A Phase 2 trial evaluating sargramostim in patients with COVID-19 associated acute hypoxemia

iLeukPulm

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Protocol Approval Signature Page

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A Phase 2 trial evaluating sargramostim in patients with COVID-19 associated acute hypoxemia

I acknowledge the study protocol contains all necessary details for me and my staff to conduct the study as described. I will conduct this study in compliance with all applicable regulations and guidelines as stated in the protocol and other information supplied to me.

Signature Principal Investigator: _____

Print name:_____

Date:_____

[redacted]

Protocol Synopsis

Sponsor:	**Name of Medical Products:**	**Study Phase:**
Partner Therapeutics	PTX-001-002	Phase 2

Study Title

A Phase 2 trial evaluating sargramostim in patients with COVID-19 associated acute hypoxemia

Study Center(s)

Up to approximately 20 sites (US)

Study Design

This Phase 2, randomized, open-label study will enroll approximately 120 patients with COVID-19 associated acute hypoxemia randomized 2:1 to evaluate sargramostim treatment plus institutional standard of care compared to institutional standard of care alone. A Data Safety Monitoring Board (DSMB) will review the safety and benefit/risk of sargramostim after approximately 10 and 20 patients are enrolled and complete the sargramostim treatment period. Enrollment of patients will be paused until the DSMB has conducted the safety review and permitted the study to proceed. An additional DSMB review will occur after approximately 60 patients (total of both treatment arms) are enrolled to the study. Upon completion of this safety review, the DSMB may request an additional review after approximately 80 patients have been randomized. Enrollment will not be paused during these reviews at approximately 60 and 80 patients.

Primary objective

The aim of the study is to determine if inhaled sargramostim, as an adjunct to institutional SOC, improves clinical outcomes in patients with COVID-19-associated acute hypoxemia.

Primary Outcome Measures:

- Change in oxygenation parameter of $P(A\text{-}a)O_2$ gradient by Day 6 and % of patients who have been intubated by Day 14

Secondary Outcome Measures:

- Change in ordinal scale
- All cause 28-day mortality
- Number of patients with treatment-emergent serious adverse events or clinically significant adverse drug reactions (ADRs)
- Survival time and causes of death
- Time to improvement in oxygenation (PaO_2/FiO_2 ratio, SpO_2/FiO_2 ratio and $P(A\text{-}a)O_2$ gradient)
- Rate of nosocomial infection (as determined by local institution practice)
- Duration of hospitalization
- Number of patients requiring initiation of mechanical ventilation

Sponsor:	**Name of Medical Products:**	**Study Phase:**
Partner Therapeutics	PTX-001-002	Phase 2

- Duration of invasive and non-invasive ventilation and/or supplemental oxygen
- Time to normalization of WBC and lymphocytes

Exploratory Outcome Measures:

- National Early Warning Score (NEWS-2)
- Sequential organ failure assessment (SOFA) scores
- ROX index
- Progression to ARDS
- Changes on chest X-ray or CT

Inclusion Criteria

1. Patients aged ≥ 18 years
2. Patients (or legally authorized decision maker) must provide informed consent
3. Test positive for SARS-CoV-2 virus by PCR (positive confirmation prior to start of sargramostim)
4. Admitted to hospital
5. Presence of acute hypoxemia defined as (either or both)
   a) saturation below 93% on ≥ 2 L/min oxygen supplementation
   b) PaO_2/FiO_2 below 350

Sponsor:	**Name of Medical Products:**	**Study Phase:**
Partner Therapeutics	PTX-001-002	Phase 2

Exclusion Criteria

1. Patients requiring invasive (mechanical ventilation) or non-invasive (CPAP, BiPAP for hypoxemia) ventilation or ECMO (Note: oxygen supplementation using high flow oxygen systems or low flow oxygen systems would not exclude patients from this study)
2. Intractable metabolic acidosis
3. Cardiogenic pulmonary edema
4. Hypotension requiring use of vasopressors
5. Hyperferritinemia (serum ferritin ≥2,000 mcg/L)
6. White blood cell count > 50,000/mm^3
7. Participation in another interventional clinical trial for COVID-19 therapy
8. Highly immunosuppressive therapy or anti-cancer combination chemotherapy within 24 hours prior to first dose of sargramostim
9. Known or suspected intolerance or hypersensitivity to sargramostim, or any component of the product
10. Previous experience of severe and unexplained side effects during aerosol delivery of any kind of medical product
11. Presence of any preexisting illness that, in the opinion of the Investigator, would place the patient at an unreasonably increased risk through participation in this study
12. Pregnant or breastfeeding females
13. Severe or uncontrolled pulmonary comorbid conditions, including systemic steroid dependent asthma, systemic steroid dependent COPD, oxygen dependent COPD, lung transplant, known interstitial lung disease, or cystic fibrosis

Sponsor:	**Name of Medical Products:**	**Study Phase:**
Partner Therapeutics	PTX-001-002	Phase 2

Patient population:

Patients with COVID-19 associated acute hypoxemia

Rationale:

GM-CSF is a critical cytokine for healthy pulmonary function. 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 (Dranoff, 2017; Guilliams, 2013). 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 (Zhan, 2019). GM-CSF is also known to reverse immunoparalysis seen in sepsis by immune activation, resulting in beneficial outcomes (Mathias, 2015; Meisel, 2009). Additionally, researchers have shown that in response to lung infection, GM-CSF signals B1a B cells to secrete polyreactive emergency immunoglobulin M (IgM) and ensures effective early frontline defense against bacteria invading the lungs (Weber, 2014).

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 (Umstead, 2020; Steinwede, 2011) and through increased production of reactive oxygen species by the alveolar macrophages (Subramanian, 2014). Pulmonary delivery of this cytokine has the potential to reduce morbidity and mortality due to viral pneumonia, potentially to include COVID-19.

The safety and efficacy of sargramostim 125 mcg has been evaluated in several clinical studies. In one clinical study of six patients with moderate to severe community-acquired pneumonia or ventilator-associated pneumonia acute respiratory distress syndrome (ARDS), 125 mcg of sargramostim was administered by an Aeroneb Solo nebulizer device (Covidien, Neustadt, Germany) at an interval of 48 hours for 2 doses (Herold, 2014). At this dose, a significant improvement of oxygenation in response to sargramostim inhalation ($p=0.0035$), with no drug-related toxicity, was observed. Inhaled sargramostim has been evaluated at doses higher than 125 mcg in patients with autoimmune pulmonary alveolar proteinosis (aPAP) (Wylam, 2006; Tazawa, 2019; Campo, 2016). Patients with aPAP have extremely high titers of anti-GM-CSF antibodies, which sequesters native GM-CSF leading to accumulation of surfactant in alveolar sacs with resultant hypoxia. To overcome the high anti-GM-CSF antibody levels in aPAP patients, sargramostim has been administered at doses of up to 500 mcg total daily dose, although daily doses of 125 mcg and 250 mcg have been used and associated with an improvement in clinical outcome (Wylam, 2006; Tazawa, 2019; Campo, 2016). Given the demonstrated efficacy of 125 and 250 mcg doses, 125 mcg is considered a minimally effective dose when treating viral-related diseases.

Sponsor:	**Name of Medical Products:**	**Study Phase:**
Partner Therapeutics	PTX-001-002	Phase 2

Number of Patients:

Approximately 120: of which 80 patients will receive sargramostim + SOC, and 40 patients who will receive SOC alone.

Duration of Therapy

All patients randomized to the sargramostim treatment arm will receive sargramostim for the duration of treatment and remain on study for the duration of study period, unless the patient has unacceptable toxicity to sargramostim or patient/legally authorized decision maker/investigator decides to discontinue the patient, or patient has rapid deterioration requiring other advanced treatments or medical care not conducive to continued treatment with sargramostim. Patients will receive sargramostim treatment for up to 5 days.

For the purpose of this study, Day 1 is the day the patient receives the first dose of sargramostim and/or SOC.

Following the sargramostim treatment period (Day 1-5), patients will continue to receive SOC as appropriate following institutional guidelines. The study period (Days 6-28) will continue until completion of all safety and efficacy assessments on Day 28 (end of study).

Patients on the control arm will receive institutional standard of care alone.

All patients, including those who discontinue treatment early, will be followed for the duration of the study period.

Duration of Follow-Up

All patients should be followed for safety evaluations for up to Day 90. Data from a final follow-up visit at day 90 (+/-30 days) should also be collected.

Duration of Study

Study completion will be defined as the date when the last patient completes the follow-up period, or has withdrawn from the study. Alternatively, the study may be stopped earlier for reasons such as feasibility, poor enrollment, ethical or safety reasons.

Test Product/Device, Dose, and Mode of Administration:

All patients randomized to the sargramostim treatment arm will be treated with 125 mcg inhaled sargramostim twice daily (with the interval between doses per institutional practice for twice a day dosing) for 5 days delivered via a mesh nebulizer.

