Clinical Study Protocol

A Phase II/III Study of Sargramostim in Patients with Coronavirus Disease-2019 (COVID-19)

Nobel Pharma Co. Ltd.

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List of Abbreviations

Abbreviation	Description
A-aDO_2	Alveolar–arterial oxygen gradient
ADR	Adverse drug reaction
AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ARDS	Acute Respiratory Distress Syndrome
AST	Aspartate aminotransferase
BUN	Blood urea nitrogen
Ca	Calcium
CCL	CC chemokine ligand
CK	Creatine kinase
CI	Confidence interval
Cl	Chlorine
COVID-19	Coronavirus disease-2019
CPK	Creatine phosphokinase
CRO	Contract research organization
CRP	C-reactive protein
CT	Computed tomography
CXCL	C-X-C motif chemokine ligand
ECG	Electrocardiography
ECMO	Extracorporeal membrane oxygenation
eCRF	Electronic case report form
EDC	Electronic Data Capture
FAS	Full Analysis Set
FiO_2	Fraction of inspiratory oxygen
GCP	Good Clincal Practice
GM-CSF	Granulocyte–macrophage colony stimulating factor
GOT	Glutamic oxaloacetic transaminase
GPT	Glutamic pyruvic transaminase
hCG	Human chorionic gonadotropin
HDL	High-density lipoprotein
HRCT	High-resolution computed tomography
IRB	Institutional Review Board
IL	Interleukin
IQR	Interquartile range
IWRS	Interactive web response system
K	Potassium
LDH	Lactate dehydrogenase
LDL	Low-density lipoprotein
MedDRA	Medical Dictionary for Regulatory Activities
MMP	Matrix metalloproteinase
Na	Sodium
P	Inorganic phosphorus
$PaCO_2$	Partial pressure of carbon dioxide
PaO_2	Partial pressure of oxygen
PB	Barometric pressure

Abbreviation	Description
PCR	Polymerase chain reaction
pH	potential of Hydrogen
PH_2O	Partial pressure of water vapor in inspired air
PT	Preferred terms
R	Respiratory quotient
rhGM-CSF	Recombinant human granulocyte-macrophage colony stimulating factor
RNA	Ribonucleic acid
SAE	Serious adverse event
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SaO_2	Arterial oxygen saturation
SOC	System organ class
SOP	Standard operating procedures
SP	Safety Population
SpO_2	Percutaneous oxygen saturation
TG	Triglyceride
TNF	Tumor necrosis factor
γ-GTP	γ-Glutamyl transpeptidase

Definition of Term

Term	Definition
Investigators	Principal investigator or sub-investigator

Table of Contents

1 Synopsis

Study title	A phase II/III study of sargramostim in patients with coronavirus disease-2019 (COVID-19)
Protocol number	NPC-26-1
Study objective	To evaluate the efficacy and safety of inhalation administration of sargramostim for 5 days, in principle (up to 10 days) as Add-on treatment to the standard treatment in COVID-19 patients.
Study design	A randomized, placebo-controlled, double-blind, group comparison, multicenter study
Study subjects	Moderate stage II[1] COVID-19 patients with clinically diagnosed pneumonia
Inclusion criteria	Japanese male or female subjects who have been confirmed to meet all the following criteria. (1) Hospitalized patients under treatment who were severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2] positive by polymerase chain reaction (PCR) test. (2) Patients with clinically diagnosed pneumonia and a percutaneous oxygen saturation [SpO_2] of 93% or less on breathing of room air at bed rest. (3) Patients for whom written informed consent has been obtained from those themselves or the legally acceptable representatives. (4) Patients aged 20 years or older and younger than 85 years at the time of obtaining informed consent.
Exclusion criteria	Subjects who meet any of the following criteria will be excluded. Unless otherwise stated, the following criteria refer to those at the time of screening. (1) Patients who have allergic reactions or hypersensitivity to the active ingredient of the test drug (sargramostim) or its additives (mannitol, sucrose and tromethamine) or yeast-derived products or others. (2) Patients who have been participating in other intervention studies, such as studies on unapproved pharmacotherapy, within 90 days prior to screening. (3) Patients who have experienced off-label use of approved drugs (including those for COVID-19 treatment other than steroids and favipiravir as standard treatment) within 7 days prior to screening. (4) Patients who are not expected to survive longer than 24 hours after commencement of study drug administration. (5) Patients who are using invasive ventilator or extracorporeal membrane oxygenation (ECMO). (6) Patients who have a chronic respiratory disease requiring continuous home oxygen therapy or ventilator use. (7) Patients with an underlying condition that is considered very unlikely to withdraw ventilator (e.g., motor neuron disease, Duchenne muscular dystrophy, rapidly progressive interstitial pulmonary fibrosis). (8) Patients who have a disease including bronchial asthma, lower respiratory tract infections, and interstitial lung diseases that may affect the assessment of the clinical study, since before the symptom onset of COVID-19.

	(9) Patients who have a disease including leukemia and leukocytosis that causes leukocytosis. (10) Patients who have a chronic kidney disease requiring dialysis. (11) Patients who have severe liver failure (Child Pugh grade C). (12) Patients who are pregnant or breastfeeding or who are positive on pregnancy test. (13) Patients aged 80 years or older who have cardiac disease, cerebrovascular disorder, BMI 30 or higher, dyslipidemia, hypertension or diabetes (14) Other patients considered inappropriate by the investigators for inclusion in the study
Test drug	Sargramostim (A lyophilized preparation containing 250 μg of sargramostim in 1 vial)
Dosage and administration	Active treatment group: 250 μg/vial of sargramostim will be dissolved in 4 mL of physiological saline and 125 μg of which will be administered using inhaler twice daily in approximately 10-15 minutes. Control group: 2 mL of physiological saline will be administered using inhaler twice daily in approximately 10-15 minutes.
Treatment period	5 days, in principle (up to 10 days)
Prohibited concomitant medications/therapies	【Prohibited concomitant medication】 Concomitant use of any unapproved drugs (including off-label use of approved drugs and use at unapproved dosage and administration, excluding steroids as standard treatment at each institution) will be prohibited throughout the study period. In addition, the combination of sargramostim with chemotherapy and radiotherapy is contraindicated. Except treatment/therapy mentioned above, there are no specific prohibited concomitant medications in this study. And steroids used as standard treatment at each institution, approved antiviral agents, or others can be used concomitantly as a part of standard treatment. 【Prohibited concomitant therapy】 If a subject has started to use invasive ventilation or ECMO, the study drug administration will be terminated after initiation of use of such devices (see "15.1 Study drug discontinuation "). The necessity to use these devices as part of the standard treatment for COVID-19 will be judged based on the subject's condition and medical necessity during the study period.
Study procedures	COVID-19 patients will be randomly assigned to either of the following two groups in a 2:1 ratio: Sargramostim (125 μg) or physiological saline will be administered by inhalation twice daily for 5 days, in principle (up to 10 days) as Add-on treatment to the standard treatment. (1) Active treatment group (standard treatment plus sargramostim) (2) Control group (standard treatment plus physiological saline)

	Stratified allocation with standard treatment as the allocation factor will be performed for randomization.
Observation period	Period until Day 28 (including the case after discharge)
Endpoints	【Efficacy endpoints】 Primary endpoint Number of days to achieve at least 2-rank improvement on a 7-point ordinal scale from baseline until Day 28. Secondary endpoints (1) Changes in alveolar-arterial oxygen partial pressure gradient (A-aDO_2) on Day 5 and at the end of administration from baseline. (2) Number of days until discharge from baseline (days of shifting to Category 7 on a 7-point ordinal scale). (3) Proportion of subjects whose category has shifted to Category 1 or 2 on a 7-point ordinal scale from baseline until Day 28. (4) Changes in pulmonary inflammatory findings on Day 5 and at the end of administration from baseline. (5) Changes in arterial blood-gas parameters (partial pressure of oxygen [PaO_2] and partial pressure of carbon dioxide [$PaCO_2$]) on Day 5 and at the end of administration from baseline. (6) Changes in inflammatory markers and cytokines at the end of administration from baseline. (7) Distribution and proportion of each category on a 7-point ordinal scale on Day 14 and Day 28. (8) Distribution and proportion of each category of oxygen requirement until Day 14. (9) Other exploratory items (details will be specified separately in the statistical analysis plan). 【Safety endpoints】 Incidence of adverse events and serious adverse event.

<table>
<tr><td>Efficacy parameters</td><td>(1) 7-point ordinal scale
<table>
<tr><th>Category</th><th>Ordinal scale</th></tr>
<tr><td>1</td><td>Have died</td></tr>
<tr><td>2</td><td>Use an invasive ventilator or ECMO</td></tr>
<tr><td>3</td><td>Use a noninvasive ventilator or high-flow oxygen supply device</td></tr>
<tr><td>4</td><td>Require oxygen supply</td></tr>
<tr><td>5</td><td>Do not require oxygen supply but require continuous therapy (related to COVID-19 or others)</td></tr>
<tr><td>6</td><td>Do not require both oxygen supply and continuous therapy</td></tr>
<tr><td>7</td><td>Have been discharged from hospital</td></tr>
</table>
(2) Arterial blood gas analysis Arterial blood gas analysis (PaO2, PaCO2, A-aDO2, others).
(3) Chest high-resolution computed tomography (HRCT) or plain chest CT or chest X-ray
(4) Inflammatory marker (C-reactive protein [CRP])
(5) Cytokines (IL-6, IL-1β, TNF-α, IL-10, CCL17, CXCL9, MMP-12).
(6) Oxygen requirement
<table>
<tr><th>Category</th><th>Ordinal scale</th></tr>
<tr><td>1</td><td>Require oxygen supply of 4 L/min or more using oxygen mask</td></tr>
<tr><td>2</td><td>Require oxygen supply of 2 L/min or more using nasal cannulas</td></tr>
<tr><td>3</td><td>Require oxygen supply of less than 2 L/min using nasal cannulas</td></tr>
<tr><td>4</td><td>Do not require oxygen supply but feel shortness of breath during walking or moving</td></tr>
<tr><td>5</td><td>Do not require oxygen supply and do not feel shortness of breath during walking or moving.</td></tr>
</table></td></tr>
<tr><td>Safety parameters</td><td>Physical examinationVital signs12-lead Laboratory tests (blood chemistry, hematology, urinalysis, and pregnancy test)Confirmation of AEs.</td></tr>
<tr><td>Target number of subjects</td><td>60 subjects (2:1 for active treatment group: control group)</td></tr>
<tr><td>Statistical analysis</td><td>【Efficacy】
Regarding the number of days to achieve at least 2-rank improvement on a 7-point ordinal scale from baseline until Day 28, the primary efficacy endpoint, estimation of the proportion of improvement by treatment group using the Kaplan-Meier method, and the comparison between groups using the log-rank test at a significance level of 5% will be performed as primary analysis. In addition, evaluation using Cox's proportional hazards model with the allocation factor as a covariate will be performed.
For other efficacy endpoints, distribution or summary statistics by treatment group will be provided, and differences between groups will be estimated and tested statistically.
【Safety】
A 95% confidence interval is calculated for the incidence of adverse events by treatment group.</td></tr>
<tr><td>Medical experts</td><td>[redacted]</td></tr>
</table>

Coordinating investigator	[redacted]
Study Period	October 2020 to October 2021

2 Observation and Examination Schedule

Item	Screening	Date of allocation	Observation period [5]											Discontinuation/ Exit Test [6]
		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11 ～14	Day 15 ～28	
Acceptable window	To Day 0	-2 days [7]	—	—	—	—	+1 day [7]	—	—	—	—	—	—	—	+1 day
Informed consent	●														
Medical history/current medical conditions	●														
Subject characteristics	●														
Height and weight	●														
Inclusion and exclusion criteria	●														
Percutaneous oxygen saturation (SpO2)	●														
Allocation		●													
Study drug administration [1]			●	●	●	●	●	○	○	○	○	○			
7-point ordinal scale [2]		●	●	●	●	●	●	●	●	●	●	●	●	●	
Oxygen requirement		●	●	●	●	●	●	●	●	●	●	●	●		
Arterial blood gas analysis (PaO2、PaCO2、A-aDO2, others)			●[3]				●								●
Inflammatory markers, cytokines			●[3]		●		●								●
Chest HRCT or plain chest CT or Chest X-ray		●					●								●
Physical examination		●					●								●
Vital sign		●					●								●
12-lead ECG		●					●								●
Laboratory tests (blood chemistry, hematology and urinalysis)		●					●								●
Pregnancy test (urine or blood sample)		●													
adverse events[4]			● →											●	
Concomitant medication/therapy	● →												●		

● : Will be performed in all the subjects ○ : Will be performed only in the subjects who continue to receive the study drug after Day 5.

