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Supplemental information

Loss of GM-CSF-dependent instruction of alveolar macrophages in COVID-19 provides a rationale for inhaled GM-CSF treatment

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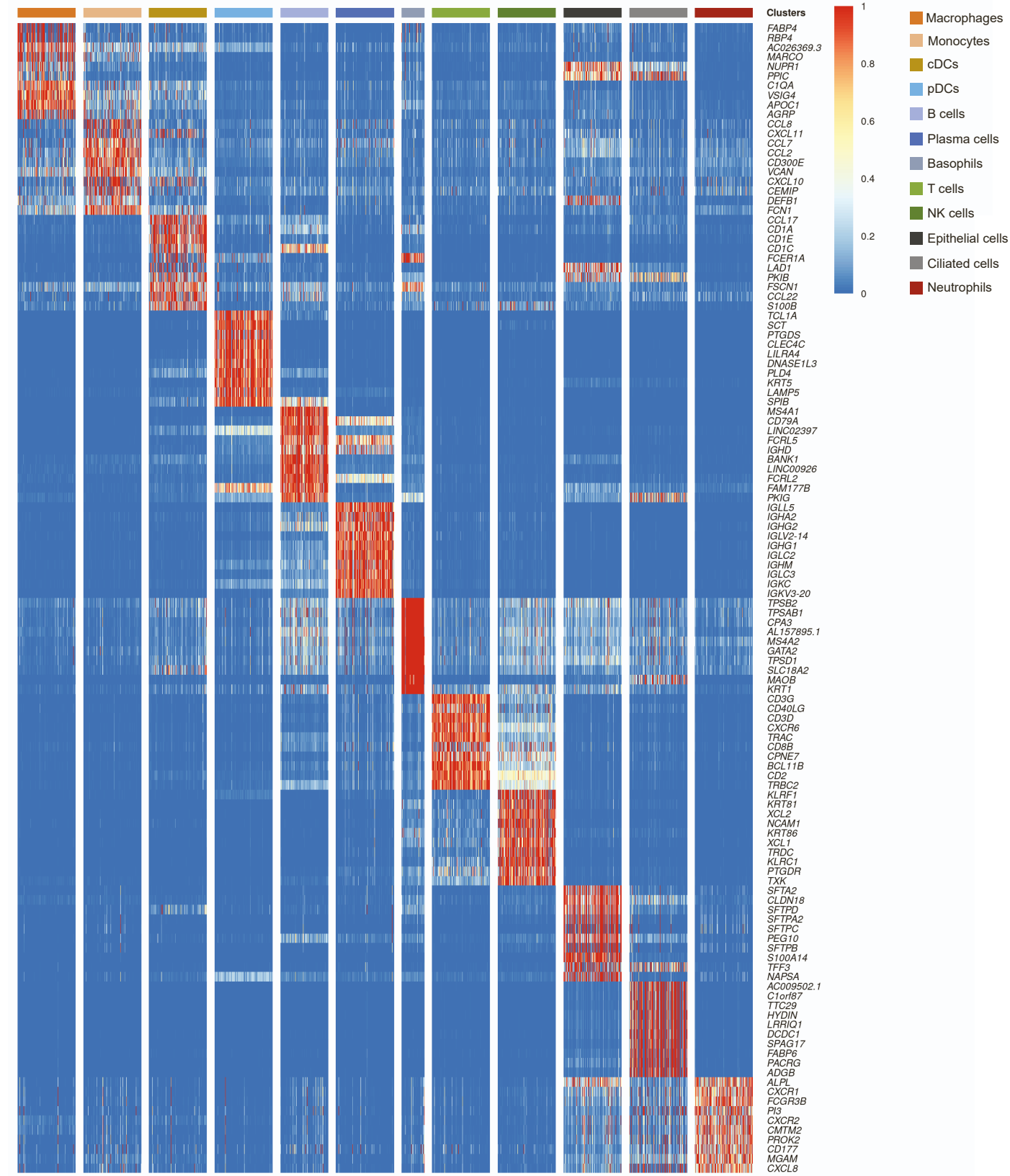
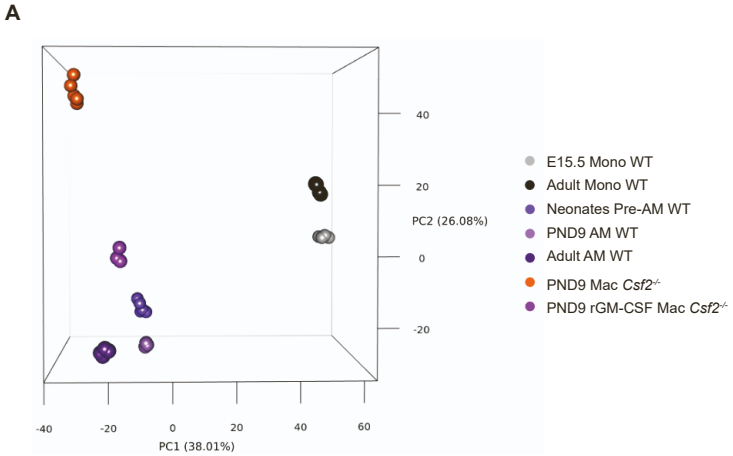
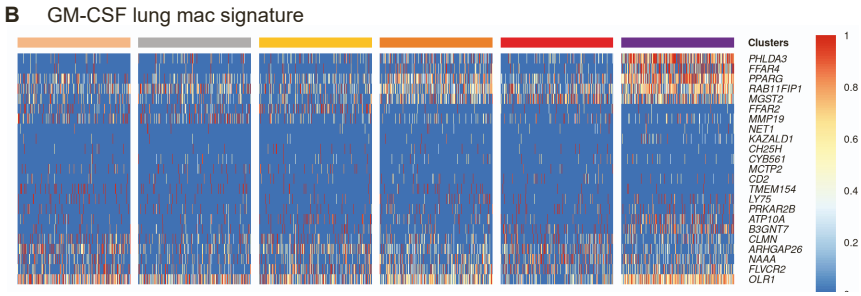
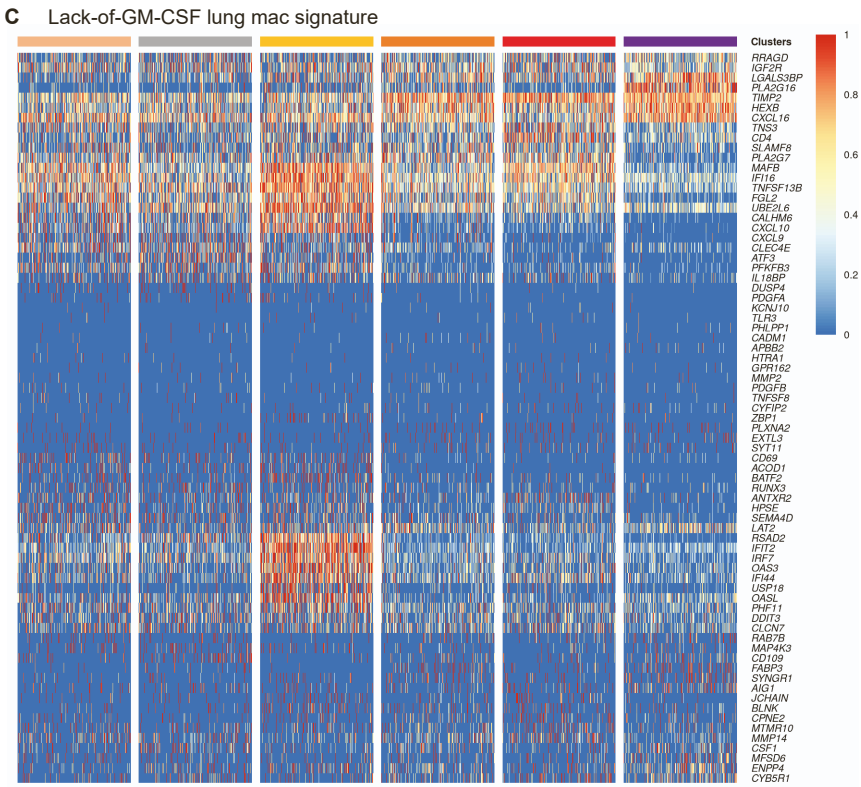
Supplementary figure 1