Patients cannot start the study using intravenous (IV) administration of sargramostim. If a patient cannot continue to receive inhaled sargramostim upon progression to an invasive mechanical ventilator, administration of sargramostim may be delivered by IV infusion to complete a total of 5 days (including days delivered via inhalation).

Patients on the control arm will receive institutional standard of care alone.

Study Design:

Randomized two-arm open-label study

Sponsor:	Name of Medical Products:	Study Phase:
Partner Therapeutics	PTX-001-002	Phase 2

Safety Assessments

Hematology laboratory parameters should include complete blood count with differential, hemoglobin, hematocrit, and absolute counts for white blood cells, platelets, neutrophils, lymphocytes, eosinophils and monocytes.

Chemistry laboratory parameters should include albumin, amylase, lipase, BUN, creatinine, ALT, AST, LDH, serum alkaline phosphatase, direct and total bilirubin, glucose, total protein, sodium, potassium, chloride, bicarbonate, calcium and uric acid.

Required laboratory measurements of ferritin, d-dimer and C-reactive protein should also be collected at screening, Days 1-6 and any other time it is performed as part of SOC.

Immune profiling, if performed, including CD4+, CD8+, HLA-DR, IL-6, IL-1, GM-CSF.

Efficacy Assessments

Efficacy data will include the following:

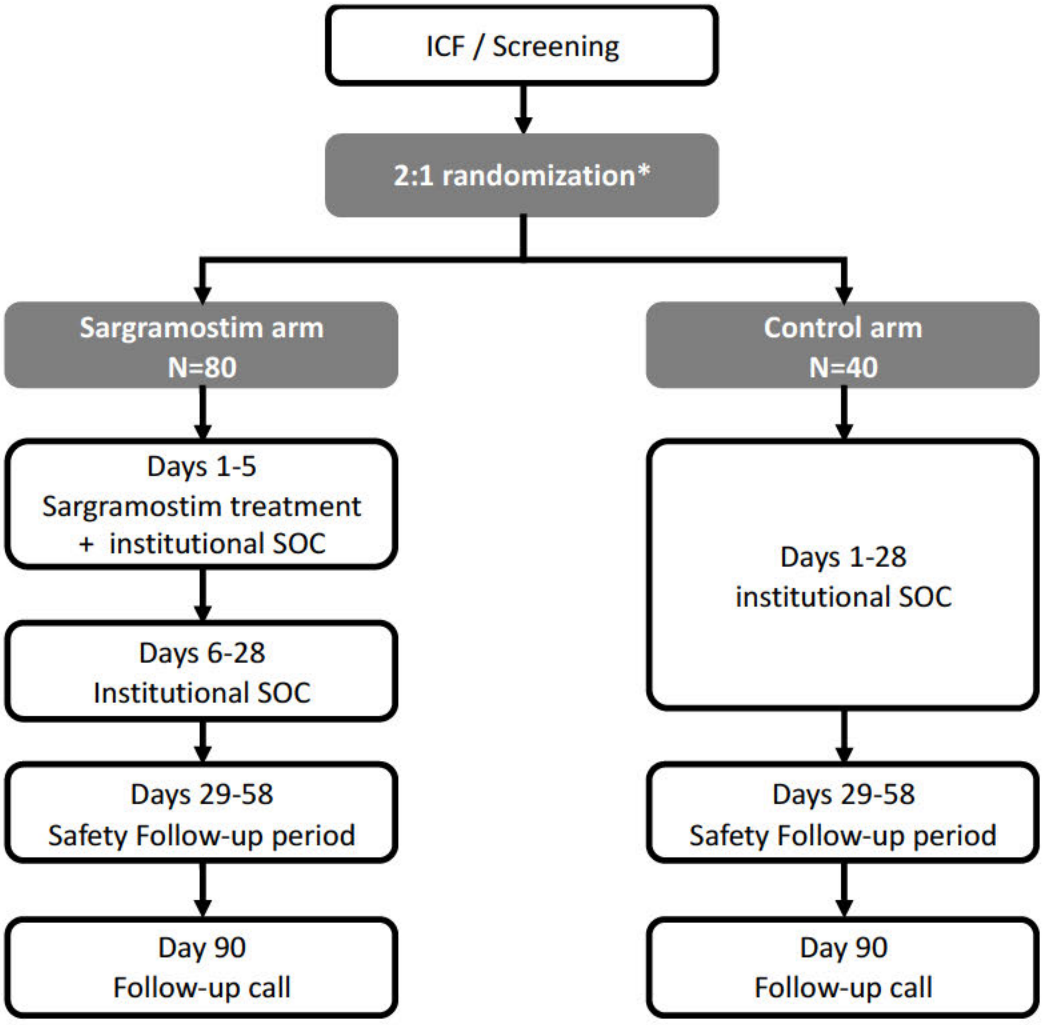
- Need for ventilation, or other means of respiratory support
- Arterial blood gases / Oxygenation parameters
- Identification / occurrence of nosocomial infections
- SOFA score, NEWS-2 score, Ordinal score, ROX Index
- Chest radiographic changes
- Mortality

Statistical Plan

Approximately 120 patients will be randomized: of which 80 will receive sargramostim + SOC, and 40 patients who will receive SOC alone. The sample size was based on practical and clinical considerations to ensure that the efficacy endpoints and safety profile could be appropriately evaluated and was not based on any statistical assumptions or hypotheses. As a result, the analyses of this study will be considered as descriptive analyses.

Derivation of two-sided 95% confidence intervals and p-values will be generated where applicable.

1 SCHEMATIC OF STUDY DESIGN



*Stratification factors include: institution, SOFA score (<6 versus ≥ 6)

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2 ABBREVIATIONS

ADR:	adverse drug reaction
ADL	activities of daily living
AE:	adverse event
AEC:	alveolar epithelial cell
ALT:	alanine aminotransferase
AM:	alveolar macrophage
aPAP:	autoimmune pulmonary alveolar proteinosis
ARDS:	acute respiratory distress syndrome
AST:	alanine aminotransferase
BAL:	bronchoalveolar lavage
BiPAP:	bilevel positive airway pressure
BUN:	blood urea nitrogen
CFR:	Code of Federal Regulations
CIOMS:	Council for International Organizations of Medical Sciences
c-Met:	tyrosine-protein kinase Met
CPAP:	continuous positive airway pressure
COPD	chronic obstructive pulmonary disease
CRF:	case report form
CRP:	C-reactive protein
Cstat:	static compliance
CT:	Computed tomography
CTCAE:	Common Terminology Criteria for Adverse Events
DC:	dendritic cell
DSMB:	Data Safety Monitoring Board
EC:	European commission
ECG:	electrocardiogram
EGFR:	epidermal growth factor receptor
ECMO:	extracorporeal membrane oxygenation
FcγR:	Fcγ receptor
FDA:	Food & Drug Administration
FiO_2:	fraction of inspired oxygen
GCP:	Good clinical practice

GCS:	Glasgow coma score
GM-CSF:	granulocyte macrophage colony stimulating factor
HGF:	hepatocyte growth factor
HLA-DR:	human leukocyte antigen – DR isotype
ICF:	Informed consent form
ICH:	International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
ICU:	intensive care unit
IgM:	immunoglobulin M
IL-1:	Interleukin-1
IL-6:	Interleukin-6
IRB:	Institutional Review Boards
IV:	Intravenous
LDH:	lactate dehydrogenase
NEWS-2:	National Early Warning Score 2
$P(A\text{-}a)O_2$:	gradient (alveolar-arterial) oxygen gradient
$PaCO_2$:	partial pressure of carbon dioxide
PAGE:	Pulmonary Alveolar Proteinosis GM-CSF Inhalation Efficacy (PAGE) trial
PaO_2:	partial pressure of oxygen
PCR:	polymerase chain reaction
PEEP:	positive end expiratory pressure
PI:	Principal Investigator
Pplat:	plateau pressure
PTX:	Partner Therapeutics
PU.1:	transcription factor PU.1
rhu-GM-CSF:	recombinant human granulocyte-macrophage colony stimulating factor
ROS:	reactive oxygen species
SAE:	serious adverse event
SOC:	Standard of care
SOFA:	sequential organ failure assessment
SpO_2:	peripheral oxygen saturation
SUSAR:	suspected unexpected serious adverse reaction
TGF-α:	transforming growth factor alpha

US:	United States
USP:	United States Pharmacopoeia
VFD:	ventilator free days
WBC:	white blood cells

3 BACKGROUND

3.1 Sargramostim

Sargramostim (Leukine) is a recombinant human granulocyte-macrophage colony stimulating factor (rhu-GM-CSF), and has been approved since 1991. GM-CSF, a pleiotropic cytokine, is an important leukocyte growth factor known to play a key role in hematopoiesis, 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. Since the initial FDA approval in 1991, [redacted] patients have been estimated to have received Leukine, providing extensive clinical and post-marketing data in a broad range of treated individuals across several disease states. This includes preterm neonates as well as the elderly, including males and females, representing a well-characterized safety profile for Leukine.