1) Inhalation administration will be started on the morning of Day 1 and performed twice daily (morning, evening) for 5 days, in principle (up to 10 days).
2) Will be evaluated every day during the period between Day 1 and Day 28. Use or no use of a ventilator will be included in this evaluation.
3) Will be performed prior to study drug administration.
4) Only the adverse events that have occurred until Day 14 after the start of study drug administration. may be collected and entered in the electronic case report form. Serious adverse events related to the study drug administration will be collected until Day 28.

5) Assessment of efficacy on the 7-point ordinal scale and oxygen requirement will be performed after completion of the second dose (inhalation) of the day.
6) Will be performed on the day of discontinuation if study drug administration has been prematurely discontinued before Day 5, or on the day of the last dose in the case that the last day is not Day 5.
7) Except 7-point ordinal scale and oxygen requirement.

3 Study Administrative Structure

Refer to Attachment 1 for the study administrative structure.

4 Background Information

Refer to the latest Investigator's Brochure[2] for more background information on sargramostim.

4.1 Introduction

As of September 7, 2020, approximately 27 million people have been affected with COVID-19 worldwide and more than 70,000 people in Japan, and the number of deaths is also continuously increasing to reach 900,000 worldwide[3,4]. While many affected individuals end with subclinical infection, those who become critically ill are complicated by pneumonia or acute respiratory symptoms, leading to use of intubation or mechanical ventilation, and may come to use ECMO, and death may occur in some cases. It is considered that acute respiratory distress syndrome (ARDS) is rapidly progressing in patients who are going to die[5]. Therapeutic agents for COVID-19 patients are currently being developed in different countries. In Japan, remdesivir (Veklury ®) was approved in May in 2020. And dexamethasone, a steroid drug, was added to the "Drugs Approved in Japan" [1] for treatment of COVID-19 patients in July[1]. However, these drugs can be used only for patients requiring oxygen inhalation or patients with more severe disease conditions as a rule, thus, it is difficult to say that adequate treatment options are available.

Granulocyte-monocyte colony-stimulating factor (GM-CSF) is a type of cytokines, and its action to promote the differentiation and increase of pluripotent hematopoietic stem cells into neutrophils, monocytes/macrophages, and dendritic cells has been known. Moreover, the therapeutic effects of GM-CSF on lung diseases infected by virus are expected as shown by various researches in recent years. For example, the following research has been published[6]; when GM-CSF conditional knock-out mouse were infected with influenza virus, ARDS developed and death occurred in untreated individuals, however, when GM-CSF has been highly expressed in those mouse after infection, shifts from M1 macrophages to M2 occurred and cytokine storm activated by M1 macrophages was inhibited, leading to prevention of ARDS development to improve survival.

Sargramostim is a recombinant human GM-CSF (rhGM-CSF) produced by yeasts. After obtaining approval in 1991 in the United States, sargramostim has been marketed by Partner Therapeutics as a trade name of Leukine ® (injectable) and has been used in several indications, including shortening time to neutrophil recovery and reducing the incidence of severe and life-threatening infections and infections resulting in death following induction chemotherapy in patients with acute myeloid leukemia. Although sargramostim has not been approved in Japan, a placebo-controlled phase III study[7] (investigator-initiated study) of inhalation administration of sargramostim has been conducted in patients with autoimmune pulmonary alveolar proteinosis by Professor Nakata and colleagues at Niigata University. In this study, alveolar-to-arterial oxygen uptake, using the alveolar air-arterial oxygen partial pressure

gradient (A-aDO_2) as an efficacy endpoint, was improved, and the efficacy and safety of inhalation administration of sargramostim has been confirmed.

Various basic studies have been reported that sargramostim is also efficacious in relieving symptoms of COVID-19. Its main pharmacological actions include:1. sargramostim promotes differentiation and increase of granulocytes and macrophages from pluripotent hematopoietic stem cells; 2.sargramostim promotes survival and activation of mature myeloid cells; 3.sargramostim shifts M1 macrophages of alveolar macrophages to M2 macrophages after viral infection; 4.sargramostim activates innate and acquired immunity by activation and proliferative stimulation of dendritic cells and T cells; 5.sargramostim accelerates viral clearance; 6.sargramostim promotes proliferation and barrier repair of respiratory epithelial cells; and 7.sargramostim ameliorates immunosuppression (immunoparalysis) caused by overproduction of anti-inflammatory cytokines.

At present, most of the drugs for treatment of COVID-19 including those approved in Japan (remdesivir) or under development (e.g., favipiravir) are antiviral drugs targeting RNA-polymerase for viral proliferation. On the other hand, sargramostim is an agent differently targeting to suppress inflammation of virally infected lungs, and not a steroid drug. In the future, intensification therapy of sargramostim combined with other drugs may also be expected.

This study will evaluate the efficacy and safety of inhalation administration of sargramostim in COVID-19 patients in collaboration with Japanese medical institutions, and aim to submit application of the drug for early market introduction.

4.2 Nonclinical studies

The nonclinical toxicity studies required to initiate clinical studies of sargramostim have been evaluated in repeated-dose toxicity studies in monkeys including a 14 days intravenous administration study, a 6 weeks subcutaneous administration study, and a 26 weeks inhalation administration (intermittent administration) study, and no specific safety concerns have been observed.

In the 26-week intermittent inhalation administration study, 3 cynomolgus monkeys/sex/group were treated with 0 (vehicle), 5, 100, or 500 μg/kg/day of sargramostim for 1 week followed by a 1-week washout cycle for 26 weeks. No deaths were reported during the observation period in either group. There were no apparent toxicities associated with sargramostim administration throughout the study, and the no-observed-adverse-effect-level under the study conditions was considered to be 500 μg/kg/day for both sexes.

4.3 Clinical studies

Inhalation administration of sargramostim has been experienced in humans in the Phase I studies in healthy adults and patients with autoimmune pulmonary alveolar proteinosis[8], a Phase II study[9] in patients with autoimmune pulmonary alveolar proteinosis (6 cycles of 125 μg inhaled twice daily for 8 days plus a 6-day washout followed by 6 cycles of 125 μg inhaled once daily for 4 days plus a 10-day

washout), and a placebo-controlled Phase III study[7] in patients with autoimmune pulmonary alveolar proteinosis (125 μg inhaled twice daily for 7 days every other week for 24 weeks). No safety concerns have been observed in these studies. Regarding the safety of high dose of inhalation administration of sargramostim, it has also been reported[10] that the maximum tolerated dose was not reached in a study in which 40 patients with lung metastases from malignant melanoma received inhalation administration of 500-2000 μg of sargramostim twice daily for 7 days repeatedly every other week (unless there was worsening of symptoms or serious adverse events).

In addition, an investigator-initiated study[11] in 80 of COVID-19 patients is ongoing in Belgium. An interim analysis based on 20 patients suggested the efficacy of sargramostim for A-aDO_2 (p=0.053), and further data supporting the efficacy and safety of sargramostim for COVID-19 will be expected by accumulating the cases in the future.

In the sargramostim development in Japan, considering the results of basic studies on sargramostim or GM-CSF as well as the tendency of COVID-19 Japanese patients becoming more severe, the efficacy and safety of inhalation administration of sargramostim will be evaluated in moderate stage II[1] COVID-19 Japanese patients with clinically diagnosed pneumonia.

4.4 Known or unknown adverse drug reactions

The most recent Investigator's Brochure[2] shall be referred to for potential risks of sargramostim administration.

When sargramostim is administered by inhalation in COVID-19 patients, no other adverse events (AEs) will be anticipated than those related to COVID-19.

COVID-19 is the most recent syndrome, and available data on this disease are limited. Usual symptoms that are considered the expected events and the symptoms observed during the natural course of the disease include dyspnea, cough, sputum production, expectoration, discomfort, myalgia, malaise, fever, anorexia, vomiting, diarrhea, headache, sore throat, chest tightness, chest pain, chest discomfort, laboratory test abnormalities [white blood cells count decreased, lymphocytes count decreased, platelets count decreased, C-reactive protein (CRP) increased], decreased oxygen saturation, progression of respiratory failure, progression to ARDS, hypotension, and progression to multiple organ failure. [12, 13, 14, 15].

5 Objective, Endpoints, and Parameters

5.1 Objective

To evaluate the efficacy and safety of inhalation administration of sargramostim for 5 days, in principle (up to 10 days) as Add-on treatment to standard treatment in COVID-19 patients.

5.2 Endpoints

5.2.1 Efficacy endpoints

Primary efficacy endpoint

Number of days to achieve at least 2-rank improvement on a 7-point ordinal scale from baseline until Day 28.

[Rationale]

A 7-point ordinal scale is one of the commonly used scales for assessment of drugs used in the clinical studies of COVID-19 and was set as an index to numerically assess the improvement effect of sargramostim administration on the disease condition. Taking the study drug administration period (5 days, in principle [up to 10 days]) in this study, the usual period for treatment and hospital stay of COVID-19 patients in Japan, and the results of other clinical studies into account, the required number of days to achieve at least 2-rank improvement on a 7-point ordinal scale from baseline until Day 28 was set as the primary efficacy endpoint.

Secondary endpoints

(1) Changes in alveolar-arterial oxygen partial pressure gradient (A-aDO_2) from baseline to Day 5 and the end of administration.
(2) Number of days until discharge from baseline (days for shifting to Category 7 on a 7-point ordinal scale).
(3) Proportion of subjects whose category has shifted to Category 1 or 2 on a 7-point ordinal scale from baseline until Day 28.
(4) Changes in pulmonary inflammatory findings from baseline to Day 5 and the end of administration.
(5) Changes in arterial blood-gas parameters (partial pressure of oxygen [PaO_2] and partial pressure of carbon dioxide [$PaCO_2$]) from baseline to Day 5 and the end of administration.
(6) Changes in inflammatory markers and cytokines from baseline to the end of administration.
(7) Distribution and proportion of each category on 7-point ordinal scale on Day 14 and Day 28.
(8) Distribution and proportion of each category of oxygen requirement until Day 14.
(9) Other exploratory items (details will be specified separately in the statistical analysis plan).