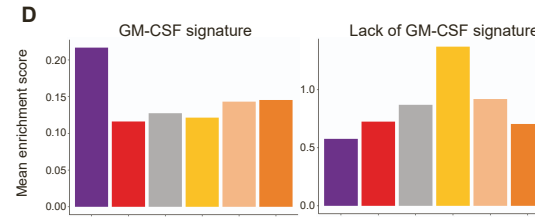


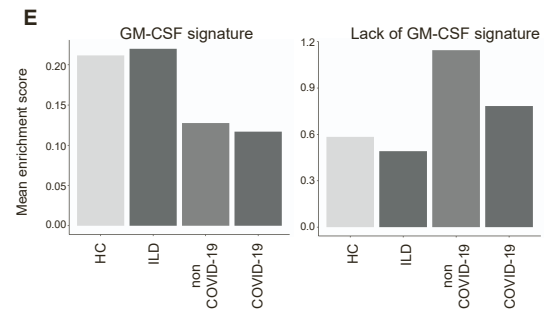
Supplementary figure 2





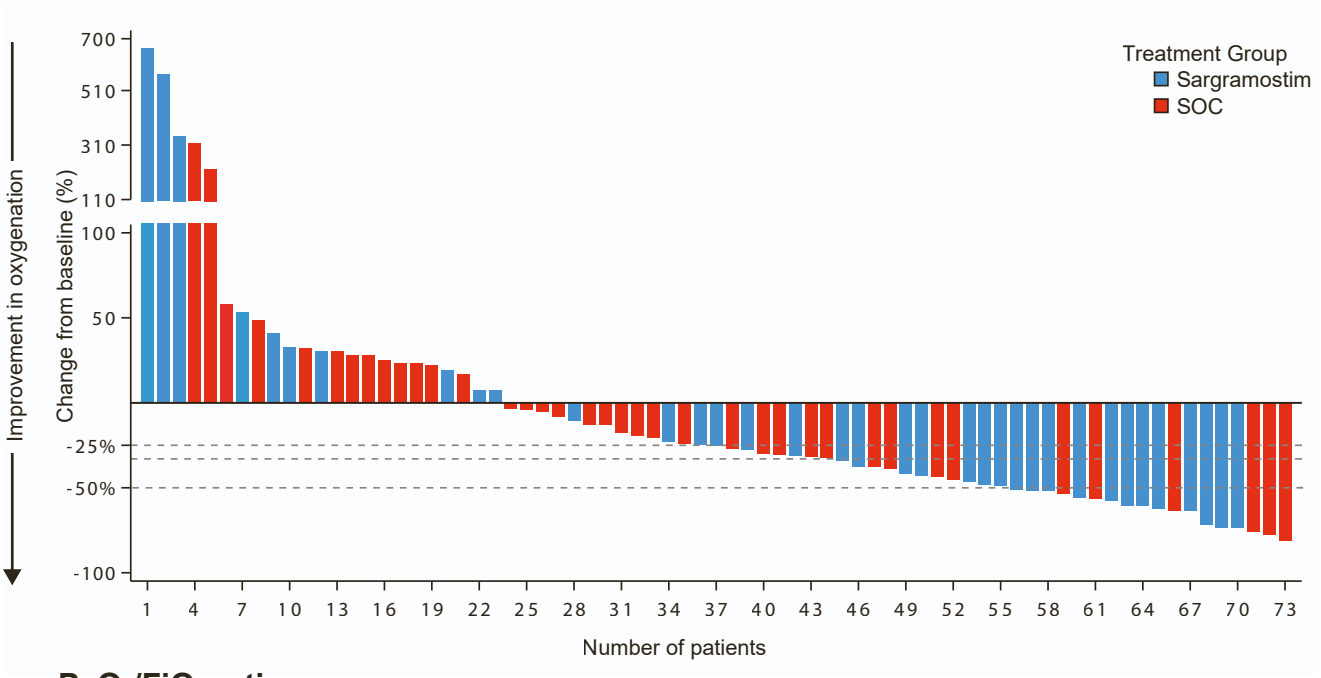




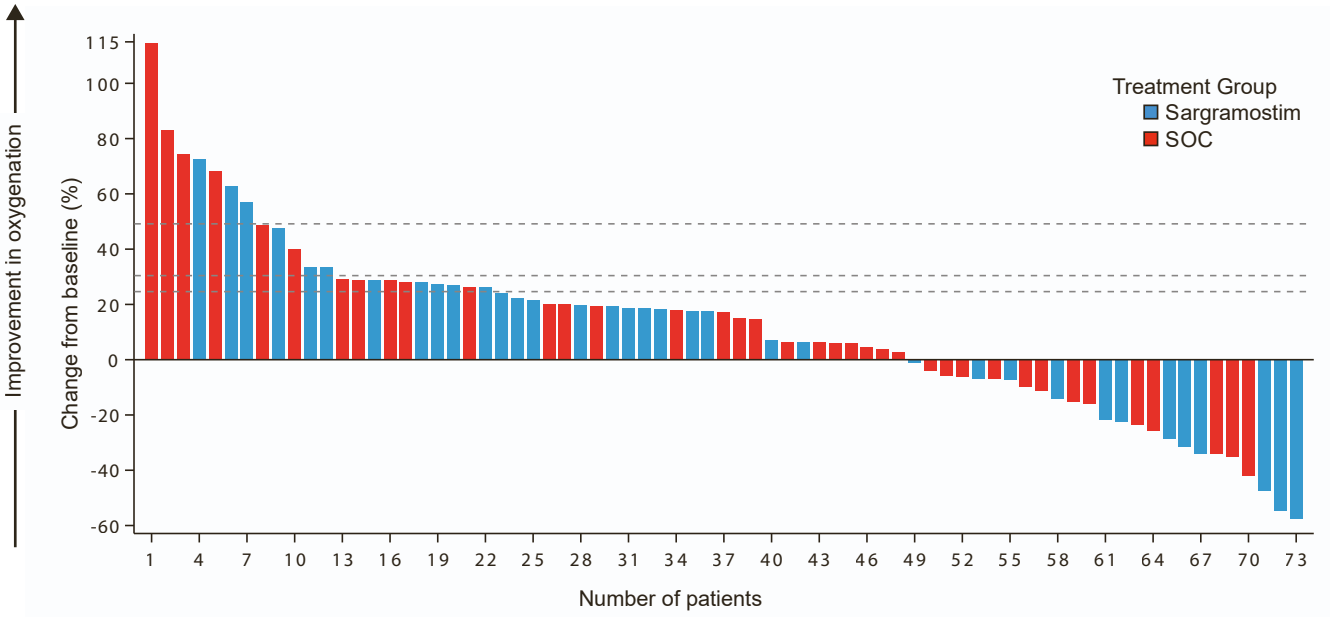


Supplementary figure 3

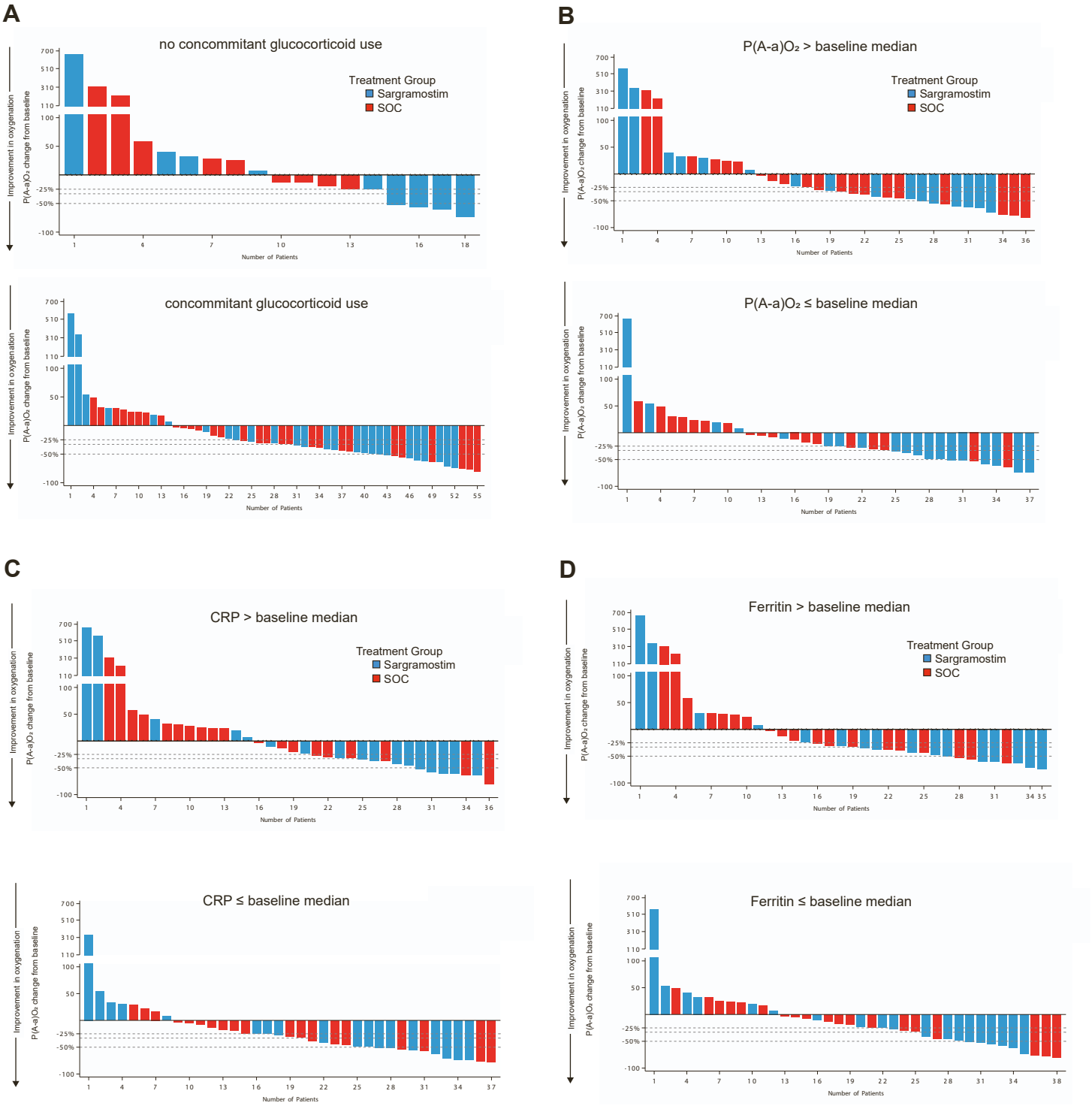
A $P(A\text{-}a)O_2$ gradient



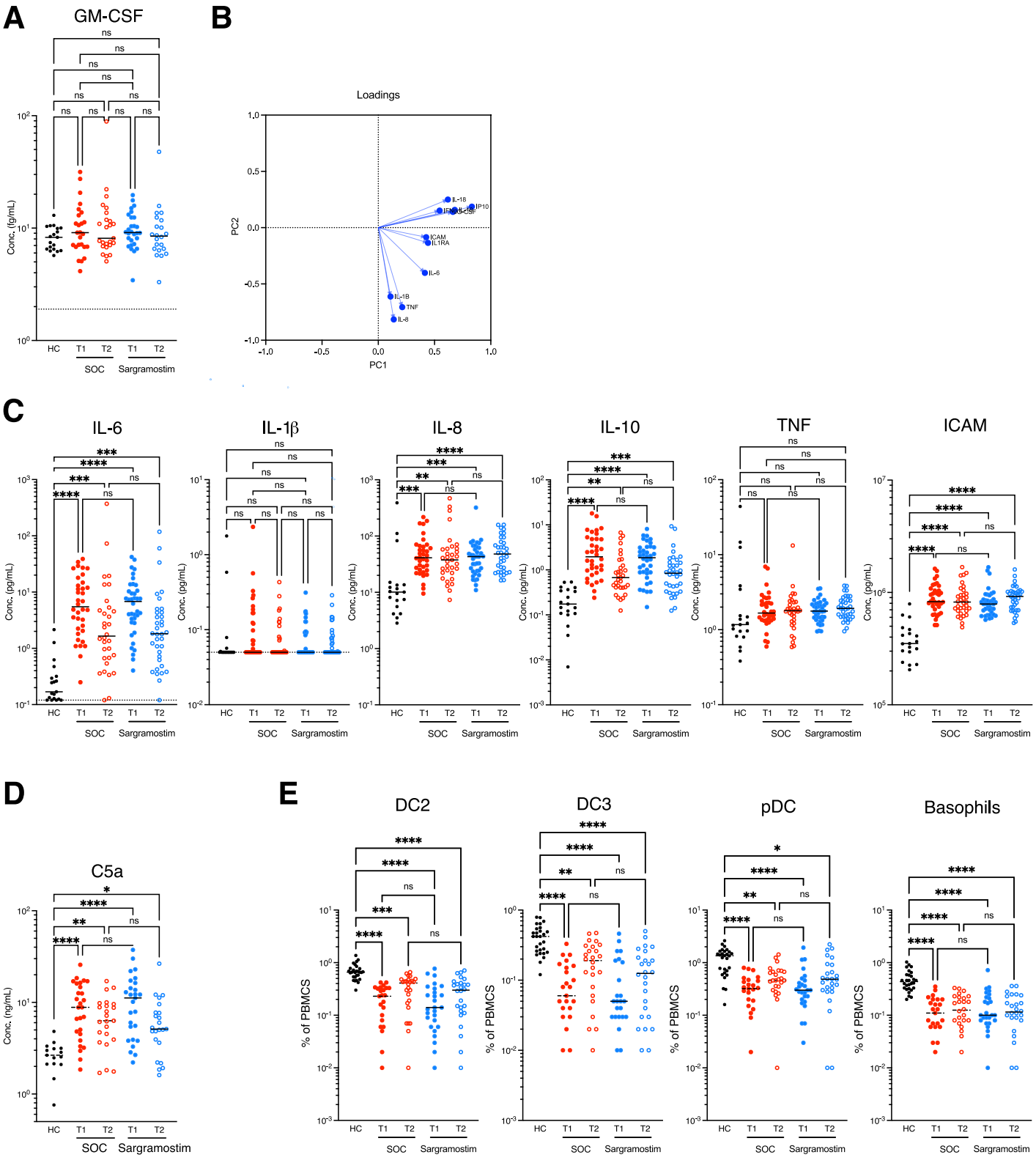
B PaO_2/FiO_2 ratio



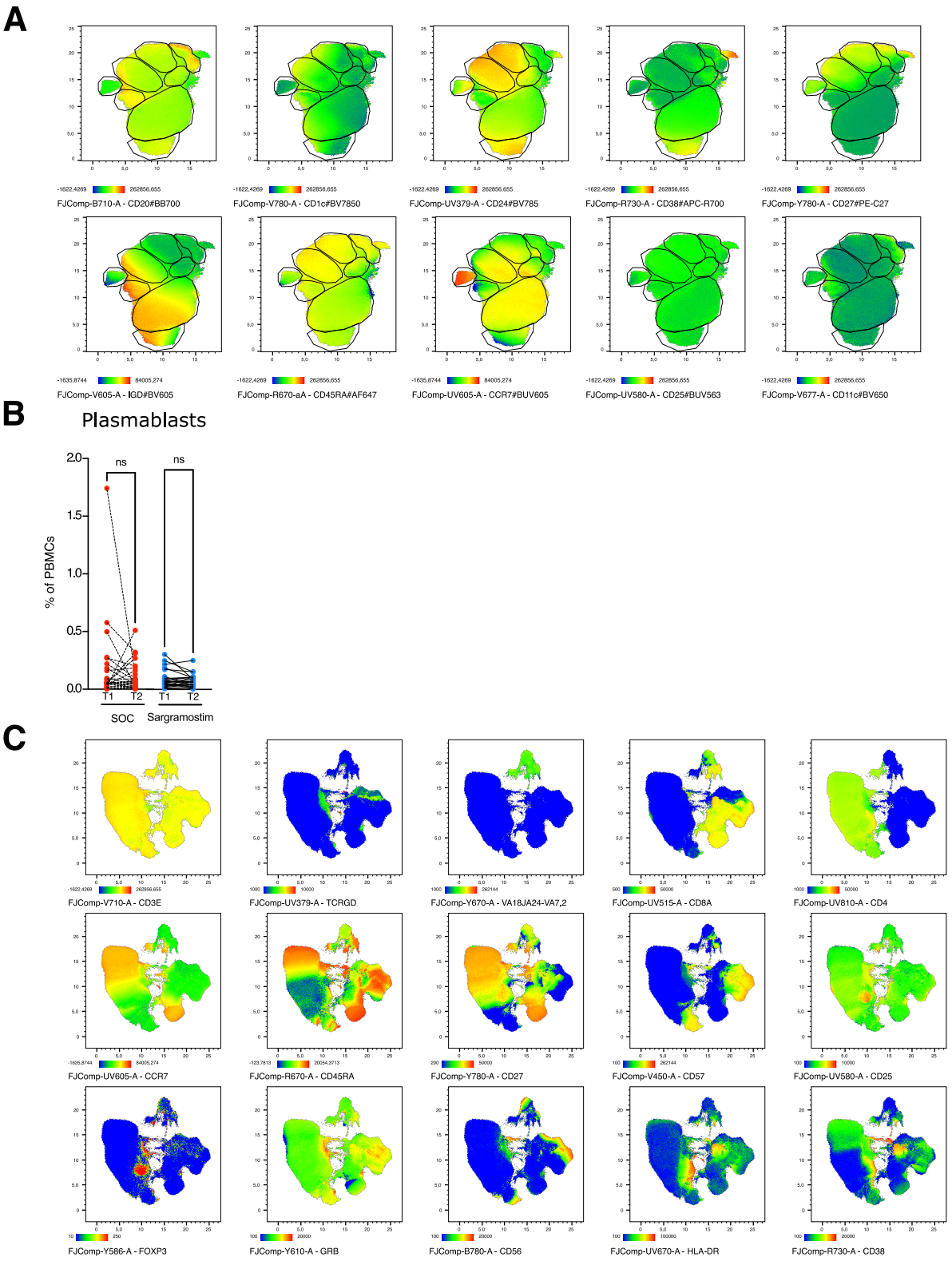
Supplementary figure 4



Supplementary figure 5



Supplementary figure 6



Supplementary figure 1 | Core transcriptional profile of cells isolated after BAL, related to Figure 1

Heatmap showing top 10 differentially expressed genes per cell cluster based on LogFC per group. Heatmap was created by comparing transcriptional data from each annotated cluster.

Supplementary figure 2 | GM-CSF instruction in monocyte and macrophage clusters, related to Figure 2

(A) Principal component (PC) analysis of transcriptional profile of the main cell populations shown in Figures 2H-I.

(B) Heatmap listing genes from the 'murine GM-CSF lung mac signature' and showing the log2 normalised expression of the gene split over the different human monocyte and macrophage clusters.

(C) Heatmap listing genes from the 'murine lack-of-GM-CSF lung mac signature' and showing the log2 normalised expression of the gene split over the different human monocyte and macrophage clusters.

(D) Mean enrichment scores (calculated via SingleCellSignatureExplorer) for the 'murine GM-CSF lung mac signature' (left plot) and 'murine lack-of-GM-CSF lung mac signature' (right plot) over the different monocyte and macrophage clusters.

(E) Mean enrichment scores (calculated via SingleCellSignatureExplorer) for the 'murine GM-CSF lung mac signature' (left plot) and 'murine lack-of-GM-CSF lung mac signature' (right plot) over healthy control group (n=2), patient with interstitial lung disease (n=1), patients with non-COVID-19 pulmonary infection (n=8) and COVID-19 patients (n=8).

Supplementary figure 3 | Primary endpoint, related to Figure 4

(A) Waterfall plot of relative change from baseline of $P(A\text{-}a)O_2$ (mmHg) on day 6. Dotted line indicates improvement by at least 25, 33 or 50% compared to baseline.

(B) Waterfall plot of relative change from baseline of PaO_2/FiO_2 ratio on day 6. Dotted line indicates improvement by at least 25, 33 or 50% compared to baseline.

Supplementary figure 4 | Subgroup analysis of primary endpoint, related to Figure 4

(A) Waterfall plot of relative change from baseline of $P(A\text{-}a)O_2$ (mmHg) on day 6 for patients on concomitant glucocorticoids (upper panel) or not on glucocorticoids (lower panel) at randomization.

(B) Waterfall plot of relative change from baseline of $P(A\text{-}a)O_2$ (mmHg) on day 6 for patients with a baseline $P(A\text{-}a)O_2$ value greater than the median baseline $P(A\text{-}a)O_2$ value per group (upper panel) or lower than the median baseline $P(A\text{-}a)O_2$ value per group (upper panel)

(C) Waterfall plot of relative change from baseline of $P(A\text{-}a)O_2$ (mmHg) on day 6 for patients with a baseline CRP value greater than the median CRP value per group (upper panel) or lower than the median baseline CRP value per group (upper panel)

(D) Waterfall plot of relative change from baseline of $P(A\text{-}a)O_2$ (mmHg) on day 6 for patients with a baseline ferritin value greater than the median CRP value per group (upper panel) or lower than the median baseline ferritin value per group (upper panel)

Supplementary figure 5 | Effect of sargramostim on immune landscape, related to Figure 5

(A) GM-CSF measured in serum of healthy control (HC; N= 19), standard of care (SOC; n(T1) = 25; n(T2) = 25) and sargramostim group (n(T1) = 28; n(T2) = 22) at baseline (T1) and after (T2) 5 days of treatment.

(B) Included variables (cytokines) denoted in the vectors in the loadings plot of the principal component (PC) analysis.

(C) Cytokines and chemokines measured in serum of healthy control (HC), standard of care (SOC) and sargramostim group at baseline (T1) and after (T2) 5 days of treatment. HC (n= 19), SOC (n(T1) = 36; n(T2) = 34) and sargramostim (n(T1) = 37; n(T2) = 39).

(D) C5a measured in serum of healthy control (HC), standard of care (SOC) and sargramostim group at baseline (T1) and after (T2) 5 days of treatment. HC (n= 15), SOC (n(T1) = 27; n(T2) = 23) and sargramostim (n(T1) = 27; n(T2) = 23).

(E) Percentage of DC2, DC3, pDC and basophils in PBMC fraction of healthy control (HC; n= 28), standard of care (SOC; n(T1) = 25; n(T2) = 26) and sargramostim group (n(T1) = 27; n(T2) = 26) at baseline (T1) and after (T2) 5 days of treatment.

The comparisons were performed by the Kruskal Wallis test with Dunn's correction for panel (A), (C), (D) and (E). The line in panel (A), (C), (D) and (E). indicates the median.

Supplementary figure 6 | Effect of sargramostim on lymphocytes, related to Figure 6 and 7

(A) Heatmap plots illustrating the signature surface markers used to identify B cell subsets.

(B) Percentage of plasmablasts in PBMC fraction of standard of care (SOC; n(T1) = 25; n(T2) = 25) and sargramostim group (n(T1) = 26; n(T2) = 26) at baseline (T1) and after (T2) 5 days of treatment.

(C) Heatmap plots illustrating the signature surface markers used to identify T cell subsets.

(D) Percentage of activated (HLA-DR^{+}CD38^{+}) CD4 T cells in PBMC fraction of healthy control (HC; n= 11), standard of care (SOC; n(T1) = 25; n(T2) = 25) and sargramostim group (n(T1) = 26; n(T2) = 26) at baseline (T1) and after (T2) 5 days of treatment.

(E) Absolute number of IFNg^{+} (left) or IFNg^{+}IL-2^{+} (right) spots detected by ELISpot after CD4 T cell stimulation with SARS-CoV-2 peptide pools in healthy control (HC; n= 22), standard of care (SOC; n(T1) = 29; n(T2) = 24) and sargramostim group (n(T1) = 30; n(T2) = 27) at baseline (T1) and after (T2) 5 days of treatment.

The comparisons were performed by the Wilcoxon test for panel (B), (D) and (E). The line in panel (E) indicates the median.

Table S1. Demographics of patients with BAL procedure, related to Figure 1 and 2

	Control (N=2)	COVID-19 (N=8)	Non-COVID-19 pulmonary infection (N=8)	ILD (N=1)
Age				
Median (IQR) – yr	64 (63-64)	53 (42-58)	65 (38-76)	47
Sex				
Male – no. (%)	0 (0.0)	5 (62.5)	5 (62.5)	1 (100.0)
Ethnicity				
White – no. (%)	2 (100.0)	7 (87.5)	8 (100)	1(100)
Asian – no. (%)	0 (0.0)	1 (12.5)	0 (0)	0 (0.0)
BMI, Median (IQR)	27.2 (25.5-28.8)	27.7 (25.5-33.9)	25.9 (23.6-27.6)	35.8 (NA)
Median no. days since symptom onset (IQR) – days	NA	17 (11-30)	9 (5-10)	5 (NA)
Median no. days since hospitalization (IQR) – days	NA	10 (6-21)	2 (2-2)	1 (NA)
Comorbidity – no. (%)				
Arterial hypertension	0 (0.0)	2 (25)	1 (12.5)	0 (0.0)
Diabetes mellitus	0 (0.0)	2 (25)	1 (12.5)	0 (0.0)
Cardiovascular disease	0 (0.0)	1 (12.5)	1 (12.5)	0 (0.0)
Chronic kidney disease	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)
Severe liver disease	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)
Chronic lung disease	0 (0.0)	2 (25.0)	4 (55.5)	1 (100.0)
Cancer	**2 (100)**	0 (0.0)	3 (37.5)	0 (0.0)
Smoking status – no. (%)				
Current	2 (100.0)	1 (20.0)	1 (12.5)	1 (100)
Former	0 (0.0)	3 (60.0)	3 (37.5)	0 (0)
Concomitant medication at randomization	NA			
Glucocorticoids	NA	1 (14.3)	1 (12.5)	0 (0.0)
Antiviral drugs (remdesivir)	NA	0 (0.0)	0 (0.0)	0 (0.0)
Hydroxychloroquine	NA	0 (0.0)	0 (0.0)	0 (0.0)
Antibiotics	NA	4 (57.1)	8 (100)	1 (100)
Oxygenation, Median (IQR)				
PaO_2/FiO_2 ratio	NA	161 (119-212)	294 (202-336)	285.7 (NA)
Aa gradient (IQR) – mmHg	NA	287 (287-289)	NA	NA
Lab values, Median (IQR)				
C-reactive protein level – mg/l	NA	94.5 (40.1-146.3)	141.6 (40.6-186.8)	62.4 (NA)
Eosinophil count – no. x 10^9/l	NA	0.024 (0.01-0.15)	0.12 (0.04-0.24)	0.32 (NA)
Lymphocyte count – no. x 10^9/l	NA	0.92 (0.68-1.15)	0.13 (0.84-1.63)	1.46 (NA)
Ferritin – µg/l	NA	505 (400-563)	299 (165-736)	208 (NA)
Lactate dehydrogenase – ukat/l	NA	6.3 (3.9-11.5)	4.1 (2.7-5.7)	5.7 (NA)
Asparate aminotransferase – ukat/l	NA	1.1 (0.5-1.3)	0.3 (0.2-0.4)	0.3 (NA)
Alanine aminotransferase – ukat/l	NA	0.9 (0.6-2.0)	0.2 (0.2-0.3)	0.3 (NA)
Creatinine – µmol/l	NA	58.4 (50.2-69.2)	69.9 (65.4-88.4)	67.2 (NA)
SOFA score, median (IQR)	NA	8.5 (3.3-10)	2 (1-3.5)	2 (NA)
6-category ordinal scale at randomization – no. (%)				
2 Hospitalized, on invasive mechanical ventilation or ECMO	0 (0.0)	6 (75.0)	0 (0.0)	0 (0.0)
3 Hospitalized, on non-invasive ventilation or high flow oxygen devices	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
4 Hospitalized, requiring supplemental oxygen	0 (0.0)	2 (25.0)	6 (75.0)	1 (100.0)
5 Hospitalized, not requiring supplement oxygen	0 (0.0)	0 (0.0)	2 (25.0)	0 (0.0)
6 Non-hospitalized	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)

Table S2. Technical details of the single-cell analysis, related to Figure 1 and 2

Patient ID	nrCells	median_genesPerCell	mean_readsPerCell	mean_percentMito
COV002	16222	1878	5727	3,66
COV004	9240	2060	7600	5,21
COV006	5751	1717	7206	3,4
COV007	1963	1720	7323	3,18
COV012	14156	2067	6480	1,6
COV013	16751	1487	5166	4,82
COV014	11568	346	932	2,15
COV015	10998	457	1818	1
COV016	5564	2406	9480	7,79
COV017	6730	2468	9100	7,77
COV021	8981	2808	11454	4,97
COV022	8428	1740	7110	3,67
COV023	17220	513	2645	2,87
COV024	4217	2126	9052	5,7
COV025	16958	436	1156	1,54
COV034	15585	608	2500	0,47
COV035	19032	407	1230	2,02
COV036	15472	429	2515	1,4
COV037	19091	552	1542	0,98

Table S3. Overview primary and secondary study endpoints, related to Figure 4 and Table 2

	SOC (n = 41)	Sargramostim (n = 40)	P Value
Primary endpoints			
Number of patients with **≥ 25%** Reduction Change from Baseline in **P(A-a) Gradient** on Day 6* – no./total no. (%)	15/38 (39.5)	22/35 (62.9)	0.0459[C]
Number of patients with **≥ 33%** Reduction Change from Baseline in **P(A-a) Gradient** on Day 6* – no./total no. (%)	10/38 (26.3)	19/35 (54.3)	0.0147[C]
Number of patients with **≥ 50%** Reduction Change from Baseline in **P(A-a) Gradient** on Day 6* – no./total no. (%)	6/38 (15.8)	12/35 (34.3)	0.1023[F]
Mean Change from Baseline in P(A-a) Gradient on Day 6* (SD)	-0.3 (55.0)	4.0 (96.3)	0.8182[T]
Median Change from Baseline in P(A-a) Gradient on Day 6* (95% CI)	-9.4 (-13.4, 6.2)	-14.6 (-23.6, -8.7)	0.1292[M]
Number of patients with **≥ 25%** Increase Change from Baseline in **PaO_2/FiO_2** on Day 6* – no./total no. (%)	11/38 (28.9)	11/35 (31.4)	0.8175[C]
Number of patients with **≥ 33%** Increase Change from Baseline in **PaO_2/FiO_2** on Day 6* – no./total no. (%)	6/38 (15.8)	6/35 (17.1)	>0.9999[F]
Number of patients with **≥ 50%** Increase Change from Baseline in **PaO_2/FiO_2** on Day 6* – no./total no. (%)	4/38 (10.5)	3/35 (8.6)	>0.9999[F]
Mean Change from Baseline in PaO_2/FiO_2 on Day 6* (SD)	+29.7 (71.8)	+25.7 (85.3)	0.8263[T]
Median Change from Baseline in PaO_2/FiO_2 on Day 6* (95% CI)	+20.0 (-10, 61)	+58.0 (-3, 64)	0.0818[M]
Secondary endpoints			
Mean change in 6-point ordinal scale change between Baseline and Day 6 (SD)	0.3 (1.0)	0.3 (1.0)	0.9893[T]
Median number of days in hospital (95% CI)	9.0 (7, 14)	8.5 (6, 12)	0.9093[M]
Incidence of nosocomial infection – no./total no. (%)	1 (2.4)	2 (5.0)	0.1655[W]
Death at 28 days – no. (%)	2 (4.9)	2 (5.0)	>0.9999[F]
Incidence of progression to mechanical ventilation and/or ARDS – no./total no. (%)	6 (14.6)	7 (17.5)	0.7254[C]
Median time (days) to clinical sign score < 6 for at least 24h (95% CI)	3.0 (1.0, 6.0)	2.0 (1.0, 3.0)	0.4171[L]
Mean change in clinical sign score between Baseline and Day 6 (SD)	-2.2 (3.0)	-2.0 (3.1)	0.8525[T]

Mean change in NEWS2 score between Baseline and Day 6 (SD)	-0.4 (3.0)	-0.6 (3.1)	0.8018[T]
Mean change in SOFA between Baseline and Day 6 (SD)	-0.4 (1.2)	-0.5 (1.5)	0.9302[T]
Median Change Ferritin level between Baseline and Day 6 (95% CI)	-112 (-259, 118)	-90 (-150, 34)	0.8974[M]
Median Change D-dimer level between Baseline and Day 6 (95% CI)	-0.44 (-2.90, 2.46)	-0.71 (-1.79, 1.33)	0.7172[M]
Median Change CRP level between Baseline and Day 6 (95% CI)	-43.1 (-73.8, -22.8)	-43.6 (-69.8, -21.7)	0.8974[M]
Median Change Lymphocyte number between Baseline and Day 6 (95% CI)	0.20 (0.02, 0.56)	0.45 (0.18, 0.70)	0.2650[M]
Median Change Eosinophil number between Baseline and Day 6 (95% CI)	0.005 (0.000, 0.073)	0.100 (0.010, 0.160)	0.1079[M]
HRCT fibrosis score at follow-up (95% CI)	102.5 (100.0, 105.8)	100.8 (100.0, 102.5)	0.5696[M]

* Day 6 or hospital discharge, whichever came first

[c] Chi-square Test

[F] Fisher's Exact Test

[M] Brown-Mood Test; [W] Wald test

[T] T Test

HRCT fibrosis score is based on the collected average of the six individual HRCT zone scores.

[L] Log-rank test

Table S4. Definition severe COVID-19, related to Figure 5

1. Antigen, PCR or serological proof of SARS-Cov2 infection
2. Presence of hypoxia defined as PaO_2/FiO_2 below 350 while breathing room air in upright position or PaO_2/FiO_2 below 280 on supplemental oxygen and immediately requiring high flow oxygen device or mechanical ventilation.
3. Signs of cytokine release syndrome defined as ANY of the following:
a. Serum ferritin concentration >1000 mcg/L and rising since last 24h b. Single ferritin above 2000 mcg/L in patients requiring immediate high flow oxygen device or mechanical ventilation. c. Lymphopenia defined as <800 lymphocytes/microliter and two of the following extra criteria: i. Ferritin > 700 mcg/L and rising since last 24h ii. Increased LDH (above 300 IU/L) and rising since last 24h iii. D-Dimers > 1000 ng/mL and rising since last 24h iv. CRP above 70 mg/L and rising since last 24h and absence of bacterial (if three of the above are present at admission, no need to document 24h rise)
4. Chest X-ray and/or CT scan showing bilateral infiltrates within last 2 days

SARPAC

A prospective, randomized, open-label, interventional study to investigate the efficacy of sargramostim (Leukine®) in improving oxygenation and short- and long-term outcome of COVID-19 patients with acute hypoxic respiratory failure.

Sargramostim in patients with acute hypoxic respiratory failure due to COVID-19 (SARPAC)

Acronym / Protocol code	SARPAC
Protocol version and date	4.0, 07 June 2021
Phase	4
EudraCT n°	2020-001254-22
Sponsor	University Hospital Ghent, C. Heymanslaan 10 9000 Ghent Belgium
Financial/Material Support:	Partner Therapeutics will provide sargramostim
Coordinating Investigator:	**Bart N. Lambrecht, MD, PhD** **Department of Internal Medicine & Pediatrics,** **Department of Respiratory Medicine** **University Hospital Ghent** **Corneel Heymanslaan** **9000 Ghent, Belgium** **+32/93329110**
Co-investigators:	Multicenter Trial in Belgium

A prospective, randomized, open-label, interventional study to investigate the efficacy of sargramostim (Leukine®) in improving oxygenation and short- and long-term outcome of COVID-19 patients with acute hypoxic respiratory failure.

Sargramostim in patients with acute hypoxic respiratory failure due to COVID-19 (SARPAC)

Protocol Co-ordinating Investigator signature page

I certify that I will conduct the study in compliance with the protocol, any amendments, GCP and the declaration of Helsinki, and all applicable regulatory requirements.

Investigator:

Name: Prof. Dr. B. Lambrecht
Function: Pulmonologist
Institution: UZ Ghent

Date: **Signature:**

A prospective, randomized, open-label, interventional study to investigate the efficacy of sargramostim (Leukine®) in improving oxygenation and short- and long-term outcome of COVID-19 patients with acute hypoxic respiratory failure.

Sargramostim in patients with acute hypoxic respiratory failure due to COVID-19 (SARPAC)

Protocol Site Principal Investigator signature page

I certify that I will conduct the study in compliance with the protocol, any amendments, GCP and the declaration of Helsinki, and all applicable regulatory requirements.