3.2 Role of GM-CSF in Lung Function

GM-CSF is a critical cytokine for healthy pulmonary function. 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 (Dranoff, 2017; Guilliams, 2013). GM-CSF has a wide array of effects on myeloid cells. It 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 (Zhan, 2019). GM-CSF is also known to reverse immunoparalysis seen in sepsis by immune activation, resulting in beneficial outcomes (Mathias, 2015; Meisel, 2009). Additionally, researchers have shown that in response to lung infection, GM-CSF signals B1a B cells to secrete polyreactive emergency immunoglobulin M (IgM) and ensures effective early frontline defense against bacteria invading the lungs (Weber, 2014). In the lung, the alveolar epithelial cells (AEC)-released GM-CSF is known to improve the innate immune responses of myeloid cells, in particular the alveolar macrophage (AM). It has been shown to enhance viral clearance and modulate surfactant lipid metabolism, preventing its accumulation in the alveoli and maintaining the optimal physiologic gas exchange. In addition, the AEC-expressed GM-CSF also has direct beneficial effects on the injured epithelial barrier by orchestrating epithelial proliferation and barrier repair (Subramaniam, 2014; Subramaniam, 2015).

There is a large body of evidence generated with GM-CSF in animal studies suggesting the potential use in ARDS and infections. 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.

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 (Halstead, 2018). 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 (Unkel, 2012). 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 (Huang, 2011).

There is evidence that inhaled GM-CSF prevents systemic bacteremia in post influenza bacterial pneumonia primarily through locally-mediated improved lung resistance during infection (Umstead,

2020; Steinwede, 2011) and through increased production of reactive oxygen species by the alveolar macrophages (Subramanian, 2014).

In conclusion, GM-CSF confers resistance to influenza by enhancing innate immune mechanisms that depend on alveolar macrophages, for their health and normal functioning. Pulmonary delivery of this cytokine has the potential to reduce morbidity and mortality due to viral pneumonia (Figure 1).

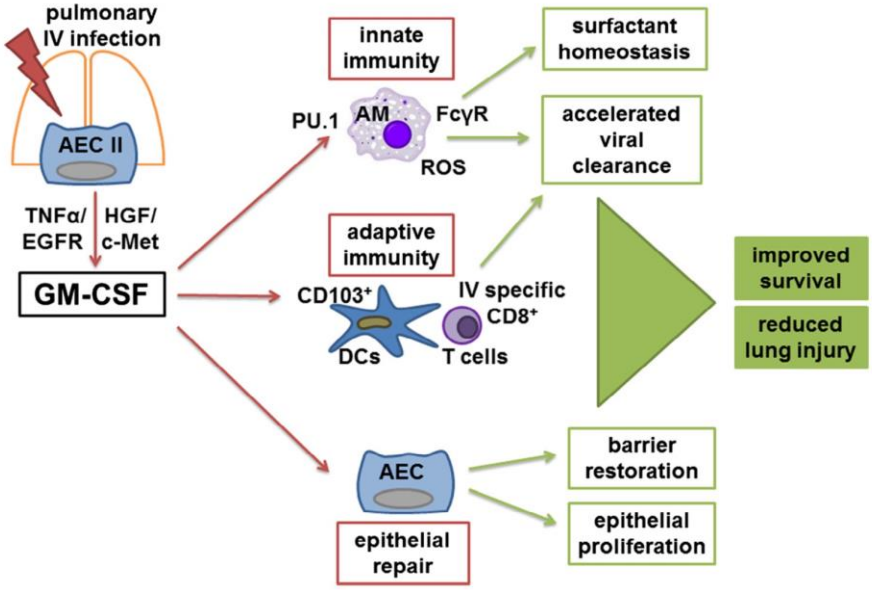

Figure 1 GM-CSF-modulated immune response to influenza virus infection (Rosler, 2016). *GM-CSF-modulated immune response to influenza virus infection. After pulmonary influenza virus infection GM-CSF is released from alveolar epithelial cell II, mediated through HGF/c-Met and TGF-α/EGFR signaling. In 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 alveolar macrophage resistance, maturation, ROS production, and phagocytosis capacity, e.g., by the FcγR-mediated opsonophagocytosis. GM-CSF also stimulates activation and proliferation of dendritic cells, especially CD103$^+$ dendritic cells, and T cells and enhances antigen priming and influenza virus-specific CD8$^+$ T cell recruitment. Altogether alveolar epithelial cells GM-CSF leads to increased survival and reduced lung injury. [AEC alveolar epithelial cells, AM alveolar macrophage, c-Met hepatocyte growth factor receptor, DC dendritic cell, EGFR epithelial growth factor receptor, FcγR Fcγ 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].*

3.3 Use of sargramostim in conditions that are similar to COVID-19 ARDS

A small (18 patient) double blind randomized placebo controlled clinical trial of low-dose (3 mcg/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 (Rosler, 2016). In a phase II study, 130 patients with severe sepsis with respiratory dysfunction were randomized to GM-CSF (250 mcg/m^2 intravenously daily for 14 days) or placebo. The results showed an improvement in 28 day mortality on GM-CSF; this did not reach statistical significance (Paine, 2012).

Herold and colleagues used sargramostim by inhalation route on a compassionate basis in six patients with moderate to severe community-acquired pneumonia or ventilator-associated pneumonia-associated acute respiratory distress syndrome (ARDS) who were not improving despite all measures and at least 6 days of mechanical ventilation (Herold, 2014). Sargramostim 125 mcg was applied by Aeroneb Solo device (Covidien, Neustadt, Germany) at an interval of 48 hours for 2 doses. 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.

Six clinical studies evaluated sargramostim administered via intravenous, subcutaneous, or inhaled routes in critically ill patients and monitored systemic cytokines, markers of inflammation, and safety (Meisel, 2009; Hall, 2011; Pinder, 2018; Paine, 2012; Herold, 2014; Rosenbloom, 2005). In these studies, there was no increase in systemic cytokines, including IL-6, IL-8, IL-1β, IL-10, TNFα, GMCSF, and IL-12-p70, nor an indication of treatment-related organ toxicity or increased rates of treatment-related serious adverse events. Indeed, in the clinical studies where IL-6, a key driver of cytokine storm, was monitored, no change in the systemic levels were observed. Importantly, there was no adverse effects of sargramostim observed in any of these studies that increased or exacerbated organ toxicity or mortality. In totality, these data suggest that sargramostim does not increase the risk of cytokine storm in critically ill patients. While these studies were of various sizes, improvements in clinical outcomes were observed.

3.4 Recent translational data in COVID-19

A recent study has shown that the replication of SARS-CoV-2 in alveolar cells can result in a limited antiviral response, void of IFN-I and IFN-III response in both a human lung alveolar carcinoma cell line and in vivo in ferrets (Blanco-Melo, 2020). In addition, by using ex vivo human lung tissue explants respectively challenged by the same inoculum of SARS-CoV-2 and SARS-CoV, it was found that SARS-CoV-2 was capable of infecting and producing significantly higher amount of virus in human lung tissues while triggering suboptimal levels of IFNs and pro-inflammatory cytokines/chemokines during that same time period. These findings could possibly implicate an escape from innate immune detection (Chu, 2020). An efficient immune suppression in the early phase may allow the SARS-CoV-2 to replicate unchecked in the respiratory tract, achieving high viral load and eventually contributing to its efficient person-to-person transmission before onset of severe clinical symptoms (Wei, 2020). Evidence of additional immune dysfunction comes from the autopsy reports of COVID-19 patients demonstrating a low number of CD8 positive T lymphocytes infiltrating lung tissue (Yao, 2020). As described in laboratory reports, along with the increased levels of cytokines in systemic circulation, a decrease of immune cell populations such as CD4, CD8 and NK cells in peripheral blood were identified as risk factor of cytokine storm in COVID-19-infected pneumonia patients (Chen, 2020; Wenjun, 2020; Ruan, 2020). Additionally, the NK cells and cytotoxic lymphocytes appeared to be exhausted with a reduced ability to produce CD107a, IFN-γ, IL-2, granzyme B, and TNF-α (Zheng, 2020). The T cells from COVID-19 patients also have significantly higher levels of the exhaustion markers PD-1 as compared to the healthy controls (Zheng, 2020). These increases in PD-1 and Tim-3 expression on T cells were reported as the patients progressed from mildly symptomatic to severe stage, further indicative of T cell exhaustion. Interestingly, the SARS-CoV protease 3CLPro-transfected lung epithelial cells were shown to have a decreased GM-CSF mRNA and protein expression suggesting that the SARS-CoV may play a role in the mechanism of lung fibrosis of SARS through the suppression of GM-CSF (Liao, 2011).