[Rationale]

(1) An interim analysis of the clinical study [11] being conducted in Belgium in COVID-19 patients (20/ target sample size of 80) suggested the efficacy of sargramostim for A-aDO_2, an index of arterial oxygen uptake (see 6.3 Rationale for dosage and administration). Therefore, changes in A-aDO_2 was set as a secondary endpoint in this study. Since the duration of the study drug administration in this study is set to be "5 days, in principle (up to 10 days) ", both the periods of "Day 5 from baseline" and "End of administration from baseline" were set for assessment.
(2) As an evaluation of the drug for COVID-19 from clinical standpoint, "number of days until discharge from hospital" considered to be equally important with " number of days to achieve at least 2-rank improvement on a 7-point ordinal scale" was set.
(3) In order to visualize the inhibitory effects of sargramostim on the shifting of disease condition to more severe condition from a simple pneumonic condition, proportion of subjects whose category

has shifted to Category 1 or 2 (worsening to use of invasive ventilator or worse) on a 7-point ordinal scale until Day 28 was set as an index.

(4) This endpoint was set in order to evaluate the effect of sargramostim on pneumonia.

(5) This endpoint was set in order to evaluate the effect of sargramostim on pulmonary function.

(6) This endpoint was set in order to evaluate the effect of sargramostim on the inflammatory changes.

(7) Data derived from the same measurement parameter as those for the primary endpoint were to be assessed secondarily from different point of view. In addition, since the disease condition requiring treatment may likely be persistent in a COVID-19 patient requiring hospitalization, the assessment time was set not only on Day 14 but also on Day 28.

(8) Oxygen supply is considered to be one of the clinical indicators to assess respiratory failure and thus was set as a categorized ordinal scale.

(9) Since the data on inhalation administration of sargramostim to Japanese population as well as the data on the pathology of COVID-19 over time in Japanese population will be valuable, an exploratory endpoint will be added as needed.

5.2.1.1 Number of days to achieve at least 2-rank improvement on a 7-point ordinal scale

The clinical condition of a subject will be comprehensively measured at the timing specified in "2 Observation and Examination Schedule" and will be evaluated according to "13.3 7-point ordinal scale" by the investigators.

Assessment on Day 0 will be used as a baseline value, and the number days to achieve at least 2-rank improvement (see Table 13.3-1) from baseline will be assessed.

5.2.1.2 Changes in alveolar -arterial oxygen gradient (A-aDO_2)

A-aDO_2 will be measured and calculated according to "13.10 Arterial blood-gas analysis (PaO_2, $PaCO_2$, A-aDO_2, others) " at the timing specified in "2 Observation and Examination Schedule."

Measurement values obtained prior to study drug administration on Day 1 will be used as baseline values, and changes of A-aDO_2 values from baseline will be assessed.

5.2.1.3 Number of days until discharge

The category of the disease condition on Day 0 of a subject is supposed to fall into one of Category 3 to 5 on a 7-point ordinal scale (see Table 13.3-1). The number of days required to have been shifted from the category on Day 0 to Category 7 will be evaluated.

5.2.1.4 Proportion of subjects whose category has shifted to Category 1 or 2 on a 7-point ordinal scale

The category of the disease condition on Day 0 of a subject is supposed to fall into one of Category 3 to 5 on a 7-point ordinal scale (see Table 13.3-1). The proportion of subjects whose category has shifted from the category on Day 0 to Category 1 or 2 at the time of evaluation will be calculated.

5.2.1.5 Changes in pulmonary inflammatory findings

Inflammatory findings in the lungs will be examined according to “13.11 Chest HRCT or plain chest CT or chest X-ray” at the timing specified in "2 Observation and Examination Schedule", and the results will be judged by the investigators.

Quantitative changes will be assessed using densitometry for chest HRCT. The judgement on Day 0 will be used as the baseline value, and changes in inflammatory changes from baseline will be assessed according to the separately defined criteria.

5.2.1.6 Changes in arterial blood gas parameters (PaO_2, $PaCO_2$)

PaO_2 and $PaCO_2$ will be measured according to "13.10 Arterial blood gas analysis(PaO_2, $PaCO_2$, A-aDO_2, others)" at the timing specified in "2 Observation and Examination Schedule".

Measurement values prior to study drug administration on Day 1 will be used as the baseline values, and the changes from baseline will be assessed.

5.2.1.7 Changes in inflammatory markers and cytokines

Inflammatory markers and cytokines will be measured according to "13.8 Inflammatory marker" and "13.9 Cytokines" at the timing specified in "2 Observation and Examination Schedule".

Measurement values prior to study drug administration on Day 1 is used as baseline values, and changes from baseline will be assessed.

5.2.1.8 Distribution and proportion of each category on a 7-point ordinal scale

Distribution of category (Category 1-7) on a 7-point ordinal scale (see Table 13.3-1) and the proportion of each category will be evaluated. Details of the categories will be defined in the statistical analysis plan separately.

5.2.1.9 Distribution and proportion of each category of oxygen requirement

Oxygen requirement will be classified into 5 categories (see Table 13.12-1) and the distribution and proportion of each category will be evaluated. Details of the categories will be defined in the statistical analysis plan separately.

5.2.2 Safety endpoints

Incidence of adverse events (AEs) and serious adverse events (SAEs)

[Rationale]

An event reported as an AE is not only the subjective symptoms reported by the subject, but also reflects the medical judgment of the investigators based on the results of the safety assessment. The incidence of AEs (or SAEs) is the most appropriate and widely accepted measure for safety evaluation in clinical studies, thus was set.

5.2.2.1 Incidence of AEs and SAEs

Information on AEs occurring from the start of study drug administration until Day 14 will be collected in this study. For SAEs related to the study drug, those occurring until Day 28 will be collected. The number of subjects with AEs and the incidence of AEs will be calculated by seriousness, system organ class (SOC) and/or preferred term (PT), and severity by treatment group.

5.3 Parameters

5.3.1 Efficacy parameters

7-point Ordinal scale, arterial blood gas analysis (PaO_2, $PaCO_2$, A-aDO_2, others), HRCT or chest simple CT or chest X-ray, inflammatory markers, cytokines, oxygen requirement.

5.3.2 Safety parameters

Physical examination, vital signs, 12-lead ECG, laboratory tests (blood chemistry, hematology, urinalysis, and pregnancy test), AEs.

6 Study Design

6.1 Overview of study design

This is a randomized, placebo-controlled, double-blind, group-comparison, multicenter study.

The efficacy and safety will be evaluated when sargramostim or physiological saline is administered by inhalation for 5 days, in principle (up to 10 days) as Add-on treatment to standard treatment in COVID-19 patients.

Enrolled subjects will be randomized in a 2:1 ratio to receive either standard treatment plus sargramostim (active treatment group) or standard treatment plus physiological saline (control group).

The study design is shown in Figure 6.1-1

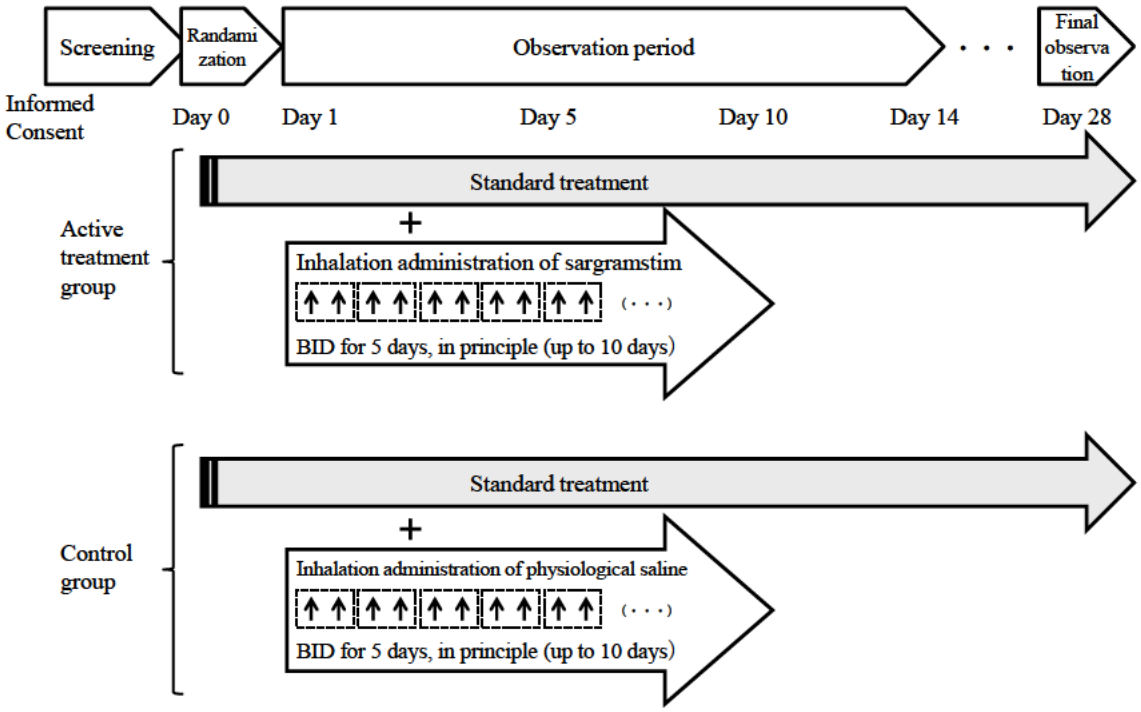

Figure 6.1-1 Study Design

6.2 Rationale for study design

The objective of the study is to verify the efficacy and safety of sargramostim, thus, a randomized, placebo-controlled, double-blind, group comparison study design has been chosen to avoid any biases including biases in assessment of the investigators.

There are currently no established therapeutic agents on the market for moderate COVID-19 patients, thus the standard treatment cannot be established for the control group. And, the control group to be administered physiological saline as a placebo by inhalation was considered appropriate in order to verify the efficacy and safety of sargramostim. In consideration of ethics and patients' safety, both the active treatment group and the control group are to be set as Add-on treatment to standard treatment in each medical institution .The use of steroids as standard treatment, approved antiviral agents, or others in each institution is to be permitted in both groups.

Because preliminary data suggesting the efficacy of sargramostim have been obtained, the allocation ratio of 2:1 was set between the active treatment group and the control group.

Since patients may cough during inhalation administration of the study drug, use of air cleaner or similar is to be allowed to prevent infection among healthcare workers.

6.3 Rationale for dosage and administration

Dosage and administration of the study drug in this study is based on the fact that the efficacy and safety in Japanese patients with autoimmune pulmonary alveolar proteinosis have been confirmed on the same dosage and administration[7,9], and that the efficacy of sargramostim was suggested in the COVID-

19 patients [p=0.053, compared with standard treatment (without sargramostim)] for A-aDO_2, the primary endpoint, in the interim analysis (20/ target number of 80 subjects) of the clinical study being conducted in Belgium [11].

Inhalation administration for 5 consecutive days was set based on the fact that inhalation administration for 7 consecutive days has been confirmed safe in the placebo-controlled phase III study in patients with autoimmune pulmonary alveolar proteinosis (investigator-initiated study) conducted in Japan[7].