Investigator:

Name:
Function:
Institution:

Date: **Signature:**

Protocol Amendment History:

Version Number	Date	Description of amendment
1.4	10APR2020	Specification of participating centers (UZ Ghent and AZ Sint Jan Brugge to multicenter trial
		Section 10.3: extra sampling only in selected centers
2.0	15APR2020	Section 6.1: Inclusion criteria 1 removed and changed to COVID-19 diagnosis confirmed by antigen detection test and/or PCR and/or positive serology, or any emerging and validated diagnostic laboratory test for COVID-19 within this period.
		Section 1.5, 6.1: Extra Inclusion criteria: In some patients, it may be impossible to get a confident laboratory confirmation of COVID-19 diagnosis after 24h of hospital admission because viral load is low and/or problems with diagnostic sensitivity. In those cases, in absence of an alternative diagnosis, and with highly suspect bilateral ground glass opacities on recent (<24h) chest-CT scan (confirmed by a radiologist and pulmonary physician as probable COVID-19), a patient can be enrolled as probable COVID-19 infected. In all cases, this needs confirmation by later seroconversion
		Section 10: redefining sampling.due to addition of extra study sites. Section 8.1.5: better definition of duration of treatment Section 13.6: Despite the known safety profile of the study medications and study design, a DSMB is foreseen.
		General: Better definition of progressive disease: Progression to ARDS requiring mechanical ventilation is removed and replaced by: progressive disease requiring mechanical ventilatory support.
		General: Safety follow-up period is 10-20 weeks.
		Section 1.6.1, 8.1.5: Nebulizing is preferably done in an isolation negative pressure chamber, and if not, personnel should use an FFP2 mask. Patient should self-administer the medication and where possible, the room should not be entered within one hour after administration.
		Section 9.4: arterial blood gas mandatory at D1, D6 and FU Section 9.2, 9.4: if arterial blood gas is taken within 24h before first dose administration, as described in point° the arterial blood gas of screening can be used as D1 value
		Section 7.1.2: If a patient decides to leave hospital before day 6 of the study, for example because of clinical improvement, the oxygenation parameters at day of discharge will be used to calculate the primary endpoint measurement.

3.0	14 May 2020	Section 9.4: Schematic overview of the data collection & interventions: lay-out was updated to improve clarity.
		Section 9.4: Added to flowchart, as per standard of care during follow-up visit: - 6 minutes walk test (Section 4.2) - HRCT scan to assess HRCT fibrosis score
		Section 10: - Clarification on study blood sampling added: EDTA only to be collected in selected sites. - processing details of samples were updated from 1500RPM or 410g to 1770 g.
		General: Typo's were corrected.
		General: "requiring invasive mechanical ventilatory support": wording "invasive" changed to "non-invasive / invasive".
		Section 9.2: "on page 36" added to "as described in point°".
		Section 9.4: clinical assessments added to flowchart: Ordinal Scale Category, Clinical Sign Sore, NEWS2 Score, SOFA Score, HScore, CURB-65, APACHE II and Glasgow Coma Scale.
		Section 3.2, 4.2: Mean change of SOFA score between day 1 and day 6 or between day 1 and day 11: updated to day 10. Mean change NEWS2 score between day 1 and day 6 or between day 1 and day 11: updated to day 10.
4.0	07 June 2021	General: Typo's were corrected.
4.0	07 June 2021	Section 1.5 and 6.2 -patients on high dose systemic steroids (> 20 mg methylprednisolone or equivalent) Replaced by -patients on high dose systemic steroids (> 20 mg methylprednisolone or equivalent) for COVID-19 unrelated disorder AND - Patients with serum ferritin >2000 mcg/ml (which will exclude ongoing HLH) Replaced by - Patients with serum ferritin >2000 mcg/L (which will exclude ongoing HLH)
4.0	07 June 2021	Section 3.3 and Sections 4.1 and 4.2 Further clarification of Primary and Secondary endpoint measurements
4.0	07 June 2021	Section 4.3: Enumeration and description of planned pharmacodynamic measurements (biomarkers, flow cytometry, immunomonitoring)
4.0	07 June 2021	Section 9.3.6: Clarification on role of VIB-UGent Center for Inflammation Research

		Clarification of which pharmacodynamic parameters, biomarkers, immunomonitoring assays will be performed
4.0	07 June 2021	Definitions of follow-up visit were made consistent.
4.0	07 June 2021	Section 11: Shipment process of optional samples was updated.
4.0	07 June 2021	Section 11.3: Typo selected centres corrected to all centres Better description of sample handling and analysis by centers
	07June 2021	Secion 11.4 Clarification of sample storage and shipment, including role of VIB
4.0	07 June 2021	Section 12.3: correction statistical analysis team Further clarification on statistical analysis performed
4.0	07 June 2021	Section 13.4: Access to data and data ownership better defined
4.0	07 June 2021	Section 14.7: Period of first DSUR reporting modified to 1 year + 60 days

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AE	=	Adverse Event
AECC	=	American-European Consensus Conference
ARDS	=	Acute Respiratory Distress Syndrome
CI	=	Coordinating Investigator
COVID-19	=	Coronavirus induced disease-2019
CT	=	Clinical Trial Unit
DSMB	=	Data Safety Monitoring Board
DSUR	=	Development Safety Update Report
EC	=	Ethics Committee
ECG	=	Electrocardiogram
eCRF	=	electronic Case Report Form
EDC	=	Electronic Data Capture
EPD	=	Electronic Patient Dossier
FAMHP	=	Federal Agency for Medicines and Health Products
FiO2	=	Fraction of inspired oxygen
FPI	=	First Patient In
FVC	=	Forced vital capacity
GCP	=	Good Clinical Practice
GDPR	=	General Data Protection Regulation
GM-CSF	=	Granulocyte-macrophage colony stimulating factor
GMP	=	Good Manufacturing Practice
HIRUZ	=	Health, Innovation and Research Institute UZ Ghent
HLH	=	Hyperferritinemia and Hemophagocytic Lymphohistiocytosis
IB	=	Investigator's Brochure
ICF	=	Informed Consent Form
ICH	=	International Council for Harmonisation
IMP	=	Investigational Medicinal Product
IMPD	=	Investigational Medicinal Product Dossier
LVLS	=	Last Visit, Last Subject
PCWP	=	Pulmonary Capillary Wedge Pressure
PEEP	=	Positive End Expiratory Pressure
PI	=	Principal Investigator
PaO_2	=	Partial pressure of oxygen
SAE	=	Serious Adverse Event
sHLH	=	secondary hemophagocytic lymphohistiocytosis
SmPC	=	Summary of Product Characteristics
SOP	=	Standard Operating Procedure
SUSAR	=	Suspected Unexpected Serious Adverse Reaction
TERENA	=	Trans-European Research and Education Networking Association
TLC	=	Total Lung Capacity
TLS	=	Transport Layer Security

1. Protocol Summary

SARPAC trial : Use of sargramostim in patients with acute hypoxic respiratory failure due to COVID-19

Title	A prospective, randomized, open-label, interventional study to investigate the efficacy of sargramostim (Leukine®) in improving oxygenation and short- and long-term outcome of COVID-19 patients with acute hypoxic respiratory failure.
Protocol number	SARPAC
Protocol version	V4.0
EudraCT number	2020-001254-22
Sponsor	University Hospital Ghent
Co-ordinating Investigator	Bart N. Lambrecht
Type of study	Interventional
Fase	IV
Methodology	prospective, randomized, open-label study
Study duration	22 weeks
Purpose of study	To study the effectiveness of additional sargramostim (GM-CSF) inhalation versus standard of care on blood oxygenation in patients with COVID-19 coronavirus infection and acute hypoxic respiratory failure
Number of participants	80
Study population and main inclusion criteria	Patients with confirmed COVID-19 infection and acute hypoxic respiratory failure Presence of hypoxic respiratory failure defined as O_2 saturation below 93% on minimal 2l/min O_2 therapy and/or ratio PaO_2/FiO_2 below 350
Investigational drug, dose, route	Sargramostim/Leukine® 125 mcg BID via inhalation, for 5 days Sargramostim/Leukine® 125 mcg/m2 once daily IV upon progression, for 5 days
Treatment duration	5 days, followed by possible 5 day extension upon deterioration

1.1. Protocol specifics

EudraCT number : 2020-001254-22

University Hospital Ghent

1.2. Study Type and Study Phase

This is phase 4 academic, prospective, randomized, open-label, interventional study designed to investigate the efficacy of sargramostim (Leukine®) in improving oxygenation and short- and long-term outcome of COVID-19 patients with acute hypoxic respiratory failure.

1.3. Aim of the study (including primary endpoints)

The **primary objective** is to investigate whether the administration of inhaled sargramostim (Leukine®) at a dose of 250 mcg daily during 5 days improves oxygenation in COVID-19 patients with acute hypoxic respiratory failure.

The **secondary objectives** are:
- to study if early intervention with sargramostim is safe (number of AEs/SAEs)
- to study if early intervention with inhaled sargramostim affects clinical outcome defined by
  duration of hospital stay, 6-point ordinal scale, clinical sign score, SOFA score, NEWS2 score
- to study if early intervention with sargramostim affects the rate of nosocomial infection
- to study if early intervention with inhaled sargramostim affects progression to mechanical ventilation and/or ARDS
- to study if treatment with sargramostim affects all-cause mortality rate at 4 and 20 weeks post inclusion
-to study if treatment with sargramostim affects features of secondary haemophagocytic lymphohistiocytosis, defined by HS score
- to study if treatment with sargramostim has a favourable effect on long term 10-20 week follow up

1.4. Subjects

1.4.1. Number of subjects

A total of 80 patients with confirmed COVID-19 and acute hypoxic respiratory failure will be enrolled, 40 in the active and 40 in the control group.

1.4.2. Target group

Confirmed COVID-19 patients with acute hypoxic respiratory failure admitted to the COVID-19 isolation ward.

1.5. Inclusion and exclusion criteria

Inclusion criteria
The following patients will be enrolled:

- Recent (≤2weeks prior to randomization) confident diagnosis of COVID-19 confirmed by antigen detection and/or PCR, and/or seroconversion or any other emerging and validated diagnostic test.
- In some patients, it may be impossible to get a confident laboratory confirmation of COVID-19 diagnosis after 24h of hospital admission because viral load is low and/or problems with diagnostic sensitivity. In those cases, in absence of an alternative diagnosis, and with highly suspect bilateral ground glass opacities on recent (<24h) chest-CT scan (confirmed by a radiologist and pulmonary physician as probable COVID-19), a patient can be enrolled as probable COVID-19 infected. In all cases, this needs confirmation by later seroconversion.
- Presence of acute hypoxic respiratory failure defined as (either or both)
    - saturation below 93% on minimal 2 l/min O2
    - PaO2/FiO2 below 350

- Admitted to specialized COVID-19 ward
- Age 18-80
- Male or Female
- Willing to provide informed consent

Exclusion criteria
- Patients with known history of serious allergic reactions, including anaphylaxis, to human granulocyte-macrophage colony stimulating factor such as sargramostim, yeast-derived products, or any component of the product.
- mechanical ventilation before start of study
- patients with peripheral white blood cell count above 25.000 per microliter and/or active myeloid malignancy
-patients on high dose systemic steroids (> 20 mg methylprednisolone or equivalent) for COVID-19 unrelated disorder
-patients on lithium carbonate therapy
- Patients enrolled in another investigational drug study
- Pregnant or breastfeeding females (all female subjects regardless of childbearing potential status must have negative pregnancy test at screening)
- Patients with serum ferritin >2000 mcg/L (which will exclude ongoing HLH)

1.6. Study Interventions

Confirmed or highly suspect COVID-19 patients with acute hypoxic respiratory failure (saturation below 93% on minimal 2 l/min O2 or PaO2/FiO2 <350) will be randomized to receive sargramostim 125mcg twice daily for 5 days as a nebulized inhalation on top of standard of care (active group), or to receive standard of care treatment (control group). Upon progression of disease requiring initiation of non-invasive or invasive mechanical ventilatory support within the 5 day period, in patients in the active group, inhaled sargramostim will be replaced by intravenous sargramostim 125mcg/m^2 body surface area once daily until the 5 day period is reached. From day 6 onwards, progressive patients in the active group will have the option to receive an additional 5 days of IV sargramostim, based on the treating physician's assessment. In the control group with progressive disease requiring non-invasive or invasive mechanical ventilatory support, from day 6 onwards, the treating physician will have the option to initiate IV sargramostim 125mcg/m^2 body surface area once daily for 5 days.
Safety data, including blood leukocyte counts, will be collected in all patients. Efficacy data will also be collected and will include arterial blood gases, oxygenation parameters, need for ventilation, lung compliance, organ function, radiographic changes, ferritin levels, triglyceride levels, etc. as well as occurrence of secondary bacterial infections.

Patients will stop the investigational drug if there is unacceptable toxicity according to investigator's judgement.

1.6.1. IMPs and dosage

LEUKINE® (sargramostim) prepared and administered for inhalation using nebulizer

LEUKINE for injection is a sterile, preservative-free lyophilized powder that requires reconstitution with 2mL normal saline solution. Once reconstituted, LEUKINE can be inhaled as an aqueous aerosol using either a vibrating mesh nebulizer (Philips InnospireGo) or jet nebulizer, per manufacturer instructions. (Nebulizers studied include: AKITA2 Apixneb, PARI LC-Plus set, PulmoAide, Pan LC, Aeroneb Solo Device). Use reconstituted LEUKINE® solution for inhalation within 16 hours following reconstitution and/or dilution.

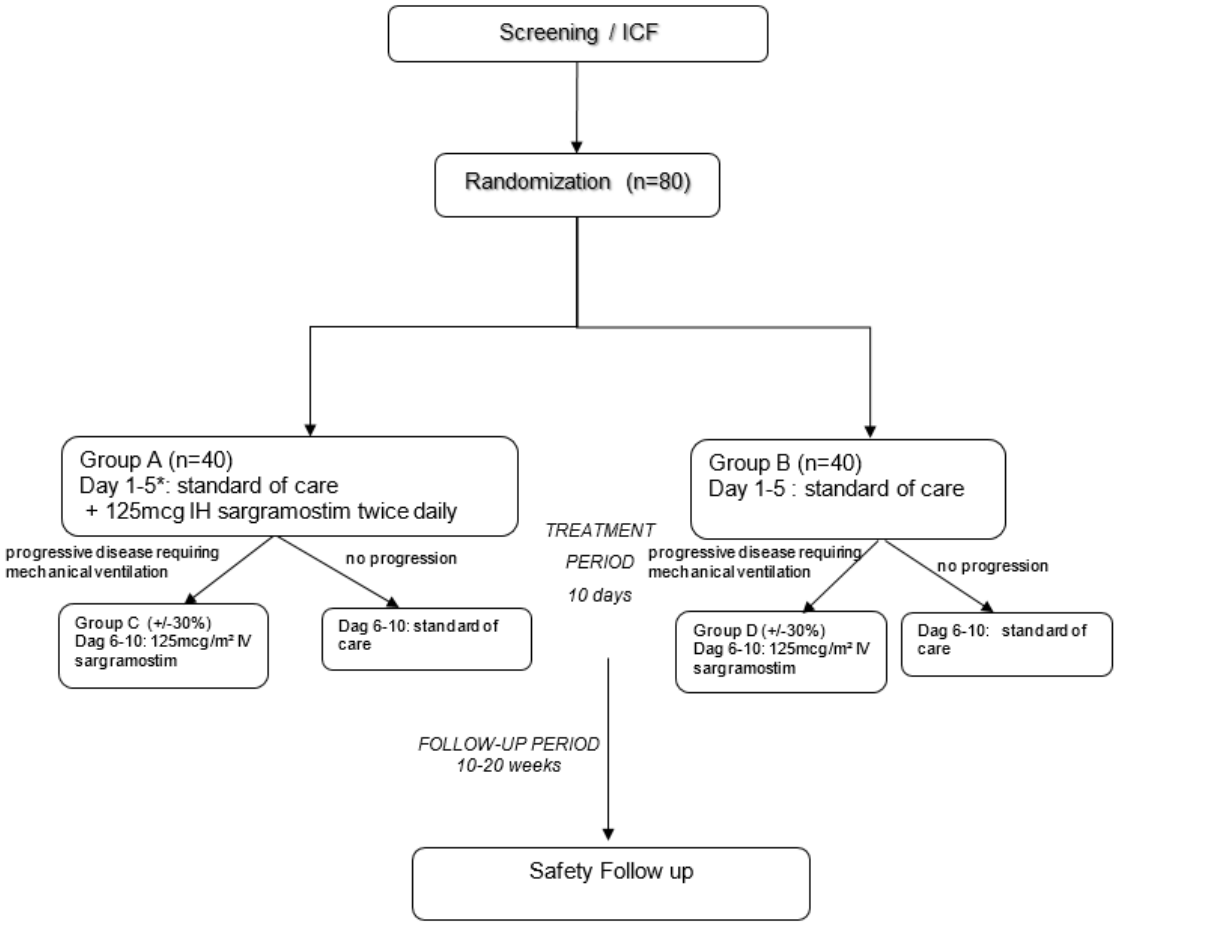
Nebulizing is preferably done in an isolation negative pressure chamber, and if not, personnel should use an FFP2 mask. Patient should self-administer the medication and where possible, the room should not be entered within one hour after administration.

LEUKINE® (sargramostim) prepared and administered intravenously

For patients that are on a mechanical ventilator and cannot be treated with LEUKINE® inhalation:

- The recommended dose is 125 mcg/m^2/day administered intravenously over a 4-hour period once daily for up to 5 days.
- For intravenous injection: Administer LEUKINE injection in 0.9% Sodium Chloride Injection, USP. Dilute LEUKINE for intravenous infusion in 0.9% Sodium Chloride Injection, USP. If the final concentration of LEUKINE is below 10 mcg/mL, add Albumin (Human) at a final concentration of 0.1% to the saline prior to addition of LEUKINE to prevent adsorption to the components of the drug delivery system. To obtain a final concentration of 0.1% Albumin (Human), add 1 mg Albumin (Human) per 1 mL 0.9% Sodium Chloride Injection, USP (e.g., use 1 mL 5% Albumin [Human] in 50 mL 0.9% Sodium Chloride Injection, USP).

1.6.2. Schematic overview of the data collection & interventions



*in case of progressive disease requiring mechanical ventilator support within the first 5 days, IV sargramostim can be initiated until the 5 day period is reached. From day 6 onwards, progressive patients in the active group will have the option to receive an additional 5 days of IV sargramostim, based on the treating physician's assessment.

1.7. Study duration

The total treatment duration of the study is 10 days, and the entire study duration is 10-22 weeks to final follow up visit.

2. Rationale and background

2.1. Rationale

Sargramostim (Leukine®) is a yeast-derived recombinant humanized granulocyte-macrophage colony stimulating factor (rhuGM-CSF, sargramostim) and the only FDA approved GM-CSF (Leucine Package Insert). GM-CSF, a pleiotropic cytokine, is an important leukocyte growth factor known to play a key role in haematopoiesis, effecting the growth and maturation of multiple cell lineages as well as the functional activities of these cells in antigen presentation and cell mediated immunity (*1*). Since its initial FDA approval in 1991, over 500,000 patients have received Leukine®, providing extensive clinical and post-marketing data in a broad range of treated individuals - from preterm neonates to the elderly and including males and females - representing a well-characterized safety profile for Leukine®. Leukine® administered as a subcutaneous or intravenous injection is approved for six indications including use as a medical countermeasure for radiation exposure. The US Government currently holds Leukine® in the Strategic National Stockpile. Leukine® may benefit patients with beginning signs of Acute Respiratory Distress Syndrome (ARDS) due to COVID-19 Infection. GM-CSF is a critical cytokine for the health of lungs. The alveolar macrophages are dependent on GM-CSF for differentiation and normal functioning. In addition, GM-CSF is an immunomodulator that plays a critical role in host defense by promoting differentiation of dendritic cells, and stimulating antiviral immunity (*2-4*).

As described in detail below, it is being studied as an adjuvant therapy in the management of life-threatening infections to boost the hosts innate immune response to fight infection, reduce the risk of secondary infection, and in varied conditions to prevent infection during critical illness (*5-8*). In addition, it has been studied in pulmonary conditions that affect alveolar macrophages, such as autoimmune pulmonary alveolar proteinosis ("aPAP"), with beneficial outcomes (*9, 10*). We propose based on preclinical and clinical data and the safety data from more than 500,000 adult and pediatric patients treated with Leukine®, that patients with beginning signs of acute lung injury and/or ARDS due to COVID-19 infection be given Leukine®. ARDS due to COVID-19 carries a high mortality rate (*11*) and Leukine® may confer benefit by both active management of this complication as well as in prevention of secondary infections.

In animal models of postviral ARDS and mortality, GM-CSF has demonstrated immunomodulatory effects that improve the clinical response and symptoms associated with influenza and other viral respiratory infections (*12-14*), and represents a promising candidate for the prevention of ARDS in patients with COVID-19.

Practical Advantages of LEUKINE® for Acute Respiratory Distress due to COVID-19

Advantage	Details
Approved for use by the FDA	- 6 indications
Safe in Pediatric and Adult populations, including elderly	- Established safety profile in pediatric and adult populations, including elderly (>500,000 patients)
Available for use in the SNS plus commercial distribution	- Current approvals provide a pre-existing distribution and stockpile resource Existing formulation safe for use with Pari LC Sprint Jet and Vectura Akita 2 nebulizers - US based manufacturing of BDS and FDP
Proven effective in fighting infection/enhancing immune response	- GM-CSF modulates immune response to influenza virus and promotes viral clearance - GM-CSF activates dendritic cells and T-cells - GM-CSF improves epithelial lung repair

2.2. Background

The proposed development plan was guided by three specific considerations:

1. Supportive Scientific Rationale:
The biology and effects of GM-CSF on the lung, specifically alveolar macrophages and epithelial cells, as well its immunomodulatory activities in stimulating antiviral immunity make GM-CSF a critical cytokine for healthy pulmonary function and defence. Detailed studies have shown that GM-CSF is necessary for the maturation of alveolar macrophages from fetal monocytes and the maintenance of these cells in adulthood (*1*).
GM-CSF has a wide array of effects on myeloid cells. GM-CSF has been shown to be a myelopoietic growth factor that has pleiotropic effects not only in promoting the differentiation of immature precursors into polymorphonuclear neutrophils, monocytes/ macrophages and dendritic cells, but also in controlling the function of fully mature myeloid cells (*15*). GM-CSF is also known to reverse immunoparalysis seen in sepsis by immune activation, resulting in beneficial outcomes (*5*).

There is a large body of evidence generated with GM-CSF in animal studies suggesting the potential use in ARDS and infections (*16*). For the purpose of brevity, we will point to the data that reflects the potential value in viral lung infections and preventing secondary bacterial infections and progression to ARDS:

Halstead and colleagues demonstrated that in vivo high airway levels of GM-CSF profoundly rescue mice from lethal influenza pneumonia. While in vitro GM-CSF is canonically described as an M1-polarizing cytokine, their data demonstrated that in vivo, during influenza A virus infection, GM-CSF instead temporizes the type II interferon-induced M1 polarization of airway macrophages and reduces inflammation induced damage (*12, 13*). Unkel and colleagues demonstrated GM-CSF–dependent cross-talk between influenza virus infected alveolar epithelial cells and CD103+ dendritic cells is crucial for effective viral clearance and recovery from injury and thus pointing to the potential use of GM-CSF treatment in severe influenza virus pneumonia (*17*). Investigations have shown that GM-CSF conferred resistance to influenza in mice via alveolar phagocytes and through alveolar macrophages which became more resistant to influenza- induced apoptosis. Delivery of intranasal GM-CSF to wild-type mice also conferred resistance to influenza (*18*). There is evidence that inhaled GM-CSF prevents bacteremia in post influenza bacterial pneumonia primarily through locally-mediated improved lung antibacterial resistance to systemic bacteremia during influenza A viral infection (*13*).

