Official Title:	**A Multicenter, Randomized, Double-blind, Placebo-Controlled, Parallel Group Study to Evaluate the Safety and Efficacy of Liquid Alpha1-Proteinase Inhibitor (Human) Plus Standard Medical Treatment (SMT) Versus Placebo Plus SMT in Hospitalized Subjects With COVID-19**
NCT Number:	NCT04547140
Document Date:	Protocol Version 6.0: 08 December 2021

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[redacted]	[redacted]	07-Dec-2021 17:32:10

Clinical Study Protocol

Protocol Title:	A Multicenter, Randomized, Double-blind, Placebo-Controlled, Parallel Group Study to Evaluate the Safety and Efficacy of Liquid Alpha$_1$-Proteinase Inhibitor (Human) plus Standard Medical Treatment (SMT) versus Placebo plus SMT in Hospitalized Subjects with COVID-19
Investigational Product:	Liquid Alpha$_1$-Proteinase Inhibitor (Human) or Liquid Alpha$_1$-PI
Sponsor's Name and Address:	Grifols Therapeutics LLC 79 TW Alexander Drive Research Triangle Park, NC 27709
Study Number:	GC2006
Additional Identifier	Liquid Alpha$_1$-Proteinase Inhibitor (Human) COVID-19
IND Number:	IND22462
Development Phase:	2
Sponsor Signatory:	[redacted] M.D. [redacted] Grifols Therapeutics, LLC. email address: [redacted] Phone: [redacted] [redacted] Grifols Bioscience Industrial Group Email address: [redacted] Telephone number: [redacted]

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Protocol Version History

Protocol Version	Date of Approval/Effective Date
6.0 Amendment 5 + Integrated Protocol	See left margin
5.0 Amendment 4 + Integrated Protocol	19 Jul 2021
4.0 Amendment 3 + Integrated Protocol	02 Mar 2021
3.0 Amendment 2 + Integrated Protocol	30 Jul 2020
2.0 Amendment 1 + Integrated Protocol	01 Jul 2020
1.0 Original	18 May 2020

Amendment 5

The protocol for GC2006 (Version 5.0, dated 19 Jul 2021) has been amended as Protocol Amendment 5, Version 6.0. See the table in Appendix 5 for a summary of changes for Protocol Amendment 5.

PROTOCOL SYNOPSIS

Title of Study: A Multicenter, Randomized, Double-blind, Placebo-Controlled, Parallel Group Study to Evaluate the Safety and Efficacy of Liquid Alpha$_1$-Proteinase Inhibitor (Human) plus Standard Medical Treatment (SMT) versus Placebo plus SMT in Hospitalized Subjects with COVID-19

Short Title: Study to Evaluate the Safety and Efficacy of Liquid Alpha$_1$-Proteinase Inhibitor (Human) in Hospitalized Subjects with Coronavirus Disease (COVID-19)

Study Number: GC2006

Phase: 2

Study Objectives:

Primary Efficacy Objective

To determine if Liquid Alpha$_1$ Protease Inhibitor (Human) (Liquid Alpha$_1$-PI) plus SMT can reduce the proportion of subjects dying or requiring intensive care unit (ICU) admission on or before Day 29 or who are dependent on high flow oxygen devices or invasive mechanical ventilation on Day 29 versus placebo plus SMT in hospitalized subjects with COVID-19.

Secondary Efficacy Objective

To compare Liquid Alpha$_1$-PI plus SMT versus placebo plus SMT with regard to clinical efficacy as assessed by clinical severity, duration of hospital stay, dependency on oxygen or new need for ventilatory support, clinical response criteria including National Early Warning Score (NEWS), and clinical status scale through Day 29 in hospitalized subjects with COVID-19.

Exploratory Efficacy Objectives

- To evaluate the effect of Liquid Alpha$_1$-PI (Human) plus SMT versus placebo plus SMT with regard to quantitative severe acute respiratory syndrome, coronavirus 2 (SARS-CoV-2) viral load and anti-SARS-CoV-2 antibodies in hospitalized subjects with COVID-19.
- To evaluate whether Liquid Alpha$_1$-PI (Human) plus SMT versus placebo plus SMT reduces the frequency of hyperinflammation based on a pre-specified biochemical definition through Day 29.
- To evaluate cytokine profile changes from baseline for Liquid Alpha$_1$-PI (Human) plus SMT versus placebo plus SMT through Day 29.
- To evaluate levels of alpha$_1$-proteinase inhibitor (alpha 1-PI, also known as alpha 1-antitrypsin [AAT]) activity and antigen through Day 29 (samples to be stored for measurement of both antigenic content and functional activity [potency] assays)

Safety Objective

To determine the safety and tolerability profile through Day 29 of Liquid Alpha$_1$-PI (Human) plus SMT versus placebo plus SMT in hospitalized subjects with COVID-19.

Overall Study Design and Description:

This is a prospective, multi-center, randomized (1:1), double-blind, placebo-controlled study of Liquid Alpha$_1$-PI (Human) plus SMT versus placebo plus SMT in subjects with COVID-19 who are hospitalized. Symptomatic subjects with positive polymerase chain reaction (PCR; reverse transcriptase [RT]-PCR) or nucleic acid amplification technology (NAT) for SARS-CoV-2 (or other commercial or public health assay approved by regulatory authorities) will receive placebo plus SMT or SMT plus Liquid Alpha$_1$-PI (Human) given as two intravenous (IV) doses of 120 mg/kg (body weight) one week apart (on Day 1 and Day 8); if the subject is discharged from hospital then the 2nd infusion is not mandatory, and is at the discretion of the Principal Investigator and subject.

Subjects will be assessed daily while hospitalized. Discharged subjects will be evaluated on Days 15 and 29. Phone Checks will occur at Day 60 and Day 90 for vital status (living or deceased), any hospital re-admissions, or serious/non-serious adverse events after the Day 29 Final Clinic Visit. Details are provided in Appendix 1 (Schedule of Study Procedures).

The first 6 subjects randomized will be staggered with an interval of no less than 1 week between subjects. If there are no definitely related serious adverse events (SAEs) reported by the time Day 8 is completed by the 6th subject, competitive enrollment would ensue thereafter. If a definitely related SAE were to be reported among these 6 subjects, and the subject was found by the Study-Independent Safety Review Committee (SISRC) to be randomized to the Liquid Alpha$_1$-PI (Human) plus SMT arm, the SISRC would carefully review and evaluate the case and make appropriate recommendations with regard to study status.

Number of Subjects Planned:

Approximately 100 subjects will be randomized (1:1) with an interim analysis after 50 subjects (approximately 25 per group). Due to the exploratory nature of this phase 2 study, the multiplicity adjustments will not be considered; full details will be described in the statistical analysis plan. This interim evaluation is part of Sponsor safety due diligence.

Interim Futility Analysis: An interim futility analysis will be conducted to assess whether the trial will be terminated due to lack of efficacy (futility) based on all available data assessable for the primary endpoint and key secondary efficacy endpoints.

Diagnosis and Main Criteria for Eligibility

Inclusion Criteria:

A subject must meet all the following inclusion criteria to be eligible for participation in this study.

1. Hospitalized male or female subject ≥ 18 years of age at time of Screening who is being treated for COVID-19. Subjects must be screened within 48 hours (≤ 48 hours) of hospital admission.
2. Has laboratory-confirmed novel coronavirus (SARS-CoV-2) infection as determined by qualitative PCR (reverse transcriptase [RT]-PCR), or other commercial or public health assay approved by regulatory authorities as a diagnostic test for COVID-19 in any specimen during the current hospital admission OR 96 hours prior to the hospital admission date and prior to randomization (the SARS-CoV-2 test results must be performed by a hospital laboratory and the documentation available).

3. COVID-19 illness (symptoms) of any duration, including **both of** the following:
    a) Radiographic infiltrates by imaging (chest X-Ray, CT scan, etc.) and/or clinical assessment (evidence of rales/crackles on exam) with SpO2 <94% on room air
    b) **Any One of** the following related to COVID-19: i. Ferritin > 400ng/mL, ii. LDH > 300 U/L, iii. D-Dimers > reference range, or iv. C-reactive protein (CRP) > 40 mg/L
4. Subject provides informed consent prior to initiation of any study procedures.
5. Female subjects of childbearing potential (and males with female partners of childbearing potential) must agree to use of acceptable contraception methods during study (e.g., oral, injectable, or implanted hormonal methods of contraception, placement of an intrauterine device or intrauterine system, condom or occlusive cap with spermicidal foam/gel/film/cream/suppository, male sterilization, or true abstinence*) throughout the study.

    *True abstinence: When this is in line with the preferred and usual lifestyle of the subject. (Periodic abstinence [e.g., calendar, ovulation, symptothermal, post-ovulation methods], declaration of abstinence for the duration of a trial, and withdrawal are not acceptable methods of contraception.)

Diagnosis and Main Criteria for Eligibility:

Exclusion Criteria

A subject meeting any of the following exclusion criteria is NOT eligible for participation in the study.

1. Subjects requiring invasive mechanical ventilation or ICU admission or with PaO2/FIO2 ≤ 150 mm Hg (i.e., arterial oxygen in mm Hg divided by fraction inspired oxygen concentration [e.g., 0.21 for room air])
2. Clinical evidence of any significant acute or chronic disease that, in the opinion of the investigator, may place the subject at undue medical risk.
3. The subject has had a known serious anaphylactic reaction to blood, any blood-derived or plasma product, or known Selective IgA Deficiency with anti-IgA antibodies.
4. A medical condition in which the infusion of additional fluid is contraindicated (e.g., decompensated congestive heart failure or renal failure with fluid overload). This includes currently uncontrolled congestive heart failure New York Heart Association Class III or IV stage heart failure.
5. Shock that is unresponsive to fluid challenge and/or multiple vasopressors and accompanied by multiorgan failure considered not able to be reversed by the Principal Investigator
6. Known alpha-1 antitrypsin deficiency for which the subject is already receiving alpha1-proteinase inhibitor augmentation therapy
7. Women who are pregnant or breastfeeding. Female subjects of child-bearing potential must have a negative test for pregnancy blood or urine human chorionic gonadotropin (HCG)-based assay at Screening/Baseline Visit.