If there is viral immune evasion, the host may be predisposed towards a more belligerent wave of cytokine and chemokine activity with marked inflammatory responses as the viral load increases. Profound lung damage due to unchecked viral replication can cause a complete breakdown of epithelial barrier function leading to diffuse alveolar damage with increased microvascular permeability. There is leakage of hallmark inflammatory cytokines such as IL-6 and IL-1 into systemic circulation and a concurrent recruitment of neutrophils and pro-inflammatory monocytes into the lung. This ensuing 'cytokine storm', with incessant proinflammatory cytokine production and accumulation of pro-inflammatory cells can eventually overwhelm the homeostatic tissue repair functions in the lung leading to irreversible tissue damage and depletion of AMs (Herold, 2011; Wong, 2019). During this process the

activated fibroblasts can deposit excess collagen to impair gas exchange in the lung (Meduri, 1993). Epithelial cell death may also expose the basement membrane to secondary microbial pathogens offering them access to the systemic circulation (Plotkowski, 1986). AMs are the sentinels of innate immune system against the respiratory pathogens. They do so via secretion of oxygen metabolites, anti-microbial proteases and by initiating an inflammatory response that can recruit activated neutrophils into alveolar spaces. They can also aid in resolving inflammation after the infectious challenge gets resolved, by phagocytosing the apoptotic neutrophils. This can reduce the pro-inflammatory cytokine secretion by macrophages and the production of anti-inflammatory cytokines such as TGF-β and IL-10 (Haslett, 1999; Knapp, 2003). Therefore, the loss of AMs could be one of the likely contributors of failed lung functions in COVID-19 patients. Importantly, while AMs were found to as the principle lung macrophages in both heathy controls and mildly infected COVID-19 patients, they were almost completely lost in the severely infected lungs in these patients (Liao, 2020).

Viral, bacterial, and fungal pulmonary infections can all cause the cytokine storm syndrome that may be challenging to differentiate on clinical grounds. A complex cytokine response that builds in infection is characterized by series of overlapping networks that may show similarities across indications but may also be different in amplitude and features, such that it is important to highlight them. A more aggressive form, labeled cytokine release syndrome (CRS) is an acute systemic inflammatory syndrome characterized by fever and multiple organ dysfunction that is associated with chimeric antigen receptor (CAR)-T cell therapy, therapeutic antibodies, and haploidentical allogeneic transplantation. Levels of inflammatory markers and cytokines may differ dramatically between this and other manifestations of cytokine storms (Vardhana, 2020). The hyperinflammatory response in COVID-19 has been likened to conditions including classical ARDS, macrophage activation syndrome or hemophagocytic lymphohistiocytosis, or simply “cytokine storm.” It is likely that none of these syndromes can precisely fit all COVID-19 patients due to the complexity and heterogeneity in inflammatory effects and diverse pathophysiology of the disease seen across different patients, however, several biomarkers (ferritin, C-reactive protein (CRP) and d-dimers) have shown a strong correlation of worsening disease or mortality (Chen 2020a; Chen, 2020b; Richardson, 2020).

3.5 Clinical experience of inhaled sargramostim

There is a large body of evidence of inhaled sargramostim 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 sargramostim at 9 pulmonary centers throughout Japan in patients with unremitting or progressive aPAP with hypoxia and symptoms (Tazawa, 2010). Patients received 250 mcg 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 7 of the 39 patients receiving GM-CSF, and these were all transient. The treatment led to an improvement in oxygenation, radiological changes as well as symptoms. Thirty five of these patients were followed for a 30-month observation period and sargramostim was shown to sustain remission in over half of the patients (Tazawa, 2014). A study using the I-neb nebulizer, conducted in 6 aPAP patients evaluated inhaled sargramostim 250 mcg once daily in a 4 days on, 4 days off schedule as long as needed (Papiris, 2014). Upon remission, the dose of sargramostim was reduced. All patients achieved remission and 3 patients maintained remission when the dose was reduced; no significant adverse events were noted (Papiris, 2014).

Following these results, a larger randomized phase 3 study (also referred to as the PAGE study) was conducted by the Japanese investigators in 12 centers. Sixty-four patients with mild to moderate aPAP with hypoxia were randomized to receive placebo or sargramostim (33 patients) at a dose of 125 mcg

twice a day for 7 days followed by a week of no treatment and this two week cycle was repeated 12 times over a period of 24 weeks. The treatment was well tolerated with no significant differences in adverse events between the two groups. The GM-CSF treated patients had a significantly improved hypoxia parameter as were the radiological changes (Tazawa, 2019).

This clinical experience of use of sargramostim in viral pneumonia and aPAP suggests salutary effects. In addition, these studies establish the safety of inhaled sargramostim and also provide evidence for activity of inhaled sargramostim.

3.6 Use of sargramostim inhalation in COVID-19

An investigator-sponsored study in Belgium [NCT04326920], "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 (Corona Virus Disease) Patients With Acute Hypoxic Respiratory Failure", was initiated on March 26, 2020. Patients were randomized to receive either inhaled sargramostim 125 mcg twice daily via a Philips© InnoSpire Go portable mesh nebulizer for 5 days (active group) together with institutional standard of care (SOC), or SOC alone (control group). SOC included: administration of antibiotics, anti-viral therapy, and use of supplemental oxygen and noninvasive or invasive ventilation (as necessary). A data safety monitoring board (DSMB) is overseeing the study. [redacted]

Based on the scientific rationale that GM-CSF is critical to maintain and restore lung function as well as the totality of clinical data in critically ill patients as well as patients with COVID-19, this study will evaluate whether inhaled sargramostim can improved clinical outcomes in patients with acute hypoxemia due to COVID-19. As discussed above in Section 3.4, the biomarkers ferritin, CRP and d-dimers are correlated with worsening of disease and high mortality in patients with COVID-19. To enable constant safety monitoring during the study, daily monitoring of these biomarkers during the sargramostim treatment period is required and will be evaluated (alongside adverse events and other routine laboratory tests) by the Data Safety Monitoring Board (see Section 12.2 for more details). After the first 9 and 22 patients were enrolled and treated with up to 5 days of sargramostim, the DMSB reviewed safety data. Following its second review, the DSMB supported a) expanding the study to 120 patients and b) excluding patients with serious baseline pulmonary comorbid conditions.

4 OBJECTIVES

4.1 Primary Objective

The aim of the study is to determine if inhaled sargramostim, as an adjunct to institutional SOC, improves clinical outcomes in patients with COVID-19-associated acute hypoxemia.

4.2 Primary Outcome Measure

- Change in oxygenation parameter of $P(A\text{-}a)O_2$ gradient by Day 6 and % of patients who have been intubated by Day 14

4.3 Secondary Outcome Measures

- Change in ordinal scale
- All cause 28-day mortality
- Number of patients with treatment-emergent serious adverse events or clinically significant adverse drug reactions (ADRs)
- Survival time and causes of death
- Time to improvement in oxygenation (PaO_2/FiO_2 ratio, SpO_2/FiO_2 ratio and $P(A\text{-}a)O_2$ gradient)
- Rate of nosocomial infection (as determined by local institution practice)
- Duration of hospitalization
- Number of patients requiring initiation of mechanical ventilation
- Duration of invasive and non-invasive ventilation and/or supplemental oxygen
- Time to normalization of WBC and lymphocytes

4.4 Exploratory Outcome Measures

- National Early Warning Score (NEWS-2)
- Sequential organ failure assessment (SOFA) scores
- ROX Index
- Progression to ARDS
- Changes on chest X-ray or CT

5 STUDY DESIGN

This study is a randomized two-arm open-label study.

5.1 Study Description

This Phase 2 study will randomize approximately 120 patients with COVID-19: of which 80 will receive sargramostim + SOC, and 40 patients who will receive SOC alone.

All patients on the sargramostim arm will be treated with 125 mcg inhaled sargramostim twice daily (with the interval between doses per institutional practice for a twice daily dosing) for 5 days delivered via a mesh nebulizer, in addition to institutional standard of care.

Patients cannot start the study using intravenous (IV) administration of sargramostim. If a patient cannot continue to receive inhaled sargramostim upon progression to an invasive mechanical ventilator, sargramostim may be delivered by IV infusion to complete a total of 5 days (including days delivered via inhalation).

Patients on the control arm will receive institutional standard of care alone.

This study is designed as a proof of concept study and will enroll 120 patients. The sample size was increased from 60 patients to 120 patients (80 sargramostim treated patients and 40 control patients) to collect additional safety and efficacy data of inhaled sargramostim in hospitalized patients. This study is not designed nor expected to show substantial evidence of effectiveness sufficient to support a label

indication. Inclusion of additional patients will enable evaluation of less common adverse events. Furthermore, enrolling more patients will also enable greater precision of estimates of safety and efficacy measures.