In the clinical study [11] in COVID-19 patients being conducted in Belgium and the U.S., 5 days was set for inhalation administration of sargramostim. If the investigator determined it necessary to administer sargramostim after the sixth day, sargramostim was to be administered intravenously (approved route of administration in the U.S.) for further five days. Based on this, the administration period in this study was set as "5 days, in principle (up to 10 days) " by the inhalation administration route.

Final observations are to be performed until Day 28 (including the case after discharge from hospital), so that not only the observations during treatment period but also the follow-up observations can be performed to observe prognosis after the end of administration.

Because it is difficult to obtain the placebo preparation of the test drug, an unblinded pharmacist is to prepare the physiological saline for the control group and the control drug is to be administered by inhalation in the same manner as in the active treatment group.

7 Subjects

Patients with moderate-II coronavirus disease-2019 (COVID-19) who were clinically diagnosed pneumonia.

[Rationale]

Considering the mechanism of action of sargramostim, patients with clinically diagnosed pneumonia whose respiratory function is suggestively decreased are to be included in the study. In addition, patient population without use of invasive ventilator yet (the category of a patient that falls into one of Category 3 to 5 on a 7-point ordinal scale (see Table 13.3-1)) is to be chosen in order that an accurate dose of study drug can be administered by inhalation.

7.1 Inclusion criteria

Japanese male or female subjects who have been confirmed to meet all the following criteria.

(1) Hospitalized patients under treatment who were severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2] positive by polymerase chain reaction (PCR) test.
(2) Patients with clinically diagnosed pneumonia and a percutaneous oxygen saturation [SpO_2] of 93% or less on breathing of room air at bed rest.
(3) Patients for whom written informed consent has been obtained from those themselves or the legally acceptable representatives.

(4) Patients aged 20 years or older and younger than 85 years at the time of obtaining informed consent.

[Rationale]

- Although overseas clinical studies have already preceded, Japanese patients were considered unlikely to be included in these studies. Japanese patients are to be enrolled in this study in order to evaluate the efficacy and safety in Japanese patients.
- Sargramostim was approved in the United States in 1991 and its clinical experience has been established in the market. Moreover, since no difference in safety profile between sexes has been observed in the clinical experience in patients with autoimmune pulmonary alveolar proteinosis in Japan, both sexes are to be included in the study.

(1) It was set in order to confirm that the patient is a COVID-19 patient.
(2) Considering the mechanism of action of sargramostim, the severity classification of the disease was set in reference to the current " Guide to new coronavirus infections (COVID-19) practice " [1] .
(3) It was set based on the Declaration of Helsinki and the GCP.
(4) The lower limit (20 years) was set for adults under the Japanese law.
Considering that the fatality due to COVID-19 becomes higher for patients who are elderly and also have underlying disease, patients aged 80 years and older with an underlying disease are excluded and the upper limit was set as < 85 years in order to enroll the patient population that is as close to actual clinical situation of COVID-19 as possible.

7.2 Exclusion criteria

Subjects who meet any of the following criteria will be excluded. Unless otherwise stated, the following criteria refer to those at the time of screening.

(1) Patients who have allergic reactions or hypersensitivity to the active ingredient of the test drug (sargramostim) or its additives (mannitol, sucrose and tromethamine) or yeast-derived products or other.
(2) Patients who have been participating in other intervention studies, such as a study on unapproved pharmacotherapy, within 90 days prior to screening.
(3) Patients who have experienced off-label use of approved drugs (including those for COVID-19 treatment other than steroids and favipiravir as standard treatment) within 7 days prior to screening.
(4) Patients who are not expected to survive longer than 24 hours after commencement of study drug administration
(5) Patients who are using invasive ventilator or extracorporeal membrane oxygenation (ECMO).
(6) Patients who have a chronic respiratory disease requiring continuous home oxygen therapy or ventilator use.
(7) Patients with an underlying condition that is considered very unlikely to withdraw ventilator (e.g., motor neuron disease, Duchenne muscular dystrophy, rapidly progressive interstitial pulmonary fibrosis).
(8) Patients who have a disease including bronchial asthma, lower respiratory tract infections, or interstitial lung diseases that may affect the assessment of the clinical study, since before the symptom onset of COVID-19.

(9) Patients who have a disease including leukemia or leukocytosis that causes leukocytosis.
(10) Patients who have a chronic kidney disease requiring dialysis.
(11) Patients who have severe liver failure (Child Pugh grade C).
(12) Patients who are pregnant or breastfeeding or who are positive on pregnancy test.
(13) Patients aged 80 years or older who have/are cardiac disease, cerebrovascular disorder, obese patient (BMI 30 or higher), dyslipidemia, hypertension or diabetes
(14) Other patients considered inappropriate by the investigators for inclusion in the study

[Rationale]

- In order to minimize the burden of the medical front for the treatment of COVID-19 patients, the assessment results at screening are to be applied as much as possible, as far as the results are considered reasonable.

(1) It was set to ensure the safety of subjects.
(2) It was set as a general item to ensure the safety of the subjects and to evaluate the study ethically and appropriately.
(3) The objective of this study is to evaluate the Add-on effect of sargramostim on the standard treatment. It was set in order to eliminate effects by other treatments that have not been recognized or established as "standard treatment".
(4) It was set because participation of patients who require emergency care with the highest priority is not considered appropriate.
(5) Subjects who entered a stage to use invasive ventilator or ECMO are to be excluded from the study because inhalation administration of the sargramostim is difficult for these subjects and this disease condition of the subject has been included in the criteria for the study drug discontinuation.
(6) Patients with underlying disease who meet this criterion are to be excluded from the study because it is difficult to accurately assess whether use of ventilator is due to COVID-19 infection or not.
(7) Patients with these diseases are to be excluded from the study because they are likely to develop more severe pneumonia resulting in death.
(8) It was set in order to exclude patients who have been having diseases which may affect the assessment of arterial blood gas analysis or others, a measurement parameter of the study, since before the onset of COVID-19.
(9) It was set in order to exclude patients with increased risk of safety concerns due to administration of the study drug.
(10) It was set in consideration that patients with chronic kidney disease requiring hemodialysis are at increased risk to become severe COVID-19 and that sargramostim is removed by hemodialysis.
(11) Patients who meet this criterion are likely to be seriously ill at the onset of infection and their mortality become higher when intubated due to respiratory failure, thus are to be excluded from the study.
(12) It was set because the safety of administration of sargramostim during pregnancy or lactation has not been established. Women of childbearing potential are to be excluded from the study if they are positive on pregnancy test.
(13) Elderly patients with an underlying disorder are excluded because they are at increased risk of becoming severe and death.

(14) Other than the predetermined criteria, it was set as a general exclusion criterion so that inclusion validity can be assessed at the discretion of the investigators.

8 Subject Information, Informed Consent, and Subject Authorization

8.1 Informed consent form

Prior to the implementation of the study, the principal investigator will collaborate with the sponsor, prepare an informed consent form, submit it to the head of the medical institution for approval by the Institutional Review Board (IRB), and submit the approved informed consent form to the sponsor. The informed consent form shall include the following information as stipulated in the GCP.

① The clinical study involves research.
② Objectives of the clinical study.
③ Study procedures (including those aspects of the study that are experimental, patient inclusion/exclusion criteria)
④ Expected duration of the patient's participation in the study.
⑤ Approximate number of patients involved in the study.
⑥ Anticipated clinical benefits and risks or inconveniences to the patient.
⑦ Other treatment that may be available to the patient, and their important potential benefits and risks.
⑧ Compensation and/or treatment available to the patient in the event of study-related injury.
⑨ The patient's participation in the clinical study is voluntary, and the patient or the patient's legally acceptable representative may refuse to participate or withdraw from the study, at any time, without penalty or loss of benefit to which the patient is otherwise entitled.
⑩ The patient or the patient's legally acceptable representative should promptly be informed if information becomes available that may be relevant to the patient's willingness to continue participation in the clinical study.
⑪ Foreseeable circumstances and/or reasons under which the patient's participation in the clinical study may be terminated.
⑫ Monitors, auditors, IRB, and regulatory authorities are given direct access to the patient's original medical records. In such cases, the confidentiality of the patient should be protected, and by signing and dating the informed consent form, the patient or the patient's legally acceptable representative is authorizing such access.
⑬ If the results of the clinical study are published, the patient's identity should remain confidential.
⑭ Anticipated expenses, if any, to the patient for participating in the clinical study.
⑮ Anticipated prorated payment, if any, to the patient for participating in the clinical study (arrangements for prorated payment).
⑯ Name, title, and address of contact of the principal investigator or subinvestigator.
⑰ Persons to contact for further information regarding the clinical study and the rights of patients, or the medical institution to refer to and contact in the event of the study-related injury, when the patient or patient's legally acceptable representative requested such information.
⑱ The patient's responsibilities.

⑲ Types of the IRB that review and discuss the appropriateness of this clinical study, items that are investigated and discussed by the IRB, and other matters related to the IRB related to this clinical study.

⑳ That the IRB's procedures, etc. may be checked. Provide public access to the IRB's procedures on the website of the medical institution, etc., by keeping the website addressed, if not publicly available, and the IRB's procedures, etc., in the office, etc. Description of “If you wish to check the IRB's procedures, please offer it”.

8.2 Timing and procedures for obtaining informed consent

8.2.1 Explanation of the clinical study and obtaining informed consent

After the clinical study agreement between the medical institution and the sponsor has been signed, the investigators shall explain the details of the clinical study, etc., using the explanation materials for informed consent form to the candidate patient, shall provide the patient with the opportunity to ask questions and sufficient time to decide whether or not to participate in the clinical study, and shall obtain voluntarily written informed consent from the patient before conducting any procedures for examinations and observations described in the protocol.

If the investigators judge that the candidate patient does not have adequate ability to provide consent due to mental retardation, etc., the investigators shall provide the legally acceptable representative (person with parental authority, guardian or similar, or person who acts in the patient's best interest) with adequate information using explanation materials for informed consent, shall give the opportunity to ask questions and shall give sufficient time to decide whether or not the candidate patient to participate in the clinical study, and shall obtain voluntarily written informed consent from the legally acceptable representative. At obtaining informed consent, a document where the relationship between the legally acceptable representative and the candidate patient has been recorded will be retained. In this case, the investigators shall explain to the candidate patient about the clinical study using the explanation materials for informed consent depending on his/her comprehensibility, and shall obtain voluntarily written confirmation for participation in the clinical study from the candidate patient, if possible. However, if the candidate patient himself/herself refuses to participate in the clinical study, the investigators are not allowed to involve the candidate patient in the clinical study even though the written consent has been obtained from the legally acceptable representative.

If it is difficult to explain to the candidate patient about the clinical study due to his/her disease condition or age, and it is not possible to obtain signed consent, the fact and the reason for not having obtained signed consent shall be recorded in the informed consent form signed by the legally acceptable representative or in the medical record.

8.2.2 Delivery of informed consent form

The investigators shall provide the candidate patient or the legally acceptable representative with a copy of the informed consent form signed and sealed or signed by the investigators and the candidate patient as well as the explanation materials. The investigators shall provide the candidate patient and the legally acceptable representative with sufficient time to ask questions and to decide whether to participate in the clinical study before obtaining informed consent. The investigators shall answer all the questions so that the candidate patient and the legally acceptable representative are satisfied with the answers.