8. Subjects for whom there is limitation of therapeutic effort such as "Do not resuscitate" status Note: If the decision is made not to apply treatments or therapeutic procedures that will provide little benefit for the suffering or agony the subject is experiencing, such a subject would not be appropriate for participation in this study and should be excluded.
9. Currently participating in another interventional clinical trial with investigational medical product or device
10. Subjects previously requiring long-term oxygen therapy (home oxygen therapy)
11. History (within the last 2 years) of myocardial infarction, unstable angina, stroke or transient ischemic attacks, pulmonary embolism or deep venous thrombosis
12. Subject has medical condition (other than COVID-19) that is projected to limit lifespan to ≤ 1 year
13. Systolic blood pressure < 100 mm Hg or > 160 mm Hg (uncontrolled hypertension) at the time of Screening
14. Alanine aminotransferase (ALT) ≥ 2 times the upper limit of normal (ULN)
15. Any elevation of total bilirubin at the time of Screening
16. Estimated glomerular filtration rate (eGFR) < 45 mL/min (or subject is dependent on dialysis/renal replacement therapy) at the time of Screening

    eGFR is calculated by the Cockcroft-Gault equation:

    For men:

$$\frac{(140 - \text{age in years})(\text{body weight [kg]})}{(72)(\text{serum creatinine } [\frac{\text{mg}}{\text{dL}}])}$$

    For women:

$$\frac{0.85 \text{ x } (140 - \text{age in years})(\text{body weight [kg]})}{(72)(\text{serum creatinine } [\frac{\text{mg}}{\text{dL}}])}$$

17. Hemoglobin < 10 g/dL at the time of Screening
18. Absolute neutrophil count < 1000/mm^3 at the time of Screening
19. Platelet count < 75,000/mm^3 at the time of Screening
20. Subject has history of drug or alcohol abuse within the past 24 months
21. Subject is unwilling to commit to follow-up visits
22. Known history of prothrombin gene mutation 20210, homozygous Factor V Leiden mutations, antithrombin III deficiency, protein C deficiency, protein S deficiency or antiphospholipid syndrome

Investigational Product, Dose and Mode of Administration

Subjects randomized to combination Liquid Alpha$_1$-PI (Human) plus SMT will receive the first dose of blinded Liquid Alpha$_1$-PI (Human) given as an IV dose of 120 mg/kg (body weight) on Day 1. A second dose of blinded Liquid Alpha$_1$-PI (Human) (120 mg/kg) will be infused on Day 8. If the subject has been discharged from the hospital at the time of the Day 8

blinded Liquid Alpha$_1$-PI (Human) infusion, the 2nd infusion is not mandatory, and is at the discretion of the Principal Investigator and subject. Liquid Alpha$_1$-PI (Human) has a concentration of alpha$_1$-proteinase inhibitor of 50 mg/mL. For a subject weighing 75 kg each dose of 120 mg/kg (on Day 1 and Day 8) will require 180 mL of Liquid Alpha$_1$-PI (Human) to achieve the specified 120 mg/kg dose.

Liquid Alpha$_1$-PI (Human) is clear, colorless or pale yellow or pale green. Do not use if discolored or cloudy. Before delivery to the subject, Liquid Alpha$_1$-PI (Human) should be brought to room temperature and administered at room temperature for comfort to the subject. Pooled Liquid Alpha$_1$-PI (Human) solution should be maintained at room temperature for administration within 3 hours.

Subjects will be randomized to either Liquid Alpha$_1$-PI (Human) 120 mg/kg or the same volume of normal saline as a placebo. Both treatment arms will filter the solution during administration using an IV administration set with a suitable 5 to 15-micron infusion filter. Infuse Liquid Alpha$_1$-PI (Human) separately, without mixing with other agents or diluting solutions. Infuse Liquid Alpha$_1$-PI (Human) IV at 0.08 mL/kg/min as determined by subject response and comfort. Both treatment arms' infusion lines can be flushed with 0.9% sodium chloride for injection. Do Not flush with heparin.

Duration of Treatment:

Subjects will receive blinded Liquid Alpha$_1$-PI (Human) or placebo on Day 1 and Day 8; for all subjects SMT will be continued throughout the subject's hospitalization. Subject participation (from Screening Visit to the Final Visit) will be approximately 30 days. After the Day 29 Final Clinic Visit, phone checks will occur at Day 60 and Day 90 for follow-up of vital status (living or deceased), any hospital re-admissions, or serious/non-serious adverse events.

Reference Therapy, Dose and Mode of Administration

Reference therapy will be standard medical treatment for COVID-19 plus sterile 0.9% sodium chloride injection, United States Pharmacopeia (0.9% NaCl, USP) or equivalent used as placebo. The infusion solution will be at a volume commensurate to that required for the appropriate weight-based dose of Liquid Alpha$_1$-PI (Human) and visually masked to maintain the blind.

Key Study Variables:

The primary efficacy variable is the proportion of subjects dying or requiring ICU admission on or before Day 29 or who are dependent on high flow oxygen devices or invasive mechanical ventilation on Day 29.

The secondary efficacy variables include:

- Assessment of Clinical Severity: Change in NEWS from baseline (Day 1 through Day 29)
- The NEWS has demonstrated an ability to discriminate patients at risk of poor outcomes. This score is based on 7 clinical parameters (respiration rate, oxygen saturation, any supplemental oxygen, temperature, systolic blood pressure, heart rate, level of

consciousness [Alert, Voice, Pain, Unresponsive]). https://www.mdcalc.com/national-early-warning-score-news (Appendix 2)

- Time to clinical response: NEWS ≤ 2 maintained for 24 hours, Day 1 through Day 29
- Time to hospital discharge: defined as duration of hospitalization from Day 1 of study
- If admitted to ICU post randomization: Duration of ICU stay through Day 29
- Duration of any oxygen use Day 1 through Day 29
- If requiring mechanical ventilation post randomization: Duration mechanical ventilation through Day 29
- Absolute value and mean change from baseline in the Ordinal scale Day 1 through Day 29

  The Ordinal scale is as follows:

  1) Death;
  2) Hospitalized, on invasive mechanical ventilation or extracorporeal membrane oxygenation (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, limitation on activities;
  7) Not hospitalized, no limitations on activities.

- Proportion (percentage) of subjects in each severity category of the 7-point Ordinal scale at Day 15 and Day 29
- Time to sustained normalization of temperature and proportion of subjects with normalization of fever at all time points, defined as temperature < 36.6 °C armpit, < 37.2°C oral, or < 37.8 °C rectal sustained for at least 24 hours
- Number of subjects who develop Acute Respiratory Distress Syndrome (ARDS) through Day 29 (Appendix 3)
- Length of time to clinical progression through Day 29 (defined as the time to death, mechanical ventilation, or ICU admission)

The exploratory efficacy variables include:

- Change from baseline in quantitative SARS-CoV-2 viral load by NAT or PCR (real-time RT-PCR) to Day 5± 1 day, Day 15± 1 day, and Day 29 ± 1 day
- Change from baseline in quantitative anti- SARS-CoV-2 IgM and IgG antibodies to Day 5± 1 day, Day 15± 1 day, and Day 29 ± 1 day
- Proportion of subjects with evidence of hyperinflammation on Day 1, Day 5, Day 15, and Day 29 defined as:
  a) Lymphocyte counts <1000 cells/µL, AND
  b) Two of the following 4 criteria:
     i) Ferritin > 500ng/mL,

ii) LDH > 300 U/L,
iii) D-Dimers > 1000 ng/mL (fibrinogen equivalent units [FEU]) or > 2 times upper normal limit,
iv) C-reactive protein (CRP) > 70 mg/L

- Change from baseline in cytokine profile (cytokine panel includes IL-1β, IL-10, IL-6, IL-8, IL-2, interferon γ, and tumor necrosis factor-α [TNF-α]) to Day 5± 1 day, Day 15± 1 day, and Day 29 ± 1 day
- Change from baseline in levels of alpha 1-PI (AAT) activity and antigen through Day 29 (both plasma [sodium citrate] and serum samples to be stored for measurement of both antigenic content and functional activity [potency])

The safety variables include:

- Cumulative incidence of treatment-emergent serious adverse events (SAEs) and potentially related SAEs through Day 90 phone check
- Cumulative incidence of Grade 3-5 treatment-emergent adverse events (TEAEs) and potentially related TEAEs through Day 29 as defined in the Common Terminology Criteria for Adverse Events (CTCAE), US Department of Health and Human Services, National Institutes of Health (NIH), and National Cancer Institute (NCI)
- Cumulative incidence of all TEAEs and potentially related TEAEs through Day 29

In this clinical study, disease progression is defined as the worsening of a subject's condition attributable to the disease for which the investigational product is being studied. It may be an increase in the severity of the targeted disease and/or increases in the symptoms of the targeted disease. Anticipated symptoms of COVID-19 include fever, cough, hypoxia, dyspnea, hemoptysis, myalgia, fatigue, pharyngitis, which may develop at any time during the course of the disease. The development of COVID-19 symptoms during the clinical study should not be recorded as individual TEAEs, unless there is evidence suggesting a causal relationship between blinded Liquid Alpha$_1$-PI (Human)/Placebo and the TEAE.

Study Assessments and Procedures:

A complete schedule of study procedures and events are located in Appendix 1.

Clinical status will be evaluated for all hospitalized subjects daily with vital signs, Ordinal Scale, and NEWS at Screening/Baseline, during and after Liquid Alpha$_1$-PI (Human) plus SMT or placebo plus SMT treatment and during Follow-up. The NEWS calculation is delineated in Appendix 2 and can be calculated using the on-line web tool at https://www.mdcalc.com/national-early-warning-score-news

All subjects will be followed daily through Day 10 (note: for Day 6 through Day 10, this stipulation is for as long as subjects are hospitalized). After Day 6, if subjects are discharged from the hospital, evaluations at Day 15 and 29 are required. Subjects remaining hospitalized will be followed daily through Day 29. Phone Checks will occur at Day 60 and Day 90 for

vital status (living or deceased), any hospital re-admissions or serious/non-serious adverse events after the Day 29 Final Clinic Visit.

Respiratory samples for quantitative measurement of SARS-CoV-2 viral load by NAT or PCR (real-time RT-PCR) will be obtained on Day 1, and subsequently on Day 5± 1 day, Day 15± 1 day, and Day 29 ± 1 day (obtained via nasopharyngeal swab). *The results of these samples are not necessary for continuing on study.* Similarly, *blood samples for* quantitative measurement of IgM and IgG antibodies to SARS-CoV-2 via enzyme linked immunosorbent assay (ELISA), Indirect Fluorescent Antibody (IFA) or other assay methodology will be drawn at the same time points: Day 1, and subsequently on Day 5± 1 day, Day 15± 1 day, and Day 29 ± 1 day. *The results of these samples are not necessary for continuing on study.* These laboratory tests will be performed at a central/external lab.

Ferritin, D-dimer, CRP and Basic chemistry analytes (creatinine, albumin, alanine aminotransferase [ALT], total bilirubin, lactate dehydrogenase [LDH]) will be assessed on Day 1 and on Day 5± 1 day, Day 15± 1 day, and Day 29 ± 1 day. An estimated glomerular filtration rate (eGFR) will also be calculated at these time points using the Cockcroft-Gault formula.