5.2 Randomization

Randomization will be performed, using a 2:1 randomization ratio, with the strata defined as:

- investigational site,
- SOFA score (<6 versus ≥ 6),

6 KEY ELIGIBILITY CRITERIA

6.1 Inclusion Criteria

1) Patients aged ≥ 18 years
2) Patients (or legally authorized decision maker) must provide informed consent form
3) Test positive for SARS-CoV-2 virus by PCR (positive confirmation prior to start of sargramostim)
4) Admitted to hospital
5) Presence of acute hypoxemic respiratory failure defined as (either or both)
   a) Saturation below 93% on ≥ 2 L/min oxygen supplementation
   b) PaO_2/FiO_2 below 350

6.2 Exclusion Criteria

1) Patients requiring invasive (mechanical ventilation) or non-invasive (CPAP, BiPAP for hypoxemia) ventilation or ECMO. (Note: oxygen supplementation using high flow oxygen systems or low flow oxygen systems would not exclude patients from this study)
2) Intractable metabolic acidosis
3) Cardiogenic pulmonary edema
4) Hypotension requiring use of vasopressors
5) Hyperferritinemia (serum ferritin ≥2,000 mcg/L)
6) White blood cell count > 50,000/mm^3
7) Participation in another interventional clinical trial for COVID-19 therapy
8) Highly immunosuppressive therapy or anti-cancer combination chemotherapy within 24 hours prior to first dose of sargramostim
9) Known or suspected intolerance or hypersensitivity to sargramostim, or any component of the product
10) Previous experience of severe and unexplained side effects during aerosol delivery of any kind of medical product
11) Presence of any preexisting illness that, in the opinion of the Investigator, would place the patient at an unreasonably increased risk through participation in this study.
12) Pregnant or breastfeeding females

13) Severe or uncontrolled pulmonary comorbid conditions, including systemic steroid dependent asthma, systemic steroid dependent COPD, oxygen dependent COPD, lung transplant, known interstitial lung disease, or cystic fibrosis

6.3 Replacement of ineligible patients

In the event a patient has been enrolled to the study pending their COVID-19 PCR result, which is subsequently confirmed as negative, they may continue on the study per protocol, provided the investigator believes continuation in the study is in the best interest of the patient, taking into account potential benefits and risks.

Any ineligible patients are to be replaced to ensure approximately 80 *eligible* patients are enrolled to the sargramostim arm and 40 *eligible* patients are enrolled to the SOC arm.

7 DRUG INFORMATION

Each investigational site is expected to follow its own institutional standards for COVID-19 treatment regimens and duration of treatment for standard of care. All patients in this study will receive standard of care per their institutional standards.

7.1 Sargramostim Treatment Regimen

All patients randomized to the sargramostim treatment arm will be treated with 125 mcg inhaled sargramostim twice daily (with the interval between doses per institutional practice for a twice daily dosing) for 5 days delivered via a mesh nebulizer.

Patients cannot start the study using IV administration of sargramostim. If a patient cannot continue to receive inhaled sargramostim upon progression to an invasive mechanical ventilator, administration of sargramostim may be delivered by IV infusion to complete a total of 5 days (including days delivered via inhalation). Sargramostim IV dose is 125 mcg/m^2 administered intravenously over 4 hours once daily.

Refer to the Pharmacy Manual for full details on sargramostim supply, preparation and accountability.

Follow local institution practice regarding inhaled medications for the administration of sargramostim to patients on ventilator.

7.2 Sargramostim Dose Modifications

7.2.1 Dose modification for AEs

Patients with documented white blood cell count > 50,000/mm^3 should stop sargramostim treatment.

7.3 Concomitant Medications and Medical Procedures

Patients should receive appropriate standard of care for COVID-19-associated acute hypoxemia, and for any other medical condition's patients may have or develop. This study does not restrict, or limit concomitant medications or medical procedures patients may need.

8 SAFETY MONITORING

All Adverse events (AE) will be managed and reported in compliance with all applicable regulations, and included in the final clinical study report.

8.1 Definitions

8.1.1 Adverse Event

An Adverse Event is any untoward medical occurrence in a patient administered with a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment.

This includes the following:

- AEs not previously observed in the patient that emerge during the reporting period, that were not present prior to the AE reporting period
- Preexisting medical conditions (other than the condition being studied) judged by the investigator to have worsened in severity or frequency or changed in character during the reporting period

8.1.2 Serious Adverse Event

A Serious Adverse Event (SAE) is any untoward medical occurrence regardless of causality that results in any of the following:

- Death
- A life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity
- Congenital anomaly/birth defect;
- An important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above

8.2 Safety Reporting

8.2.1 Adverse Event Reporting

All AEs regardless of seriousness, starting from informed consent (i.e., occurring during the baseline period even in the absence of any administration of treatment), up to 30 days following the last dose of treatment, are to be recorded on the case report form (CRF). Any SAEs experienced after this 30 day period should only be reported if the investigator suspects a causal relationship to the study drug.

Whenever possible, diagnosis or single syndrome should be reported instead of symptoms. The Investigator should specify the date of onset, severity, action taken with respect to study drug, corrective treatment/therapy given, additional investigations performed, outcome, and his/her opinion as to whether there is a reasonable possibility that the AE was caused by the study drug. Adverse events should be followed until the event has resolved, stabilized, has decreased in severity to the same or less than baseline (pre-treatment), or patient has completed the follow-up period.

Vital signs or ECG abnormalities are to be recorded as AEs only if they are symptomatic and/or requiring corrective treatment and/or leading to treatment discontinuation and/or modification of dosing and/or fulfilling a seriousness criterion.

Laboratory abnormalities are to be recorded as AEs only if they lead to treatment discontinuation, modification of dosing, fulfill a seriousness criterion, additional concomitant therapy, and/ or additional medical procedures.

Treatment-emergent adverse events are adverse events that begin or worsen after the start of treatment.

8.2.2 Attribution of the AE

The relationship or attribution of an adverse event to treatment is defined as follows:

- *Definite* - The AE is clearly related to the study treatment
- *Probable* - The AE is likely related to the study treatment
- *Possible* - The AE may be related to the study treatment
- *Unlikely* - The AE is doubtfully related to the study treatment
- *Unrelated* - The AE is clearly NOT related to the study treatment

8.2.3 Grading of AEs

All adverse events will be graded using the Common Terminology Criteria for Adverse Events (CTCAE), version 5.0 or later. Grade refers to the severity of the AE. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline:

Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated

Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL; i.e. preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc)

Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL (i.e. bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden)

Grade 4: Life-threatening consequences; urgent intervention indicated

Grade 5: Death related to AE

CTCAE resources: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

Quick reference guide:
https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf

8.2.4 Serious Adverse Event Reporting

In the case of a SAE, a pregnancy report, or an overdose, the Investigator must immediately:

- REPORT (**within 24 hours** by fax or e-mail) the CIOMS information related to the SAE, pregnancy or overdose to PTx

e-mail: [redacted]
fax number: [redacted]

ENTER the information related to the SAE in the appropriate SAE form and AE CRF and include a photocopy of all examinations carried out and the dates on which these examinations were performed. Care should be taken to ensure that the patient's identity is protected and the patient's identifiers in the clinical trial are properly

mentioned on any copy of source document provided to PTx. For laboratory results, include the laboratory normal ranges.

- All further data updates should be recorded in the SAE form and AE CRF as appropriate, and further documentation as well as additional information (for laboratory data, concomitant medication, patient status) should be reported (by fax or e-mail as detailed above) to PTx within 24 hours of knowledge. In addition, effort should be made to further document each SAE that is fatal or life threatening within the week (7 days) following initial notification.
- An SAE occurring at a different time interval or otherwise considered completely unrelated to a previously reported one should be reported separately as a new event.

8.3 Expected Toxicities

For inhaled sargramostim use in patients with COVID-19, no serious adverse events are considered to be expected except for COVID-19 disease-related adverse events. If the investigator considers a COVID-19 disease related event to be related to inhaled sargramostim, or if the event is more severe than anticipated for the disease, and the event is serious, it must be reported as a suspected unexpected serious adverse reaction (SUSAR).

COVID-19 is a very recent syndrome, on which few data are available. Clinical features that will be considered as events due to the disease, include dyspnea, cough, sputum production, expectoration, pneumonia, malaise, myalgia, fatigue, fever, anorexia, vomiting, diarrhea, headache, sore throat, chest tightness, chest pain, chest discomfort, abnormal laboratory values (leukopenia, lymphopenia, thrombocytopenia, elevated levels of C-reactive protein), drop in oxygen saturation, progression to respiratory failure, progression to acute respiratory distress syndrome, hypotension, or progression to multi-organ failure (Wang, 2020; Huang, 2020; Chen, 2020a; Yang, 2020). Severe COVID-19 may lead to death.

8.4 Unexpected Toxicities

Suspected Unexpected Serious Adverse Reactions (SUSARs) will be collected and reported to the competent authorities and relevant ethics committees in accordance with Directive 2001/20/EC or as per national regulatory requirements in participating countries.