Conclusions: GM-CSF confers resistance to influenza by enhancing innate immune mechanisms that depend on alveolar macrophages, which are dependent on GM-CSF for their health and normal functioning. Pulmonary delivery of this cytokine has the potential to reduce morbidity and mortality due to viral pneumonia. This is summarized in the diagram below:

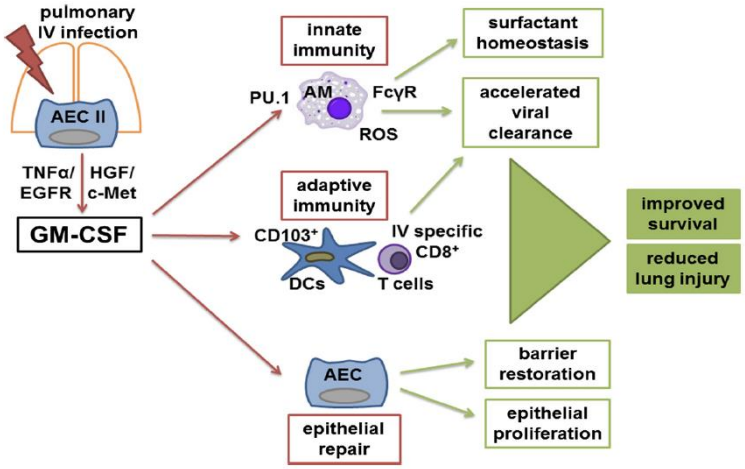


Fig. 1 GM-CSF-modulated immune response to IV infection. After pulmonary IV infection GM-CSF is released from AEC II, mediated through HGF/c-Met and TGF-α/EGFR signaling. In an autocrine manner, it stimulates epithelial repair, including epithelial proliferation and barrier restoration. Innate and adaptive immunity are activated, resulting in accelerated viral clearance. Via PU.1, GM-CSF improves AM resistance, maturation, ROS production, and phagocytosis capacity, e.g., by the FcγR-mediated opsonophagocytosis. GM-CSF also stimulates activation and proliferation of DCs, especially $CD103^+$ DCs, and T cells and enhances Ag priming and IV-specific $CD8^+$ T cell recruitment. Altogether AEC GM-CSF leads to increased survival and reduced lung injury. *AEC* alveolar epithelial cells, *Ag* antigen, *AM* alveolar macrophage, *c-Met* hepatocyte growth factor receptor, *DC* dendritic cell, *EGFR* epithelial growth factor receptor, *FcγR* Fcɣ receptor, *GM-CSF* granulocyte and macrophage colony stimulating factor, *HGF* hepatocyte growth factor, *PU.1* transcription factor PU.1, *ROS* reactive oxygen species, *TGF-α* transcriptional growth factor α

2. Experience: Use of Leukine® has beneficial effect in the treatment of conditions that are similar to ARDS seen with COVID-19.

A small (18 patient) double blind randomized placebo controlled clinical trial of low-dose (3mcg/kg daily for 5 days) intravenous GM-CSF treatment in adult patients with severe sepsis and respiratory dysfunction, led to the conclusion that GM-CSF treatment was associated with improved gas exchange and might play a homeostatic role (*6*). In a phase II study, 130 patients with severe sepsis with respiratory dysfunction were randomized to GM-CSF (250mcg/m^2 intravenously daily for 14days) or placebo. The results showed an improvement in 28day mortality on GM-CSF; this did not reach statistical significance due to the small sample size (*7*).

Herold and colleagues used Leukine® by inhalation route on a compassionate basis in six patients with moderate to severe community-acquired pneumonia or ventilator-associated pneumonia ARDS who were not improving despite all measures and at least 6 days of mechanical ventilation(*8*). 125mcg of Leukine® were applied by Aeroneb Solo device (Covidien, Neustadt, Germany) at an interval of 48 hours. Compared to historical controls, the authors observed significant improvement in oxygenation and lung compliance with GM-CSF therapy. This resulted in improved morbidity using standard scoring systems and 4 of the six patients recovered and were discharged from the hospital. There is an ongoing study of inhaled GM-CSF across multiple centers in Germany (GI HOPE; NCT02595060) recruiting patients with diagnosis of pneumonia associated ARDS.

There is a large body of evidence of inhaled Leukine® in autoimmune pulmonary alveolar proteinosis (aPAP), which results in accumulation of surfactant in alveolar sacs with resultant hypoxia. Tazawa and colleagues conducted a phase II study of inhaled Leukine® at 9 pulmonary centers throughout Japan in patients with unremitting or progressive aPAP with hypoxia and symptoms (*9*). Patients received 250mcg daily by inhalation, using an LC-PLUS nebulizer with a manual interrupter valve connected to a PARI Turbo BOY compressor, for 7 days and this cycle was repeated every other week for six cycles (total 12 weeks). The treatment was well tolerated with no serious adverse events. Adverse events were reported in just 7 of the 39 patients oxygenation, radiological changes as well as symptoms. Following these results, a larger randomized phase 3 study (PAGE study) was conducted by the Japanese investigators in 12 centers. 64 patients with mild to moderate aPAP with hypoxia were randomized to receive placebo or Leukine® (33 patients) at a dose of 125mcg twice a day for 7days

followed by a week of no treatment. This two-week cycle was repeated 12 times over a period of 24 weeks. The treatment was again well tolerated with no significant differences in adverse events between the two groups. The GM-CSF treated patients had significantly improved hypoxia parameters and radiographic changes (*10*). This clinical experience of use of Leukine® in viral pneumonia suggests salutary effects. In addition, these studies establish the safety of inhaled Leukine® and provide evidence for activity of inhaled Leukine®.

3. Expediency: Toxicology, pharmacologic and safety data supports the immediate clinical use of Leukine® in hypoxic respiratory failure with acute lung injury leading to ARDS due to COVID-19. Investigator brochure is available and contains detailed information on toxicity.

2.3. Risk/Benefit Assessment

COVID-19 poses a very significant risk of mortality of 3-7% and this percentage rises to mortality of 20% in patients with co-morbidity (*11, 19*). Of all infected patients, some 15-20% develop severe respiratory symptoms necessitating hospital admission. Around 5% of infected patients will require invasive mechanical ventilation, and many of those (40-50% will die). The current world-wide pandemic of COVID-19 is putting unforeseen stress on the entire primary, secondary and tertiary medical system, leading to unseen triage of patients that potentially benefit or not from admission to ICU units when they develop respiratory failure.

GM-CSF (sargramostim, Leukine®) has been given systemically to almost 500.000 patients in the past. It is therefore a well characterized product. Inhalation of GM-CSF has also been used to treat patients with interstitial lung disease and reduced oxygen saturation (i.e. partial acute hypoxic respiratory failure) with few significant side effects above the placebo arm. The protocol is set up to give twice daily inhalation with GM-CSF, followed by intravenous administration if the patient would move to the ICU unit on mechanical ventilation.

Although GM-CSF has been given systemically and via inhalation to patients with pneumonia-associated ARDS, there are no current data on the safety profile of this drug in patients with COVID-19. Given the severity of the clinical syndrome caused by COVID-19, and the prior triage of patients before hospital admission to the COVID-19 ward, this trial will be performed in a hospital setting on a COVID-19 ward with close monitoring of vital parameters (continuous ECG, oxygen saturation, temperature, vital clinical signs), which will allow intermediate intervention should serious side effects occur. Once on the ICU unit, patients will be intensively monitored for all vital parameters, as part of the routine ICU monitoring.

There are currently no treatments directed at improving lung repair and local immunity in COVID-19 patients, and no treatment that attempt to halt the progression from manageable acute hypoxic respiratory failure to ARDS. Preventing such progression to ARDS could have a huge impact on the foreseeable overflow of the ICU units. We therefore believe the benefits of administering inhaled GM-CSF treatment in early stage COVID-19 acute hypoxic respiratory failure outweighs the risks associated with a phase 4 IMP administration via a different route and unknown indication.

2.4. Limitations

There is a large number of COVID-19 infected patients that are currently being hospitalized across the globe. In just 9 days time, our COVID-19 ward at Ghent University Hospital has admitted 25 confirmed cases, of which a significant portion (50%) already fulfill eligibility criteria for the current proposed protocol. We therefore believe that given the current ascending part of the epidemiology curve, with numbers of patients rising sharply, there will be no shortage of patients that are eligible.
Partner Therapeutics has offered to give (free of charge) enough GM-CSF to treat 20 patients for a 10 day period and an additional 20 controls for 5 days (should deterioration occur). There are large

amounts of GM-CSF in the United States strategic national stockpile, so should this therapy work, there might be immediate worldwide application of a GM-CSF inhalation therapy.

3. Objectives

3.1. Primary Objectives

This is phase 4 academic, prospective, randomized, open-label, interventional study designed to investigate the efficacy of sargramostim (Leukine®) in improving oxygenation and short- and long-term outcome of COVID-19 patients with acute hypoxic respiratory failure. There are currently no treatments directed at improving lung repair and local immunity in COVID-19 patients, and no treatment that attempt to halt the progression from manageable acute hypoxic respiratory failure to ARDS in patients with COVID-19 infection.

Justification for our objective is that preventing progression from early acute hypoxic respiratory failure to ARDS could have a huge impact on the foreseeable overflow of the ICU units, that is already happening in some countries and is bound to happen on a global scale. The outcome of our study could thus have large impact from a medical, ethical and economic perspective.

The **hypothesis of the proposed intervention** is that GM-CSF has profound effects on antiviral immunity, can provide the stimulus to restore immune homeostasis in the lung with acute lung injury post COVID-19, and can promote lung repair mechanisms, that lead to a 25% improvement in lung oxygenation parameters.

This hypothesis is based on experiments performed in mice showing that GM-CSF treatment can prevent mortality and prevent ARDS in mice with post-viral acute lung injury.

To address our hypothesis, we will randomize patients with confirmed COVID-19 with acute hypoxic respiratory failure (saturation below 93% on minimal 2 l/min O2 or PaO2/FiO2 <350) to receive sargramostim 125mcg twice daily for 5 days as a nebulized inhalation on top of standard of care (active group), or to receive standard of care treatment (control group). Upon progression of disease requiring initiation of non-invasive or invasive mechanical ventilatory support within the 5 day period, in patients in the active group, inhaled sargramostim will be replaced by intravenous sargramostim 125mcg/m^2 body surface area once daily until the 5 day period is reached.

To measure the effectiveness of sargramostim on restoring lung homeostasis, the **primary endpoint** of this intervention is **measuring oxygenation** after 5 DAYS of inhaled (and intravenous) treatment through assessment of pretreatment and post-treatment ratio of PaO2/FiO2 and through measurement of the P(A-a)O2 gradient, which can easily be performed in the setting of clinical observation of patients admitted to the COVID -19 ward or ICU COVID-19 unit. During the 5 day treatment period, we will perform daily measurements of oxygen saturation (pulse oximetry) in relation to FiO2, and the slope of alterations in these parameters could also be an indicator that our hypothesis is correct.

Comparison will be between active group A receiving sargramostim on top of standard of care and control group B receiving standard of care.

Data from the Wuhan COVID-19 epidemic show that patients that deteriorate are facing a prolonged period of mechanical ventilation. Therefore, from day 6 onwards, progressive patients in the active group will have the option to receive an additional 5 days of IV sargramostim, based on the treating physician's assessment. This group will be called group C. In the control group, for patients with

progressive disease requiring (non-) invasive mechanical ventilatory support, from day 6 onwards, the treating physician will have the option to initiate IV sargramostim 125mcg/m^2 body surface area once daily for 5 days. This group will be called group D. Comparisons of Group A (early 5 day intervention with sargramostim) with Group D (late 5 day intervention with sargramostim) will also be very informative.

3.2. Secondary Objectives

- to study if early intervention with sargramostim is safe (number of AEs/SAEs)

- **to study if early intervention with inhaled sargramostim affects clinical outcome defined by**
Duration of hospital stay
Mean and median change in 6-point ordinal scale between day 1 and day 6
Mean and median change in clinical sign score between day 1 and day 6
Time to clinical sign score <6 maintained for 24h
Mean and median change of SOFA score between day 1 and day 6 or between day 1 and day 10.
Mean and median change NEWS2 score between day 1 and day 6 or between day 1 and day 10.
Time to NEWS2 score less than 2 for at least 24h

- **to study if early intervention with sargramostim affects the rate of nosocomial infection**

- to study if early intervention with inhaled sargramostim affects progression to mechanical ventilation and/or ARDS

-**to study if treatment with sargramostim affects all cause mortality rate at 28 days and 20 weeks post inclusion**

-**to study if treatment with sargramostim affects features of secondary haemophagocytic lymphohistiocytosis, as defined by Hs score (temp, organomegaly, cytopenia, triglycerides, fibrinogen, ferritin, AST and known immunosuppression)**

- **to study if treatment with sargramostim has a favourable effect on long term 10-20 week follow up**

4. End Points + Time Points

4.1. Primary End Points + Time Points

To measure the effectiveness of sargramostim on restoring lung homeostasis, the **primary endpoint** of this intervention is **measuring oxygenation** after 5 DAYS of inhaled (and intravenous) treatment through assessment of pretreatment (day 0) and post-treatment (day 5) ratio of PaO2/FiO2 and through measurement of the P(A-a)O2 gradient, which can easily be performed in the setting of clinical observation of patients admitted to the COVID -19 ward or ICU COVID-19 unit. Preferentially, this measurement should be done in the upright position, while breathing room air for a minimum of 3 minutes. If this is impossible due to need for supplemental oxygen, FiO2 and oxygen supplementation method should be recorded in patient record, so that A-a gradient can be normalized for age expected normal A-a gradient while on supplemental oxygen use.
During the 5 day treatment period, we will perform daily measurements of oxygen saturation (pulse oximetry) in relation to FiO2, and the slope of alterations in this parameters could also be an indicator that our hypothesis is correct.
If the patient leaves hospital prior to the day 6 analysis point, oxygenation at day of discharge will be used as value for measuring primary endpoint.

Improvement will be expressed as % of patients showing an improvement in P(A-a)O_2 gradient and PaO_2/FiO_2 ratio between day 6 and day 1 of at least 25%; at least 33% and at least 50% in each treatment arm and expressed also as mean and median change in P(A-a)O2 gradient and PaO2/FiO2 ratio comparing D6 to D1 in both treatment arms.

4.2. Secondary End Points + Time Points

- to study if early intervention with sargramostim is safe (number of AEs/SAEs)
Although sargramostim has been given previously by inhalation to patients with ARDS and interstitial lung disease, data on safety in patients with COVID-19 infection are currently lacking. Since we are randomizing against 5 days of no sargramostim treatment, comparison of AEs and SAEs between group A and group B will be very informative.

-**to study if early intervention with inhaled sargramostim affects clinical outcome** defined by
Length of hospital stay
Mean and median change in 6-point ordinal scale change between day 1, day 6
Mean and median change in clinical sign score between day 1 and day 6
Time to clinical sign score <6 maintained for 24h
Mean and median change of SOFA score between day 1 and day 6 or between day 1 and day 10.
Mean and median change NEWS2 score between day 1 and day 6 or between day 1 and day 10.
Time to NEWS2 score less than 2 for at least 24h

- to study if early intervention with sargramostim affects the rate of nosocomial infection
Patients with viral respiratory infection are at risk of secondary bacterial infections. As part of routine clinical care, sputum samples will be collected in patients suspected of secondary bacterial pneumonia, and checked for the presence of bacteria.

-to study if early intervention with inhaled sargramostim affects progression to mechanical ventilation and/or ARDS
Decreasing oxygenation often leads to the need for non-invasive or invasive mechanical ventilation, and if severe enough to a diagnosis of ARDS. We will therefore as a secondary endpoint also study if early intervention with inhaled sargramostim prevents progression to criteria-defined ARDS (according to the American-European Consensus Conference (AECC) diagnostic criteria for ARDS: acute onset; ratio of partial pressure of arterial oxygen to fraction of inspired oxygen (PaO_2/FiO_2) of 200 or less, regardless of positive end-expiratory pressure; bilateral infiltrates seen on frontal chest radiograph; and pulmonary artery wedge pressure of 18 mm Hg or less when measured, or no clinical evidence of left atrial hypertension), requiring high-flow oxygen devices, non-invasive mechanical ventilation, mechanical ventilation, by measuring the day from admission when this diagnosis is made or therapies are initiated.

-**to study if treatment with sargramostim affects all-cause mortality rate at 4 and 20 weeks post inclusion.**

-**to study if treatment with sargramostim affects features of secondary haemophagocytic lymphohistiocytosis**.
A large subset of patients with severe COVID-19 developing respiratory failure might have a cytokine storm syndrome, designated as secondary haemophagocytic lymphohistiocytosis (sHLH). sHLH is an under-recognised, hyperinflammatory syndrome characterised by a fulminant and fatal hypercytokinemia with multi-organ failure. Cardinal features of sHLH include unremitting fever,

cytopenias, and hyperferritinaemia; hypertriglyceridemia, pulmonary involvement can present as ARDS. A cytokine profile resembling sHLH is associated with COVID-19 disease severity, characterised by increased interleukin (IL)-2, IL-7, granulocyte-colony stimulating factor, interferon-γ inducible protein 10, monocyte chemoattractant protein 1, macrophage inflammatory protein 1-α, and tumour necrosis factor-α.
Predictors of fatality from a recent retrospective, multicentre study of 150 confirmed COVID-19 cases in Wuhan, China, included elevated ferritin (mean 1297·6 ng/ml in non-survivors vs 614·0 ng/ml in survivors; p<0·001) and IL-6 (p<0·0001), suggesting that mortality might be due to virally driven hyperinflammation.
To address the effect of sargramostim treatment on sHLH, we will measure levels of ferritin, these chemokines and cytokines at the beginning of the trial day 0 and after the initial 5 day treatment. PBO including leukocytes and lymphocytes are performed on a routine clinical basis in these patients.

- **to study if treatment with sargramostim has a favourable effect on long term 10-20 week follow up**

At 10-20 weeks after Day 1, patients will be seen on routine check-up by pulmonologist, who will perform a clinical exam, pulmonary function tests (including FVC, TLC and diffusion capacity), a laboratory (ferritin, lymphocytes, leukocytes) and a 6 minutes walk test and HRCT if done per standard of care.

4.3. Pharmacodynamic and Pharmacokinetic endpoints

Pharmacodynamic endpoints:
Plasma and serum samples will be collected for summary and exploratory analysis by the Primary Immunodeficiency lab at UZ Gent and the VIB-UGent Inflammation Research Center (IRC) as appropriate (e.g. descriptive statistics, compare change from baseline between the two treatment arms), may include but are not limited to:

• anti-drug antibodies (ADA) – D1 and long-term follow-up serum samples (Summary only to indicate presence or not)
• Local safety labs such as D-dimers, LDH, ferritin, CRP, fibrinogen, eosinophils, lymphocytes
• Cytokines and biomarkers (from selected centres) on D1, and D6 (or at hospital discharge, if earlier) and follow up
    o Cytokines may include, but not limited to:
        ▪ IL-1beta, IL-1RA, IL-2, IL-8, IL-6, TNFa, GM-CSF, G-CSF, IP-10, MCP-1, MIP-1a, IFNg, and IL-10
    o Biomarkers may include, but are not limited to
        ▪ sRAGE, Angiopoietin-2, KL6, GDF-15, suPAR.
• Immunomonitoring (from selected centers) on D1, and D6 (or at hospital discharge, if earlier) and follow up will include
    ○ flow cytometry analysis of numbers of peripheral blood lymphocyte and monocyte subsets, and their activation status by flow cytometry
    ○ ELISPOT assays to measure the number of IFNg, TNFa, IL-2 and GM-CSF producing CD4 and CD8 T cells following restimulation of frozen and thawed peripheral blood mononuclear cells (PBMC) with a SARS-CoV2 megapool of immunogenic peptides.

5. Study design

5.1. Description of study design

This is phase 4 academic, prospective, randomized, open-label, interventional study designed to investigate the efficacy of sargramostim (Leukine®) in improving oxygenation and short- and long-term outcome of COVID-19 patients with acute hypoxic respiratory failure. There are currently no treatments directed at improving lung repair and local immunity in COVID-19 patients, and no treatment that attempt to halt the progression from manageable acute hypoxic respiratory failure to ARDS in patients with COVID-19 infection. **Justification for our objective** is that preventing progression from early acute hypoxic respiratory failure to ARDS could have a huge impact on the foreseeable overflow of the ICU units, that is already happening in some countries and is bound to happen on a global scale.

The **hypothesis of the proposed intervention** is that GM-CSF has profound effects on antiviral immunity, can provide the stimulus to restore immune homeostasis in the lung with acute lung injury post COVID-19, and can promote lung repair mechanisms, that lead to a 25% improvement in lung oxygenation parameters. This hypothesis is based on experiments performed in mice showing that GM-CSF treatment can prevent mortality and prevent ARDS in mice with post-viral acute lung injury.

We will randomize patients with confirmed COVID19 with acute hypoxic respiratory failure (saturation below 93% on minimal 2 l/min O2 or PaO2/FiO2 <350) to receive sargramostim 125mcg twice daily for 5 days as a nebulized inhalation on top of standard of care (active group), or to receive standard of care treatment (control group). Upon progression of disease to requiring non-invasive or invasive mechanical ventilatory support within the 5 day period, in patients in the active group, inhaled sargramostim will be replaced by intravenous sargramostim 125mcg/m^2 body surface area until the 5 day period is reached.
Dosing of inhaled and systemic sargramostim are based on prior experience of this drug in patients with pulmonary alveolar proteinosis (inhaled) and with pneumonia associated ARDS (inhaled and intravenous). The inhaled route is preferred first, because high local concentrations of GM-CSF have a favourable effect on lung immunity, lung homeostasis and lung repair. The switch to intravenous treatment with deterioration requiring initiation of mechanical ventilation is necessitated by the fact that patients with COVID-19 poorly tolerate ventilation in the absence of high level positive end expiratory pressure (PEEP), especially when they develop ARDS. For giving the sargramostim via inhalator in a ventilated patient, this would involve PEEP-free ventilation for at least 10-15 minutes, which will not be tolerated in COVID-19 associated severe hypoxic respiratory failure and/or ARDS according to expert opinion (Prof. Dr. Pieter Depuydt, Intensive Care Unit, UZ Ghent).