Hematology (hemoglobin, hematocrit, platelet count, absolute neutrophil & lymphocyte count, leukocyte count with differential) will be assessed on Day 1, on Day 5± 1 day, Day 15± 1 day, and Day 29 ± 1 day. A cytokine panel will be performed on Day 1, and on Day 5± 1 day, Day 15 ± 1 day, and Day 29 ± 1 day. Cytokine panel includes IL-1β, IL-10, IL-6, IL-8 IL-2, interferon γ, and TNF-α. Note: serum samples must be stored at -70° C for later analysis at a reference laboratory. Levels of alpha 1-PI (AAT) activity and antigen will be evaluated on Day 1, on Day 8± 1 day, Day 15± 1 day, and Day 29 ± 1 day; both plasma (sodium citrate) and serum samples to be stored at - 70° C for measurement of both antigenic content and functional activity (potency) (all samples are mandatory at all time points regardless of hospital discharge status, except for the Day 8 AAT activity and antigen which is not required if subjects are discharged from the hospital and no 2nd blinded study drug infusion is administered).

Statistical Methods:

Descriptive statistics will include the number of non-missing observations, mean, standard deviation (SD), median, minimum, and maximum values for the continuous/quantitative data or absolute and relative frequency counts and percentages for categorical/qualitative data. All statistical tests will be 2-sided at a significance level of 0.05.

An interim analysis will be conducted after 50 subjects (25 per group) for safety variables. Due to the exploratory nature of this phase 2 study, the multiplicity adjustments will not be considered; full details will be described in the statistical analysis plan. This interim evaluation is part of Sponsor safety due diligence.

Interim Futility Analysis: An interim futility analysis will be conducted to assess whether the trial will be terminated due to lack of efficacy (futility) based on all available data assessable for the primary endpoint and key secondary efficacy endpoints.

Determination of Sample Size

Because of the urgency and lack of previous prospective COVID-19 data, sample size estimation remains incompletely defined; however, the size of this pilot study is

commensurate with other Phase 2 investigations ongoing during the COVID-19 pandemic. Approximately 100 subjects are allowed to be randomized as part of a humanitarian effort against COVID-19.

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GLOSSARY AND ABBREVIATIONS

AAT	$alpha_1$-antitrypsin
ACE2	Angiotensin-converting enzyme 2
ADE	antibody dependent enhancement
ADR	adverse drug reaction
AE	adverse event
ALI	acute lung injury
$alpha_1$-PI	$alpha_1$-proteinase inhibitor
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
ANOVA	analysis of variance
AR	adverse reaction
ARDS	acute respiratory disease syndrome
ARMA	Acute Respiratory Management of ARDS (database)
AMS	aseptic meningitis syndrome
BAFF	B-cell-activating factor
CathG	cathepsin G
CI	confidence interval
CoVs	coronaviruses
COVID-19	Coronavirus disease 2019
CRF	case report form
CTCAE	Common Terminology Criteria for Adverse Events
DVT	deep vein thrombosis
EEA	European Economic Area
eGFR	estimated glomerular filtration rate
ELISA	Enzyme-linked immunosorbent assay
EU	European Union
FcR	Fc receptors
FDA	US Food and Drug Administration
FEV1	forced expiratory volume in 1 second
FiO_2	inspired oxygen concentration
GCP	Good Clinical Practice
hAAT	human $alpha_1$-antitrypsin
HCG	human chorionic gonadotropin
HCoVs	highly pathogenic CoVs
HFI	hereditary fructose intolerance
HIV	human immunodeficiency virus
HR	heart rate
IB	investigator's brochure
ICF	informed consent form
ICH	The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICU	intensive care unit
IFA	indirect fluorescent antibody
IgG	immunoglobulin G
IL-1β	interleukin-1β
IL-6	interleukin-6
IP	investigational product

IQR	interquartile range
IRB/EC	Institutional Review Board/Ethics Committee
ITT	intention to treat
IV	intravenous
LD_{50}	lethal dose, 50%
MABP	mean arterial blood pressure
MCP-1	monocyte chemoattractant protein-1
MedDRA	Medical Dictionary for Regulatory Activities
MERS	Middle East respiratory syndrome
NaCl	Sodium chloride
NAT	nucleic acid amplification technology
NCI	National Cancer Institute
NE	neutrophil elastase
NEWS	National Early Warning Score
NF-κB	Nuclear Factor-kappaB
NIH	National Institutes of Health
p38	mitogen-activated protein kinase
PCR	polymerase chain reaction
PE	pulmonary embolism
PEEP	positive end-expiratory pressure
PI	primary immunodeficiency
PICs	pro-inflammatory cytokines
PK	pharmacokinetics
PP	Per Protocol
PR3	proteinase 3
RR	respiratory rate
RT-PCR	reverse transcriptase PCR
SAE	serious adverse event
SaO_2	arterial oxygen saturation
SAP	statistical analysis plan
SARS	severe acute respiratory syndrome
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SBP	systolic blood pressure
SD	standard deviation
SISRC	Study-Independent Safety Review Committee
SMT	standard medical treatment
SOFA	Sequential Organ Failure Assessment
SpO_2	Peripheral oxygen saturation by pulse oximetry
T	Temperature
T1DM	type 1 diabetes mellitus
TEAE	treatment-emergent adverse event
TGF-β1	transforming growth factor β 1
Th17	T helper 17 cells
TNF-α	tumor necrosis factor-α
TRALI	transfusion-related acute lung injury
US	United States
USP	United States Pharmacopeia
WBC	white blood cell
WHO	World Health Organization

1 GENERAL INFORMATION

Protocol title and other key study information are provided on the title page. Information regarding additional key personnel and organizations involved in the conduct of the study, including names and contact details of participating investigators, monitors, clinical laboratories, technical departments and/or institutions, as well as information on members of additional study committees, will be found in the study files of the sponsor and at the investigator sites within the study reference manual/file.

Investigators and staff will receive training in appropriate individual site training session(s) depending on what is feasible, given the emergency epidemic situation.

2 BACKGROUND INFORMATION

Coronavirus disease 2019 (COVID-19) is a respiratory tract infection caused by a newly emergent coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), that was first recognized in Wuhan, China, in December 2019 (WHO Interim guidance 13 March 2020). Genetic sequencing of the virus suggests that SARS-CoV-2 is a betacoronavirus closely linked to the severe acute respiratory syndrome (SARS) virus (Team NCPERE 2020). While most people with COVID-19 develop mild or uncomplicated illness, approximately 14% develop severe disease requiring hospitalization and oxygen support and 5% require admission to an intensive care unit (Team NCPERE 2020). In severe cases, COVID-19 can be complicated by acute respiratory disease syndrome (ARDS), sepsis and septic shock, multiorgan failure, including acute kidney injury and cardiac injury (Yang 2020). Older age and co-morbid disease have been reported as risk factors for death, and recent multivariable analysis confirmed older age, higher Sequential Organ Failure Assessment (SOFA) score and d-dimer > 1 µg/L on admission were associated with higher mortality. This study also observed median duration of viral RNA detection was 20.0 days (interquartile range [IQR] 17.0–24.0) in survivors, but SARS-CoV-2 virus was detectable until death in non-survivors. The longest observed duration of viral shedding in survivors was 37 days (Huang 2020; Zhou 2020).

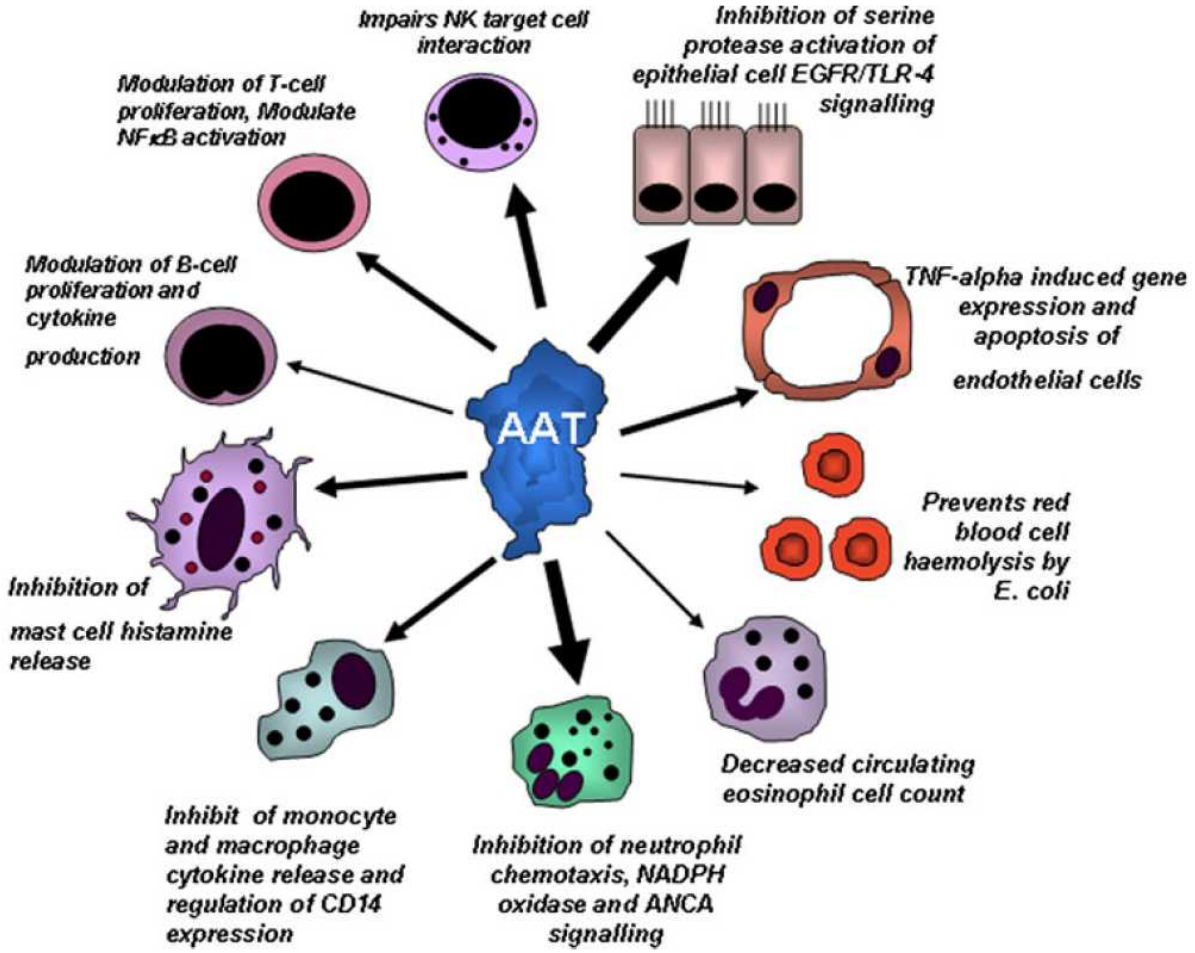
Currently there are no approved treatments for COVID-19 and no approved prophylactic, post-exposure, or therapeutic treatment modalities exist for SARS-CoV-2. This study will enroll subjects who have COVID-19 requiring hospital admission in order to evaluate treatment with Prolastin®-C Liquid, as it is considered that it may provide therapeutic benefit via its modulating and potentially anti-inflammatory properties in this critical situation.