8.3 Obtaining new information and revision of informed consent form

If new information has been obtained that may affect the willingness of the subject and the legally acceptable representative to continue participating in the clinical study, the investigators shall revise the informed consent form based on the new information and obtain approval from the IRB.

In addition, the investigators shall promptly inform the subject and the legally acceptable representative of the relevant information, shall confirm again the willingness of the subject and the legally acceptable representative whether to continue participating in the clinical study, and shall record the result in the medical record, etc. Moreover, the investigators shall explain the details of the revised informed consent form approved by the IRB to the subject and the legally acceptable representative, and again shall obtain a written informed consent form as specified in "8.2 Timing and procedures for obtaining informed consent."

8.4 Others

The investigators shall comply with the following items when obtaining informed consent from the candidate patient and the legally acceptable representative.

① The investigators shall not give unjustified effect such that they force to participate or continue participation in a clinical study to the candidate patient and the legally acceptable representative.

② The informed consent form, the explanation materials and the information provided verbally shall not contain any phrases that cause or suspect to waive the right of the candidate patient and the legally acceptable representative, or that cause or suspect to exempt the investigators, the medical institution, or the sponsor from their legal responsibility.

③ The informed consent form and the information provided verbally shall be understandable by the candidate patient and the legally acceptable representative and shall be available in non-professional language as far as possible.

④ Regardless of the reason, the clinical study shall not be initiated based on the only informed consent obtained orally.

9 Study Drug

9.1 Test drug

Non-proprietary name：sargramostim (Code name: NPC-26)

Dosage form/Strength：A lyophilized preparation containing 250 μg of sargramostim in 1 vial

Storage conditions：4℃ (2 to 8℃)

9.2 Packaging and labeling of test drug

The vials will be placed in individual packaging boxes. The label and package will contain the following information. Expiration date will be specified in a separate written procedure.

- A statement that the test drug is for clinical study use.
- Name and address of the sponsor.
- Identification number.
- Manufacturing number.
- Storage condition.

9.3 Physiological saline for dissolution of the test drug and for the control group

In the active treatment group in the study, the test drug will be dissolved in physiological saline and administered by inhalation. In the control group, the physiological saline itself will be administered by inhalation (see "10.3 Administration of study drug"). The physiological saline used in the study will not be supplied by the sponsor, but purchased by the medical institution.

Therefore, the records of storage, management, and delivery of physiological saline will not be applicable to "9.4 Management of test drug" in the next section.

9.4 Management of test drug

(1) The sponsor will deliver the test drug and the " Investigational product handling procedures" to the Investigational product manager after a contractual agreement with the medical institution has been signed.

(2) The Investigational product manager will store and manage the test drug in accordance with the " Investigational product handling procedures", and record the prescription at the medical institution, usage in each subject, disposition, and return of the test drug to the sponsor onto the Investigational product management sheet.

(3) The Investigational product manager will store the used test drug, unused test drugs, and package, outer box at the appropriate time in accordance with the " Investigational product handling procedures " based on the rule of the medical institution until they are retrieved by the sponsor. Moreover, a copy of the Investigational product management sheet will be provided to the sponsor appropriately.

10 Study Procedures

This is a randomized, placebo-controlled, double-blind, group-comparison, multicenter study. Refer to Figure 6.1-1 for the study design.

10.1 Enrollment of subject

The investigators will assign a subject identification code to subjects in the order of giving informed consent, and enter the information of the eligible randomized subjects in the electronic case report form (eCRF). The subject identification code will be composed of a two-digit number assigned to each medical institution and a three-digit number assigned to each subject in ascending order from 001 within the medical institution connecting with a hyphen (ex. the subject identification code of the first subject in medical institution 01 is "01-001"). Moreover, a duplicable "Screening list" will be prepared. The following information will be included in the Screening list.

［First sheet］

Date of informed consent, subject's name, medical record number, subject identification code, date of screening test, and screening test results (by eligibility) will be recorded and stored at the medical institution.

［Second sheet］

Information other than the subject's name and the medical record number on the first sheet will copied on the second sheet. After the clinical study has been terminated, the principal investigator will confirm the contents, sign and seal, or sign, and submit it to the sponsor.

The investigators will conduct observations/examinations on the day of allocation (Day 0) for eligible subjects based on the screening test results, ensure that the inclusion criteria are met, and enter the required information in the eCRF. This electronic entry will be regarded as enrollment of the subject, then the investigators will instruct the unblinded pharmacist to randomize the subject.

10.2 Randomization

Randomization will be performed by the unblinded pharmacist using the web-automated responsiveness system (IWRS) of the eCRF. The unblinded pharmacist shall be specifically in charge of allocation, preparation and arrangement of the study drug and must not perform any other study-related duties other than those assigned. The unblinded pharmacist must not disclose any information on allocation to others except the unblinded monitor. When the allocation code is recorded or duplicated for the purpose of preparation and arrangement of the study drug, the unblinded pharmacist shall strictly control these documents so that they are not seen by others. The unblinded pharmacist shall discard these documents in an appropriate manner as soon as possible after use.

Stratified allocation using standard treatment as the allocation factor will be performed at the randomization. Enrolled subjects will be randomized in a 2:1 ratio to either the active treatment group or the control group.

The investigators and the study collaborator other than the unblinded pharmacist should not be present at the operations for preparation and dispensing of the study drug performed by the unblinded pharmacist.

10.3 Administration of study drug

Administration of study drug will start in the morning of Day 1 (twice daily, in the morning and evening between Day 1 and Day 5).

In the active treatment group, 250 μg/vial of sargramostim will be dissolved in 4 mL of physiological saline, and 2 mL of which will be administered twice daily (in the morning and evening) using an inhaler for 5 days, in principle (up to 10 days). In the control group, 2 mL of physiological saline will be administered twice daily (in the morning and evening) using an inhaler for 5 days, in principle (up to 10 days).

Approximately 10-15 minutes will be taken for one inhalation administration which shall be continue until the prepared solution has been depleted. During the inhalation administration, air cleaner or similar will be available for prevention against infection for healthcare workers and other patients.

The start date and time of the study drug administration should be recorded in the eCRF. Details of the preparation and dispensing of the study drug should be in accordance with the procedures specified separately.

All subjects may continue to receive inhalation administration of the study drug at the discretion of the investigators for up to Day 10 from Day 6 onward. After Day 6, the dosage and administration should be the same as that in the first 5 days.

Occurrence of serious allergic or anaphylactic reactions after administration of sargramostim has been reported. If a serious allergic or anaphylactic reaction occurs, administration should be discontinued and appropriate therapy should be initiated. The investigators shall follow up at least 1 hour after inhalation administration to ensure that no clinical symptoms such as acute hypersensitivity reactions (e.g., facial or extremity swelling, cough, urticaria, decreased consciousness, respiratory distress) are observed.

10.4 Disclosure of allocation code in an emergency

When disclosure of allocation code in an emergency is requested by the investigators through the sponsor in the event of a serious adverse event, the unblinded pharmacist will disclose the allocation code of the subject in accordance with the procedures provided by the sponsor separately.

10.5 Unblinding after completion of clinical study

The allocation code for this study will be stored in the EDC system, but only the unblinded pharmacist and the unblinded monitors will be allowed to review the code during the study period. After completion of the clinical study, the data will be locked in the blinded condition according to the predetermined procedures,then the allocation code will be outputted from the EDC system and unblinded.

11 Concomitant Medication/Therapy

A standard treatment for each subject against COVID-19 will be chosen and performed appropriately based on medical judgment, with reference to current knowledge and guideline[1, 16, 17].

Concomitant use of any unapproved drugs (including off-label use of approved drug and use at unapproved dosage and administration, excluding steroids as standard treatment at each medical institution) will be prohibited throughout the study period (with an exception of concomitant medication/therapy given after study discontinuation when a subject meets any of the criteria for the study drug discontinuation and is removed from the study. See “15.1 Study drug discontinuation”). In addition, the combination of sargramostim with chemotherapy and radiotherapy is contraindicated. Except treatment/therapy mentioned above, there are no specific prohibited concomitant medications in this study. And steroids as standard treatment at each medical institution and approved antivirals, or others can be concomitantly used as part of standard treatment.

If a subject initiates use of invasive ventilator or ECMO, administration of the study drug will be terminated after use of these devices has been initiated (see "15.1 Study drug discontinuation"). The necessity to use these devices during the study period will be judged as part of the standard treatment against COVID-19 according to the disease condition and medical necessity of the subject.

The investigators will record and report the concomitant medications/therapies used during the study (see 13.14 Confirmation of concomitant medication/therapy).

12 Management of Subject

If a subject is discharged from the hospital during the study period, the subject shall be appropriately instructed to receive the observation/examination at the timing specified in the "2 Observation and Examination Schedule" and to avoid pregnancy or breastfeeding until the last observation for a female subject of childbearing potential.

13 Measurement of Parameters

In accordance with “2 Observation and Examination Schedule”, observations/examinations and evaluations will be performed, and the date and the results of observations/examinations will be recorded in the source documents such as the medical record. In addition, the prespecified data will be entered in the eCRF.

After obtaining written informed consent from the candidate patient and the legally acceptable representative in accordance with "8.2 Timing and procedures for obtaining informed consent”, the investigators will perform a screening test. The screening test results will be entered in the eCRF. The data at the screening test may be used if they have been collected within 7 days prior to informed consent. In addition, when the examinations scheduled on Day 0 have been collected prior to informed consent, the data may be used if they were collected within 2 days prior to Day 0.

Additional observations/examinations may be performed at the discretion of the investigators depending on the results of the scheduled observations/examinations, even if those additional observations/examinations are not included in the scheduled observations/examinations. If the scheduled observations/examinations were not performed, the reason should be clarified.

The study period will be from the day of informed consent to the day of the last observation (Day 28) (including the case of discharge from hospital). After completion of the final observations/examinations, completion of the study and the date of completion will be recorded in the eCRF.

Refer to "15 Criteria for Discontinuation of Study, Subject and Study Drug " for premature termination of study participation of a subject or discontinuation of study drug.

Follow-up examination will be conducted according to "14.8 Observation of AEs".

13.1 Subject characteristics

The date of informed consent, date of birth, sex, height, weight, alcohol consumption, smoking history, presence or absence of allergies (drugs, food, etc.), date of COVID-19 onset, date of collection of a sample where SARS-CoV-2 was detected positive by PCR test, past medical history/current medical condition, prior medication/therapy, concomitant medication/therapy.

The date and the results of the observations/examinations will be entered in the eCRF.

13.2 Physical examination

The investigators will conduct physical examination (medical interview, inspection, palpation, and auscultation, etc.) according to the standard procedures of the medical institution, and the results will be recorded. Also, the date and the results of the examinations will be entered in the eCRF.

13.3 7-point ordinal scale

The investigators will evaluate the category on a 7-point scale, referring to Table 13.3-1. The evaluation results will be recorded. Also, the date and the results of the evaluation will be entered in the eCRF.

Table 13.3-1 7-Point Ordinal Scale

Category	Ordinal scale
1	Have died
2	Use an invasive ventilator or ECMO
3	Use a noninvasive ventilator or high-flow oxygen supply device
4	Require oxygen supply
5	Do not require oxygen supply but require continuous therapy (related to COVID-19 or others).
6	Do not require both oxygen supply and continuous therapy
7	Have been discharged from hospital

13.4 Vital signs

Blood pressure (systolic, diastolic), pulse rate, body temperature and respiratory rate will be measured and recorded. The date and the results of the measurements will be entered in the eCRF.