To measure the effectiveness of sargramostim on restoring lung homeostasis, the **primary endpoint** of this intervention is **measuring oxygenation** after 5 days of inhaled (and intravenous) treatment through assessment of pretreatment and post-treatment ratio of $PaO2/FiO_2$ and through measurement of the $P(A\text{-}a)O_2$ gradient, which can easily be performed in the setting of clinical observation of patients admitted to the COVID -19 ward or ICU COVID-19 unit. Supplemental oxygen use will be recorded, and if needed A-a gradient will be normalized against expected age- and supplemental oxygen dependent A-a gradient. During the 5 day treatment period, we will perform daily measurements of oxygen saturation (pulse oximetry) in relation to FiO2, and the slope of alterations in this parameters could also be an indicator that our hypothesis is correct. If the patient leaves hospital prior to the day 6 analysis point, oxygenation at day of discharge will be used as value for measuring primary endpoint.

Comparison will be between active group A receiving sargramostim on top of standard of care and control group B receiving standard of care.

Data from the Wuhan COVID-19 epidemic show that patients that deteriorate are facing a prolonged period of mechanical ventilation. Therefore, from day 6 onwards, progressive patients in the active group will have the option to receive an additional 5 days of IV sargramostim, based on the treating physician's assessment. This group will be called group C. In the control group with progressive disease requiring non-invasive or invasive mechanical ventilatory support or developing ARDS, from day 6 onwards, the treating physician will have the option to initiate IV sargramostim 125mcg/m^2 body surface area once daily for 5 days. This group will be called group D. Comparisons of Group A (early 5 day intervention with sargramostim) with Group D (late 5 day intervention with sargramostim) will also be very informative.

5.2. End of Study Definition

5.2.1. For an individual subject

The subject has completed the study if he or she has completed all phases of the study, including the last visit (week 10-20 clinical follow up visit) or the last scheduled procedures, as described in this protocol (see section "9. Study Specific Procedures").

5.2.2. For the whole study

Overall, the end of the study is reached when the last study procedure for the last subject has occurred: last subject, last visit (LSLV).
As soon as the whole study has ended (cfr. the definition above), the co-ordinating Investigator shall notify the HIRUZ Clinical Trial Unit, so that the Competent Authority and the Ethics Committee can be informed in a timely manner according to the regulatory requirements (within 90 days after end of the study, or if the study had to be terminated early, this period must be reduced to 15 days and the reasons should clearly explained).

5.3. Estimated duration of the study

There is a large number of COVID-19 infected patients that are currently being hospitalized across the globe. In just 9 days time, our COVID-19 ward at Ghent University Hospital has admitted 25 confirmed cases, of which a significant portion (50%) already fulfill eligibility criteria for the current proposed protocol. We therefore believe that given the current ascending part of the epidemiology curve, with numbers of patients rising sharply, there will be no shortage of patients that are eligible. We estimate the study to terminate in 30 weeks, including last clinical follow up visits.

6. Inclusion and Exclusion Criteria

6.1. Inclusion Criteria

The following patients will be enrolled

Recent (≤2weeks prior to randomization) - Confident COVID-19 diagnosis confirmed by antigen detection test and/or PCR and/or positive serology, or any emerging and validated diagnostic laboratory test for COVID-19 within this period.

-In some patients, it may be impossible to get a confident laboratory confirmation of COVID-19 diagnosis after 24h of hospital admission because viral load is low and/or problems with diagnostic sensitivity. In those cases, in absence of an alternative diagnosis, and with highly suspect bilateral ground glass opacities on recent (<24h) chest-CT scan (confirmed by a radiologist and pulmonary physician as probable COVID-19), a patient can be enrolled as probable COVID-19 infected. In all cases, this needs confirmation by later seroconversion.
-Presence of acute hypoxic respiratory failure defined as (either or both)
saturation below 93% on minimal 2 l/min O2
PaO2/FiO2 below 350
-Admitted to specialized COVID-19 ward
-Age 18-80
-Male or Female
-Willing to provide informed consent

6.2. Exclusion Criteria

-Patients with known history of serious allergic reactions, including anaphylaxis, to human granulocyte-macrophage colony stimulating factor such as sargramostim, yeast-derived products, or any component of the product.

-mechanical ventilation before start of study

-Patients enrolled in another investigational drug study

-Pregnant or breastfeeding females (all female subjects regardless of childbearing potential status must have negative pregnancy test at screening)

- patients with peripheral white blood cell count above 25.000 per microliter and/or active myeloid malignancy

-patients on high dose systemic steroids (> 20 mg methylprednisolone or equivalent) for COVID-19 unrelated disorder

-patients on lithium carbonate therapy

-Patients with serum ferritin >2000 mcg/L (which will exclude ongoing HLH)

6.2.1. Screen failures

Screen failures are defined as subjects who consent to participate in the clinical study but are not subsequently randomly assigned to the study intervention or entered in the study. A minimal set of screen failure information will be kept to ensure transparent reporting of screen failure subjects.

7. Target Population

7.1. Subjects

7.1.1. Number of subjects and planned recruitment rate

There is a large number of COVID-19 infected patients that are currently being hospitalized across the globe. In just 9 days time, our COVID-19 ward at Ghent University Hospital has admitted 25 confirmed cases, of which a significant portion (50%) already fulfill eligibility criteria for the current proposed protocol. Similar numbers of patients are currently being seen in all centers. We therefore believe that given the current ascending part of the epidemiology curve, with numbers of patients rising sharply, there will be no shortage of patients that are eligible.

The number of subjects that will be included in this study is: 80.
These are divided into following sub-groups:

Group A : active **sargramostim** treatment group, treatment for initial 5 days, no deterioration after 5 days
Number of patients : 40

Group B : control group : no treatment with sargramostim in first 5 days
Number of patients : 40

Group C and D :
Data from the Wuhan COVID-19 epidemic show that patients that deteriorate are facing a prolonged period of mechanical ventilation. Therefore, from day 6 onwards, progressive patients in the active group will have the option to receive an additional 5 days of IV sargramostim sargramostim 125mcg/m2 body surface area once daily, based on the treating physician's assessment. This group will be called group C. It is estimated that some 30% of patients might deteriorate and require non-invasive or invasive mechanical ventilation, giving potentially rise to 12 patients that progress from group A to group C, if the clinician decides to move forward with the drug.

In the control group progressing to requiring non-invasive or invasive mechanical ventilatory support, from day 6 onwards, the treating physician will have the option to initiate IV sargramostim 125mcg/m^2 body surface area once daily for 5 days. This group will be called group D. It is estimated that some 30% of patients might deteriorate to mechanical ventilation or ARDS, giving potentially rise to 12 patients that progress from group A to group C, if the clinician decides to move forward with the drug

Comparisons between group A (early sargramostim) versus group B (no sargramostim) at day 6 will be important for reaching primary endpoint, and for key secondary endpoints. Comparisons of Group A (early 5 day intervention with sargramostim) with Group D (late 5 day intervention with sargramostim) will also be very informative for secondary endpoint analysis.

7.1.2. Withdrawal and replacement of subjects

Subjects are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue or withdraw a subject from the study for the following reasons:

- allergic reactions (anaphylactic shock) to sargramostim
- Pregnancy
- Progression to non-invasive or invasive mechanical ventilation and/or ARDS between screening and randomization
- Significant study intervention non-compliance
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject
- If the subject meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation

In all cases, the reason why subjects are withdrawn must be recorded in detail in the eCRF and in the subject's medical records.

If a patient decides to leave hospital before day 6 of the study, for example because of clinical improvement, the oxygenation parameters at day of discharge will be used to calculate the primary endpoint measurement.

The following actions must be taken if a subject fails to return to the clinic for a required study visit (visit at 10-20 weeks after Day 1) :

- The site will attempt to contact the subject and reschedule the missed visit within 4 weeks and counsel the subject on the importance of maintaining the assigned visit schedule and ascertain if the subject wishes to and/or should continue in the study.
- Before a subject is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the subject (where possible, 3 telephone calls and, if necessary, a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record or study file.
- Should the subject continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

7.2. Method of recruitment

Subjects will be recruited at the COVID-19 hospitalization ward at the participating centers. The study will be proposed by the treating physician to all subjects with PCR-Confirmed COVID-19 infection and a presence of acute hypoxic respiratory failure.

There will be no compensation for study participation. Partner Therapeutics Inc. is providing sargramostim to the study subjects, free of charge.

Since this is a hospital based trial, taking place over a minimum of five days in which patients are severely ill, we suspect the retention in the trial to be high.

7.3. Screening

Patients will be informed about the study by the treating physician.

After receiving full explanation, having received sufficient time to considerer the trial, asking questions and receiving satisfying responses to all questions, patients will be asked to sign ICF.

A serum pregnancy test will be done (female patients only).
Medical history will be checked for review of exclusion criteria and relevant subject information.

Patients will be continuously monitored on the COVID-19 ward.
Exams (standard of care) include, but are not limited to:

- ECG
- Chest X-Ray, and CT-scan
- Laboratory tests for leukocyte formula, kidney and liver function, ferritin levels
- Vital signs
- Pulse oximetry, Arterial blood gas, capnography

As soon as all in- and exclusion criteria are checked and patient is considered eligible, patient can be randomized. There is no minimal window to randomize the patient.

8. Investigational Medicinal Product (IMP)

8.1. Name of the IMP

LEUKINE®

8.1.1. Composition and active substance of the IMP

Sargramostim, Granulocyte macrophage colony-stimulating factor (GM-CSF), is the active substance of Leukine®.

8.1.2. Producer and Distributor of the IMP

The producer and distributor of Leukine® is Partner Therapeutics Inc, an integrated commercial-stage biotech company focused on the development and commercialization of therapeutics that improve health outcomes in the treatment of cancer. The distribution of IMP will be done by Tanner Pharma.

8.1.3. Preparation + Dosage + administration of the IMP

For inhalation: LEUKINE® is a sterile, preservative-free lyophilized powder that requires reconstitution with 4mL normal saline solution, to reach a concentration of 62,5 mcg/ml. Once reconstituted, LEUKINE® can be inhaled as an aqueous aerosol using either a vibrating mesh nebulizer or jet nebulizer, aerosolizing 2 ml twice daily. Reconstituted LEUKINE® solution for inhalation should be used within 16 hours following reconstitution and/or dilution. Dosage for inhalation: 125mcg twice daily via nebulizer. Nebulizing is preferably done in an isolation negative pressure chamber, and if not, personnel should use an FFP2 mask. Patient should self-administer the medication and where possible, the room should not be entered within one hour after administration.

For intravenous injection: LEUKINE® injection in 0.9% Sodium Chloride Injection, USP. Dilute LEUKINE® for intravenous infusion in 0.9% Sodium Chloride Injection, USP. If the final concentration of LEUKINE®

is below 10 mcg/mL, add Albumin (Human) at a final concentration of 0.1% to the saline prior to addition of LEUKINE to prevent adsorption to the components of the drug delivery system. To obtain a final concentration of 0.1% Albumin (Human), add 1 mg Albumin (Human) per 1 mL 0.9% Sodium Chloride Injection, USP (e.g., use 1 mL 5% Albumin [Human] in 50 mL 0.9% Sodium Chloride Injection, USP). Once diluted for infusion, LEUKINE® is stable for 6h. Dosage for intravenous injection: 125mcg/m^2/day over a 4-hour period for up to 5 days.

8.1.4. Permitted dose adjustments and interruption of treatment

No dose adjustments and interruptions are permitted during this trial. In case of anaphylaxis or severe AE, the drug will be immediately interrupted.

8.1.5. Duration of treatment

LEUKINE® will be administered for 5 days, with a possible 5 day extension to a maximum of 10 days in case of progression of disease and need for mechanical ventilation.

8.1.6. Packaging and Labeling of the IMP

LEUKINE® (sargramostim) for injection is a sterile, preservative-free, white lyophilized powder supplied in a carton containing five 250 mcg single-dose vials.

LEUKINE® (sargramostim) injection is a sterile, clear, colorless solution preserved with 1.1% benzyl alcohol supplied in a carton containing one 500 mcg/mL multiple-dose vial and a carton containing five 500 mcg/mL multiple-dose vials (NDC 0024-5844-05).

Storage and Handling : Leukine should be stored at 4 °C.

Drug will be labeled by Pharmacy UZ Ghent (for UZ Ghent enrolment) for inhaled or intravenous use.

8.1.7. Storage conditions of the IMP

Store LEUKINE® vials refrigerated at 2°C-8°C (36°F-46°F) in the original carton to protect from light. Do not freeze or shake. Do not use beyond the expiration date printed on the vial.
Leukine® is to be shipped refrigerated at 2°C-8°C (36°F-46°F). The medication will be delivered to the pharmacy of the participating centers. Temperature during shipment and storage is to be monitored continuously. Whenever a temperature deviation occurs, Partner Therapeutics Inc. should be contacted. Partner Therapeutics Inc. might allow further use of the medication vials depending on the duration and intensity of the temperature excursion. The co-ordinating investigator should be informed of this deviation as well.

8.1.8. Known side effects of the medication

To date, there have been no new safety signals associated with LEUKINE® (sargramostim). Observed side effects with aerosolized LEUKINE® at 250mcg dose and in at least one evaluation have included: bronchospasm, cough, dyspnea, a decrease in vital capacity and/or forced expiratory volume associated with bilateral infiltrates, pleural effusions, increased phlegm, throat irritation, and back pain.

8.2. Concomitant / Rescue Medication

There are no restrictions regarding concomitant/rescue medication.

9. Study Specific Procedures

Patients will be informed about the study by the treating physician.
After receiving full explanation, having received sufficient time to considerer the trial, asking questions and receiving satisfying responses to all questions, patients will be asked to sign ICF.
The ICF process will be performed before any other study related procedure.

9.1. Randomization

In this open label trial patients will be randomized in a 1:1 ratio. Randomization in Belgium will be done using REDCap (electronic IVRS system).

9.2. Study specific interventions

This is a hospital based intervention trial, in which patients with COVID-19 will be treated at least for 5 days with sargramostim. Patients with COVID-19 infection and respiratory failure are severely ill, and will require multiple daily clinical exams, blood sampling, vital parameter measurements, blood oxygenation measurements, and chest X-rays. These are all part of the clinical management plan of the patients, and data stored in the electronic patient file will be used as part of the assessment of efficacy and safety profile of sargramostim.

On screening, blood sample will be taken, preferentially during routine blood sampling, to determine exclusion criteria (pregnancy, high ferritin level).

On day 1, prior to sargramostim treatment in group A, and during the day in group B control patients, a tube of blood serum (5 ml) and an EDTA tube (10 ml) will be collected for measuring blood cytokine and chemokine levels, and activation of immune cells in selected centers. Also in each center, an arterial blood gas determination via arterial puncture will be taken. This sample should be taken in an upright position, while breathing room air for a minimum of 3 minutes. If this is impossible due to dependency on supplemental oxygen, FiO2, oxygen flow rate, and method of oxygen delivery should be noted in the patient file. If arterial blood gas is taken within 24h before first dose administration, the arterial blood gas of screening can be used as D1 value.

Method	O2 flow (l/min)	Estimated FiO2 (%)
Nasal	<= 1,5	24
	> 1,5 and <= 2,5	28
	> 2,5 and <= 3,5	32
	> 3,5 and <= 4,5	36
	> 4,5 and <= 5,5	40
	> 5,5 and <= 15	44
Nasal pharyngal cannula	<= 0,5	24
	> 0,5 and <= 1	28
	> 1 and <= 1,5	30
	> 1,5 and <= 2,5	32
	> 2,5 and <= 3,5	36
	> 3,5 and <= 4,5	40
	> 4,5 and <= 5,5	50
	> 5,5 and <= 6	60
Face mask	<= 1,5	24
	> 1,5 and <= 2	28
	> 2 and <= 2,5	30
	> 2,5 and <= 3,5	32
	> 3,5 and <= 4	36
	> 4 and <= 4,5	38
	> 4,5 and <= 5,5	40
	> 5,5 and <= 7	50
	> 7	60
Face mask with reservoir	<= 2,5	28
	> 2,5 and <= 3,5	32
	> 3,5 and <= 4,5	40
	> 4,5 and <= 5,5	50
	> 5,5 and <= 6,5	60
	> 6,5 and <= 7,5	70
	> 7,5 and <= 8,5	80
	> 8,5 and <= 9,5	90
	> 9,5	95

On day 6 or on day of discharge before day 6 , a tube of blood serum (5ml) and an EDTA tube (10 ml) will be collected for measuring blood cytokine and chemokine levels, and activation of immune cells in selected centers. Also in each center, an arterial blood gas determination via arterial puncture will be taken.

On days 1-5, patients in group A will inhale sargramostim 125mcg twice daily for 5 days as a nebulized inhalation using a Philips InnoSpire Go portable mesh nebulizer on top of standard of care. This device is a handheld mesh nebulizer that can be fitted with a facial mask. Patients will be instructed prior to receiving the first dose on how to use this simple device, by a physician. This procedure is finished in 5-10 minutes, and will be performed twice daily, in the morning (between 6 a.m. and 11 a.m.) and evening (between 6 p.m. and 11 p.m.).

Upon progression of disease requiring initiation of non-invasive or invasive mechanical ventilatory support within the 5 day period, in patients in the active group, inhaled sargramostim will be replaced by intravenous sargramostim 125mcg/m^2 body surface area once daily until the 5 day period is reached. This administration will occur via a centrally placed catheter or peripheral catheter, that will be in place as part of routine medical care at the ICU.

On a final clinical visit between week 10-20 an additional serum tube (5ml) and an EDTA tube (10 ml) will be taken in selected centers.

9.3. Overview of collected data

1. patient demographics
   age, sex, ethnicity, day of admission
2. day of COVID-19 PCR positivity, and conversion to negative
2. patient biometry
   weight, length, BMI, body surface area
3. Clinical and laboratory parameters on screening day and during trial
   -first day of illness, potential source of infection
   -clinical examination findings (cyanosis, crepitation's and rales, heart murmurs, peripheral edema)
   -vital signs (temperature, blood pressure, heart rate, breathing rate)
   -pulse oximetry data (SaO2)
   -clinical blood gas sampling (PaO2, PaCO2, HCO3)
   -clinical chemistry sampling (ferritin, leukocyte formula, platelets, kidney and liver function, fibrinogen, triglycerides)
   -Chest X-ray and/or CT characteristics and radiology clinical report
   -in case of admission to ICU : invasive monitoring data (arterial blood pressure, PCWP, continuous O2 saturation, continuous ECG, ventilatory parameters (tidal volume, FiO2, PEEP pressure, peak pressure, minute ventilation)
4. All standard care drugs used during the trial and on day of enrolment of the trial, including oxygen flow rate.
5. Basic clinical data on prior medical history (prior lung diseases, smoking history, prior lung function measurements (preferentially within 5 preceding years), prior gas exchange measurements) and medication use will be collected from electronic medical record.

6. Study specific measurements
On serum samples from selected centers, at each of the indicated timepoints exploratory analysis may include but is not limited to:

- Anti-drug antibodies (ADA) (D1-D6-FU) using ELISA
- Quantification of sRAGE (D1-D6-FU) using ELISA
- Quantification of Ang2 (D1-D6-FU) using ELISA
- Quantification of MUC1 (D1-D6- FU) using ELISA
- Quantification of GDF15 (D1-D6- FU) using ELISA
- Quantification of suPAR (D1-D6-FU) using ELISA
- Quantification of cytokines GM-CSF, IL-1b, IL1RA, IL-6, IL-8, IL-10, IL12p40, IL17A, IL-18, IL23p19, CCL2, CXCL9, CXCL10, INFgamma, TNF (D1-D6-FU) using Luminex at VIB-UGhent.
- Quantification of IFNalpha2 (D1-D6-FU) using SIMOA
- Quantification of GM-CSF (D1-D6-FU) using MSD.
- Quantification of S1 specific IgG and IgA antibodies (D1-D6-FU) using ELISA
- Quantification of NCP specific IgG (D1-D6-FU) using ELISA

- will be measured on samples collected at the various centers at day 1 or day 6 or discharge (whichever comes first) and at 12-22 weeks follow up visit.

Peripheral blood mononuclear cells will be prepared at UZ Gent, following shipment of EDTA-blood samples by selected centers, for Immunomonitoring purposes on D1, and D6 (or at hospital discharge, if earlier) and follow up visit. PBMC samples will be vitally frozen until analysis. When all samples are available from the selected centers, they will be thawed and PBMCs will be analyzed by the Primary Immunodeficiency Lab at UZ Ghent, in collaboration with the VIB-UGent Center for Inflammation Research. VIB-UGhent will perform some these assays as a service to UZGhent labs. Analyseswill include :

- flow cytometry analysis of numbers of peripheral blood lymphocyte and monocyte subsets, and their activation status by flow cytometry
- ELISPOT assays to measure the number of IFNg, TNFa, IL-2 and GM-CSF producing CD4 and CD8 T cells following restimulation of frozen and thawed peripheral blood mononuclear cells (PBMC) with a SARS-CoV2 megapool of immunogenic peptides.

9.4. Schematic overview of the data collection & interventions

10. Procedure	Screening	D1	D2-D5	D6/discharge	D7-D9	D10	Follow-up (10-20 weeks after D1)
Informed consent	X						
Inclusion/exclusion criteria	X						
Randomization		X					
Medical history & biometry	X						
Anamnesis and (S)AE inquiry	X	→	→	→	→	→	X
Concomitant medication	X	→	→	→	→	→	X
Physical examination[l]	X	→	→	→	→	→	X
6 Minutes Walk Test [o, l]		→	→	→	→	→	X
Vital signs [a]	X	→	→	→	→	→	X
ECG[l]	On clinical grounds						
Chest X-ray and/or (HR)CT scan	On clinical grounds						X [m]
Lung function[l]							X
Routine laboratory assessments[l] on clinical grounds, except: - screening/day 1 and day 6/discharge	X	X[b]		X[c]			X[c]
Serum pregnancy test	X						
Study blood sampling - 5 ml serum tube - 10 ml EDTA tube **(selected centers only)**		X		X			X
Arterial blood gas [d]		X		X			X
Score assessments							
- 6-point ordinal scale [e]	→	→	→	→	→	→	X
- Clinical sign score [f]	→	→	→	→	→	→	X
- NEWS2 score [g]	→	→	→	→	→	→	X
- SOFA score [h]		X		X		X	X
- HScore [i]		X		X			X
- CURB-65 [j]		X					

- APACHE II [k]		X					
- Glasgow Coma Scale	→	→	→	→	→	→	X
- HRCT fibrosis score[m]							X
IMP[n]		(X)	(X)	(X)	(X)	(X)	
Drug compliance		→	→	→	→	→	

[a] Includes morning assessment (7-10 am) of T°C (actual and highest last 24h), Pulse rate, Blood Pressure, Respiratory Rate, SpO2 by pulsoximetry. Time point assessment (7-10 am) is not applicable for the Follow-Up visit.