Prolastin-C Liquid is a formulation of alpha$_1$-proteinase inhibitor (alpha$_1$-PI), also known as alpha$_1$-antitrypsin (AAT), which has a number of anti-inflammatory properties that have been demonstrated in vitro and in vivo animal models. AAT is a member of the Serpin (serine protease inhibitors) family of protease inhibitors and is one of many plasma proteins that comprise the ‘‘acute phase proteins’’ although AAT also demonstrates unique anti-inflammatory properties (Bergin 2012). The primary role of AAT is to function as a serine protease inhibitor, and it has been shown to inhibit a range of proteases derived from:

- Degranulating neutrophils including neutrophil elastase (NE), cathepsin G and proteinase 3 (PR3) (Duranton 2003).

- Mast-cell-derived tryptase and chymase (Chen 2004; He 2004)
- Lymphocyte-derived granzyme B (Mahrus 2004)
- The epithelial-cell membrane- bound protease matriptase (Janciauskiene 2008).
- Other serine proteases associated with coagulation (Gallimore 1975; Mast 1991), digestive enzymes (Beatty 1980), kalkriens derived from serum and tissue (Luo 2006; Patston 1990) and urokinase (Clemmensen 1976).
- Other classes of proteases including neutral and aspartic-cysteine proteases (Al-Omari 2011; Petrache 2006)
- The matrix metalloprotease, ADAM-17 (Bergin 2010).

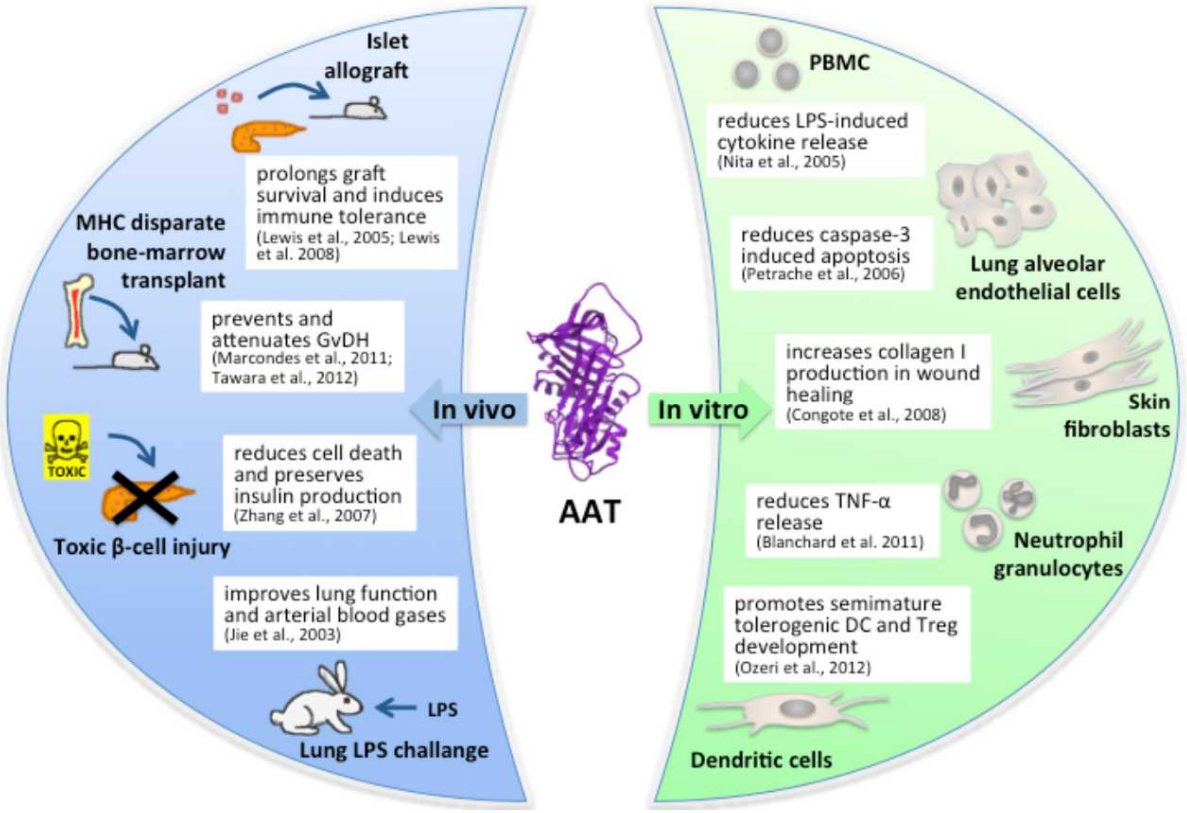
The various modulatory effects of AAT are illustrated in Figure 2-1 wherein the thickness of the arrow reflects the perceived depth of knowledge (thicker indicates more data available).



Source: Bergin 2012

Figure 2-1 AAT modulation of different cell types during inflammation .

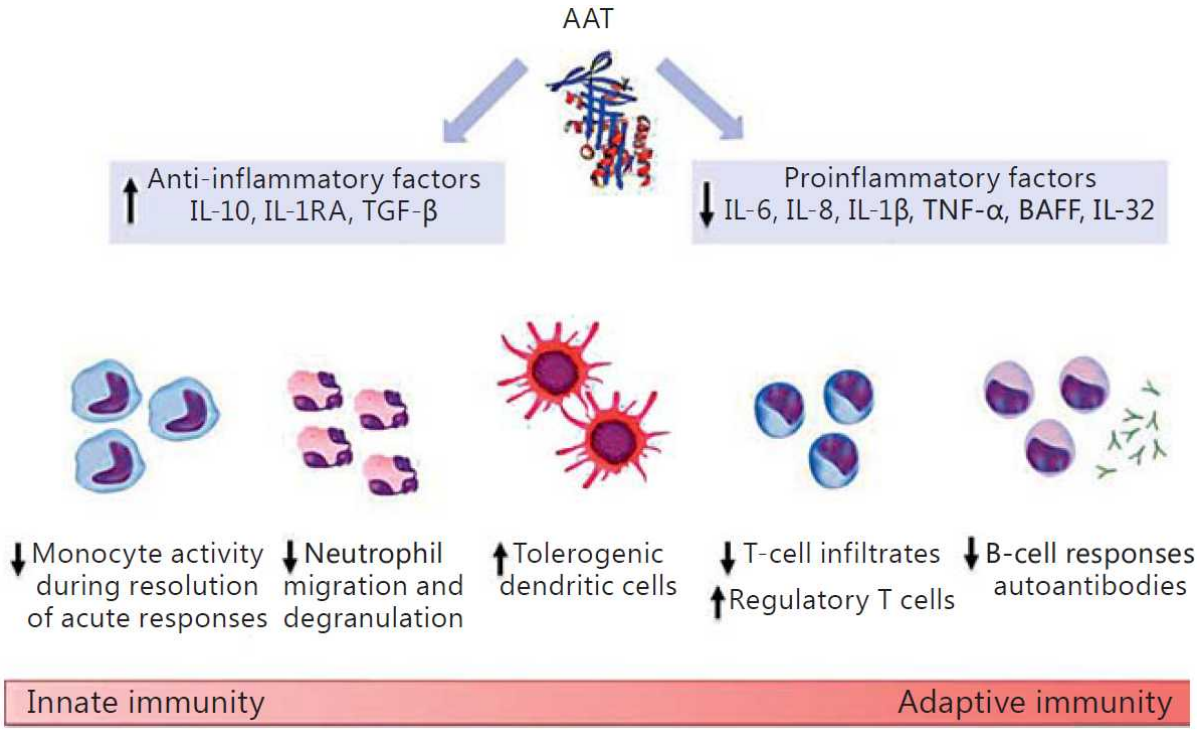
In addition to in vitro results, in vivo models of anti-inflammatory properties and reported modulating effects of AAT are shown in Figure 2-2. In murine models, exogenous human AAT protected islet cell allografts from rejection and increased survival in an allogeneic marrow transplantation model (Janciauskiene 2013). In other models AAT therapy protected against tumor necrosis α (TNF-α) / endotoxin induced lethality, cigarette smoke induced emphysema and inflammation and appeared to suppress bacterial proliferation during infections (Lewis 2012). Furthermore, human AAT given to mice during renal ischemia–reperfusion (I/R) injury lessened tissue injury and attenuated organ dysfunction (Daemen 2000).



Source: Janciauskiene 2013

Figure 2-2 Selected anti-inflammatory and immunoregulatory activities of AAT.

In terms of inflammatory cytokines, the influence of AAT is multifaceted. AAT changes the cytokine milieu in the tissue microenvironment influencing both innate and adaptive immune responses; it downregulates proinflammatory cytokines (interleukin IL-6, IL-8, IL-1β, tumor necrosis factor-α [TNF-α], B-cell-activating factor [BAFF] and IL-32) while promoting anti-inflammatory mediators [IL-10, IL-1RA and transforming growth factor-β. These effects are displayed in Figure 2-3 (Baraldo 2016).



Source: Baraldo 2016

Figure 2-3 Schematic representation of the immunomodulating effects of AAT

The severity of COVID-19 in vulnerable individuals in the setting of the ongoing pandemic warrants investigation regarding whether Liquid Alpha$_1$-PI (Human) may therapeutic benefit and impact illness severity and mortality. While the majority of affected patients will recover, a significant number require hospitalization; morbidity and sequelae can be severe (Guan 2020).

In addition to the information provided below, please also refer to the Investigator's Brochure (IB) and any additional data supplied by the sponsor.

2.1 Name and Description of the Investigational Product(s)

See Section 4.4 Study Treatments for detail.

The investigational product (IP) is Liquid Alpha$_1$-PI (Human).

The following IP will be used in this clinical trial (Table 2-1):

Table 2-1 Investigational product

INVESTIGATIONAL PRODUCT	
Investigational Product: Liquid Alpha$_1$-PI (Human)	Liquid Alpha$_1$-PI (Human) is supplied in glass vials as a sterile, stable, liquid concentrate of alpha$_1$-PI (Human) (nominally 50 mg/mL of functionally active alpha$_1$-PI (Human)) and has a purity of ≥ 90% alpha$_1$-PI (Human). The product is a sterile solution containing 0.013-0.025M sodium phosphate and is stabilized with 0.20-0.30M alanine. The total sodium concentration is ≤ 100 mEq/L. Liquid Alpha$_1$-PI (Human) must be refrigerated at 2°C to 8°C (36°F to 46°F). Do not freeze.

