±XX.XX	XX.XX±XX.XX
Male	XXX(XX.X%)	XXX(XX.X%)
Body temperature, ℃[#]	XX.XX±XX.XX	XX.XX±XX.XX
Fever on admission[#]	XXX(XX.X%)	XXX(XX.X%)
Respiratory rate, .min[#]	XX.XX±XX.XX	XX.XX±XX.XX
PaO_2:FiO_2 ratio, mmHg†	XX.XX(XX.XX~XX.XX)	XX.XX(XX.XX~XX.XX)
Blood pressure, mmHg[#]		
Systolic	XX.XX±XX.XX	XX.XX±XX.XX
Diastolic	XX.XX±XX.XX	XX.XX±XX.XX
Lymphocyte count, per mm^3†	XX.XX(XX.XX~XX.XX)	XX.XX(XX.XX~XX.XX)
White-cell count, per mm^3†	XX.XX(XX.XX~XX.XX)	XX.XX(XX.XX~XX.XX)
Platelet count, per mm^3†	XX.XX(XX.XX~XX.XX)	XX.XX(XX.XX~XX.XX)
C-reactive protein, mg/liter†	XX.XX(XX.XX~XX.XX)	XX.XX(XX.XX~XX.XX)
Procalcitonin, ng/ml†	XX.XX(XX.XX~XX.XX)	XX.XX(XX.XX~XX.XX)
Aspartate aminotransferase, U/liter†	XX.XX(XX.XX~XX.XX)	XX.XX(XX.XX~XX.XX)
Alanine aminotransferase, U/liter†	XX.XX(XX.XX~XX.XX)	XX.XX(XX.XX~XX.XX)
Lactate dehydrogenase, U/liter†	XX.XX(XX.XX~XX.XX)	XX.XX(XX.XX~XX.XX)
Creatine kinase, U/liter†	XX.XX(XX.XX~XX.XX)	XX.XX(XX.XX~XX.XX)
Medical history		

	rhG-CSF	Standard-of-care
	(n = XXX)	**(*n* = XXX)**
Shortness of breath	XXX(XX.X%)	XXX(XX.X%)
Cyanosis	XXX(XX.X%)	XXX(XX.X%)
Fatigue	XXX(XX.X%)	XXX(XX.X%)
Hemoptysis	XXX(XX.X%)	XXX(XX.X%)
Seven-category scale		
Grade 3	XXX(XX.X%)	XXX(XX.X%)
Grade 4	XXX(XX.X%)	XXX(XX.X%)
Grade 5	XXX(XX.X%)	XXX(XX.X%)
……	XXX(XX.X%)	XXX(XX.X%)
Viral load		
ORF1ab†	XX.XX(XX.XX~XX.XX)	XX.XX(XX.XX~XX.XX)
N†	XX.XX(XX.XX~XX.XX)	XX.XX(XX.XX~XX.XX)
Medication		
Interferon α	XXX(XX.X%)	XXX(XX.X%)
Lopinavir–ritonavir	XXX(XX.X%)	XXX(XX.X%)
Arbidol	XXX(XX.X%)	XXX(XX.X%)
Treatment		
Non-invasive mechanical ventilation	XXX(XX.X%)	XXX(XX.X%)
Invasive mechanical ventilation	XXX(XX.X%)	XXX(XX.X%)
Extracorporeal membrane oxygenation	XXX(XX.X%)	XXX(XX.X%)
Glucocorticoid therapy	XXX(XX.X%)	XXX(XX.X%)
Others	XXX(XX.X%)	XXX(XX.X%)

	rhG-CSF (n = XXX)	Standard-of-care (*n* = XXX)
Duration of glucocorticoid therapy†	XX.XX(XX.XX~XX.XX)	XX.XX(XX.XX~XX.XX)

No of Missing: XXX of XXX.