[b] Should minimally include haemoglobin, platelets, WBC count, eosinophils, lymphocytes, CD4 and CD8 T cell count, CRP, bilirubin, AST, ALT, LDH, creatinine, fibrinogen, triglycerides, ferritin, ureum, troponin, D-dimers.

[c] Should minimally include haemaglobin, hematocrit, platelets, WBC count, neutrophils, eosinophils, lymphocytes, CD4 and CD8 T cell count, CRP, bilirubin, AST, ALT, LDH, creatinine, fibrinogen, triglyceride, ferritin, troponin, D-dimers, ESR, CK

[d] Patient sitting upright breathing room air for a minimum of 3 minutes. If this is impossible due to dependency to supplemental oxygen, FiO2, oxygen flow rate, and method of oxygen delivery should be noted in the patient file. If arterial blood gas is taken within 24h before first dose administration, the arterial blood gas of screening can be used as D1 value.

[e] Defined as 1 = Death; 2 = Hospitalized, on invasive mechanical ventilation or ECMO; 3 = Hospitalized, on non-invasive ventilation or high flow oxygen devices; 4 = Hospitalized, requiring supplemental oxygen; 5 = Hospitalized, not requiring supplemental oxygen; 6 = Not hospitalized.

[f] (0-18) by scoring 6 clinical signs from 0 to 3 (0 = absent, 1 = mild, 2 = moderate and 3 = severe): Fever (0 = <37°C; 1 = 37.1-38°C; 2 = 38.1-39°C; 3 = >39°C) last 24h; Cough; Fatigue; Shortness of breath; Diarrhea; Body pain.

[g] NEWS2 (see https://www.mdcalc.com/national-early-warning-score-news-2): requires RR, SpO2, T°C, SBP and pulse.

[h] SOFA score (see https://www.mdcalc.com/sequential-organ-failure-assessment-sofa-score): requires PaO2, FiO2, platelet count, GCS, bilirubin, MAP and creatinine.

[i] HScore (see https://www.mdcalc.com/hscore-reactive-hemophagocytic-syndrome): requires T°C, haemoglobin, WBC count, platelets, ferritin, triglycerides and AST (BM aspirate is not required).

[j] CURB-65 (see https://www.mdcalc.com/curb-65-score-pneumonia-severity): requires confusion, BUN, RR, blood pressure and age.

[k] APACHE II (see https://www.mdcalc.com/apache-ii-score): requires age, T°C, MAP, pH, pulse, RR, sodium, potassium, hematocrit, creatinine, WBC count, GCS and FiO2.

[l] Intervention is standard of care and is being performed regardless of inclusion in the study.

[m] Preferably HRCT if done per standard of care, which will be used to evaluate fibrosis at follow visit. This is a subjective assessment of the overall extent of normal attenuation, reticular abnormalities, honeycombing and traction bronchiectasis will be performed. A reticular abnormality is defined as a collection of innumerable areas of small linear opacity. Honeycombing is defined as the presence of a cystic airspace measuring 3‑10 mm in diameter, with 1- to 3-mm thick walls. Traction bronchiectasis is defined as irregular bronchial dilatation within the surrounding areas showing parenchymal abnormalities. The morphological criteria on HRCT scans include bronchial dilatation with respect to the accompanying pulmonary artery, a lack of tapering of the bronchi and the identification of bronchi within 10 mm of the pleural surface. The HRCT findings will be graded on a scale of 1‑4 based on the classification system: 1. normal attenuation; 2. reticular abnormality; 3. traction bronchiectasis; and 4. honeycombing. The presence of each of the above four HRCT findings will be assessed independently in three (upper, middle and lower) zones of each lung. The upper lung zone is defined as the area of the lung above the level of the tracheal carina, the lower lung zone is defined as the area

of the lung below the level of the inferior pulmonary vein and the middle lung zone is defined as the area of the lung between the upper and lower zones. The extent of each HRCT finding will be determined by visually estimating the percentage (to the nearest 5%) of parenchymal involvement in each zone. The score for each zone will be calculated by multiplying the percentage of the area by the grading scale score. The six zone scores will be averaged to determine the total score for each patient. The highest score is 400 points and the lowest score is 100 points using this calculation method. The total score is the "HRCT fibrosis score". (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3922654/pdf/1465-9921-15-10.pdf)

[n] Patients randomized in the treatment group will receive inhaled sargramostim from D1 untill D5. In case of progression requiring mechanical ventilation within the first 5 days, IV sargramostim can be initiated until the 5 day period is reached. From day 6 onwards, progressive patients in the active group will have the option to receive an additional 5 days of IV sargramostim, based on the treating physician's assessment. Patients in the control group will have the option to receive 5 days of IV sargramostim in case of progression requiring mechanical ventilation, based on the treating physician's assessment. IMP should always be administered after other assessments, where possible.

[o] 6 MWT: to assess the distance walked over 6 minutes as submaximal test of aerobic capacity/endurance.

10.1. Restrictions for subjects during the study

There are no subject restrictions during this trial.

11. Sampling

11.1. Types and number of samples

D1: serum blood sample 5ml, EDTA blood sample 10 ml
D6 or discharge before day 6 : serum blood sample 5ml, EDTA blood sample, 10 ml
W10-20 follow-up visit : serum blood sample 5ml, EDTA blood sample, 10 ml

EDTA blood samples should only be collected in selected sites.

11.2. Timepoints of sampling

These samples are to be taken on D1 and D6 (or discharge if before day 6) and on final follow up visit between week 10 and 20. There's no time window allowed.

11.3. Sample Handling & Analysis

In all centers samples will be taken during hospitalization together with the blood draw for standard of care.

After clotting for 30-60 minutes the samples will be processed at 1770 g during 10 minutes at room temperature. 3 aliquots will be filled and frozen at -80°C until further analysis.
Centrifugation and storage will be done by qualified personal.

EDTA blood samples (only for selected sites) will be processed to purify peripheral blood mononuclear cells (PBMC) by gradient centrifugation and stained for flow cytometric analysis of number of monocytes, HLA-DR expression on monocytes and dendritic cells, and lymphocyte activation, described under 4.3. Flow cytometry will be performed on paraformaldehyde fixed samples.

Multiple cytokines and chemokines will be measured by multiplex bead based ELISA assay, described under 4.3. Development of anti-drug antibodies (ADA) will be measured using protocol developed by PartnerTherapeutics on serum samples taken at day 1 and follow up visit.

11.4. Sample Storage and/or shipping

Serum samples will be stored at minus 20 degrees temperature at the participating research centers. These samples will be shipped to sponsor at regular intervals, and in any case at the end of the study. In selected samples, EDTA blood will be collected for flow cytometry analysis and shipped to the PID lab at UZGhent for purification of PBMCs, freezing and later flow cytometry. These samples will be shipped same day (with a 24h tolerance) to UZGhent.
Frozen PBMC's may also be analyzed in specific participating centers if this was agreed with PI of the study. Storage conditions of frozen PBMCs is at -80°C prior to thawing and flow cytometry analysis.

For all sites where PBMC manipulation is being done at site for analysis at that site, samples will be destroyed at the end of the study. For samples which go directly to PID lab at UZGhent, for purification of PBMCs, freezing and later analysis. VIB shall help in the analysis of flow cytometry experiments, but

will not become the owner of the samples, nor of the data ensuing from those samples. At all times, UZGhent remains the owner of the samples and data resulting from these analyzes by VIB.

11.5. Future use of stored samples

Initially samples will be stored for the use as described within this protocol. If at a later time point samples will be stored for future use, they will be stored in a FAGG certified biobank. In that scenario, the Ethics comite of UZGhent will be asked for permission to store the material in a FAGG certified biobank. If permission is not granted, samples will be destroyed after completion of the study.

12. Statistical Considerations

12.1. Sample size calculation

The outcome(s) on which the sample size calculation is based upon, is the primary endpoint measurement of oxygenation, defined as ratio of Pa02/FiO2 and P(A-a)O2.
Sample calculation and power analysis have been performed using Genstat. The target difference is the difference measured at the primary endpoint (at day 6) between the control and the treated group. Given a sample size of 40 patients each, a minimal improvement of 10% in the treated group relative to the control group will be detected as significant at a significance level of 0,01 with a power of 0.90. The error variance was set at 100 units, corresponding with a standard deviation of 10 units.
The post-treatment evaluations should be assessed within 24 hours of the last dose of treatment. That is, Day 6 will be the timepoint for measures of efficacy endpoints based on 5 days of treatment, and Day 10 for patients who complete 10 days of treatment. If the patient is discharged from hospital prior to the day 6 (or day 10) efficacy evaluations , the values at day of discharge will be used as value for measuring efficacy endpoints.

12.2. Type of statistical methods

All endpoints will be summarized and where relevant represented graphically

A detailed statistical analysis plan (SAP) has been set up by EffectStats LLC, Cambridge Massachusetts, USA. The statistical tests to be used to look at improvements in oxygenation between treatment groups will be a Chi-square test. Mortality frequencies will be analyzed using Wald test.

Key timepoints of interest for endpoints include Day 6 and Day 11, where data are available. All available efficacy data will be tabulated and presented for all patients in the mITT Population.

Oxygenation after 5 days of Sargramostim Intervention

To measure the effectiveness of sargramostim on restoring lung homeostasis, the primary endpoint of this intervention is measuring oxygenation after 5 days of inhaled (and intravenous) treatment through assessment of pretreatment and post-treatment ratio of PaO_2/FiO_2, SpO_2/FiO_2 and through measurement of the $P(A\text{-}a)O_2$ gradient. During the 5 day treatment period, daily measurements of oxygen saturation (pulse oximetry) in relation to FiO_2 will be performed. Negative value of $P(A\text{-}a)O_2$ gradient would be removed from the primary analysis.
$P(A\text{-}a)O_2$ gradient, PaO_2/FiO_2 ratio and SpO_2/FiO_2 are defined as:
$P(A\text{-}a)O_2$ gradient=$[(FiO_2) \times (\text{Atmospheric Pressure} - H_2O \text{ Pressure}) - (PaCO_2/0.8)] - PaO_2$
PaO_2/FiO_2=Partial Pressure Oxygen/Fraction of Inspired Oxygen*100
SpO_2/FiO_2 = Oxygen Saturation/ Fraction of Inspired Oxygen*100
Comparison will be between active group A receiving sargramostim on top of standard of care and control group B receiving standard of care. The change from baseline and daily change from baseline in oxygenation/respiratory parameter of $P(A\text{-}a)O_2$ gradient, ratio of PaO_2/FiO_2 and ratio of SpO_2/FiO_2 will be evaluated and summarized between group A and group B at Day 6. The difference of change from baseline between two groups will be tested by a t-test. In general, the last observed measurement prior to the first dose of study treatment occurred on Day 1 visit will be considered the baseline measurement. Day 6 is the primary analysis point. If the patient leaves hospital prior to the day 6 analysis point, oxygenation at day of discharge will be used as value for measuring primary endpoint.
The analyses described above will also be performed between group A (early 5 day intervention with sargramostim) and group D (late 5 day intervention with sargramostim). Change from baseline (D1) to Day 10, daily change from baseline (D1) at Day 10 in oxygenation/respiratory parameter of $P(A\text{-}a)O_2$

gradient, ratio of PaO_2/FiO_2 and ratio of SpO_2/FiO_2 will be summarized as well. A t-test will be conducted to compare the difference between the groups.
The same analyses will be repeated for pathological oxygenation parameter of $P(A\text{-}a)O_2$ gradient, ratio of PaO_2/FiO_2 and ratio of SpO_2/FiO_2 including summary of their value and change from baseline at Day 6 and Day 10. A waterfall plot will be used to respent the change from baseline and percentage change from baseline on Day 6 for 1)oxygenation parameter of $P(A\text{-}a)O_2$ gradient; 2) pathological oxygenation parameter of $P(A\text{-}a)O_2$ gradient; 3) ratio of PaO_2/FiO_2; 4) ratio of SpO_2/FiO_2. Percentage change from baseline of $P(A\text{-}a)O_2$ gradient and ratio of PaO_2/FiO_2 on Day 6 and follow-up will be summarized.
Reasons of missing AA gradient values on Day 6 will also be summarized.
The normal value of AA gradient for room air is calculated as: 2.5+(0.21×Age). All the results including change from baseline, and maximum change from baseline after 5 day intervention with sargramostim, normal AA gradient value for room air and flag of abnormality on Day 6will be listed.
At least 25% reduction from baseline, at least 33% reduction from baseline, and at least 50% reduction from baseline in $P(A\text{-}a)O_2$ gradient and pathological gradient on Day 6 will be summarized for mITT population.

Survival status will be collected up to follow-up period (20 weeks after day 1). Death is considered as an event. All the mortality events and cause of death will be listed by treatment group and by patient. Number of patients died and survival time will be summarized by treatment group. Survival time will also be listed by patient. Risk and risk difference of all causes mortality by Day 28 and during the study period will also be summarized.
The hazard ratio will be estimated by the Cox proportional hazards model with treatment group as a covariate in the model. Relevant hazard rates, hazard ratio between treatment groups and associated p-values will be tabulated.

12.3. Statistical analysis team

The statistical analysis will be performed by EffectStats LLC, Cambridge Massachusetts, USA, under guidance of Dr Ella Li, statistician.

13. Data handling

13.1. Method of data collection

Subjects that are included in the study , will be assigned a unique study number upon their registration in REDCap.. On all documents submitted to the coordinating center, sponsor or CI, patients will only be identified by their study number. The subject identification list will be safeguarded by the site. The name and any other directly identifying details will not be included in the study database.

13.1.1. Case Report Form

An electronic data capture (EDC) system, i.e. REDCap, will be used for data collection. Data reported on each eCRF should be consistent with the source data. If information is not known, this must be clearly indicated on the eCRF. All missing and ambiguous data will be clarified.
Only the data required by the protocol are captured in the eCRF. The eCRFs and the database will be developed, based on the protocol. The final eCRF design will be approved by the Co-ordinating Investigator.

All data entries and corrections will only be performed by study site staff, authorized by the investigator. Data will be checked by trained personnel (monitor, data manager) and any errors or inconsistencies will be clarified. The investigator must verify that all data entries in the eCRF are accurate and correct.

REDCap is provided and maintained by Vanderbilt University; a license for use was granted to the Health, Innovation and Research Institute (HIRUZ). REDCap is a web-based system.
The study site staff is responsible for data entry in REDCap.

13.1.2. Data directly collected in the CRF (no source available)

N.A.

13.2. Data storage

The data is accessed through a web browser directly on the secure REDCap server. The server is hosted within the UZ Ghent campus and meets hospital level security and back-up requirements.

Privacy and data integrity between the user's browser and the server is provided by mandatory use of Transport Layer Security (TLS), and a server certificate issued by TERENA (Trans-European Research and Education Networking Association). All study sites will have access to REDCap. Site access is controlled with IP restriction.

13.3. Archiving of data

The investigator and sponsor specific essential documents will be retained for at least 25 years. At that moment, it will be judged whether it is necessary to retain them for a longer period, according to applicable regulatory or other requirement(s).

13.4. Access to data

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit study-related monitoring, audits and inspections.

Login in REDCap is password controlled. Each user will receive a personal login name and password and will have a specific role which has predefined restrictions on what is allowed in REDCap. Furthermore, users will only be able to see data of subjects of their own site. Any activity in the software is traced and transparent via the audit trail and log files.

For access to the pseudonymized data needed for statistical analysis, a separate data transfer agreement is in place between UZGhent and EffectStats LLC. UZGhent owns the data, and the results of the statistical analysis, and EffectStats LLC is not allowed to share these data with third parties. Data generated at the VIB Center for Inflammation research (results of ELISA analysis, results of flow cytometry analysis) remain property of UZ Ghent.

14. Safety

14.1. Definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a subject to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Unexpected Adverse Event	An adverse event, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational product or package insert/summary of product characteristics for an approved product).
Adverse Reaction (AR)	An untoward and unintended response in a subject to an investigational medicinal product which is related to any dose administered to that subject. The phrase "response to an investigational medicinal product" means that a causal relationship between a study medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the study medication qualify as adverse reactions.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • results in death • is life-threatening • requires inpatient hospitalisation or prolongation of existing hospitalisation • results in persistent or significant disability/incapacity • consists of a congenital anomaly or birth defect Other 'important medical events' may also be considered serious if they jeopardise the subject or require an intervention to prevent one of the above consequences. NOTE: The term "life-threatening" in the definition of "serious" 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 which hypothetically might have caused death if it were more severe.
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the study treatments, based on the information provided.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out: • in the case of a product with a marketing authorisation, in the summary of product characteristics (SmPC) for that product • in the case of any other investigational medicinal product, in the investigator's brochure (IB) relating to the study in question

Attribution definitions
An adverse event is considered associated with the use of the drug if the attribution is possible, probable or definitive.

Not related
An adverse event which is not related to the use of the drug.

Unlikely
An adverse event for which an alternative explanation is more likely - e.g. concomitant drug(s), concomitant disease(s), and/or the relationship in time suggests that a causal relationship is unlikely.

Possible
An adverse event which might be due to the use of the drug. An alternative explanation - e.g. concomitant drug(s), concomitant disease(s), - is inconclusive. The relationship in time is reasonable; therefore the causal relationship cannot be excluded.

Probable
An adverse event which might be due to the use of the drug. The relationship in time is suggestive (e.g. confirmed by dechallenge). An alternative explanation is less likely - e.g. concomitant drug(s), concomitant disease(s).

Definitely
An adverse event which is listed as a possible adverse reaction and cannot be reasonably explained by an alternative explanation - e.g. concomitant drug(s), concomitant disease(s). The relationship in time is very suggestive (e.g. it is confirmed by dechallenge and rechallenge).

14.2. Reporting requirements

14.2.1. AE reporting

AE's will be recorded from the first drug administration until the end of the study, as defined in section 5.2.
Special attention will be given to those subjects who have discontinued the study for an AE, or who experienced a severe or a serious AE. All AE's should be recorded in the patient's file and in the CRF.

14.2.2. SAE reporting

SAE's occurring during the entire study period will be reported as below.

All serious adverse events (initial and follow up information) and pregnancies occurring during this study must be reported by the local Principal Investigator within 24 hours after becoming aware of the SAE to:

- The local ethics committee (it is the responsibility of the local PI to report the local SAE's to the local EC)
- HIRUZ CTU of the University Hospital Ghent
- The National Coordinating Investigator (in case of multicenter studies)
  The company Partner Therapeutics that provides the IMP

This reporting is done by using the appropriate SAE form. For the contact details, see below.

14.2.3. SUSAR reporting

In case the Coordinating Investigator, in consultation with HIRUZ CTU, decides the SAE is a SUSAR (Suspected Unexpected Serious Adverse Reaction), HIRUZ CTU will report the SUSAR to the Central EC and the FAMHP within the timelines as defined in national legislation. The Coordinating Investigator reports the SUSAR to all local PI's.
In case of a life-threatening and fatal SUSAR the entire reporting process must be completed within 7 calendar days. In case of a non life-threatening SUSAR the reporting process must be completed within 15 calendar days.

14.3. List of contact details for safety reporting

HIRUZ CTU:
Ghent University Hospital
C. Heymanslaan 10, 1K5
9000 Ghent, Belgium
e-mail: hiruz.ctu@uzgent.be
Tel: +32 9 332 05 00
Fax: +32 9 332 05 20

Coordinating Investigator:
Prof. dr. Bart Lambrecht
Ghent University Hospital
Department of pneumology
C. Heymanslaan 10, 1K5
9000 Ghent, Belgium
email: bart.lambrecht@ugent.be
Tel: +32 9 332 91 10

Marketing Authorisation Holder:
Partner Therapeutics,
Dr. Debasish Roychowdhury
e-mail: Debasish.Roychowdhury@partnertx.com
Tel: +16107721703

14.4. Flowchart Reporting

Type of Adverse Event	*Action to be taken*
AE	List all AE's per subject in the patient's file and add this information to the CRF.
SAE	Notify to HIRUZ CTU within 24 hours after becoming aware of the SAE + add the SAE to a list that will be reported yearly (see section 13.8)
SAR	Notify to HIRUZ CTU within 24 hours after becoming aware of the SAE → HIRUZ CTU will submit to the central EC

	→ study team informs company that provides the IMP
SUSAR	Notify to HIRUZ CTU within 24 hours after becoming aware of the SUSAR → HIRUZ CTU will submit to the central EC. → HIRUZ CTU will submit to the FAMHP → study team informs company that provides the IMP

In case the (SU)SAR occurs at a local participating site, the local PI or study team should also contact:

- The local Ethics Committee
- The Co-ordinating Investigator

14.5. Events, excluded from reporting

COVID-19 infection is a very recent syndrome, on which few data are available. Normal symptoms and natural disease course symptoms that will not be reported as adverse events are dyspnea, coughing, malaise, fever, drop in oxygen saturation, progression to respiratory failure, progression to ARDS, severe drop in blood pressure in the ICU,progression to multi-organ failure.

14.6. Data Safety Monitoring Board (DSMB)

All study medication is registered and used in current practice. Despite the known safety profile of the study medications and study design, a DSMB is foreseen.

14.7. Development Safety Update Report

The Coordinating Investigator will provide DSURs once a year throughout the entire duration of the clinical study, or on request, to the Competent Authority (FAMHP in Belgium), Ethics Committee and Sponsor. This DSUR will include all SAE's (who were not categorized as SAR's and were not immediately reported to the EC).

The report will be submitted 1 year (+ maximum 60 days) after the 'Development International Birth Date (DIBD)' , and will subsequently be submitted each year until the study is declared ended. This DIBD is the date of the sponsor's first overall authorisation to conduct the clinical trial in any country worldwide. HIRUZ CTU can provide a template that can be used to complete this DSUR.

15. Monitoring/Auditing/Inspection

15.1. Monitoring

15.1.1. General

Monitoring of the study will be performed in compliance with GCP E6(R2) and the applicable regulatory requirements. The study team will be trained in an initiation visit by the monitor. A training and delegation log will be held. A detailed description of the monitoring tasks can be found in the latest version of the (study-specific) 'Monitoring plan'.

15.1.2. Monitoring team

Monitoring services will be provided by HIRUZ CTU. All relevant contact details (e.g. primary contact person, can be found in the 'Monitoring plan'.