Liquid Alpha$_1$-PI (Human) will be the investigational product being tested in combination with standard medical treatment (SMT) versus placebo plus SMT in subjects with COVID-19.

Liquid Alpha$_1$-PI (Human) is indicated for chronic augmentation and maintenance therapy in adults with clinical evidence of emphysema due to severe hereditary with alpha$_1$-PI deficiency (e.g., phenotypes PiZZ, PiZ(null), Pi (null,null) and PiSZ).

Prior to infusion visually inspect parenteral drug products for particulate matter and discoloration. The product may contain a few protein particles. The solution is clear, colorless or pale yellow or pale green. Do not use if discolored or cloudy. Before delivery to the subject, Liquid Alpha$_1$-PI (Human) should be brought to room temperature and administered at room temperature for comfort to the subject. Liquid Alpha$_1$-PI (Human) is for intravenous (IV) administration only. Pooled Liquid Alpha$_1$-PI (Human) solution should be maintained at room temperature for administration within 3 hours.

Filter the solution during administration using an IV administration set with a suitable 5 to 15-micron infusion filter. Infuse Liquid Alpha$_1$-PI (Human) separately, without mixing with other agents or diluting solutions. Infuse Liquid Alpha$_1$-PI (Human) IV at 0.08 mL/kg/min as determined by subject response and comfort.

2.2 Relevant Findings from Nonclinical and Clinical Trials

Single and repeat dose toxicology studies of IV-administered Prolastin have demonstrated a wide margin of safety for alpha$_1$-PI in mice, rats, and rabbits. Toxicology studies of IV Prolastin did not reveal any toxicologically relevant findings. Single-dose IV toxicology (lethal dose, 50% [LD_{50}]) studies in mice, rats and rabbits failed to demonstrate alpha$_1$-PI toxicity from administered alpha$_1$-PI at doses ranging from 517.5 to 3900 mg/kg. IV administration of 5 daily doses of Liquid Alpha$_1$-PI (Human) to rabbits at a dose up to 600 mg/kg per day (10-fold higher dose than a human dose of 60 mg/kg administered weekly), did not result in any signs of toxicity. Further, there were no differences in safety and tolerability of Prolastin-C and Liquid Alpha$_1$-PI (Human) in nonclinical testing.

The augmentation therapeutic dose of 60 mg/kg body weight for patients with inherited AAT deficiency (AATD) and emphysema is well established. Prolastin was licensed for use as augmentation therapy for AATD, at a dose of 60 mg/kg weekly, in the United States (US) in 1987 and in Germany in 1988. Prolastin has been administered intravenously for over 30 years and has a well-defined safety profile. It is authorized in 14 European Union countries as well as in Switzerland.

Grifols Prolastin-C which has a modified process with a higher concentration of AAT (50 mg/mL when lyophilized product reconstituted) has been approved for marketing since 2009 for weekly IV administration, at a dose of 60 mg/kg, for the treatment of patients with severe alpha$_1$-antitrypsin deficiency (AATD) and clinically evident emphysema. Grifols Liquid alpha$_1$-proteinase inhibitor (Prolastin-C Liquid) manufacturing process is the same as that for Prolastin-C until the final formulation step where alanine is added as a stabilizer versus sodium chloride for the formulation of the lyophilized Prolastin-C product.

Prolastin-C Liquid has been approved for marketing since September 2017 for weekly IV administration, at a dose of 60 mg/kg, for chronic augmentation and maintenance therapy in adults with clinical evidence of emphysema due to severe hereditary AATD; it is approved in the United States and Canada. Prolastin-C Liquid is a sterile, stable, liquid preparation of purified human alpha$_1$-PI prepared from pooled human plasma by modification and refinements of the cold ethanol fractionation method of Cohn using purification by polyethylene glycol precipitation, anion-exchange chromatography, and cation-exchange chromatography.

A multicenter, randomized, double-blind, crossover study (GTI1402) was conducted in which Prolastin-C was used as a comparator to assess the safety and pharmacokinetics (PK)of a Liquid formulation of alpha$_1$-PI, Liquid Alpha-1 PI (Prolastin-C Liquid). The safety profile of Prolastin-C Liquid (n=32 subjects with AATD) administered weekly at 60 mg/kg for 8 weeks either before or after 8 weeks of infusions with Prolastin-C was consistent with that found in previous studies. The primary bioequivalence analysis indicated that steady-state serum exposure to alpha$_1$-PI was bioequivalent between Liquid Prolastin-C 60 mg/kg/week and Prolastin-C 60 mg/kg/week as determined by $AUC_{0\text{-}7days}$ (antigenic content) values among 31 subjects in the PK population. The safety profile of both formulations of Prolastin-C was good and was well tolerated among subjects.

Grifols has conducted a number of clinical studies at higher dose levels of alpha$_1$-PI than 60 mg/kg body weight per week. While these studies were performed with Grifols Prolastin-C®, the results are directly applicable to the Liquid Alpha$_1$-PI (Human) product as well. All studies have demonstrated a good tolerability profile at doses up to 180 mg/kg per week in clinical trials. These studies are summarized in Table 2-2.

Table 2-2 Tabular Summary of Clinical Trials Utilizing Higher than 60 mg/kg/week Dose Levels of Alpha$_1$-PI

Study Title (Acronym)	Number of Subjects[a]	Study Design, Duration of Treatment	Treatment(s)	Important Findings
Completed Clinical Trials				
A Multi-center, Randomized, Double-blind, Crossover Study to Assess the Safety and Pharmacokinetics of Two Different Doses of Weekly Intravenous Administration of Alpha$_1$-Proteinase Inhibitor (Human) in Subjects with AATD (SPARK)	30	Randomized, cross-over (two 8-week double-blind treatment periods)	60 mg/kg alpha$_1$-PI; 120 mg/kg alpha$_1$-PI	The steady-state mean serum trough concentration of alpha$_1$-PI following weekly doses of 60 mg/kg alpha$_1$-PI was above the historical "protective threshold" of 11 µM in all subjects. In addition, all subjects after the 120 mg/kg dose had an average steady-state serum trough value that was greater than the lower limit (20 µM) of the range of alpha$_1$-PI serum levels in non-AATD individuals. The weekly dose of 120 mg/kg alpha$_1$-PI provided an average steady state mean trough concentration of alpha$_1$-PI (27.7 µM) that was within the reported range of alpha$_1$-PI serum levels (20 to 53 µM) in non-AATD individuals. Alpha$_1$-PI administered at doses of 60 mg/kg and 120 mg/kg for 8 weeks was safe and well-tolerated; no SAEs were reported and there were no discontinuations due to an AE. TEAEs that were assessed as being drug-related after starting treatment with 60 mg/kg alpha$_1$-PI were cognitive disorder, rosacea, and subcutaneous nodule; cognitive disorder was the only TEAE that was considered to be drug-related after starting treatment with 120 mg/kg alpha$_1$-PI. There were no mean changes from baseline that were of clinical concern for any laboratory parameter after starting alpha$_1$-PI treatment.

Study Title (Acronym)	Number of Subjects[a]	Study Design, Duration of Treatment	Treatment(s)	Important Findings
Clinical Trial Ended Early (futility/insufficiently powered for efficacy)				
A Multicenter, Randomized, Partial-Blinded, Placebo-Controlled Study to Evaluate the Safety and Efficacy of a Human Plasma-Derived Alpha$_1$-Proteinase Inhibitor in Subjects with New-Onset Type 1 Diabetes Mellitus (GTI1302)	60	Randomized, partial-blind, placebo-controlled 13 or 26 weeks of treatment	180 mg/kg weekly infusions of alpha$_1$-PI for 26 weeks 90 mg/kg weekly infusions of alpha$_1$-PI for 26 weeks 180 mg/kg weekly infusions of alpha$_1$-PI for 13 weeks 90 mg/kg weekly infusions of alpha$_1$-PI for 13 weeks Placebo (Saline, 0.9% Sodium Chloride Solution) 26 weeks of weekly infusions or 13 weeks of weekly infusions	Treatment with Alpha-1 MP (Prolastin-C) was well tolerated. The most frequently reported TEAEs were upper respiratory infection, headache, nasopharyngitis, and vomiting. No clinically meaningful differences in TEAE incidences were noted among treatment groups. One subject in the high dose alpha$_1$-PI treatment group received a 2.2-fold overdose without any associated AEs reported. Three subjects reported SAEs, all in the 13-week high dose alpha$_1$-PI treatment group (1 subject each: completed suicide, spontaneous abortion, and hyperglycemia). All SAEs started several weeks after starting treatment, all were considered unrelated to study treatment, and all except the suicide resolved. One subject in the 26-week high dose alpha$_1$-PI treatment group was discontinued due to a nonserious, moderate TEAE (migraine), which started on Day 45, resolved on Day 49, and was considered possibly related to study treatment.
Ongoing Clinical Trials				
A Randomized, Double-Blind, Placebo-Controlled Study to Assess the Efficacy and Safety of Two Dose Regimens (60 mg/kg and 120 mg/kg) of Weekly Intravenous Alpha$_1$-Proteinase Inhibitor (Human) in Subjects with Pulmonary Emphysema due to Alpha$_1$-Antitrypsin Deficiency (GTi1201, SPARTA)	339 planned; 224 enrolled and dosed	Randomized, double-blind, placebo-controlled 156 weeks of treatment	60 mg/kg/week alpha$_1$-PI by IV administration 120 mg/kg/week alpha$_1$-PI by IV administration Placebo, weekly, by IV administration	Treatments in this ongoing study remain blinded. A total of 130 treatment-emergent SAEs have been reported in 70/224 (31.1%) randomized subjects. All reported SAEs were unrelated to study drug with 2 exceptions: 1 SAE of supraventricular tachycardia that was considered unlikely related to study drug and 1 SAE of thrombocytopenia that was considered related to study drug and led to subject withdrawal from the study.

a Number of subjects who received any amount of study drug.

2.2.1 Dose Response for Inflammatory Biomarkers – Preclinical Data

The strongest available data pertaining to a well-defined dose response for effects of specific doses of AAT on inflammatory biomarkers stems from preclinical investigations with Prolastin-C®.

Preclinical data of different internal Grifols studies support the use of high dose human alpha$_1$-antitrypsin (hAAT) as a therapy for inflammation related diseases.