Blood pressure and pulse rate will be measured after resting for about 5 minutes. The blood pressure may be measured in either supine or sitting position, but the posture for blood pressure measurement should remain constant throughout the study whenever possible.

The site (forehead, ear, axilla, etc.) for body temperature measurement in the same subject should remain constant throughout the study whenever possible.

13.5 12-lead Electrocardiography

Twelve-lead electrocardiography (ECG) will be performed after resting for at least 5 minutes in accordance with the standard procedure of the medical institution.

The investigators or the designated, will confirm and assess the electrocardiogram, and record. The date of assessment, heart rate, and assessment results will be entered in the eCRF.

13.6 Laboratory tests (blood chemistry, hematology, urinalysis, and pregnancy test)

The following items will be measured at the medical institution. If the measurement results deviate from the reference value, the investigators will assess whether the deviation is clinically significant. Pregnancy test will be performed only for women of childbearing potential. Pregnancy test will not necessarily be required for a female subject considered unable to become pregnant due to the following reasons: menopause (amenorrhea for at least 12 months without medical reasons), total hysterectomy, or that she does not get the first menstruation yet. The date of the sapmle collection and the test results will be entered in the eCRF.

Abnormal changes in laboratory test values that are judged by the investigators to be applicable to adverse events will be reported separately as adverse events (see "14.1 Definitions of AE").

Table 13.6-1 List of Laboratory Parameters

Hematology	Red blood cells count, white blood cells count, differential white leucocytes (neutrophils, lymphocytes, monocytes, eosinophils, basophils), platelets count, hemoglobin, hematocrit
Blood chemistry	Total protein, albumin, AST (GOT), ALT (GPT), γ-GTP, AlP, LDH, CK (CPK), total bilirubin, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides (TG), BUN, creatinine, blood glucose, Ca, Na, K, P, Cl
Urinalysis	Occult blood, protein, glucose, urobilinogen
Pregnancy test	Urine hCG or serum hCG

13.7 Percutaneous oxygen saturation (SpO_2)

Percutaneous oxygen saturation (SpO_2) will be measured using a pulse oximeter on breathing of room air in bed rest. The date and the test results will be entered in the eCRF

13.8 Inflammatory marker

C-reactive protein (CRP) will be measured at the medical institution. The date of sample collection and the test results will be entered in the eCRF.

13.9 Cytokines

The following cytokines will be measured at the central laboratory. Measurement results will not be disclosed until the code breaking after the clinical study has been completed. The date of sample collection will be entered in the eCRF : IL-6, IL-1β, TNF-α, IL-10, CCL17, CXCL9, MMP-12.

Sample collection and storage will be performed in accordance with the procedures specified separately.

13.10 Arterial blood gas analysis (PaO_2, $PaCO_2$, A-aDO_2, others)

The subject will take a rest for at least 5 minutes under breathing of room air (when oxygen is being inhaled, the device will be temporarily removed) in a supine position on bed in a physicians room or in an examination room, then SpO_2 will be confirmed to be stable on a pulse oximeter and the blood sample will be collected from the artery using a blood collection kit designated by the medical institution. After blood sample is collected, the sample will be mixed adequately, and blood gas will be measured within 30 minutes using a blood gas analyzer. If it takes more than 10 minutes from blood collection to measurement, the blood sample will be kept in ice water. Whether the examination is possible or not will be determined to secure the subject safety considering the following criteria:

(1) SpO_2 94% or lower under the use of nasal high flow or mask

(2) SpO_2 93% or lower under oxygen supply of 4 L/min or more using nasal cannulas

A-aDO_2 will be automatically calculated using the following formula and automatically entered in the eCRF:

$A\text{-}aDO_2 = (PB - PH_2O) \times FiO_2 - PaCO_2/R + \{PaCO_2 \times FiO_2 \times (1\text{-}R)/R\} - PaO_2$

, where

PB: barometric pressure (measured at each medical institution)

PH_2O: partial pressure of water vapor in inspired air (47 mmHg)

R: respiratory quotient (0.80)

FiO_2: fraction of inspiratory oxygen (0.21)

The date of sample collection and the measurement values of PBs and PaO_2, $PaCO_2$, pH, SaO_2 will be entered in the eCRF

13.11 Chest HRCT or plain chest CT or chest X-ray

Chest high-resolution computed tomography (HRCT), plain chest CT, or chest X-ray will be performed in each subject (or at each medical institution) by choosing one of the above-mentioned examinations, and the same examination will be performed throughout the study period.

Details of image capturing condition and CT values for performing chest HRCT will be described in the HRCT testing procedures, whereas other image capturing procedures will be performed according to the standard procedures of the medical institution. Quantitative assessment of findings such as ground-glass opacity for the chest HRCT images will be performed using densitometry according to the procedures specified separately. The date of examination and the assessment results will be entered in the eCRF.

Image capturing of plain chest CT and chest X-ray will be performed according to the standard procedures of the medical institution, and those images will be assessed by the radiologist or the investigators of the medical institution. The assessment results will be recorded. The date of examination and the assessment results will be entered in the eCRF.

13.12 Oxygen requirement

The investigators will evaluate the category of oxygen requirement of a subject classified in 5 categories, referring to Table 13.12-1. The date of evaluation and the results will be entered in the eCRF.

Table 13.12-1 Oxygen Requirement

Category	Ordinal scale
1	Require oxygen supply of 4 L/min or more using oxygen mask
2	Require oxygen supply of 2 L/min or more using nasal cannulas
3	Require oxygen supply of less than 2 L/min using nasal cannulas
4	Do not require oxygen supply but feel shortness of breath during walking or moving
5	Do not require oxygen supply and do not feel shortness of breath during walking or moving.

13.13 Confirmation of AEs

If an AE is reported by a subject at any time during medical interview, or at voluntary visit, or on telephone contact, information on the name of the AE, date and time of onset, severity, seriousness, causality, treatment, outcome, and date of outcome will be obtained and recorded. In the case of an SAE, the SAE should be reported in accordance with "14.10 Reporting of SAEs".

13.14 Confirmation of concomitant medication/therapy

Regarding concomitant medication, information on the name of the concomitant medication, route of administration, treatment duration, and reason for use of the concomitant medication will be recorded and entered in the eCRF. Regarding concomitant therapy, information on the name of the therapy, start and end dates of implementation, and reason for implementation will be recorded and entered in the eCRF.

14 AEs and adverse drug reactions (ADRs)

14.1 Definition of AE

An AE is any unfavorable or unintended sign (including abnormal laboratory changes), symptom, or disease occurring in an assigned subject from the start of study drug administration, whether or not related to the study drug. Events occurring before the start of study drug administration will be treated as a current medical condition in this study.

AEs required to be entered in the eCRF will be those occurring from the start of study drug administration to Day 14 in this study. SAEs related to the study drug should be collected until Day 28.

Moreover, the subject's current medical condition is not considered an AE unless the severity worsens or the incidence increases after study drug administration. If the investigators judge that the change in a laboratory test value is an unfavorable event for the subject, the event will be assessed as an AE.

14.2 Definition of ADR

ADR is at least reasonably likely to be related to the study drug and a causal relationship cannot be ruled out (see "14.6 Causality").

14.3 Severity of AEs

The severity of an AE will be assessed by classifying the AE into three grades: mild, moderate, and severe, referring to the following criteria and Pharmaceutical Safety Notification No. 80 (June 29, 1992) "Criteria for Classification of Seriousness of Adverse Drug Reactions". Grades 1 ,2, and 3 defined in the above mentioned notification correspond approximately to mild, moderate, severe, respectively.

■Criteria

1) Mild：Symptoms are mild and easily tolerated by the subject.
2) Moderate：The event causes the subject discomfort and interrupts the subject's usual activities and some treatment is required.
3) Severe：The event makes daily activities severely.

■Reference: Cited from Pharmaceutical Safety Notification No. 80 "Criteria for Classification of Seriousness of Adverse Drug Reactions"

Grade 1	The event considered to be a minor adverse drug reaction
Grade 2	The event considered to be neither a serious nor a minor adverse drug reaction
Grade 3	The event considered to be a serious adverse drug reaction. In other words, it may result in death or permanent dysfunction that interferes with daily life depending on the subject's nature and condition at the time of onset.

14.4 SAEs

The following AEs are defined as SAEs. Scheduled hospitalization for surgery or examination, educational hospitalization, hospitalization and prolongation of hospitalization due to the convenience of the home caregiver, hospitalization for disease control, and hospitalization for health care (medical examination) check will not be regarded as hospitalization and prolongation of the hospitalization.

1) Results in death.
2) Is life threatening.※1
3) Hospitalized to hospital or clinic※2 or require prolongation of existing hospitalization for treatment.
4) May result in disability/incapability.
5) May likely result in disability/incapability.
6) Is serious according to items 1) through 5)※3 above.
7) Is a congenital anomaly/birth defect

※1： The term “life threatening” refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it was more severe.

※2： "Hospitalization" in this clinical study refers to staying at a medical institution for at least one night. And prolonged treatment in an outpatient setting, such as infusion, or staying at a medical institution intended for examination will not be regarded as hospitalization. If a patient has been treated for a long time on the emergency visit, the investigators will judge whether the case is a hospitalization case or not, taking the situation into account.

※3： In case of a significant event that does not result in immediate life-threatening or death or hospitalization but may jeopardize the subject or require intervention to avoid the consequences described above, whether expedited reporting, as described below, is required or not should be judged based on the medical and scientific reasons, however, it should usually be considered serious. For example, blood disorders, renal and hepatic disorders, and central nervous system disorders which are considered medically important, bronchospasm requiring intensive care in the emergency room, or convulsions that do not result in hospitalization, drug dependency or substance abuse, etc. are included in this criterion.

14.5 Significant AEs

A significant AE is not an SAE, but is judged to be of special interest due to its clinical significance. A non-SAE leading to study drug discontinuation will be regarded as a significant AE.

14.6 Causality

The causal relationship between AE and study drug will be assessed as follows. If an AE is applicable to 2), the AE will be regarded as an ADR.

1) Not related: The temporal sequence of the AE to the administration of the study drug is not reasonable, or there is another obvious cause including current disease at onset, other medicinal product, or environmental factors or others.
2) Related: Events other than those "not related"

14.7 Predictability of AEs

Of AEs (including ADRs previously reported to the MHLW), those of which nature, severity, or frequency, etc. cannot be predicted according to the Investigator's Brochure [2]will be regarded as “unknown” and those which can be predicted will be regarded as “known”.

14.8 Observation of AEs

The investigators shall observe the occurrence of AEs carefully through medical interview, medical examination, etc. from the start of study drug administration. If any AE is observed, appropriate measures should be taken, and the AE shall be followed up by the investigators until the AE disappears

or resolves as far as possible. However, if the investigators judge that further follow-up observation is not necessary and discontinue follow-up observation, or if the subject refuses it, the reason shall be entered in the eCRF.

For any AEs that have occurred, information of the name of the event, date and time of onset (or date and time when the event was found), severity, seriousness (serious/non-serious), action taken with the study drug (if yes, its contents), treatment for the event (yes or no), outcome, date and time of outcome (when the event disappeared, the date and time of disappearance), and causality to the study drug should be entered in detail in the eCRF.