9 RISKS AND TOXICITIES

9.1 Overdose

There is no clinical experience with overdose of sargramostim, and no specific antidote or detoxification measures can be recommended to date. If accidental overdose is suspected, the patient should be treated symptomatically.

9.2 Pregnancy or Lactation

There are limited data in pregnant and breastfeeding women. There is potential for increased risk of spontaneous abortion based on rabbit studies. Investigators should counsel patients of the risk to the fetus. Do NOT use diluents with preservatives.

Note - this information is based on systemic administration of sargramostim. There are no data available for inhaled sargramostim.

9.2.1 Pregnancy Risk Summary

The limited available data on sargramostim use in pregnant women are insufficient to inform the drug-associated risk of adverse developmental outcomes. Based on animal studies sargramostim may cause embryofetal harm. In animal reproduction studies, administration of sargramostim to pregnant rabbits during organogenesis resulted in adverse developmental outcomes including increased spontaneous abortion at systemic exposures ≥1.3 times the human exposure expected at the recommended human dose. Advise pregnant women of the potential risk to a fetus.

The estimated background risk of major birth defects and miscarriage for the indicated population is unknown. All pregnancies have a background risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risks of major birth defects and miscarriage in clinically recognized pregnancies are 2%-4% and 15%-20%, respectively.

9.2.2 Lactation Risk Summary

There is no information regarding the presence of sargramostim in human milk, the effects on the breastfed child, or the effects on milk production. Administration of sargramostim to rabbits during lactation resulted in reduction in postnatal offspring survival. Because of the potential for serious adverse reactions advise a lactating woman not to breastfeed during treatment and for at least 2 weeks after the last dose.

10 SCHEDULE OF EVENTS AND STUDY PARAMETERS

The following safety and efficacy parameters are anticipated to be collected as standard patient care. This protocol aims to minimize protocol-specific procedures and additional data collection that would not be routinely performed as standard patient care.

All procedures should follow institutional practice for managing patients with COVID-19. There may be procedures defined in the schedule below that are not performed or collected as part of an institution's SOC for managing patients with COVID-19. For procedures that are not SOC, these can be marked not done when not performed or not collected.

Table 1: Schedule of Events

Procedure	Screening Period Within 48 hours of randomization	Treatment Period	Post-treatment	Follow-up Period			
		Days 1-5	Day 6 or day of Discharge (whichever is earlier) [1]	Day 7-27 or Until Discharge [2]	Day 28 (End of Study)	Day 58	Day 90 (+/- 30 days)
Visit Location	Hospital	Hospital	Hospital	Hospital	Hospital, Virtual Visit, or Phone Call [3]	Virtual visit or Phone call	Hospital, Virtual Visit, or Phone Call [4]
Informed consent	X*						
Medical History	X						
COVID-19 PCR	X						
Physical Examination	X	X	X	X	X		X
Vital signs	X	X	X	X	X		X
Ordinal Scale, NEWS-2, SOFA, ROX scales	X	X	X	X	X		X
ECG	X	X	X	X			X
Documentation of ventilation and Supplemental Oxygen	**X***	**X***	**X***	**X*[7]**	**X***		**X***
Oxygen saturation	X	X	X	X	X		X
Serum pregnancy test	X*						
Hematology	X	X	X	X	X		X
Ferritin, d-dimer, CRP	**X***	**X***	**X***	X	X		X
Chemistry	X	X	X	X	X		X
Arterial blood gases [8] (P(A-a)O2 gradient, etc.)	**X***	**X***	**X***	X	X		X
Immune profiling	X	X	X	X	X		X
Chest X-ray / CT	X	X	X	X	X		X
Ventilator parameters	X	X	X	X	X		
Sargramostim administration		X*					
Concomitant medications		X	X	X	X		
Adverse events*		**X***	**X***	**X***	**X***	**X*[5]**	**X*[5]**
Survival status					**X*[6]**	**X*[6]**	**X*[6]**

***Indicates <u>required</u> study-specific procedures considered beyond institutional SOC for patients with COVID-19.**

(1) Day 6 study assessments will be conducted up to 24 hours after the last dose of sargramostim. Should the patient be discharged before Day 6, the last day of study drug administration will be recorded, and post-treatment assessments will be conducted within 24 hours of the last dose, before the patient is discharged from the hospital.
(2) During Days 7-27, study procedures and data will be collected until Day 27 or discharge, whichever is earlier.
(3) If a virtual visit or telephone call is conducted, collect adverse events, and documentation of ventilation and supplemental oxygen.
(4) Collection of final follow-up on Day 90 can occur anytime between Day 60-120. If the patient had a hospital visit during this period, collect all relevant data as indicated. If patient had a virtual visit or phone call during this period collect adverse events, survival status , and documentation of ventilation and supplemental oxygen.
(5) AE data should be collected up to 30 days following the last dose of treatment. Any SAEs experienced after this 30 day period should only be reported if the investigator suspects a causal relationship to the study drug.
(6) Survival status can also be obtained using public records, such as obituaries or vital records search, where country and local regulations permit.
(7) Documentation of ventilation (and supplemental oxygen if admitted) is required on Day 14 only.
(8) Arterial blood gases should be performed via arterial puncture on Day 1 or within 24 hours before first sargramostim and/or standard of care and at Day 6 or day of discharge, whichever is earlier. On all other days, if arterial blood gas assessment is not done, PaO2 will be estimated from percent O2 saturation (SpO2) by pulse oximetry. For sites located at higher altitudes (> 1000m above sea level), an altitude correction to the PaO2/FiO2 ratio should be made by multiplying the PaO2/FiO2 ratio using average ambient to sea level barometric pressure correction. See Section 10.2 for more details.

10.1 Safety Evaluations

Laboratory data, as performed and collected as part of SOC (see Table 1), will be collected and include the following:

- Hematology laboratory parameters: complete blood count with differential, hemoglobin, hematocrit, and absolute counts for white blood cells, platelets, neutrophils, lymphocytes, eosinophils and monocytes.
- Chemistry laboratory parameters: albumin, amylase, lipase, BUN, creatinine, ALT, AST, LDH, serum alkaline phosphatase, direct and total bilirubin, glucose, total protein, sodium, potassium, chloride, bicarbonate, calcium and uric acid.
- Required laboratory measurements of ferritin, d-dimers and C-reactive protein should also be collected at screening, Days 1-6 and any other time it is performed as part of SOC.
- Immune profiling, if performed, including CD4+, CD8+, HLA-DR, IL-6, IL-1, GM-CSF

10.2 Efficacy Evaluations

Efficacy data, as performed and collected as SOC (see Table 1), will be collected and include the following:

- The need for and duration of mechanical ventilation, or other means of respiratory support.
- Evaluation of arterial blood gases for oxygenation parameters including PaO_2 (partial pressure of oxygen), FiO_2 (fraction of inspired oxygen), PaO_2/FiO_2ratio, SpO_2 (peripheral oxygen saturation), $PaCO_2$ (partial pressure of carbon dioxide), bicarbonate, pH and $P(A\text{-}a)O_2$ gradient (alveolar-arterial) oxygen gradient.
    - For sites located at higher altitudes (> 1000m above sea level), an altitude correction to the PaO2/FiO2 ratio should be made by multiplying the PaO2/FiO2 ratio using average ambient to sea level barometric pressure correction (e.g. correction of 0.86 for sites located in Salt Lake City, Utah or correction of 0.84 for sites located in Denver, Colorado).
    - Arterial blood gases should be performed via an arterial puncture taken on Day 1 (or within 24 hours) prior to the first dose of sargramostim and/or standard of care and at Day 6 (or day of discharge, whichever is earlier). Assessment of oxygenation on all other days can be determined using a noninvasive measurement by estimating PaO2 from percent saturation of hemoglobin with oxygen as measured by pulse oximetry (SpO2). The table below provides the imputed PaO2/FiO2 ratio (cells) for combinations of SpO2 (rows) and FiO2 (columns).

	0.30	0.35	0.40	0.45	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95	1.00
80%	148	127	111	98	89	81	74	68	63	59	55	52	49	47	44
81%	151	129	113	101	91	82	76	70	65	60	57	53	50	48	45
82%	155	132	116	103	93	84	77	71	66	62	58	55	52	49	46
83%	158	136	119	106	95	86	79	73	68	63	59	56	53	50	47
84%	162	139	122	108	97	89	81	75	70	65	61	57	54	51	49
85%	167	143	125	111	100	91	83	77	71	67	63	59	56	53	50
86%	171	147	129	114	103	94	86	79	73	69	64	61	57	54	51
87%	177	151	132	118	106	96	88	81	76	71	66	62	59	56	53
88%	182	156	137	121	109	99	91	84	78	73	68	64	61	58	55
89%	189	162	141	126	113	103	94	87	81	75	71	67	63	60	57
90%	196	168	147	130	117	107	98	90	84	78	73	69	65	62	59
91%	203	174	153	136	122	111	102	94	87	81	76	72	68	64	61
92%	213	182	159	142	128	116	106	98	91	85	80	75	71	67	64
93%	223	191	168	149	134	122	112	103	96	89	84	79	74	71	67
94%	236	202	177	157	142	129	118	109	101	94	89	83	79	75	71
95%	252	216	189	168	151	138	126	116	108	101	95	89	84	80	76
96%	273	234	205	182	164	149	136	126	117	109	102	96	91	86	82

Table from: Brown et al. *Nonlinear Imputation of Pao2/Fio2 From Spo2/Fio2 Among Patients With Acute Respiratory Distress Syndrome*. Chest 2016; 150(2): 307-313.