Pharmacokinetic studies in mice (C57BL/6 strain) treated twice a week with intraperitoneal hAAT were performed, following two dosing regimens:

- hAAT increasing dose (60 mg/kg from Day 0 to Day 15, and 60 mg/kg to 180 mg/kg, with 15 mg/kg twice a week increment from Day 15 to Day 39)
- hAAT constant dose (60 mg/kg) from Day 0 to Day 39

The obtained data demonstrated that animals treated with the increasing dose regimen of hAAT reached higher anti-elastase activity plasma levels than the ones treated with a hAAT constant dose of 60 mg/kg:

- hAAT 180 mg/kg: anti-elastase activity at Day 39: 3.0±0.1 mg/mL
- hAAT 60 mg/kg: anti-elastase 1.7±0.0 mg/mL
- Non-treated animals anti-elastase activity: 1.3±0.1 mg/mL

Using this increasing dose regimen of hAAT, a positive effect was observed in an experimental model of type 1 diabetes (a transgenic mice expressing IFN-β in pancreatic β-cells that present a marked lymphocytic infiltration) (Casellas et al., 2006). hAAT treated animals showed a reduced infiltration of lymphocytes in pancreatic islets resulting in an approximately half reduction of overall insulitis index score.

Furthermore, in an experimental mice model of neonatal hypoxic ischemic encephalopathy, a disease characterized by cerebral inflammation and profound brain injury, hAAT (25 to 200 mg/kg, intraperitoneal daily for 7 days) increased in a dose dependent manner the preserved area after the hypoxia damage.

Specifically, a high dose of human AAT (100 mg/kg) reduced the neutrophil infiltration in different brain areas, $p<0.001$ hAAT vs. vehicle in both areas. Results for CD25 positive cells were the following:

- in cortex 35±1% (vehicle) vs. 15±1% (hAAT)
- in hippocampus 39±3% (vehicle) vs. 17±1% (hAAT)

In relation to the levels of proinflammatory cytokines, high dose of hAAT (100 mg/kg) decreased TNFα, IL-1 and IL-6 in brain cortex, $p<0.001$ in the 3 cytokines. Specific results (% in respect to healthy animals were as follows:

- TNFα: vehicle 257±17% vs. hAAT 153±10.7%
- IL 1: vehicle 223±16% vs. hAAT 147±9.9%
- IL 6: vehicle 227±13% vs. hAAT 139±11%

Finally, similar results were obtained in a mice preclinical model of spinal cord injury. Human AAT (25 to 200 mg/kg, intraperitoneal, daily for 14 days) decreased in a dose dependent manner the lesioned area in mice after weigh drop injury. Specific results were the following:

- A high dose of hAAT (100 mg/kg) reduced TNFα and IL6 levels in this model at the end of treatment (Day 14), $p<0.001$ for both TNFα and IL-6. Results for % in respect to healthy animals were the following:
  - TNFα: vehicle 257±14.6% vs. hAAT 138.9±10.1%
  - IL 6: vehicle 231±7.9% vs. hAAT 111±9.1
- This anti-inflammatory effect lasted 14 days after treatment end (Day 28); results (% in respect to healthy animals) were the following:
  - TNFα: vehicle 276±17.1% vs. hAAT 201±7.1% ($p<0.01$);
  - IL 6: vehicle 201±11.3% vs. hAAT 159±8.7 % ($p<0.05$).

Altogether, these pre-clinical data support a dose depending effect of human AAT with an anti-inflammatory effect specially at high doses.

2.3 Known and Potential Risks and Benefits to Human Subjects

2.3.1 Benefits

No approved prophylactic, post-exposure, or therapeutic treatment modalities exist for SARS-CoV-2. The subjects enrolled in this study have COVID-19 requiring hospital admission, and it is considered that Liquid Alpha$_1$-PI (Human) may provide therapeutic benefit via its potential anti-inflammatory properties in this critical situation.

One of the primary functions of alpha$_1$-PI (AAT) in the human body is to irreversibly bind NE. NE is an omnivorous serine protease released by activated or disintegrating neutrophils. NE levels and activity are markedly increased in the airways of patients with ARDS, and previous studies have shown that IV administration of AAT in humans results in augmented anti-elastase capacity of airway epithelial lining fluid, reduced NE activity in bronchoalveloar lavage fluid and decreased breakdown of lung tissue (Campos 2019; Chapman 2015; McElvaney 2017; Wewers 1987). Although these studies were performed in subjects with congenital AAT deficiency. While AAT chiefly inhibits NE, it also other exerts an antiprotease effect on chymotrypsin, cathepsin G (CathG) and proteinase 3 (PR3) (Greene 2009).

In addition to its antiprotease effects (Matheson 1981; Ogushi 1987; Sinden 2015), AAT is a potent anti-inflammatory and immunomodulator (Bergin 2010; Bergin 2014; Hurley 2014; O'Dwyer 2015; Jonigk 2013). Of particular relevance to COVID-19, it has been shown to modulate suppress the production and activity of several key pro-inflammatory cytokines, including interleukin IL-1, IL-6, IL-8 and TNF-α (Bergin 2010; Bergin 2014; Pott 2009; McCarthy 2018), while preserving the production of the anti-inflammatory cytokine IL-10 (Janciauskiene 2007). As such, it is feasible that exogenous administration of alpha$_1$-PI (AAT) may alleviate the hyperinflammatory component of COVID-19 which can cause accentuating respiratory impairment. For this reason, the current randomized, controlled study in hospitalized

COVID-19 subjects is being undertaken to evaluate whether therapeutic benefit is feasible to attain following IV administration of Liquid Alpha$_1$-PI (Human).

Decreasing morbidity and mortality remain the theoretical benefit and aim of administration of Liquid Alpha$_1$-PI (Human) in patients with COVID-19. In this study, therapeutically high doses of Liquid Alpha$_1$-PI (Human) will be administered to those patients hospitalized with COVID-19 in an effort to reduce their symptoms and improve outcomes by leveraging the modulatory and anti-inflammatory effects of Prolastin described above. These doses are commensurate with prior clinical experience with alpha$_1$-PI.

2.3.2 Risks

There are no known drug interactions with IV alpha$_1$-PI. No known drug interactions have been encountered in clinical studies of Prolastin.

Individuals with known selective or severe IgA deficiency who have known antibodies against IgA should not receive Alpha$_1$-PI since these subjects may experience severe reactions, including anaphylaxis, to IgA which may be present in the preparation.

When medicines are made from human plasma, certain measures are put in place to prevent infections being passed on to patients. These include:

- Careful selection of blood and plasma donors to make sure those at risk of carrying infections is excluded
- The testing of each donation and pools of plasma for signs of virus/infections
- The inclusion of steps in the manufacturing of plasma derivatives with virus inactivation/removal capacity

Despite these measures, when medicines prepared from human blood or plasma are administered, the possibility of passing on infection cannot be totally excluded. This also applies to any unknown or emerging viruses or other types of infections.

In clinical trials the most common adverse reactions observed at a rate of >5% in subjects receiving Liquid Alpha$_1$-PI were diarrhea and fatigue, each of which occurred at a rate of 6% (two subjects each).

The post-marketing adverse reactions for Prolastin®-C Liquid (the formulation of Liquid Alpha$_1$ -Proteinase Inhibitor used in this study) are as follows:

- General/Body as a whole: fatigue, malaise, influenza-like illness, pain, asthenia
- Immune system: hypersensitivity including anaphylactoid/anaphylactic reactions
- Cardiovascular: tachycardia
- Musculoskeletal: arthralgia, myalgia

- Gastrointestinal: vomiting, diarrhea
- Investigation: blood pressure increased

2.4 Description of and Justification for the Route of Administration, Dosage, Dosage Regimen, and Treatment Periods

2.4.1 Administration of Investigational Product

Subjects randomized to the combination arm Liquid Alpha$_1$-PI (Human) plus standard medical treatment (SMT), will receive IV Liquid Alpha$_1$-PI (Human) and SMT.

Subjects in the placebo plus SMT arm will receive all standard of care interventions required and Placebo.

2.4.2 Justification for Selection of Doses/Timing of Investigational Product

The route of administration and the dosage regimen of 60 mg/kg body weight once weekly IV of Liquid Alpha$_1$-PI (Human) is recommended for chronic augmentation and maintenance therapy in in adults with clinical evidence of emphysema due to severe AAT deficiency (e.g., phenotypes PiZZ, PiZ [null], Pi [null] [null]; PiSZ) (Prolastin-C Liquid- alpha1-proteinase inhibitor [human] injection, solution US prescribing information, 2020).

Augmentation therapy with weekly IV infusions of 60 mg/kg AAT may be sufficient to slow progression of lung density decline in patients with AATD patients (Dirksen 2009); however, other dosing regimens have been also widely used: 120 mg/kg every 2 weeks, 180 mg/kg every 3 weeks, 250 mg/kg once a month (Stoller 2003; Miravitlles 1999, Vidal 1995). The safety profile of AAT has not been modified according to the dosing regimen, and there has been no indication of any dose-dependent toxicity in AAT deficient subjects. AAT is a normal constituent of human plasma, which does not contribute significantly to plasma osmolality, and suffers no appreciable glomerular filtration.

Higher doses than 60 mg/kg at weekly intervals have been well tolerated with no safety issues. Campos and colleagues evaluated weekly doses of 120 mg/kg in patients with AATD which had the salutary effect of bringing AAT levels to within the normal range and improvement in biomarkers (Campos 2019). In the SPARK clinical study in subjects with AATD, weekly doses of 120 mg/kg alpha$_1$-PI resulted in an average steady state mean trough concentration of AAT (27.7 µM) that was within the reported normal range of AAT serum levels (20 to 53 µM) in non-AATD individuals. The SPARK study was conducted by Grifols to assess the safety and PK of weekly IV infusions of alpha$_1$-PI at 120 mg/kg compared to the approved (product labeling) dose of 60 mg/kg in 30 subjects. In this study, a total of 30 subjects received weekly doses of 120 mg/kg for 8 weeks, and the results demonstrated that alpha$_1$-PI, was also safe and well-tolerated at this dose (Campos 2013). Additionally, Grifols is currently investigating the efficacy of 60 mg/kg and 120 mg/kg alpha$_1$-PI using CT densitometry as the primary efficacy endpoint in a randomized, placebo-controlled trial in subjects with pulmonary emphysema due to AATD; safety and tolerability of the 120 mg/kg/week dose has been good in this ongoing clinical trial. Data from an additional proof-of-concept study in 75 subjects with new onset type 1 diabetes mellitus (T1DM) with residual β cell function evaluated the safety and potential therapeutic

effect of weekly 90 mg/kg and 180 mg/kg alpha$_1$-PI for 13 or 26 weeks. This study (GTI1302) showed that both doses were well-tolerated, however it was not powered adequately to demonstrate efficacy of alpha$_1$-PI at Week 52, and additional data would not change the outcome, so for reasons of futility the study was discontinued. However, safety of all doses (including weekly alph$_1$-PI at 180 mg/kg) was shown.