14.9 Pregnancy

When a female subject has been known or suspected to be pregnant during the study period, the investigators shall immediately discontinue the study drug administration and shall report the prespecified information (at least subject identification code, date of acknowledgment of pregnancy, dates of start and discontinuation of the study drug administration) to the sponsor. Pregnancy is not handled as AE, but the follow-up observation shall be conducted until the outcome (delivery, etc.) is confirmed.

Data on sargramostim in pregnant and lactating women are limited, and there are no data on inhalation administration of sargramostim, thus the safety of sargramostim has not been established. Risks for spontaneous abortion and harm to the embryo and fetus have been reported in the rabbit studies.

There is no information on sargramostim regarding its presence in human breast milk, its effects on breastfeeding children, or its effects on milk production. It has been reported that administration of sargramostim to rabbits during lactation resulted in decreased survival of offspring after birth.

14.10 Reporting of SAEs

14.10.1 Principal investigator

If an SAE occurs during the period from the start of study drug administration to Day 14, the investigators will take the necessary measures with the highest priority given to the safety of the subject, regardless of whether it is related to the study drug or not. The principal investigator will report the event to the sponsor. However, if the event is an SAE and related to the study drug, the event that have occurred until Day 28 will be reported to the sponsor in the same manner even after completion of the above-mentioned period.

The procedures are outlined below.

(1) The sponsor should be informed of the occurrence of an SAE within 24 hours of acknowledgment of the occurrence (A form of “Report on serious adverse event (initial announcement)” shall be used, whenever possible). In addition, the SAE should be immediately reported to the head of the medical institution.

(2) A form of "Report on serious adverse event" shall be prepared and reported to the sponsor and the head of the medical institution at the earliest possible time within 7 days after the first announcement has been submitted to the sponsor, and a copy of the report will be retained.
(3) The investigators shall make efforts to collect more detailed information, and report it to the sponsor and the head of the medical institution if new information becomes available.

Emergency Contact (Sponsor)
Nobel Pharma Co., Ltd. Research and Development Division 17-24, Shinkawa 1-chome, Chuo-ku, Tokyo〒104-0033 TEL：03-6670-3812、FAX：03-6670-5051 Night and holiday： [redacted]

14.10.2 Sponsor

The sponsor shall follow the following procedures when the occurrence of an SAE has been reported by the investigators.

(1) "Report on serious adverse event" shall be obtained at the earliest possible time within 7 days after the first announcement has been received from the investigators.
(2) The sponsor will check details of the "Report on serious adverse event", and refer to the investigators as appropriate if any additional information is considered necessary.
(3) If the report is judged to meet the requirements of Article 273 of the Enforcement Regulations of the Pharmaceuticals and Medical Devices Act, the sponsor should report the SAE to the regulatory authorities within the specified period. In addition, the sponsor will report the relevant SAE in writing to each principal investigator and the head of each medical institution.

15 Criteria for Discontinuation of Study, Subject and Study Drug

15.1 Study drug discontinuation

If the subject meets any of the following criteria at any time before the second dose on Day 5, study drug administration for the subject shall be discontinued.

(1) The subject has started to use invasive ventilator or ECMO.
(2) The WBC count of the subject exceeds 25,000/μL.
(3) An AE has occurred and the investigators judged it difficult to continue study drug administration.
(4) The subject was found to be pregnant or suspected to be pregnant, or the subject wished to be pregnant or breastfeeding.
(5) The subject was discharged from the hospital (including transfer to another hospital) and cannot continue to receive the study drug.

[Rationale]

(1) It was set because accurate specified dose of the study drug is thought difficult.
(2) and (3) It was set in order to ensure the safety of the subject.
(4) It was set because the safety of sargramostim administration during pregnancy or lactation has not been established.
(5) The possibility of discontinuation due to inevitable accident was considered.

Discontinuation of study drug administration does not necessarily preclude discontinuation of participation of the subject in the clinical study, and a policy was decided to follow-up and to collect the data on Japanese COVID-19 patients who participated in the clinical study whenever possible.

If an event meets the criteria for study drug discontinuation, the investigators shall follow the following procedures.

- The investigators shall immediately discontinue the study drug administration for the subject and perform observations/examinations at the time of discontinuation within 1 day of the discontinuation of study drug administration.
- If the event does not meet the criteria of "15.2 Withdrawal of subject from the study" and the observations/examinations can be performed, the subject will continue to participate in the study and continue the scheduled study procedures other than the administration of the study drug.
- The date and reason for discontinuation of study drug administration, as well as comments on discontinuation as appropriate, will be entered in the eCRF.

15.2 Withdrawal of a subject from the study

A subject who meets any of the following criteria shall be withdrawn from study participation.
(1) The subject was found not to meet the inclusion criteria or to meet the exclusion criteria and to be ineligible for the clinical study according to the results of medical examinations, etc. performed after start of the clinical study.
(2) The subject or the legally acceptable representative has requested to terminate the clinical study or to withdraw his/her consent.
(3) The subject was dead.
(4) The investigators judged that continuation of the clinical study is not desirable for the subject due to other reasons.

[Rationale]
(1) It was set as a general item to ensure safety of the subject and to properly evaluate the study.
(2) It was set in accordance with the principles of the Declaration of Helsinki and the GCP.
(3) Since continuous observation cannot be performed, it was set as a discontinuation criterion due to inevitable accident.
(4) In addition to above mentioned criteria, it was set as a general item for discontinuation criteria so that the investigators can judge appropriateness to continue the clinical study.

If an event occurs to meet the criteria for withdrawal of subjects from the study, the investigators shall follow the following procedures.

- The date of discontinuation (the date on which discontinuation was judged and the date of last dose, if possible) and the reason for discontinuation, as well as comments on discontinuation if necessary, will be entered in the eCRF.

15.3 Premature termination of entire or a part of the study

(1) If the sponsor decides to discontinue a part of or entire clinical study, the sponsor should promptly report the details of the discontinuation and the reasons to all the principal investigators, the heads of all the medical institution, and the regulatory authorities involved in the clinical study in writing.
(2) When the head of the medical institution received a report to discontinue a part of or entire clinical study from the sponsor, he/she shall promptly inform the principal investigator and the IRB in writing and provide a detailed explanation.
(3) When the clinical study is to be discontinued, the investigators shall promptly notify the subject of the discontinuation of the clinical study and provide appropriate medical care and take other necessary measures.

16 Protocol Compliance and Amendment

16.1 Protocol compliance and deviation

(1) This study shall be conducted in compliance with the protocol in agreement between the principal investigator and the sponsor.
(2) The investigators shall not deviate from or change the protocol without prior written agreement with the sponsor and prior approval by the IRB. However, this does not apply to cases where medical treatment is unavoidable (emergency deviation) such as avoiding the immediate hazard of the subject.
(3) In case of emergency deviation, the principal investigator shall submit the emergency deviation report to the sponsor and the head of the medical institution, then approval/agreement by the IRB, the head of the medical institution, and the sponsor shall be obtained.
(4) The investigators shall document any actions that deviate from the protocol.

16.2 Protocol amendment

(1) The sponsor must revise the protocol in consultation with the medical experts when applicable to the following items.
   - The sponsor has known information related to the quality, efficacy, and safety of the test drug or other information important for proper conduct of the clinical study.
   - a change in the protocol becomes necessary due to unavoidable medical reason.
(2) The sponsor shall promptly inform the principal investigator of the finalized amendment in writing.
(3) The principal investigator will fully review the ethical and scientific appropriateness to conduct the clinical study in accordance with the revised protocol.

(4) After discussing the revised protocol between the sponsor and the principal investigator, they shall sign, or sign and seal each other on the two copies of the revised protocol or equivalent as a proof of agreement. In addition, an approval shall be obtained from the IRB.
(5) When the sponsor receives an instruction to make a change in the protocol from the IRB or the secretariat of the clinical study, the sponsor will discuss the change with the medical experts as appropriate and report the conclusion of discussion to the principal investigator.

17 Statistical Analysis

Statistical analysis will be performed in accordance with "Statistical Principles for Clinical Studies"(Notification No. 1047 of the Evaluation and Licensing Division, PMSB dated November 30, 1998). The analysis items and technical details described in this section should be described separately in the statistical analysis plan.

17.1 Analysis sets

17.1.1 Efficacy analysis set

The Full Analysis Set (FAS) is defined as any of the assigned subjects, excluding those who were not administered with the study drug or for whom no information on efficacyafter allocation was available. Analysis in FAS will be performed in this study.

17.1.2 Safety population

All subjects who received the assigned study drug will be included in the Safety Population (SP).

17.2 Statistical analysis items and analytical method

Details of the analysis items and analytical methods will be provided in the statistical analysis plan.

17.2.1 Subject characteristics

Regarding the background parameters of the subjects, the frequency distribution (number of subjects, %) for categorical data, summary statistics (mean, standard deviation, median, minimum, maximum, etc.) for continuous data, and the frequency distribution as necessary when data are categorized, will be determined by treatment group.

17.2.2 Efficacy evaluation

Regarding the number of days to achieve at least 2-rank improvement on a 7-point ordinal scale from baseline until Day 28, an efficacy endpoint, the improvement rate by treatment group will be estimated using Kaplan-Meier method, and an analysis for comparison between groups at a significance level of

5% using the log-rank test will be performed as the primary analysis. In addition, Cox's proportional hazards model using the allocation factor as a covariate will be used for evaluation.

Regarding other efficacy endpoints, distribution or summary statistics by treatment group will be provided, and differences between groups will be estimated and statistically analyzed.

17.2.3 Safety evaluation

(1) AEs and ADRs

Regarding all AEs and ADRs that occurred, the number of subjects and the number of events will be tabulated by event and by severity of the event by treatment group. In addition, a 95% confidence interval will be calculated for the incidence of AEs by treatment group.

Moreover, the names of AEs entered in the eCRF will be tabulated by preferred term (PT) coded using MedDRA. SAEs and significant AEs will be listed for each subject, then the clinical course and causality will be narrated.

(2) Laboratory test and vital signs

Regarding data on laboratory test and vital signs, summary statistics will be calculated by treatment group and observation time for continuous data, and frequency distribution (number of subjects, %) will be calculated for categorical data. The changes over time in laboratory test values will be shown in graph as needed, and a list of laboratory test values exceeding the reference value of the medical institution will be prepared.

17.3 Exploratory analysis

Additionally, exploratory analysis will be conducted as needed. Moreover, graphs and/or tables will be prepared as necessary.

17.4 Handling procedures for missing, non-adopted and abnormal data

(1) If measurement error, other artificial error, etc. are scientifically proven in the laboratory measurement, re-measurement results will be adopted.
(2) if the reason of occurrence of abnormal data is not clear, such data will be used for evaluation.
(3) In the event of missing data not stipulated above, handling of such data will be discussed with the medical experts as necessary before fixing the data.

17.5 Significance Level

As a rule, the significance level will be set at two-sided 5% and the confidence coefficient (1-α) for interval estimation will be set at two-sided 95%.