15.1.3. Scope

Monitoring services will consist of the following (non-exhaustive list):
- review of informed consents and the followed process
- check on recruitment status
- checking for protocol deviations/violations
- checking GCP compatibility
- check on safety reporting compliance
- IMP handling and storage
- review of study data

…

15.2. Inspection

This study can be inspected at any time by regulatory agencies during or after completion of the study. Therefore access to all study records, including source documents, must be accessible to the inspection representatives. Subject privacy must be respected at all times, in accordance to GDPR, GCP and all other applicable local regulations.
The investigator/study team should immediately notify the sponsor if he or she has been contacted by a regulatory agency concerning an upcoming inspection.

15.3. Protocol Deviation policy

Sponsor and all investigators agree to take any reasonable actions to correct protocol deviations/violations noted during monitoring/inspection, in consultation with the monitoring team. All deviations must be documented on a protocol deviation log by the study team that is kept available at any time for monitoring/inspection purposes. Under emergency circumstances, deviations from the protocol to protect the rights, safety or well-being of human subjects may proceed without prior approval of the sponsor and the EC.

15.4. Serious breach to GCP and/or the protocol

Critical issues that significantly affect patient safety, data integrity and/or study conduct should be clearly documented and will be communicated with the Coordinating Investigator, HIRUZ CTU and possibly both the applicable Ethics Committee(s) and Competent authority. (Please contact HIRUZ CTU asap in case of a serious breach: hiruz.ctu@uzgent.be and/or +3293320500).
Early determination of the study (in a specific center or overall) may be necessary in case of major non-compliance.

16. Ethical and legal aspects

16.1. Good Clinical Practice

The study will be conducted cfr the latest version of the ICH E6 (R2) GCP guidelines, creating a standard for the design, conduct, performance, monitoring, auditing, recording, analyses and reporting of clinical studies that provides assurance that the data and reported results are accurate and that the rights, integrity and confidentiality of study subjects are protected.

16.2. Informed Consent

Eligible subjects may only be included in the study after providing written (witnessed, if needed) Ethics Committee-approved informed consent, or, if incapable of doing so, after such consent has been provided by a legally acceptable representative(s) of the subject.
Informed consent must be obtained before conducting any study-specific procedures (as described in this protocol).

Prior to entry in the study, the investigator must explain to potential subjects or their legal representatives the study and the implication of participation. Subjects will be informed that their participation is voluntary and that they may withdraw consent to participate at any time. Participating subjects will be told that their records may be accessed by competent authorities and by authorized persons without violating the confidentiality of the subject, to the extent permitted by the applicable law(s) and/or regulations. By signing the Informed Consent Form (ICF), the subjects or legally acceptable representatives are authorizing such access.
After this explanation and before entry to the study, written, dated and signed informed consent should be obtained from the subject or legally acceptable representative. The ICF should be provided in a language sufficiently understood by the subject. Subjects must be given the opportunity to ask questions.
The subject or legally acceptable representative will be given sufficient time to read the ICF and to ask additional questions. After this explanation and before entry to the study, consent should be appropriately recorded by means of either the subject's or his/her legal representative's dated signature or the signature of an independent witness who certifies the subject's consent in writing. After having obtained the consent, a copy of the ICF must be given to the subject.
In case the subject or legally acceptable representative is unable to read, an impartial witness must attest the informed consent.
Subjects who are unable to comprehend the information provided or pediatric subjects can only be enrolled after consent of a legally acceptable representative.
The following information should be added to the electronic patient dossier (EPD):

- which version of the ICF was obtained
- who signed the ICF
- if sufficient time has been given to consider participation into the study
- which investigator obtained ICF with the date of signature
- if a copy was provided to the patient
- start and end of participation in the study

16.3. Approval of the study protocol

16.3.1. General

The protocol has been reviewed and approved by the Ethics Committee of the Ghent University (Hospital), designated as the central Ethics Committee, after consultation with the local Ethics Committees, and the Federal Agency for Medicine and Health Products (FAMHP). This study cannot start before both approvals have been obtained.

16.3.2. Protocol amendments

Any significant change or addition to the protocol can only be made in a written protocol amendment that must be approved by the Central Ethics Committee (and the FAMHP if applicable).
Only amendments that are intended to eliminate an apparent immediate safety threat to patients may be implemented immediately.
Notwithstanding the need for approval of formal protocol amendments, the investigators are expected to take any immediate action, required for the safety of any subject included in this study, even if this action represents a deviation from the protocol. These actions should always be notified to the sponsor.

16.4. Confidentiality and Data Protection

All study data will be handled in accordance with the law on General Data Protection Regulation (GDPR) and institutional rules
[Belgian law dated on 30 July 2018 and 22 Aug. 2002].

The collection and processing of personal data from subjects enrolled in this study will be limited to those data that are necessary to fulfill the objectives of the study. These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations.

Appropriate technical and organizational measures to protect the personal data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be put in place. Sponsor and site personnel whose responsibilities require access to personal data agree to keep the identity of subjects confidential.
The informed consent obtained from the subject includes explicit consent for the processing of personal data and for the investigator/institution to allow direct access to his or her original medical records (source data/documents) for study-related monitoring, audit, Ethics Committee review and regulatory inspection. This consent also addresses the transfer of the data to other entities, if applicable.

Privacy and confidentiality of data generated in the future on stored samples will be protected by the same standards applicable to all other clinical data.
Stored samples will be pseudonymized throughout the sample storage and analysis process and will not be labeled with personal identifiers.

16.5. Liability and Insurance

The sponsor has taken a no fault insurance for this study, in accordance with the relevant legislation (article 29, Belgian Law of May 7, 2004).
Sponsor: Ghent University Hospital
Insurance Details: Allianz Global Corporate & Specialty; Uitbreidingstraat 86, 2600 Berchem; Tel: +32 33 04 16 00
Polis number: BEL000862

16.6. End of Study Notification

If all subjects have completed the study, a notification of the end of the study should be submitted to the (Central) Ethics Committee and FAMHP. This notification should be made within 90 days of the end of the clinical study. In case of early termination (definition in CT-1, 4.2), this is reduced to 15 days.

17. Publication policy

This study will be registered at ClinicalStudies.gov, and results information from this study will be submitted to ClinicalStudies.gov. In addition, every attempt will be made to publish results in peer-reviewed journals.

18. Reference List

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Appendices

Appendix 1: USPI (US Package insert)

Statistical Analysis Plan

Study Protocol Number: SARPAC

Study Title:

A prospective, randomized, open-label, interventional study to investigate the efficacy of sargramostim (Leukine®) in improving oxygenation and short- and long-term outcome of COVID-19 patients with acute hypoxic respiratory failure.

Study Sponsor:

University Hospital Ghent.

Protocol Version 3.0 Date:
14 May 2020

Prepared by:

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77 Lakeview Ave
Cambridge, MA 02138 USA

Draft v0.7
05 March 2021

Table of Contents

1 Signature Page

Documents Prepared by:

Ruoxuan Qi, MS Statistician EffectStats, LLC.	Date

Reviewer

Printed Name	Title
Signature	dd-mmm-yyyy

Approval

The undersigned have reviewed and approved the Statistical Analysis Plan and find the document to be consistent with the requirements of the Protocol.

Printed Name	Title
Signature	dd-mmm-yyyy
Printed Name	Title
Signature	dd-mmm-yyyy

2 Amendment/Modification History

The following table documents any changes made to the previously approved versions of the document.

Version #	Page # / Section	Summary of Changes	Date
1.0	Original	NA	xx-xxx-2020

3 Abbreviations

AE : Adverse Event

AECC : American-European Consensus Conference

ALT: Alanine Aminotransferase

ARDS : Acute Respiratory Distress Syndrome

AST: Alanine Aminotransferase

CK: Creatine Kinase

COVID-19 : Coronavirus induced disease-2019

CRP: C-reactive protein

DSMB : Data Safety Monitoring Board

ECG : Electrocardiogram

ESR: Erythrocyte Sedimentation Rate

eCRF : electronic Case Report Form

FiO2 : Fraction of inspired oxygen

FVC : Forced vital capacity

GM-CSF : Granulocyte-macrophage colony stimulating factor

ICF : Informed Consent Form

ICU: Intensive Care Unit

LDH: Lactate Dehydrogenase

PaO_2 : Partial pressure of oxygen

SAE : Serious Adverse Event

SUSAR : Suspected Unexpected Serious Adverse Reaction

TLC : Total Lung Capacity

WBC: White Blood Cells

4 Introduction

The purpose of this statistical analysis plan (SAP) is to describe the procedures and statistical methodologies that will be used in the analysis and reporting of results for Protocol SARPAC.

This document is prepared based on the following documents:

- the study protocol version 3.0 dated 14 May 2020;
- the Case Report Form version 2.0 dated 08 April 2020.

Readers are referred to the final study protocol (and any amendments or addenda), the case report form (CRF), and CRF completion guidelines for details of the study design, conduct and data collection. Any significant changes to these documents in terms of the principle features of the study analyses may result in a SAP amendment; any other changes will be denoted in the Clinical Study Report as changes to the planned analyses.

This SAP must be finalized prior to the locking of the clinical database for this study. The mock summary tables, figures and by subject data listings (TFLs) are provided in a separate document.

5 Study Objectives and Endpoints

5.1 Primary Objective

The primary objective is to investigate whether the administration of sargramostim (Leukine®) improves oxygenation and short and long-term outcomes in COVID-19 patients with acute hypoxic respiratory failure.

5.1.1 Primary Endpoint

To measure the effectiveness of sargramostim on restoring lung homeostasis, the primary endpoint of this intervention is measuring oxygenation after 5 DAYS of inhaled (and intravenous) treatment through assessment of pretreatment (day 1) and post-treatment (day 6) ratio of PaO2/FiO2 and through measurement of the P(A-a)O2 gradient.

During the 5 day treatment period, daily measurements of oxygen saturation (pulse oximetry) in relation to FiO2 will be performed, and the slope of alterations in this parameters could also be an indicator of correctness of study hypothesis.

The post-treatment evaluations should be assessed within 24 hours of the last dose of treatment. That is, Day 6 will be the timepoint for measures of efficacy endpoints based on 5 days of treatment, and Day 10for patients who complete 10 days of treatment. If the patient is discharged from hospital prior to the day 6 (or day 10) efficacy evaluations , the values at day of discharge will be used as value for measuring efficacy endpoints.

5.1.2 Secondary Objectives & Endpoints

- to study if early intervention with sargramostim is safe (number of AEs/SAEs)
    - Incidence of AEs/SAEs.
- to study if early intervention with inhaled sargramostim affects clinical outcome
    - Length of hospital stay.
    - Mean change in 6-point ordinal scale between day 1 and day 6.
    - Mean change in clinical sign score between day 1 and day 6.
    - Time to clinical sign score<6 maintained for 24h.
    - Mean change of SOFA score between day 1 and day 6 or between day 1 and day 10.
    - Mean change NEWS2 score between day 1 and day 6 or between day 1 and day 10.
    - Time to NEWS2 score less than 2 for at least 24h.
- to study if early intervention with sargramostim affects the rate of nosocomial infection
    - Rate of nosocomial infection.
- to study if early intervention with inhaled sargramostim affects progression to mechanical ventilation and/or ARDS
    - Number of patients requiring initiation of mechanical ventilation.
    - Duration of invasive and non-invasive ventilation and/or supplemental oxygen.
- to study if treatment with sargramostim affects all cause mortality rate at 20 weeks post inclusion
    - All-cause mortality rate at 4 and 20 weeks post inclusion.
- to study if treatment with sargramostim affects features of secondary haemophagocytic lymphohistiocytosis, as defined by Hs score (temp, organomegaly, cytopenia, triglycerides, fibrinogen, ferritin, AST and known immunosuppression)

  - Features of secondary haemophagocytic lymphohistiocytosis.
- to study if treatment with sargramostim has a favourable effect on long term 10-20 week follow up
  - Clinical exams performed at 10-20 weeks follow up.
  - Pulmonary function tests (including FVC, TLC and diffusion capacity) performed at 10-20 weeks follow up.
  - Laboratory tests (ferritin, lymphocytes, leukocytes) performed at 10-20 weeks follow up.

6 Study Design

6.1 Study Design Overview

This is phase 4 academic, prospective, randomized, open-label, interventional study designed to investigate the efficacy of sargramostim (Leukine®) in improving oxygenation and short- and long-term outcome of COVID-19 patients with acute hypoxic respiratory failure.

Patients with confirmed COVID19 with acute hypoxic respiratory failure (saturation below 93% on minimal 2 l/min O2 or PaO2/FiO2 <350) will be randomizied to receive sargramostim 125mcg twice daily for 5 days as a nebulized inhalation on top of standard of care (active group), or to receive standard of care treatment (control group). Upon progression of disease to requiring initiation of non-invasive or invasive mechanical ventilatory support within the 5 day period, in patients in the active group, inhaled sargramostim will be replaced by intravenous sargramostim 125mcg/m2 body surface area until the 5 day period is reached.

The number of subjects that will be included in this study is: 80.

These are divided into following sub-groups:

Group A: active sargramostim treatment group, treatment for initial 5 days, no deterioration after 5 days

Number of patients : 40

Group B: control group : no treatment with sargramostim in first 5 days

Number of patients : 40

Group C and D:

From day 6 onwards, progressive patients in the active group (Group A) will have the option to receive an additional 5 days of IV sargramostim 125mcg/m2 body surface area once daily, based on the treating physician's assessment. This group will be called group C. It is estimated that some 30% of patients might deteriorate and require noninvasive or invasive mechanical ventilation, resulting potentially in 12 patients that progress from group A to group C, if the clinician decides to move forward with the drug.

In the control group (Group B) progressing to requiring invasive or non-invasive mechanical ventilatory support, from day 6 onwards, the treating physician will have the option to initiate IV sargramostim 125mcg/m2 body surface area once daily for 5 days. This group will be called group D. It is estimated that some 30% of patients might deteriorate to mechanical ventilation or ARDS, resulting potentially in 12 patients that progress from group B to group D, if the clinician decides to move forward with the drug.

Comparisons between group A (early sargramostim) versus group B (no sargramostim) at day 6 will be important for reaching primary endpoint, and for key secondary endpoints. Comparisons of Group A (early 5 day intervention with sargramostim) with Group D (late 5 day intervention with sargramostim) will also be very informative for secondary endpoint analysis.

Refer to Section 5.1 of the Study Protocol for a more detailed description of the study. Section 1.6.2 of the Study Protocol is a schematic of the study design.

6.2 Randomization

In this open label trial patients will be randomized in a 1:1 ratio. Randomization in Belgium will be done using REDCap (electronic IVRS system).

6.3 Study Schedule

The scheduled assessments will be carried out during the study as described in Section 9.4 of the Study Protocol.

6.4 Duration of Treatment and Study

The total treatment duration of the study is 5 days, followed by possible 5 day extension upon deterioration. The entire study duration is 10-22 weeks to final follow up visit.

6.5 End of Study Definition

The subject has completed the study if he or she has completed all phases of the study, including the last visit (week 10-20 clinical follow up visit) or the last scheduled procedures (refer to protocol section “9. Study Specific Procedures”).

6.6 Study Drug Administration

Refer to Sections 8 and 9.2 of the Study Protocol.

6.7 Study Assessments

6.7.1 Safety Evaluations

The safety and tolerability of study drug in each dosing cohort will be evaluated through:

- Incidence of AEs/SAEs.
- Pulmonary function tests
- Laboratory tests
- Physical examination
- ECG
- Chest X-ray
- Vital signs (including height and weight)

Adverse events (AEs) will be collected from the signing of informed consent form (ICF) to last subject contact/visit/end of post-treatment follow-up period.

Clinical exam, pulmonary function tests (including FVC, TLC and diffusion capacity), and a laboratory test (ferritin, lymphocytes, leukocytes) will be performed on routine check-up by pulmonologist at 10-20 weeks after discharge from hospital. Safety data, including blood leukocyte counts, will be collected in all patients.

Physical examination and vital signs will be tested from screening to last subject contact/visit/end of post-treatment follow-up period.

ECG and chest X-ray will be collected on clinical ground.

Refer to study protocol section 9.4 for the detailed schematic overview of the data collection & interventions.

6.7.2 **Efficacy Evaluations**

To measure the effectiveness of sargramostim on restoring lung homeostasis, the primary endpoint of this intervention is measuring oxygenation after 5 DAYS of inhaled (and intravenous) treatment through assessment of pretreatment (day 0) and post-treatment (day 5) ratio of PaO2/FiO2 and through measurement of the P(A-a)O2 gradient.

The post-treatment evaluations should be assessed within 24 hours of the last dose of treatment. That is, Day 6 will be the timepoint for measures of efficacy endpoints based on 5 days of treatment, and Day 10 for patients who complete 10 days of treatment. If the patient is discharged from hospital prior to the day 6 (or day 10) efficacy evaluations , the values at day of discharge will be used as value for measuring efficacy endpoints.

Efficacy data will also be collected and will include arterial blood gases, oxygenation parameters, need for ventilation, lung compliance, organ function, radiographic changes, ferritin levels, triglyceride levels, etc. as well as occurrence of secondary bacterial infections.

7 Statistical Analysis Methods

7.1 General Considerations

All safety analyses will be based on Safety Population; all efficacy analyses will be based on mITT population, unless otherwise specified. Some specific sensitivity analyses of efficacy may be based on ITT (for primary endpoints only).

All analyses will be considered as descriptive analyses. Derivation of two-sided 95% confidence intervals and p-values will be generated where applicable.

Time to event endpoints will be defined as the start date/time to the end date/time; censoring dates will be the last date/time the patient was determined to be event-free. Kaplan Meier methods will be used for time to event endpoint analyses; a log-rank test will be performed to compare the two survival curves. Timepoints estimates and median survival will be derived from the Kaplan Meier analysis. A Cox proportional hazards model may also be used to compare the treatment groups using a hazard ratio.

Categorical endpoints will be calculated as the percentage of patients with the event, relative to the number of patients treated. Logistic regression approaches and/or repeated measures statistical approaches may be used to compare patients on the sargramostim and control arms, in addition to Fisher's Exact or Chi2 tests (as appropriate).

Continuous endpoints will be summarized by n, means, medians, minimum, maximum, and 25^{th} and 75^{th} percentiles. F-test and two sample t-test may be used to compare patients on the sargramostim and control arms.

In the event that the underlying assumptions and/or distributions for a given statistical method are not satisfied, alternative statistical methods will be employed.

Additional exploratory analyses may be performed to evaluate the robustness and sensitivity of the study results, including but not limited to the analysis populations, subgroup analyses, treatment interactions, adjusted or stratified analyses, and/or alternative statistical methods.

7.1.1 Study Day

Study day will be calculated as follows:

- For the sargramostim arm, first dose date is the first sargramostim dose date.
- For the control arm, the randomization date will be used as the first dose date.
- Assessments/events prior to the first dose date, study day will be the assessment date minus the first dose date. Assessments/events on or after the first dose date, study day will be the assessment date minus the first dose date plus one.

7.1.2 Baseline Definition

In general, the last observed measurement prior to the first dose of study treatment will be considered the baseline measurement unless otherwise specified. For assessments on the day of first dose where time is not captured, a nominal pre-dose indicator, if available, will serve as sufficient evidence that the assessment occurred prior to first dose.

Assessments on the day of the first dose where neither time nor a nominal pre-dose indicator are captured, will be considered prior to the first dose.

In all summaries change from baseline variables will be calculated as the post-treatment value minus the value at baseline. The % change from baseline will be calculated as (post-baseline value - baseline value) / baseline value x 100.

7.1.3 Analysis Period

The post-treatment evaluations should be assessed within 24 hours of the last dose of treatment. That is, Day 6 will be the timepoint for measures of efficacy endpoints based on 5 days of treatment, and Day 10 for patients who complete 10 days of treatment. If the patient is discharged from hospital prior to the day 6 (or day 10) efficacy evaluations , the values at day of discharge will be used as value for measuring efficacy endpoints.

The main analysis period would be the Treatment Emergent period which is defined as the period from the date of the first dose until the end of earliest of the following 1) the date of Day 28; or 2) Date of Early discontinuation from study. This analysis period will be used for all treatment emergent adverse event, laboratory evaluations and efficacy parameters.

For the primary endpoints, the data collected within first 6 days will be used for analysis.

7.1.4 Missing Data Handling

Missing data may be imputed using last-observation-carried forward, or other advanced statistical imputation methods for sensitivity analysis. For the primary endpoint, if assessments on Day 6 is not available, assessments on Day 5 will be used for analysis. If the patient leaves hospital prior to the day 6 analysis point, oxygenation at day of discharge will be used as value for measuring primary endpoint. Imputation for intubation rate and ordinal scale will not be performed.

When using last observation carried forward, a missing follow-up visit value will be imputed as that patient's previously observed value.

Regarding to time-to-event data, if no other specification in each section, the following rules will be used for missing data imputation:

- Patients who are not lost to followed up or experienced the event will be censored at the actual date of end of study visit.
- Patients who received no drug or standard of care will be excluded from analysis.
- Patients without an event but lost to follow-up will be censored at last date of follow-up.

7.2 Sample Size

The outcome(s) on which the sample size calculation is based upon, is the primary endpoint measurement of oxygenation, defined as ratio of Pa02/FiO2 and P(A-a)O2.

Sample calculation and power analysis have been performed using Genstat. The target difference is the difference measured at the primary endpoint (at day 6) between the control and the treated group. Given a sample size of 40 patients each, a minimal improvement of 10% in the treated group relative to the control group will be detected as significant at a significance level of 0,01 with a power of 0.90. The error variance was set at 100 units, corresponding with a standard deviation of 10 units.

The post-treatment evaluations should be assessed within 24 hours of the last dose of treatment. That is, Day 6 will be the timepoint for measures of efficacy endpoints based on 5 days of treatment, and Day 10 for patients who complete 10 days of treatment. If the patient is discharged from hospital prior to the day 6 (or day 10) efficacy evaluations , the values at day of discharge will be used as value for measuring efficacy endpoints.

7.3 Data Safety Monitoring Board

Despite the known safety profile of the study medications and study design, a DSMB reviewed the data.

7.4 Analysis Population

The following analysis populations will be used to summarize the results from this study.

- **Safety Population** includes all patients who received at least one dose of sargramostim and/or SOC based on actual treatment received. Patients who did not receive any study treatment (either sargramostim and/or SOC) will be excluded from Safety Population. All safety analyses will be based on the Safety Population.
- **Intent-to-treat Population (ITT)** includes all patients who were randomized. Selected efficacy analysis ($P(A\text{-}a)O_2$) will be performed based on ITT population for the purpose of sensitivity, unless otherwise specified.
- **Modified Intent-to-treat Population (MITT)** includes all patients who were randomized and received at least one dose of sargramostim and/or standard of care based on the treatment assigned at randomization. All efficacy analysis will be performed based on modified ITT population, unless otherwise specified.
- **Enrolled Population** includes all patients who were eligible and signed informed constent form (ICF).