Subjects randomized to combination with SMT will receive 2 doses of 120 mg/kg of Liquid Alpha$_1$-PI (Human) administered with the first infused on Day 1 and the second on Day 8.

2.5 Compliance Statement

This study will be conducted under the conditions described in this protocol and in compliance with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) and all applicable regulatory requirements.

2.6 Study Population

The purpose of this study is to determine if Liquid Alpha$_1$-PI (Human) plus SMT can reduce the proportion of subjects dying or requiring intensive care unit (ICU) admission on or before Day 29 or who are dependent on high flow oxygen devices or invasive mechanical ventilation on Day 29 versus placebo plus SMT in hospitalized subjects with COVID-19.

Approximately 100 subjects will be randomized (1:1) with an interim analysis after 50 subjects (approximately 25 per group). There will be no interruption in study conduct during interim analysis as this simply represents sponsor safety due diligence during the COVID-19 pandemic.

Interim Futility Analysis: An interim futility analysis will be conducted to assess whether the trial will be terminated due to lack of efficacy (futility) based on all available data assessable for the primary endpoint and key secondary efficacy endpoints.

2.7 Relevant Data and Literature Review

2.7.1 COVID-19 Therapeutic Approaches

Coronaviruses (CoVs) typically affect the respiratory tract of mammals, including humans, and lead to mild to severe respiratory tract infections. In the past two decades, two highly pathogenic human CoVs (HCoVs), including severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), emerging from animal reservoirs, have led to global epidemics with high morbidity and mortality.

Several studies demonstrate that cells infected by SARS-CoV produce elevated levels of pro-inflammatory cytokines (PICs) in order to combat the invading viruses. However, the overproduced PICs (including monocyte chemoattractant protein-1 [MCP-1], transforming growth factor β 1 [TGF-β1], tumor necrosis factor-α [TNF-α], interleukins- 1β and -6 [IL-1β, and IL-6]), may cause immuno-mediated damage to the lungs and other organs, resulting in acute lung injury (ALI) and ARDS (He 2006; DeDiego 2014).

The elevated levels of PICs in blood released from the SARS-CoV-infected cells and the uninfected cells stimulated by viral antigens or some PIC-regulatory factors, e.g. Nuclear Factor-kappaB (NF-κB) and p38 (a mitogen-activated protein kinase) (He 2006; DeDiego 2014), may damage the cells in the lungs and other organs. In addition, blood hypo-oxygenation due to ARDS and disseminated intravascular coagulation resulting from impairment of microvessel endothelial cells may further damage the structure and function of different organs in SARS patients, resulting in multi-organ dysfunction.

The ACE2 expressing cells are the primary targets for SARS-CoV infection in vivo humans initiating a massive cascade of pro-inflammatory cytokines via macrophages over-activation causing ARDS. High levels of PICs are expressed in the SARS-CoV-infected ACE2+ cells, but not in the uninfected cells (He et al., 2006).

Therefore, application of PIC antagonists may reduce the severity and mortality of SARS and may potentially reduce other pathogenic human coronaviruses (He 2006; DeDiego 2014; Tse 2004). In the current study it is considered that the modulatory and anti-inflammatory properties of Liquid Alpha$_1$-PI (Human) may attenuate the deleterious effects of cytokine release syndrome engendered in COVID-19.

Currently, there are no approved treatments for COVID-19 in Europe or the United States. The lack of disease-directed therapeutic options has led to urgent interventions in anticipation of some potentially promising effects. Some antivirals are currently under evaluation. These include favipirivir (AVIGAN) manufactured by Fujifilm in Japan, Gilead's remdesivir, and Kaletra® (lopinavir/ritonavir) commercially available for human immunodeficiency virus (HIV). Although Kaletra® did not show demonstrable efficacy in a recently reported study in China (Cao et al., 2020), additional trials are underway. There are also investigations of chloroquine and hydroxychloroquine as treatment modalities and potential applications for post-exposure prophylaxis according to Clinicaltrials.gov and other clinical trial registries. These and other potential therapeutic agents are described on the World Health Organization (WHO) website file: WHO Landscape Therapeutics under investigation 17 Feb 2020.pdf (accessed 19 March 2020).

To date there are no confirmed specific disease modifying agents. Therefore, the potential therapeutic benefit conveyed by the immunomodulatory effects of Liquid Alpha$_1$-PI (Human) warrants clinical investigation. This is particularly true given the urgency and extent of the COVID-19 pandemic.

3 STUDY OBJECTIVES AND PURPOSE

3.1 Efficacy Objectives

3.1.1 Primary Efficacy Objective

To determine if Liquid Alpha$_1$-PI (Human) plus SMT can reduce the proportion of subjects dying or requiring intensive care unit (ICU) admission on or before Day 29 or who are dependent on high flow oxygen devices or invasive mechanical ventilation on Day 29 versus placebo plus SMT in hospitalized subjects with COVID-19.

3.1.2 Secondary Efficacy Objective

To compare Liquid Alpha$_1$-PI (Human) plus SMT versus placebo plus SMT with regard to clinical efficacy as assessed by clinical severity, duration of hospital stay, dependency on oxygen or new need for ventilatory support, clinical response criteria including National Early Warning Score (NEWS), and clinical status scale through Day 29 in hospitalized subjects with COVID-19.

3.1.3 Exploratory Efficacy Objectives

- To evaluate the effect of Liquid Alpha$_1$-PI (Human) plus SMT versus placebo plus SMT with regard to quantitative severe acute respiratory syndrome, coronavirus 2 (SARS-CoV-2) viral load and anti-SARS-CoV-2 antibodies in hospitalized subjects with COVID-19.
- To evaluate whether Liquid Alpha$_1$-PI (Human) plus SMT versus placebo plus SMT reduces the frequency of hyperinflammation based on a pre-specified biochemical definition through Day 29.
- To evaluate cytokine profile changes from baseline Liquid Alpha$_1$-PI (Human) plus SMT versus placebo plus SMT through Day 29.
- To evaluate levels of alpha 1-PI (AAT) activity and antigen through Day 29

3.2 Safety Objective

To determine the safety and tolerability profile through Day 29 of Liquid Alpha$_1$-PI (Human) plus SMT versus placebo plus SMT in hospitalized subjects with COVID-19.

4 STUDY DESIGN

4.1 Primary Endpoint and Secondary Endpoints

4.1.1 Primary Efficacy Endpoint

The primary efficacy endpoint is the proportion of subjects dying or requiring ICU admission on or before Day 29 or who are dependent on high flow oxygen devices or invasive mechanical ventilation on Day 29.

4.1.2 Secondary Efficacy Endpoints

Secondary efficacy endpoints include the following:

- Assessment of Clinical Severity: Change in NEWS from baseline (Day 1 through Day 29)
  The NEWS has demonstrated an ability to discriminate patients at risk of poor outcomes. This score is based on 7 clinical parameters (respiration rate, oxygen saturation, any supplemental oxygen, temperature, systolic blood pressure, heart rate, level of consciousness [Alert, Voice, Pain, Unresponsive]). https://www.mdcalc.com/national-early-warning-score-news (Appendix 2)

- Time to clinical response: NEWS ≤ 2 maintained for 24 hours, Day 1 through Day 29
- Time to hospital discharge: defined as duration of hospitalization from Day 1 through Day 29
- If admitted to ICU post randomization: Duration of ICU stay through Day 29
- Duration of any oxygen use Day 1 through Day 29
- If requiring mechanical ventilation post randomization: Duration mechanical ventilation through Day 29
- Absolute value and mean change from baseline in the Ordinal scale Day 1 through Day 29

  The Ordinal scale is as follows:

  1) Death;
  2) Hospitalized, on invasive mechanical ventilation or extracorporeal membrane oxygenation (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, limitation on activities;
  7) Not hospitalized, no limitations on activities.
- Proportion (percentage) of subjects in each severity category of the 7-point Ordinal scale at Day 15 and Day 29
- Time to sustained normalization of temperature and proportion of subjects with normalization of fever at all time points, defined as temperature < 36.6 °C armpit, < 37.2 °C oral, or < 37.8 °C rectal sustained for at least 24 hours
- Number of subjects who develop Acute Respiratory Distress Syndrome (ARDS) through Day 29 (Appendix 3)
- Length of time to clinical progression through Day 29 (defined as the time to death, mechanical ventilation, or ICU admission)

4.1.3 Exploratory Efficacy Endpoints

Exploratory efficacy endpoints include:

- Change from baseline in quantitative SARS-CoV-2 viral load by nucleic acid amplification technology (NAT) or polymerase chain reaction (PCR; real-time reverse transcriptase [RT]-PCR) to Day 5± 1 day, Day 15± 1 day, and Day 29 ± 1 day
- Change from baseline in quantitative anti- SARS-CoV-2 IgM and IgG antibodies to Day 5± 1 day, Day 15± 1 day, and Day 29 ± 1 day

- Proportion of subjects with evidence of hyperinflammation on Day 1, Day 5, Day 15, and Day 29 defined as:
    a) Lymphocyte counts <1000 cells/μL, AND
    b) Two of the following 4 criteria:
        i) Ferritin > 500ng/mL,
        ii) LDH > 300 U/L,
        iii) D-Dimers > 1000 ng/mL (fibrinogen equivalent units [FEU]) or > 2 times upper normal limit,
        iv) C-reactive protein (CRP) > 70 mg/L
- Change from baseline in cytokine profile (cytokine panel includes IL-1β, IL-10, IL-6, IL-8, IL-2, interferon γ, and tumor necrosis factor-α [TNF-α]) to Day 5± 1 day, Day 15± 1 day, and Day 29 ± 1 day
- Change from baseline in levels of alpha 1-PI (AAT) activity and antigen through Day 29 (both plasma [sodium citrate] and serum samples to be stored for measurement of both antigenic content and functional activity [potency])

4.1.4 Safety Endpoints

The safety endpoints include:

- Cumulative incidence of treatment-emergent serious adverse events (SAEs) and potentially related SAEs through Day 90 phone check
- Cumulative incidence of Grade 3-5 treatment-emergent adverse events (TEAEs) and potentially related severe TEAEs through Day 29 as defined in the Common Terminology Criteria for Adverse Events (CTCAE), US Department of Health and Human Services, National Institutes of Health (NIH), and National Cancer Institute (NCI)
- Cumulative incidence of all TEAEs and potentially related TEAEs through Day 29

In this clinical study, disease progression is defined as the worsening of a subject's condition attributable to the disease for which the investigational product is being studied. It may be an increase in the severity of the targeted disease and/or increases in the symptoms of the targeted disease. Anticipated symptoms of COVID-19 include fever, cough, hypoxia, dyspnea, hemoptysis, myalgia, fatigue, pharyngitis, which may develop at any time during the course of the disease. The development of COVID-19 symptoms during the clinical study should not be recorded as individual TEAEs, unless there is evidence suggesting a causal relationship between blinded Liquid Alpha$_1$-PI (Human)/Placebo and the TEAE.