17.6 Changes of analysis plan

(1) When the analysis plan is to be changed, the person in charge for statistical analysis and the statistical analysis manager will prepare a revised statistical analysis plan after clarifying the timing of the change, its purpose, and the effect of the change on the interpretation of the study results.
(2) The person in charge for statistical analysis and the statistical analysis manager will state the date and the reasons of the change of analysis plan in the statistical analysis report.
(3) The sponsor will state the time and the reasons of the change of analysis plan as well as the discussion of impact of the additional analysis on the interpretation of the study results in the clinical study report.

18 Target Number of Subjects

60 subjects (randomized in a 2:1 ratio to either of the active treatment group or the control group)

[Rationale]

In a preliminary analysis of the ACTT study[18] of remdesivir among other preceding studies in COVID-19 patients, no data on the number of days to achieve at least 2-rank improvement on a 7-point ordinal scale was reported, but the median time to remission (no treatment or discharge) of clinical symptoms was 11 days (95%CI:9-12 days) in the remdesivir group and 15 days (95%CI:13-19 days) in the placebo group. In an open-label study[19] of mavrilimumab, a monoclonal antibody of GM-CSF receptor, the median recovery days was 8 days (IQR, 5-11 days) in the mavrilimumab group and 19 days (IQR, 11-28 days) in the standard therapy group. Given the above results and the small difference between the number of patients with at least 2-rank improvement and the number of recovered patients in any of the studies, and assuming that the median values of the active treatment group and the control group are 10 days and 14 days, respectively, and when a significance test for two groups is conducted using the log-rank test of the Kaplan-Meier estimates, the required number of patients with a significance level of 5% and a power of at least 80% was calculated to be 40 in the active treatment group and 20 in the control group (82.1% power from a simulation when 1000 times of random number is generated from normal distribution where the mean values are 10 in the active treatment group and 14 in the control group and a common standard deviation is 4.5).

19 Study Period

October 2020 to October 2021

20 eCRF and Others

20.1 Data entry in eCRF and reporting

An eCRF will be applied for the case report form. Based on the source documents, data entry will be performed by the investigators or study collaborator using the Electronic Data Capture (EDC) system designated by the sponsor, thus the eCRF will be prepared. Data entry by the study collaborator is allowed only for the transcription of the data described in the source documents. Data entry, changes, and revision in the eCRF will be performed in accordance with the “Data entry manual for eCRF” provided separately by the sponsor. The subinvestigator or study collaborator who enters the eCRF must be a person registered in the "List of subinvestigators and study collaborators".

20.2 Confirmation of eCRF by the principal investigator

The details entered by the subinvestigator or study collaborator will be checked by the principal investigator at each time or before data fixation.

The principal investigator will check the details entered in the EDC system, confirm that all the entered data (including audit trails and query responses) are accurate and complete, and will electronically make a sign.

If there is any discrepancy between the source documents and the data entered in the EDC system, the principal investigator will prepare a record explaining the reason for the discrepancy and appropriately store the record. A copy of the record will be promptly submitted to the sponsor.

Regardless of whether the principal investigator checks or not, if the sponsor and the person in charge of data management of the CRO or the monitor recognize that the entered data are inconsistent or suspected during data cleaning process or by direct access, they will issue a query and request to reconfirm, add, change, or amend the entered data as necessary.

20.3 Data lock and unlock of eCRF

After data cleaning of the eCRF has been completed by the sponsor and the CRO, then all the entered data in the eCRF will be confirmed and electronically signed by the principal investigator. After above mentioned procedures have been completed, the person in charge of data management will perform data lock operations according to the data management procedures. No additions, changes or corrections can be made after data lock. If correction, etc. is required after data lock, the person in charge of data management will unlock the data at the request of the sponsor. The sponsor will notify the principal investigator that the data has been unlocked. After unlocking, the task of adding, changing, or correcting will become possible.

21 Source Documents

The source documents are the records including medical records, laboratory test records, and treatment records of the study drug, and others necessary for the reproducibility and evaluation of the factual process of the clinical study. Also, the source documents include the documents, data, and records using which the eCRF is prepared. In this clinical study, the following documents will be specifically defined as the source documents. In addition to these documents, each medical institution may include additional documents agreed in writing in the source documents. They will be stored together with other study related documents.

- Medical records; laboratory test data; nursing records
- Informed consent form
- Screening list
- Drug accountability logs
- Electronic medium in which the image file is stored
- Confirmation slip for checking calls, etc.

21.1 Direct access to source data and documents

The principal investigator and the medical institution will give direct access upon request from the sponsor and CRO monitors, auditors, IRB, and regulatory authorities so that they can investigate and confirm the documents including the source documents and the study related documents which have to be retained in accordance with the clinical study agreement, etc.

21.2 Data to be handled as source document in the eCRF

If the following data are not written in the source document, the information directly entered in the eCRF will be regarded as the source document.

(1) Confirmation of inclusion and exclusion criteria.
(2) Judgement of the severity and seriousness of AEs, causality, and related detailed description.
(3) Detailed description of follow-up of discontinued (dropped out) subject.
(4) Comment
(5) A-aDO_2

22 Quality Control and Quality Assurance

The sponsor will control and ensure quality of the study based on the standard operating procedures (SOP) to verify whether the quality of the clinical study is ensured.

22.1 Quality control

The sponsor will confirm that the following major activities are appropriately performed according to each SOP.

(1) In order to standardize the methods of the clinical study, the sponsor will explain the procedures for selection, enrollment, examination, evaluation, and others of subjects to the principal investigator and other relevant persons involved in the conduct of the clinical study before the start of the clinical study.
(2) The monitor will periodically make monitoring visits during the study period to confirm the written informed consent and the conduct of the clinical study and others in compliance with the protocol.
(3) The monitor will collect information on AEs during monitoring.
(4) The monitor will check the entered data in the eCRF based on source documents such as medical records.
(5) The data management manager or the person in charge of data management will check the entered data in the eCRF.
(6) If there are any deficiencies or inquiries regarding (4) and (5), the monitor will issue a query, and request the investigators or study collaborator to reconfirm, add, change, or amend the entered data as necessary. When the subinvestigator or study collaborator makes additions, changes, or amendments, the principal investigator will finally check the details.
(7) The sponsor will conduct a case review as needed, check the content and appropriateness of the entered data in the eCRF, and examine how to the handle the subject.
(8) The data management manager and the person in charge of data management will operate data processing using a computer and ensure the reliability of data in accordance with the SOP.
(9) The sponsor will check the descriptions when documents such as notification form for study plan, clinical study request form, contract form, and dispensing/retrieving records of the study drug and others are prepared.

22.2 Quality assurance

The audit manager will inspect whether the clinical study is being conducted in compliance with the protocol, SOPs, the Pharmaceuticals and Medical Devices Act, and related regulations such as GCP in accordance with the sponsor's SOP. Organizations to be audited will be the sponsor, CRO, and the medical institution.

23 Ethics

23.1 Compliance of GCP and others

This clinical study will be conducted in compliance with the ethical principles based on the Declaration of Helsinki (1964) and its revised editions, the standards prescribed in Article 14, Paragraph 3 and Article 80-2 of the Pharmaceuticals and Medical Devices Law, the Ministerial Ordinance on Standards for Conduct of Clinical studys of Drugs (MHW Ordinance No. 28 of March 27, 1997), and other relevant laws and regulations, and the protocol.

23.2 Review by IRB

Prior to conducting a clinical study, the IRB established by the medical institution will obtain the protocol, informed consent form, investigator's brochure, and other documents requested by the IRB, judge the conduct and continuation of the clinical study from the viewpoint of ethical , scientific, and medical appropriateness, and notify the head of the participating medical institution of its opinion in writing.

23.3 Subject confidentiality

The investigators shall give due consideration to the confidentiality of the subject. Only the subject identification code is to be entered in the eCRF. If confidential information such as the name of the subject is displayed in other documents/materials to be submitted to the sponsor, such information should be deleted. In addition, when the test results are being published for academic purposes, the privacy protection of subjects shall be taken into consideration.

The sponsor and CRO monitor and auditor should not inform a third person of any personal information obtained on the job related to the clinical study. The same shall apply after leaving from the job.

23.4 Compensation for health damages

The sponsor will be insured and take other measures to ensure the costs required for the treatment of study-related health damage and other loss compensation measures.

23.5 Payment policy

Payments of money related to the clinical study will be described separately in a written agreement or contract agreement between the sponsor and the medical institution.

24 Retention of Record

24.1 Retention of records and others

24.1.1 Sponsor

The study related documents that have to be retained will be specified in the GCP and SOP.

24.1.2 Medical institution

The study related documents that have to be retained will be specified in the GCP and the SOP of the medical institution.

24.2 Retention period

24.2.1 Sponsor

The sponsor will retain the specified study related documents until the day specified as (1) or (2), whichever comes later.

(1) The day 5 years after the date of the manufacturing and marketing approval of the test drug. However, in the case of the drug that must be reexamined according to Article 14-4, Paragraph 1 of the Pharmaceuticals and Medical Devices Act (only the drug for which the period between the date of approval and the completion of reexamination is more than 5 years), the day on which the reexamination has been completed.
(2) The day 3 years after discontinuation or completion of the clinical study.

When retention of the specified study related documents is no longer required, the said effect will be notified to the head of the medical institution or the founder of the IRB.

When the sponsor commissions duties, the sponsor will discuss the documents to be retained, the site, and the period with the outsourcing contractor.

24.2.2 Medical institution

The head of the medical institution will retain the prespecified study related documents until the day specified as (1) or (2), whichever comes later. If the sponsor requires a longer time period for retention, the head of the medical institution will discuss how long and how to retain the documents with the sponsor. When the records are retained, the responsible person for each record will be specified.

(1) The date 3 years after the manufacturing and marketing approval of the test drug is obtained (or the date 3 years after notification of discontinuation of development is received).
(2) The day 3 years after discontinuation or completion of the clinical study.

24.2.3 Founder of IRB

The founder of the IRB will retain the prespecified study related documents until the day specified as (1) or (2), whichever comes later. If the sponsor requires a longer time period for retention, the founder of the IRB will discuss how long and how to retain the documents with the sponsor. When the records are retained, the responsible person for each record will be specified.

(1) The date 3 years after the manufacturing and marketing approval of the test drug is obtained (or the date 3 years after notification of discontinuation of development is received)
(2) The day 3 years after discontinuation or completion of the clinical study.

25 Publication Policy

The sponsor has the copyright for publications prepared or delegated on the basis of the results of the sponsored clinical study. The sponsor also possesses ownership of the information contained in this protocol (especially unpublished data). Therefore, even if the information is provided by the sponsor, the principal investigator who is willing to participate in the clinical study, other relevant persons involved in the clinical study, medical institution, IRB and others may not disclose the information to a third party without written agreement from the sponsor, except the case when the procedures to obtain informed consent from the subject are being taken. Moreover, data obtained from this clinical study are proprietary asset of the sponsor. When a part or all the results of this clinical study are to be published outside academic societies, medical journals, etc. (excluding disclosure to regulatory authorities), prior approval by the sponsor will be required.

The results of this clinical study may be used by the sponsor as publications and submission documents for application to regulatory authorities.

The outline of the clinical study will also be registered with the Clinical Research Protocol and Summary Disclosure System (https://jrct.niph.go.jp/) and/or the U.S. Clinical studys Database (https://Clinical studys.gov)

26 List of Attachments

Attachment 1： Study Administrative Structure, Medical institutions, and Principal investigators

27 Reference

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