- Identification / occurrence of nosocomial infections through the evaluation of BAL (bronchoalveolar lavage), sputum, skin, urine and blood culture results or other microbiology results.
- Glasgow Coma Score (GCS), as a component of the Sequential Organ Failure Assessment (SOFA) score, see Appendix A for assessments.
- Sequential Organ Failure Assessment (SOFA) based on six different scores, one each for the respiratory, cardiovascular, hepatic, coagulation, renal, and neurological systems, see Appendix B for assessments.
- Ordinal score based on an 8-point scoring system, see Appendix C for assessments.
- National Early Warning Score (NEWS-2), see Appendix D for assessments.
- ROX Index, see Appendix E for assessments.
- Radiographic changes on a chest X-ray or CT of thorax region to assess ARDS progression.
- Mortality.

11 STUDY DURATION AND EARLY TERMINATION

11.1 Patient Discontinuation Criteria

Patients will receive sargramostim and/or SOC for the duration of treatment and remain on study for the duration of study period, unless:

- Patient withdraws consent or decision by the patient (or his/her legally authorized decision maker) or investigator/attending physician to stop treatment
- Unacceptable toxicity to sargramostim and/or SOC
- Occurrence of a treatment-related serious adverse event (see Section 8.2.2 for treatment attribution)

- Occurrence of a treatment-related Grade ≥ 3 adverse event (see Section 8.2.2 for treatment attribution)
- Rapid deterioration requiring other advanced treatments or medical care not conducive to continued treatment with sargramostim
- Death

Patients who discontinue treatment early, should remain on study and continue follow-up per the schedule of events (unless consent is withdrawn from study participation).

11.2 Study Completion

Study completion will be defined as the date when the last patient completes study treatment (including SOC), completes the follow-up period, or has withdrawn from the study. Alternatively, the study may be stopped earlier for reasons such as feasibility, poor enrollment, ethical or safety reasons.

Refer to Table 1, for a complete list of assessments required through the clinical trial.

11.3 Duration of Treatment (Days 1-5)

All patients randomized to the sargramostim treatment arm will receive sargramostim treatment for up to 5 days. For the purpose of this study, Day 1 is the day the patient receives the first dose of sargramostim and/or SOC. All patients will receive SOC per their institutional standards; SOC may vary in duration.

If at any time the constraints of this protocol are detrimental to the patient's health, protocol treatment should be discontinued. This includes any clinical adverse event, laboratory abnormality or intercurrent illness which, in the opinion of the investigator, indicates that continued treatment with study therapy is not in the best interest of the patient.

11.4 Duration of Study Period (Days 6-28)

Following the sargramostim treatment period (Days 1-5), patients should continue to receive SOC as appropriate following institutional guidelines. The study period (Days 6-28) will continue until completion of all safety and efficacy assessments on Day 28 (end of study).

All patients, including those who discontinue treatment early, should be followed for the duration of the study period.

11.5 Duration of Follow-up (up to Day 90)

All patients, including those that have withdrawn from the study or discontinued sargramostim, should be followed for safety evaluations for up to Day 90. Data from a final follow-up visit at day 90 (+/-30 days) should also be collected.

Only patients that have withdrawn their consent to participate in the trial are excluded from further follow-up after their consent has been withdrawn. Any available data up to the date of withdrawal of consent will be used for the purpose of this study.

12 STATISTICAL PLAN

12.1 Sample size consideration

Approximately 120 patients will be randomized: of which 80 will receive sargramostim + SOC, and 40 patients who will receive SOC alone to evaluate the clinical efficacy and safety of sargramostim in patients with COVID-19 associated acute hypoxemia.

The sample size was based on practical and clinical considerations to ensure that the efficacy endpoints and safety profile could be appropriately evaluated and was not based on any statistical assumptions or hypotheses. As a result, the analyses of this study will be considered as descriptive analyses.

12.2 Data Safety Monitoring Board

The DSMB will comprise of independent external experts to review the safety and benefit/risk data using the following guidelines:

- after the first approximately 10 patients are treated for 5 days with sargramostim; enrollment will be paused after the 10th patient is enrolled until the safety review is completed
- after the first approximately 20 patients are treated for 5 days with sargramostim; enrollment will be paused after the 20th patient is enrolled until the safety review is completed
- after approximately 60 patients (total of both treatment arms) are enrolled to the study. Upon completion of this safety review, the DSMB may request an additional review after approximately 80 patients have been randomized. Enrollment will not be paused during these reviews.
- any reported significant SAE can also trigger a safety review, including, but not limited to ≥2 patients experiencing a similar SAE or grade ≥3 AE, ≥2 patients discontinue for similar safety reasons; an unplanned safety review may require an enrollment pause

The safety review will occur as a real-time review of patient data, using the data available as well as a cumulative safety review as the data accrues. That is, the available data will be accepted as is, and may not have gone through any data cleaning processes. The DSMB will review adverse events and serious adverse events, key laboratory parameters such as ferritin, d-dimers, and CRP, and other routine laboratory values (hematology, chemistry, coagulation) as well as oxygenation parameter P(A-a)O_2 gradient.

The DSMB will make recommendations on pausing enrollment, modifying and/or stopping the study, including assessing whether randomization should continue, or whether additional changes to the treatment arms is warranted.

The DSMB can also modify the planned schedule of safety reviews based on the observed data, taking into account the study enrollment patterns, observed safety profile of the ongoing study, and/or any other external data that may emerge.

Refer to the Data Safety Monitoring Board Charter for additional details.

12.3 General Statistical Methods

All patients who receive at least one dose of sargramostim and/or SOC will be included in the efficacy and safety analyses. An additional analyses will be performed on the intent-to-treat population defined as all randomized patients.

A per protocol population will be defined as all patients who receive sargramostim and/or standard of care and meet the eligibility criteria of this study. Violations of the eligibility criteria have the potential to influence the efficacy or safety results of the study (see Section 6 for eligibility criteria).

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 logrank 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 two treatment arms 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 Chi^2 tests (as appropriate).

Continuous endpoints will be summarized by n, means, medians, minimum, maximum, and 25^{th} and 75^{th} percentiles. Analysis of variance, analysis of covariance, and/or repeated measures statistical approaches 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.

Missing data may be imputed using last-observation-carried forward, or other advanced statistical imputation methods.

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.

A statistical analysis plan will contain further details of the planned statistical analyses. Any important changes to the planned statistical analyses will be documented as amendments to the statistical analysis plan and/or documented in the study report.

12.3.1 Primary Endpoint(s): Oxygenation at Day 6, and Intubation by Day 14

The primary endpoint of change in oxygenation parameter of $P(A\text{-}a)O_2$ gradient will be evaluated, using a repeated measure analyses of variance methods. Key timepoints of interest include Day 6 (primary measure) and Day 14 (secondary measure).

Another key primary endpoint will be the rate of intubation by Day 14. That is, any occurrence of intubation at any point time in time between Days 1 and 14, will be considered to be an event. The treatment arms will be compared using a Fisher's Exact or Chi^2 tests (as appropriate), and/or by logistic regression approaches.

Handling of missing data is specified above in Section 12.3.

12.3.2 Secondary and Exploratory Endpoints

The secondary and exploratory endpoints described in Sections 4.3 and 4.4 above will be analyzed using the general statistical methods as described above in Section 12.3. Key timepoints of interest for endpoints include Day 6, Day 14, and Day 28, where data are available. Assessments of ventilator usage will consider alternative devices, in light of possible ventilator shortages. Refer to FDA Guidance for further advice:

> Enforcement Policy for Ventilators and Accessories and Other Respiratory Devices During the Coronavirus Disease 2019 (COVID-19) Public Health Emergency: https://www.fda.gov/media/136318/download
>
> Ventilator Supply Mitigation Strategies: Letter to Health Care Providers: https://www.fda.gov/medical-devices/letters-health-care-providers/ventilator-supply-mitigation-strategies-letter-health-care-providers

13 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

The Principal Investigator and/or designee, will prepare and maintain adequate and accurate participant case histories with observations and data pertinent to the study. Study specific Case Report Forms (CRFs) will document safety and treatment outcomes for safety monitoring and data analysis. All study data will be entered into standardized CRFs in accordance with the overall study schedule.