If the ITT is identical, or less than 10% different compared to the MITT population, then the selected efficacy analyses may not be repeated across the ITT analysis population.

7.5 Patient Disposition

Descriptive statistics by treatment will be used to summarize the number of patients screened, the number of screening failures, the number of patients enrolled, the number of patients in Safety Population, ITT, and mITT, number of patients who completed treatment period, completed the study, withdrawal from treatment, and withdrawal from the study, and reasons for withdrawals. The descriptive statistics will include numbers and percentages of patients in each identified category by treatment groups. A patient's data listing will be provided for disposition that includes patients who are excluded from the analysis populations; who prematurely withdrew from the study and reasons for excluding each analysis set, and for early discontinuation (from treatment and from the study).

For study completion status, the following logic will be used:

- Patient will be considered completed the study, if all of the following met: 1) complete the study treatment, 2) complete the first 5 study days, and 3) have a follow-up visit;
- Patient study completion status will be considered ongoing, if 1) patient started the study, and 2) the data cutoff date is less than 140 days away from the patient's first visit date in the Day 1-5 period;
- Patient will be considered discontinued from the treatment, if any of the following met: 1) the last date of drug taken is before Day 6 if the patients are in Group A, 2) the last date of drug taken is before Day 10 if the patients are in Group C/ D, 3)the last visit date is before Day 6 if the patients are in the control arm.
- Patient will be considered discontinued from the study, if any of the following met: 1) the patient is considered discontinued from the treatment, or 2) didn't complete the study treatment and the study status is not ongoing, or 3) didn't have a follow-up visit and the study status is not ongoing.

For withdrawal from study reason, the following logic will be used:

- If a patient was discharged before Day 6, the withdrawal from study reason would be "Discharged before Day 6";
- If a patient completed study treatment but did not have a follow-up visit, the withdrawal from study reason would be "Lost to follow-up";
- Else, the withdrawal from study reason would be the same as the withdrawal from treatment reason.

Patients will also be summarized by enrollment calendar time and treatment group.

7.6 Protocol Deviations

A subject listing of protocol deviations data will be presented.

The following general categories will be considered important deviations and be listed and discussed in the CSR as appropriate for the study:

- Deviation 1: Patients randomized but who did not receive study drug or standard of care
- Deviation 2: Patients who deviate from the following key entry criteria:
    - Inclusion:

- Exclusion:

- Deviation 3: Patients randomized who received treatment other than that to which they were randomized to.

7.7 Demographics and Baseline Disease Characteristics

Demographic information for Safety Population will be summarized based on the first treatment that patients have received in the study. The demographic data consists of age, gender, race, ethnicity, along with baseline height, body weight, body mass index (BMI), Body Surface Area (BSA). The baseline the disease assessment scales, including the SOFA score (including categorization of <6 versus ⩾ 6) , ordinal scale, Hs Score, Clinical sign score, NEWS2 Score, CURB-65 score and APACHE II will be summarized.

Individual demographics and other baseline factors will be listed by patient.

Continuous variables (for example, age, height, body weight, body mass index, body surface area, disease assessment scales) will be summarized by n, means, medians, minimum, maximum, and 25^{th} and 75^{th} percentiles. Number of patients and percentages will be used to describe categorical (discrete) variables (for example, gender, race and ethnicity).

Individual demographics and baseline factrors will be listed by patient.

7.8 Medical History

Medical condition and/or significant medical history will be coded using Medical Dictionary for Regulatory Activities (MedDRA) Version 23.1, and listed by reported term, System Organ Class (SOC), and Preferred Term (PT). The number and percentage of patients will be summarized by SOC and PT by treatment group for Safety Population.

Baseline medical history will be summarized separately.

7.9 Concomitant Medications

All medications received after the consent to the study until the end of study will be coded using WHO Drug Enhanced Dictionary (WHODrug_20200901_B3) and categorized as Prior Medication, Concomitant Medication, or Post Medication based on the following:

- Prior medications include medications that have a start date and end date before the date/time of the first dose of the study treatment;
- Concomitant medications include medications that start date prior to, or after the date of first dose, that continues while the patient is on treatment (Day 10) and could continue on into follow up period;
- Post medications include medications that have a start date after the end date of the study treatment (Day 10).

Depending on the start and end date, a medication could be categorized as prior, concomitant, or post, or fall into more than 1 categories. For example, a medication with a start date prior to the first dose of study drug can be both prior and concomitant:if its end date is before the first dose of study drug, it would be prior medication; or it would be the concomitant medication only if the end date of the medication is after the first dose of the study drug.

Concomitant medications will be summarized by treatment group, Anatomical Therapeutic Chemical (ATC) classes, and Preferred Term. Prior and Post Medications will be included in the patient's listing of medications including the start and end dates, prior/concomitant/post flag, whether it is ongoing, dose, unit and indication.

7.10 Extend of Exposure and Treatment Compliance

Treatment duration and treatment compliance will be summarized by treatment group. For each patient, the treatment duration is defined as the number of days from the first treatment date to the last treatment date, and can be calculated as:

Treatment Duration (Days) = Date of Last Treatment – Date of First Treatment +1.

For the purpose of Day 1-5 treatment compliance though, the Date of Last treatment will be censored on Day 5 for patients who have taken sargoramostim beyond Day 5 (progressed and switched onto IV). For the overall treatment duration, the date of the last treatment will be either the date for the end of treatment record or the last treatment date in the database. For each patient the treatment compliance is defined as the actual treatment received as percentage of the planned treatment (K days). It can be calculated as:

Treatment Compliance = 100*(Treatment Duration– Number of Days without Sargramostim within the period)/K

In these calculations, the study treatment would be sargramostim. The study specified treatment duration of sargramostim is 5 days. Therefore, K = 5, in general. For discontinued patients, K would be equal to the number of days from the date the sargramostim study drug is first received to the date of early discontinuation. For patients who received sargramostim after day 5 (e..g, groups C and D), these data will be presented in a separate treatment duration and compliance table. The compliance for standard of care will not be calculated or summarized.

7.11 Efficacy Evaluations

Key timepoints of interest for endpoints include Day 6 and Day 11, where data are available. All available efficacy data will be tabulated and presented for all patients in the mITT Population.

7.11.1 Oxygenation after 5 days of Sargramostim Intervention

To measure the effectiveness of sargramostim on restoring lung homeostasis, the primary endpoint of this intervention is measuring oxygenation after 5 days of inhaled (and intravenous) treatment through assessment of pretreatment and post-treatment ratio of PaO_2/FiO_2, SpO_2/FiO_2 and through measurement of the $P(A\text{-}a)O_2$ gradient. During the 5 day treatment period, daily measurements of oxygen saturation (pulse oximetry) in relation to FiO_2 will be performed. Negative value of $P(A\text{-}a)O_2$ gradient would be removed from the primary analysis.

$P(A\text{-}a)O_2$ gradient, PaO_2/FiO_2 ratio and SpO_2/FiO_2 are defined as:

$P(A\text{-}a)O_2$ gradient=$[(FiO_2) \times (\text{Atmospheric Pressure} - H_2O \text{ Pressure}) - (PaCO_2/0.8)] - PaO_2$

PaO_2/FiO_2=Partial Pressure Oxygen/Fraction of Inspired Oxygen*100

SpO_2/FiO_2 = Oxygen Saturation/ Fraction of Inspired Oxygen*100

Comparison will be between active group A receiving sargramostim on top of standard of care and control group B receiving standard of care. The change from baseline and daily change from baseline in oxygenation/respiratory parameter of $P(A\text{-}a)O_2$ gradient, ratio of PaO_2/FiO_2 and ratio of SpO_2/FiO_2 will be evaluated and summarized between group A and group B at Day 6. The difference of change from baseline between two groups will be tested by a t-test. In general,

the last observed measurement prior to the first dose of study treatment occurred on Day 1 visit will be considered the baseline measurement. Day 6 is the primary analysis point. If the patient leaves hospital prior to the day 6 analysis point, oxygenation at day of discharge will be used as value for measuring primary endpoint.

The analyses described above will also be performed between group A (early 5 day intervention with sargramostim) and group D (late 5 day intervention with sargramostim). Change from baseline (D1) to Day 10, daily change from baseline (D1) at Day 10 in oxygenation/respiratory parameter of $P(A\text{-}a)O_2$ gradient, ratio of PaO_2/FiO_2 and ratio of SpO_2/FiO_2 will be summarized as well. A t-test will be conducted to compare the difference between the groups.

The same analyses will be repeated for pathological oxygenation parameter of $P(A\text{-}a)O_2$ gradient, ratio of PaO_2/FiO_2 and ratio of SpO_2/FiO_2 including summary of their value and change from baseline at Day 6 and Day 10. A waterfall plot will be used to respent the change from baseline and percentage change from baseline on Day 6 for 1)oxygenation parameter of $P(A\text{-}a)O_2$ gradient; 2) pathological oxygenation parameter of $P(A\text{-}a)O_2$ gradient; 3) ratio of PaO_2/FiO_2; 4) ratio of SpO_2/FiO_2. Percentage change from baseline of $P(A\text{-}a)O_2$ gradient and ratio of PaO_2/FiO_2 on Day 6 and follow-up will be summarized.

Reasons of missing AA gradient values on Day 6 will also be summarized.

The normal value of AA gradient for room air is calculated as: $2.5+(0.21\times Age)$. All the results including change from baseline, and maximum change from baseline after 5 day intervention with sargramostim, normal AA gradient value for room air and flag of abnormality on Day 6will be listed.

At least 33% reduction from baseline, and at least 50% reduction from baseline in $P(A\text{-}a)O_2$ gradient and pathological gradient on Day 6 will be summarized for mITT population.

Sensitivity analysis:

For the purpose of evaluating the sensitivity and robustness of the primary analysis using the mITT population, the above analyses will be repeated in the ITT Population.

7.11.2 Mean Change in Ordinal Scale Between Day 1 and Day 6

Ordinal scale will be assessed at the Screening and Days 1-10. Baseline for the following analyses is defined as the last ordinal scale prior to administration of study drug.

Ordinal Score at screening, day 1, day 6 and mean change between day 1 and day 6 will be summarized and listed between group A and group B.

7.11.3 Effects on Progression to Mechanical Ventilation and/or ARDS

Decreasing oxygenation often leads to the need for non-invasive or invasive mechanical ventilation, and if severe enough to a diagnosis of ARDS. We will therefore as a secondary endpoint also study if early intervention with inhaled sargramostim prevents progression to criteria-defined ARDS (according to the American-European Consensus Conference (AECC) diagnostic criteria for ARDS: acute onset; ratio of partial pressure of arterial oxygen to fraction of inspired oxygen (PaO2/FiO2) of 200 or less, regardless of positive end-expiratory pressure; bilateral infiltrates seen on frontal chest radiograph; and pulmonary artery wedge pressure of 18 mm Hg or less when measured, or no clinical evidence of left atrial hypertension), requiring high-flow oxygen devices, non-invasive mechanical ventilation, mechanical ventilation, by measuring the day from admission when this diagnosis is made or therapies are initiated.

Respiratory support includes high-flow oxygen devices, non-invasive mechanical ventilation, mechanical ventilation. The durations of respiratory support (days) are defined as:

$$\text{Duration of respiratory support} = \sum_{k_i=0}^{N_i} (\text{End Date/time on Day } k_i - \text{ Start Date/time on Day} k_i)/24$$

Where, N_i is the total number of available study days for any patient i.

Number of patients with ARDS onset, ratio of partial pressure of arterial oygen to fraction of inspired oxygen (PaO2/FiO2), number of patients with bilateral infiltrates seen on frontal chest radiograph and duration of respiratory support will be summarized by treatment group.

Date of ARDS onset, ratio of partial pressure of arterial oygen to fraction of inspired oxygen (PaO2/FiO2), present of bilateral infiltrates seen on frontal chest radiograph and details regarding respiratory support will also be listed by patient.
Time to progression to invasive ventilation (days) is calculated as:

Time to invasive ventilation (Days) = Date of first invasive ventilation/Censoring– Date of randomization +1

All patients without progression to invasive ventilation during the study will be censored on the date of end of the follow up period, early discontinuation or death whichever is earlier.

The probability function of progression to invasive ventilation will be estimated by Kaplan-Meier method. The median time and its 95% confidence interval for each group will be reported. Cumulative progression rates estimated by the KM method for day 6, 10, and available visits during the follow up period and the 95% confidence intervals will be reported.

7.11.4 Nosocomial Infections

As part of routine clinical care, sputum samples will be collected in patients suspected of secondary bacterial pneumonia, and checked for the presence of bacteria.

Identification / occurrence of nosocomial infections through the evaluation of BAL (bronchoalveolar lavage), sputum, skin, urine and blood culture results or other microbiology results, as well as adverse events, or via other medical procedures performed will be listed and summarized by treatment group using descriptive analyses.

Nosocomial infection rate per 1000 patient per day will be calculated as:

Nosocomial infection rate = total number of nosocomial infection cases / total number of hospitalization days for all patients * 1000

Wald test will be used to compare nosocomial infection rate between treatment groups.

7.11.5 Sequential Organ Failure Assessment (SOFA)

Overall calculated SOFA score will be summarized by treatment group for each available timepoint. Mean change of SOFA score between day 1 and day 6 and between day 1 and day 10 will be summarized by treatment group as well.

Individual GCS and SOFA scores will be provided in a patient data listing.

7.11.6 National Early Warning Score (NEWS-2)

National Early Warning Score (NEWS-2) in each available measure and the calculated overall NEWS-2 score will be summarized by treatment group for each available timepoint using descriptive analyses. Mean change of NEWS2 score between day 1 and day 6 and between day 1 and day 10 will be summarized by treatment group as well. Individual measures and overall score will be provided in patient data listing.

NEWS2 score less than 2 for at least 24h will be condisered as an event.

Time to NEWS score<2 for at least 24h (days) is calculated as:

Time to NEWS score<2 (Days) = Date of first NEW2 score<2 for at least 24h/Censoring– Date of randomization +1

All patients without achieving NEWS2 score less than 2 for at least 24h during the study will be censored on the date of end of the follow up period, early discontinuation or death whichever is earlier.

The probability function of progression to invasive ventilation will be estimated by Kaplan-Meier method. The median time and its 95% confidence interval for each group will be reported. Cumulative progression rates estimated by the KM method for day 6, 10, and available visits during the follow up period and the 95% confidence intervals will be reported.

7.11.7 Clinical Sign Score

Clinical sign score ranges from 0-18 based on 6 parameters each scored 0-3 (by patient, except T°C). Overall clinical sign score and score on each parameter will be summarized by treatment group for each available timepoint using descriptive analyses. Mean change of clinical sign score

between day 1 and day 6 will be summarized by treatment group as well. Individual overall clinical sign score and score on each parameter will be provided in patient data listing.

Clinical sign score less than 6 maintained for 24h will be considered as an event.

Time to clinical sign score<6 maintained for 24h (days) is calculated as:

Time to clinical sign score<6 (Days) = Date of first clinical sign score<6 maintained for 24h/Censoring – Date of randomization +1

All patients without achieving clinical sign score less than 6 maintained for 24h during the study will be censored on the date of end of the follow up period, early discontinuation or death whichever is earlier.

The probability function of progression to invasive ventilation will be estimated by Kaplan-Meier method. The median time and its 95% confidence interval for each group will be reported. Cumulative progression rates estimated by the KM method for day 6, 10, and available visits during the follow up period and the 95% confidence intervals will be reported.

7.11.8 Mortality

Survival status will be collected up to follow-up period (20 weeks after day 1). Death is considered as an event. All the mortality events and cause of death will be listed by treatment group and by patient. Number of patients died and survival time will be summarized by treatment group. Survival time will also be listed by patient. Risk and risk difference of all causes mortality by Day 28 and during the study period will also be summarized.

The hazard ratio will be estimated by the Cox proportional hazards model with treatment group as a covariate in the model. Relevant hazard rates, hazard ratio between treatment groups and associated p-values will be tabulated.

Survival Time (days) is calculated as:

Survival Time (Days) = Date of Death/Censoring - Date of randomization +1

It is defined as the number of days from the date of first dose of study drug to the date of death or censoring. For patients who did not have deaths within 140 days' follow up time, the date of

censoring is the earliest of the following (1) early study distontinuations; (2) end of 140 days after the first dose; (3) last record in the datebase for those who were lost to follow up.

The probability function of death will be estimated by Kaplan-Meier method. The median time and its 95% confidence interval for each group will be reported. Cumulative progression rates estimated by the KM method for day 6, 10, and available visits during the follow up period and the 95% confidence intervals will be reported.

7.11.9 Hospitalization

The duration of hospitalization (days) is defined as:

Duration of hospitalization (Days) = Date of Discharge – Date of Randomization + 1.

Duration of hospitalization will be listed for each patient, and summarized by treatment group.

The duration of ICU (days) is defined as:

Duration of ICU(Days) = Date of Discharge from ICU – Date of ICU Admission + 1.

Number of patients who ever went to ICU and went to ICU on or before Day 28, along with ICU stay duration will be summarized and listed.

7.11.10 Feature of Secondary Haemophagocytic

Features of secondary haemophagocytic lymphohistiocytosis is defined by Hs score including temperature, organomegaly, cytopenia, triglycerides, fibrinogen, ferritin, AST and known immunosuppression. Hs score will be summarized by treatment group for each available timepoint using descriptive analyses. Individual Hs score will be provided in patient data listing.

7.11.11 Favourable Effect on long term follow up

At 10-20 weeks after discharge from hospital, patients will be seen on routine check-up by pulmonologist, who will perform a clinical exam (cyanosis, crepitation’s and rales, heart murmurs, peripheral edema), pulmonary function tests (including FVC, TLC and diffusion capacity), and a laboratory (ferritin, lymphocytes, leukocytes). All the results will be listed by paitent.

7.12 Safety Evaluations

Safety assessments will include monitoring of vital signs, adverse events (AEs), clinical laboratory tests, 12-lead electrocardiograms (ECG) and physical examinations. The main analysis period would be the Treatment Emergent period which is defined as the period from the date of the first dose until the end of earliest of the following 1) Date of Study Day 6 if a patient was not enrolled to Group C or D, or Date of Study Day 10 if a patient was determined to be enrolled in next 5 day treatment period; or 2) Date of Early discontinuation from study. This analysis period will be used for all treatment emergent adverse event, laboratory evaluations.

Safety variables will be tabulated and presented for all patients in the Safety Population and Intent-to-treat Population.

7.12.1 Adverse Event

Refer to protocol section 13.1 to see the definitions of adverse event. All adverse events will be coded using Medical Dictionary for Regulatory Activities (MedDRA) version 23.1. Adverse event will be categorized as prior events, treatment emergent adverse event, and post treatment adverse event:

- Prior Events includes all adverse event with a start date before the first dose of the study drug or date of on study treatment (for SOC arm) regardless of the end date.
- Treatment-emergent adverse events (TEAE) are defined as adverse events with a start date (or date of worsening) on or after the date of on-study treatment.

TEAE will be summarized by: 1) treatment group; 2) system organ classification (SOC) and preferred term (PT); 3) PT; 4) SOC, PT and the maximum severity.

Treatment-related TEAE, serious TEAE and treatment-related serious TEAE will be summarized by SOC and PT coded by the most current version of MedDRA dictionary, and the maximum severity. SAEs and deaths will be listed by patient.

The frequency of treatment-emergent serious adverse event (TESAEs) will be summarized by treatment group, SOC, and preferred term. Severity (using CTCAE) and relationship of TEAEs to treatment (sargramostim, sargramostim inhalation device or standard of care) will be based on the scales as as recorded on eCRF (also refer to Section 13.1 of the Study Protocol for definitions).

If a patient experiences more than one TEAE within a preferred term, the patient will be counted only once in the calculation of incidence of TEAE within that preferred term. Similarly, if a patient experiences more than one TEAE within a SOC, the patient will be counted only once in the calculation of incidence of TEAE within that SOC. If a patient experiences more than one TEAE within a preferred term (or SOC), the occurrence with the highest severity will be used in the calculation of the incidence of TEAE within that preferred term (or SOC) by severity. If a patient experiences more than one TEAE within a preferred term (or SOC), the occurrence considered most closely related to study drug will be used in the calculation of the incidence of TEAE with that preferred term (or SOC) by relationship to study drug.

A data listing for all AEs will be provided with flags for TEAE, relatedness and CTCAE severity. AEs related to sargramostim treatment will be listed. Any serious AEs and deaths will be listed.

7.12.2 Laboratory data

Laboratory data, as performed and collected as part of SOC (see Section 9 in the Study Protocol), will be collected and include hemoglobin, WBC, Eosinophil count, lymphocyte count, CD4+, CD8+, TBC, ESR, CRP, Creatinine, AST, ALT, Bilirubin, LDH, Troponins, CKs, Ferritin, Fibrinogen, Triglycerides, beta-HCG, D-Dimers and so on.

Descriptive statistics for baseline value, actual value and change from baseline to each scheduled postbaseline visit will be provided by treatment group for clinical hematology, chemistry laboratory, and immune profiling (where available) tests. Baseline for these tests is defined as the last assessment prior to administration of study drug. Conventional Units will be used for reporting the laboratory test results.

Serum pregnancy test results will be listed. Values for any chemistry, hematology, and immune profiling values outside the clinical reference ranges will be flagged on the individual patient data listings.

7.12.3 Physical Examination

Physical examination findings will be listed by treatment group and patient.

7.12.4 Vital Signs

Vital signs (including temperature, respiratory rate, blood pressure and pulse) will be measured during the Screening visit,Treatment period, Post-treatment period (within 24hrs), Study period, End of study and Follow-up period.

Baseline for vital signs is defined as the last assessment prior to administration of study drug.

All vital sign data including unscheduled records will be listed. Unscheduled records will be excluded from the summary statistics. Vital sign data including baseline value, actual value, and change from baseline to each post-baseline visit will be summarized by treatment group and timepoint.

7.12.5 Electrocardiogram (ECG)

Electrocardiogram examination findings for ECGs will be listed by treatment group and patient for each ECG parameter.

7.12.6 Patient Profiles

Key lab parameters for patients with SAEs, discontinuation due to AE, and deaths will be presented in patient profiles, in which the demographics and treatment data will be included. Additional profiles may be generated for any identified suspected unexpected serious adverse reactions (SUSARs).

8 Data Presentation

8.1 Insufficient Data for Presentation

Some of the TFLs may not have sufficient numbers of patients or data for presentation. If this occurs, the blank TFL shell will be presented with a message printed in the table, such as, “None reported”.

9 Revison History

10 Reference