Table 2: Results primary and secondary outcomes

Outcomes	rhG-CSF (n = XXX)	Standard-of-care (*n* = XXX)	Difference (95% CI)
Time to clinical improvement	XX.XX(XX.X~XX.X)	XX.X(XX.X~XX.X)	X.X(X.X~X.X)
Day 21 clinical improvement	XXX(XX.X%)	XXX(XX.X%)	X.X(X.X~X.X)
Time to Death	XX.XX(XX.X~XX.X)	XX.X(XX.X~XX.X)	X.X(X.X~X.X)
Day 21 mortality	XXX(XX.X%)	XXX(XX.X%)	X.X(X.X~X.X)
Time to progressing to critical condition	XX.XX(XX.X~XX.X)	XX.X(XX.X~XX.X)	X.X(X.X~X.X)
Day 21 patients progressing to critical condition	XXX(XX.X%)	XXX(XX.X%)	X.X(X.X~X.X)
Oxygen support days	XX.XX(XX.X~XX.X)	XX.X(XX.X~XX.X)	X.X(X.X~X.X)
Hospital stay days	XX.XX(XX.X~XX.X)	XX.X(XX.X~XX.X)	X.X(X.X~X.X)
Seven-category scale at day 7			
Grade 2	XXX(XX.X%)	XXX(XX.X%)	
Grade 3	XXX(XX.X%)	XXX(XX.X%)	
……	XXX(XX.X%)	XXX(XX.X%)	
Seven-category scale at day 14			
Grade 2	XXX(XX.X%)	XXX(XX.X%)	
Grade 3	XXX(XX.X%)	XXX(XX.X%)	
……	XXX(XX.X%)	XXX(XX.X%)	
Viral load at day 7			
ORF1ab	XX.XX(XX.X~XX.X)	XX.X(XX.X~XX.X)	X.X(X.X~X.X)
N	XX.XX(XX.X~XX.X)	XX.X(XX.X~XX.X)	X.X(X.X~X.X)
Viral load at day 14			
ORF1ab	XX.XX(XX.X~XX.X)	XX.X(XX.X~XX.X)	X.X(X.X~X.X)
N	XX.XX(XX.X~XX.X)	XX.X(XX.X~XX.X)	X.X(X.X~X.X)

Table 3: Comparison of adverse reactions

Adverse event	rhG-CSF (n = XXX)		Standard-of-care (*n* = XXX)	
	Any Grade	Grade 3 or 4	Any Grade	Grade 3 or 4
Any adverse event				
Over all	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)
Neutrophilia	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)
Osteodynia	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)
Muscular soreness	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)
Rash	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)
Fatigue	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)
Nausea	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)
Vomiting	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)
Facial flushing	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)
Tachycardia	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)
Increased lactate dehydrogenase	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)
Increased alkaline phosphatase	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)
Increased aspartate aminotransferase	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)
Increased alanine transaminase	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)
Increased creatinine	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)
Serious adverse event				
Over all	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)
Sepsis or septic shock	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)
Respiratory failure	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)
Acute respiratory distress syndrome	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)
Acute kidney injury	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)
Pneumothorax	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)
Disseminated intravascular coagulation	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)
Acute heart failure	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)	XXX(XX.X%)

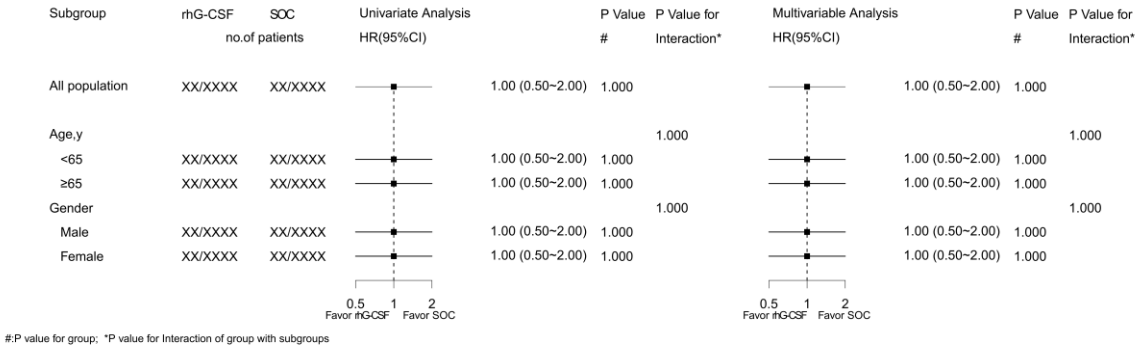
Appendix 2: Figures for the main results paper

Figure 1: Flowchart of inclusion and follow-up

Figure 2: KM plots of time to clinical improvement

Figure 3: Changes in lymphocyte and viral loads during the follow-up period

Figure 4: Forest plot of subgroups (sample plot)



Appendix 3: Statement of contribution of the authors

Chongyang Duan and Pingyan Chen participated in writing the first draft and all revisions of the SAP. All the members of this study participated in critical reviews of the SAP. The SAP was prepared without knowledge of the data.