The information collected on CRFs shall be identical to that appearing in original source documents. Source documents will be found in the patient's medical records maintained by study site personnel. All original source documentation must be maintained at the study site.

In accordance with federal regulations, the Investigator is responsible for the accuracy and authenticity of all clinical and laboratory data entered onto CRFs. The PI will approve all completed CRFs to attest that the information contained on the CRFs is true and accurate.

In signing this protocol, the investigator understands and agrees to give access to the necessary source documentation and files to enable study monitoring by employees or representatives of Partner Therapeutics.

Monitoring of the study will be performed in compliance with 21 CFR 312. Alternative methods of monitoring may need to be employed in light of the COVID-19 pandemic, and in accordance with FDA guidance on "FDA Guidance on Conduct of Clinical Trials of Medical Products during COVID-19 Public Health Emergency", available here: https://www.fda.gov/media/136238/download.

14 QUALITY CONTROL AND QUALITY ASSURANCE

To ensure compliance with GCP and all applicable regulatory requirements, a quality assurance audit may be conducted. Regulatory agencies may also conduct a regulatory inspection of this study. Such audits/inspections can occur at any time during or after completion of the study. If an audit or inspection occurs, the investigator and institution agree to allow the auditor/inspector direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and any relevant issues. In the case of an audit or inspection, the Investigator or a designee will alert Partner Therapeutics as soon as he/she becomes aware of the audit or inspection.

The investigator and study staff are responsible for maintaining a comprehensive and accurate filing system of all study-related documentation that will be suitable for inspection at any time by Partner Therapeutics and its designees, and/or regulatory agencies.

15 ETHICS

This study will be conducted in compliance with Institutional Review Board (IRB) and ICH GCP Guidelines; United States (US) Code of Federal Regulations (CFR) dealing with clinical studies (21 CFR § 50, 56, 312); the Declaration of Helsinki, and with applicable ICH guidelines regarding scientific integrity . This study will also adhere to all Food and Drug Administration (FDA), state and local regulatory requirements, and requirements for data protection.

15.1 Informed consent

Investigators must follow local IRB approved institutional practice for obtaining either written or verbal informed consent which should be obtained after adequate, thorough and clear explanation of the study objectives, procedures, as well as the potential hazards of the study. The investigator must use the most current IRB-approved consent form. In cases where the patient is incapable of providing consent and the

patients legally authorized decision maker is providing consent, the patient should be informed about the study to the extent possible given his/her understanding.

16 DATA HANDLING AND RECORD KEEPING

The collection and processing of personal data from patients 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. Records related to the study will be retained for a period of fifteen (15) years after the completion or discontinuation of the study, unless otherwise notified by the Sponsor.

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 patients confidential.

The informed consent obtained from the patient 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, as well as for research purposes unrelated to this study. This consent also addresses the transfer of the data to other entities, if applicable.

17 FINANCING AND INSURANCE

Before study initiation, each investigator must provide a protocol signature page and a fully executed and signed Form FDA 1572 to Partner Therapeutics. Financial Disclosure Forms must be completed by all investigators and sub investigators who will be directly involved in the treatment or evaluation of research patients in this study. The investigator(s) are required to disclose any financial arrangement whereby the value of the compensation for conducting the study could be influenced by the outcome of the study following information; any significant payments of other sorts from Partner Therapeutics, such as a grant to fund ongoing research, compensation in the form of equipment, retainer for ongoing consultation, or honoraria; any proprietary interest in sargramostim; any significant equity interest in Partner Therapeutics as defined in the US Code of Federal Regulations (21 CFR 54 2(b)).

18 PUBLICATION POLICY

The investigator may publish the data pertaining to patients enrolled at their institute on an accelerated timeline given the urgency of the COVID-19 pandemic. The investigator should provide advance notification of intent for accelerated disclosure and provide Partner Therapeutics any proposed publication or presentation along with information about the scientific journal or presentation forum, at least 2 business days prior to submission of the publication or presentation for the initial disclosure.

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APPENDIX A: Glasgow Coma Score

Feature	Response	Score
Best eye response	Open spontaneously	4
	Open to verbal command	3
	Open to pain	2
	No eye opening	1
Best verbal response	Orientated	5
	Confused	4
	Inappropriate words	3
	Incomprehensible sounds	2
	No verbal response	1
Best motor response	Obeys commands	6
	Localized pain	5
	Withdrawing from pain	4
	Flexion to pain	3
	Extension to pain	2
	No motor response	1

APPENDIX B: SOFA score

System	Measure	SOFA Score
Respiratory PaO2/FiO2 [mmHg (kPa)]	≥ 400 (53.3)	0
	< 400 (53.3)	+1
	< 300 (40)	+2
	< 200 (26.7) **and** mechanically ventilated	+3
	< 100 (13.3) **and** mechanically ventilated	+4
Neurological Glasgow Coma Score	15	0
	13–14	+1
	10–12	+2
	6–9	+3
	< 6	+4
Cardiovascular Mean arterial pressure OR administration of vasopressors required	MAP ≥ 70 mmHg	0
	MAP < 70 mmHg	+1
	dopamine ≤ 5 µg/kg/min or dobutamine (any dose)	+2
	dopamine > 5 µg/kg/min OR epinephrine ≤ 0.1 µg/kg/min OR norepinephrine ≤ 0.1 µg/kg/min	+3
	dopamine > 15 µg/kg/min OR epinephrine > 0.1 µg/kg/min OR norepinephrine > 0.1 µg/kg/min	+4
Hepatic Bilirubin (mg/dl) [µmol/L]	< 1.2 [< 20]	0
	1.2–1.9 [20-32]	+1
	2.0–5.9 [33-101]	+2
	6.0–11.9 [102-204]	+3
	> 12.0 [> 204]	+4
Coagulation Platelets×10^3/µl	≥ 150	0
	< 150	+1
	< 100	+2
	< 50	+3
	< 20	+4
Renal Creatinine (mg/dl) [µmol/L	< 1.2 [< 110]	0
	1.2–1.9 [110-170]	+1
	2.0–3.4 [171-299]	+2
	3.5–4.9 [300-440] (or < 500 ml/d)	+3
	> 5.0 [> 440] (or < 200 ml/d)	+4

APPENDIX C: Ordinal Score

Measure	Ordinal Score
Death	8
Hospitalized, mechanical ventilation + additional organ support (i.e. requiring ECMO, pressors, renal replacement therapy)	7
Hospitalized, requiring intubation and mechanical ventilation	6
Hospitalized, requiring high-flow oxygen therapy, non-invasive mechanical ventilation, or both	5
Hospitalized, requiring supplemental oxygen by mask or nasal prongs	4
Hospitalized, not requiring supplemental oxygen	3
Not hospitalized, limitation of activities	2
Not hospitalized, no limitation of activities	1
Not hospitalized, no clinical or virological evidence of infection	0

APPENDIX D: NEWS-2 score

Measure	Parameters	NEWS-2 Score
Respiratory rate, breaths per minute	≤8	+3
	9-11	+1
	12-20	0
	21-24	+2
	≤25	+3
SpO2 if patient has hypercapnic respiratory failure	≤83%	+3
	84-85%	+2
	86-87%	+1
	88-92%, ≥93% on room air	0
	93-94% on supplemental O2	+1
	95-96% on supplemental O2	+2
	≥97% on supplemental O2	+3
SpO2 on room air or supplemental O2	≤91%	+3
	92-93%	+2
	94-95%	+1
	≥96%	0
Room air or supplemental O2	Supplemental O2	+2
	Room air	0
Temperature	≤35.0oC (95oF)	+3
	35.1-36.0oC (95.1-96.8oF)	+1
	36.1-38.0oC (96.9-100.4oF)	0
	38.1-39.0 oC (100.5-102.2oF)	+1
	≥39.1oC (102.3oF)	+2
Systolic BP, mmHg	≤90	+3
	91-100	+2
	101-110	+1
	111-219	0
	≥220	+3
Pulse, beats per minute	≤40	+3
	41-50	+1
	51-90	0
	91-110	+1
	111-130	+2
	≥131	+3
Consciousness	Alert	0
	New-onset confusion (or disorientation/agitation), responds to voice, responds to pain, or unresponsive	+3

APPENDIX E: ROX Index

ROX Index calculation = SpO_2/FiO_2*, % / Respiratory rate, breaths/min

*Estimating FiO_2 from oxygen flow/delivery rates:

Type of O_2 delivery	Flow rates, L/min	FiO_2
Nasal cannula	1-6	~4% FiO_2 added above room air** per 1 L/min Room air = 21% 1 L/min = 25% 2 L/min = 29% 3 L/min = 33% 4 L/min = 37% 5 L/min = 41% 6 L/min = 45%
Simple face mask	~6-12	35-60%*
Non-rebreather mask	10-15	~70-90%
High-flow nasal cannula	Up to 60	30-100%

**Varies based on respiratory rate and minute ventilation.