4.2 Study Design and Plan

This is a prospective, multi-center, randomized (1:1), double-blind, placebo-controlled study of Liquid Alpha$_1$-PI (Human) plus SMT versus placebo plus SMT in subjects with COVID-19 who are hospitalized. The first 6 subjects randomized will be staggered, so randomization is no closer than 1 week apart. If there are no definitely related serious adverse events (SAEs) reported by the time Day 8 is completed by the 6th subject, competitive enrollment would ensue thereafter. If a definitely related SAE were to be reported among these 6 subjects, and the subject was found by

the Study-Independent Safety Review Committee (SISRC) to be randomized to the Liquid Alpha$_1$-PI (Human) plus SMT arm, the SISRC would carefully review and evaluate the case and make appropriate recommendations with regard to study status.

In this study, symptomatic subjects with positive polymerase chain reaction (PCR; reverse transcriptase [RT]-PCR) or nucleic acid amplification technology (NAT) for SARS-CoV-2 (or other commercial or public health assay approved by regulatory authorities) will receive placebo plus SMT *or* SMT plus Liquid Alpha$_1$-PI (Human) given as two intravenous (IV) doses of 120 mg/kg (body weight) one week apart (on Day 1 and Day 8); if the subject is discharged from the hospital then the 2nd infusion is not mandatory, and is at the discretion of the Principal Investigator and subject.

Specifically, subjects randomized to combination Liquid Alpha$_1$-PI (Human) will receive the first IV infusion of blinded Liquid Alpha$_1$-PI (Human) (120 mg/kg body weight) or Placebo on Day 1. A second Placebo/ Liquid Alpha$_1$-PI (Human) dose of 120 mg/kg body weight will be administered a week later on Day 8. If the subject has been discharged from the hospital at the time of the Day 8 blinded Placebo/Liquid Alpha$_1$-PI (Human) infusion, the 2nd infusion is not mandatory, and is at the discretion of the Principal Investigator and subject.

Approximately 100 subjects will be randomized (1:1) with an interim analysis after 50 subjects (approximately 25 per group). There will be no interruption in study conduct during interim analysis as this simply represents sponsor safety due diligence during the COVID-19 pandemic. Interim Futility Analysis: An interim futility analysis will be conducted to assess whether the trial will be terminated due to lack of efficacy (futility) based on all available data assessable for the primary endpoint and key secondary efficacy endpoints.

All subjects will be followed daily through Day 10 (note: for Day 6 through Day 10, this stipulation is for as long as subjects are hospitalized). After Day 6, if subjects are discharged from the hospital, evaluations at Day 15 and 29 are required. Berlin criteria for ARDS (Appendix 3) will be assessed on Day 1, 5, 15, and 29. Subjects remaining hospitalized will be followed daily through Day 29. Phone Checks will occur at Day 60 and Day 90 for vital status (living or deceased), any hospital re-admissions or serious/non-serious adverse events after the Day 29 Final Clinic Visit. Details are provided in Appendix 1 (Schedule of Study Procedures). Clinical status will be evaluated for all hospitalized subjects daily with vital signs, Ordinal Scale (7 categories analogous to Cao et al. 2020), and NEWS at Screening/Baseline and during Liquid Alpha$_1$-PI (Human) plus SMT or placebo plus SMT treatment and during Follow-up. The NEWS calculation is delineated in Appendix 2 and can be calculated using the on-line web tool at https://www.mdcalc.com/national-early-warning-score-news.

For all subjects, all laboratory samples must be drawn prior to infusion of Placebo/Liquid Alpha$_1$-PI (Human), if laboratory assessments are required for a day in which Placebo/Liquid Alpha$_1$-PI (Human) is being infused.

Respiratory samples for quantitative measurement of SARS-CoV-2 viral load by NAT or PCR (real-time RT-PCR) will be obtained on Day 1, and subsequently on Day 5± 1 day, Day 15± 1 day, and Day 29 ± 1 day (obtained via nasopharyngeal swab). The results of these samples are not necessary for continuing on study. Similarly, blood samples for quantitative measurement of

IgM and IgG antibodies to SARS-CoV-2 via enzyme linked immunosorbent assay (ELISA), Indirect Fluorescent Antibody (IFA) or other assay methodology will be drawn at the same time points: Day 1, and subsequently on Day 5± 1 day, Day 15± 1 day, and Day 29 ± 1 day. The results of these samples are not necessary for continuing on study.

Ferritin, D-dimer, CRP and Basic chemistry analytes (creatinine, albumin, alanine aminotransferase [ALT], total bilirubin, lactate dehydrogenase [LDH]) will be assessed on Day 1 and on Day 5± 1 day, Day 15± 1 day, and Day 29 ± 1 day. An estimated glomerular filtration rate (eGFR) will also be calculated at these time points using the Cockcroft-Gault formula. Hematology (hemoglobin, hematocrit, platelet count, absolute neutrophil & lymphocyte count, leukocyte count with differential) will be assessed Day 5± 1 day, Day 15± 1 day, and Day 29± 1 day. All routine laboratory tests will be assessed locally at the hospital/site's laboratory. A cytokine panel will be performed at the same time points. Cytokine panel includes IL-1β, IL-10, IL-6, IL-8, IL-2, interferon γ, and TNF-α. Note: serum samples must be stored at -70° C for later analysis at a reference laboratory. Levels of alpha 1-PI (AAT) activity and antigen will be evaluated on Day 1, on Day 8± 1 day, Day 15± 1 day, and Day 29 ± 1 day; both plasma (sodium citrate) and serum samples to be stored at - 70° C for measurement of both antigenic content and functional activity (potency) (all samples are mandatory at all time points regardless of hospital discharge status, except for the Day 8 AAT activity and antigen which is not required if subjects are discharged from the hospital and no 2nd blinded study drug infusion is administered).

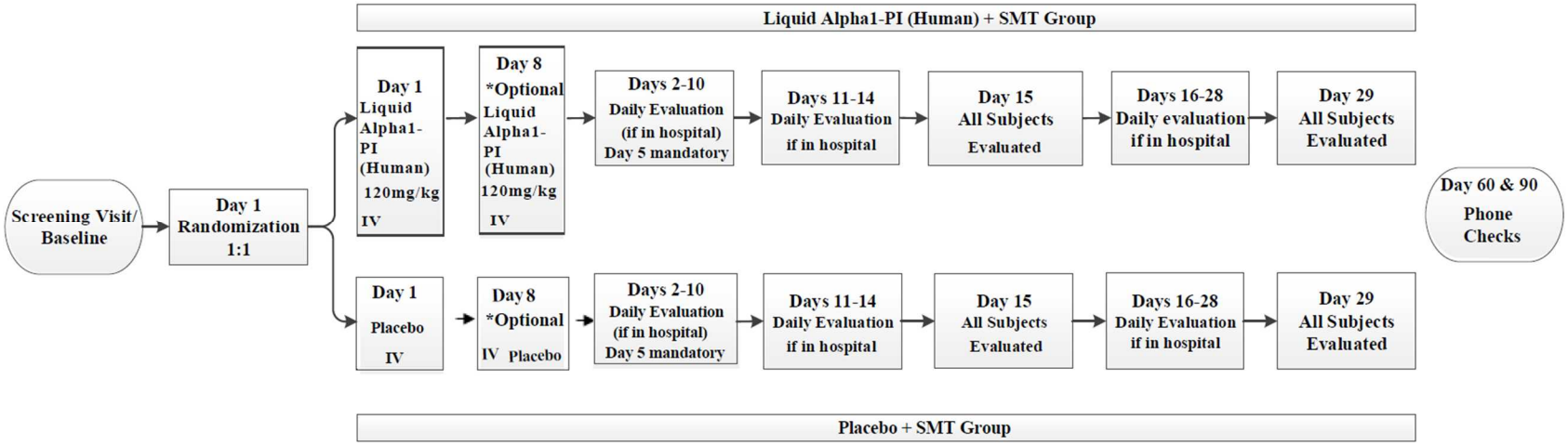
This clinical trial will consist of the following phases:

- Screening/Baseline (Day 1) during which eligibility criteria are reviewed. Once all eligibility criteria are met and all pre-infusion assessments are completed, blinded Liquid Alpha$_1$-PI (Human) or Placebo administration can begin as expeditiously as feasible. On Day 8 a second IV dose of blinded Liquid Alpha$_1$-PI (Human) or Placebo will be administered on Day 8. If the subject has been discharged from the hospital at the time of the Day 8 blinded Liquid Alpha$_1$-PI (Human) or Placebo infusion, the 2nd infusion is not mandatory, and is at the discretion of the Principal Investigator and subject.

- SMT, Observations, and Follow-up (Days 2 to Day 29): SMT will be provided to all subjects with daily assessments while hospitalized and follow-up from Day 2 through Day 29±1 day (inclusive).

- Phone Checks will occur at Day 60 and Day 90 for vital status (living or deceased), any hospital re-admissions or serious/non-serious adverse events after the Day 29 Final Clinic Visit.

The overall study schema is shown in Figure 4-1.



IV = intravenous; SMT = standard medical treatment

*Note: If the subject has been discharged from the hospital at the time of the Day 8 blinded Liquid Alpha-1 PI infusion, the 2nd infusion is not mandatory, and is at the discretion of the Principal Investigator and subject

Figure 4-1 Overall Study Schema

4.3 Measures Taken to Minimize/Avoid Bias

4.3.1 Subject Numbering

Within each study site, subjects in the study will receive a consecutive subject number. Subject numbers are generated beginning with the study center number (3 digits, assigned by the sponsor) followed consecutively with a unique number for each subject (4 digits, including leading zeros). For example, if the investigator's center number is 301, subject number will be 3010001, 3010002, 3010003, etc., in consecutive order. Subject numbers, once assigned, will not be reused at any center.

4.3.2 Randomization

Subjects will be randomized 1:1 to receive either combination treatment with Liquid Alpha$_1$-PI (Human) plus SMT or placebo plus SMT.

4.3.3 Blinding

The unblinded study pharmacist or designee, will be the only unblinded study person at each site. Preparation and pooling of study drug (Liquid Alpha$_1$-PI (Human) and placebo) will be the responsibility of the local unblinded pharmacist or designee. The placebo is sterile 0.9% sodium chloride injection, United States Pharmacopeia (0.9% NaCl, USP) or equivalent. The same total volume of placebo will be prepared so that there are no differences in infusion volume.

Since this is a double-blind, placebo-controlled study, measures will be taken to assure that the placebo infusion will be indistinguishable in terms of commensurate volume from Liquid Alpha$_1$-PI (Human) infusion volume to that required for the appropriate weight-based dose of Liquid Alpha$_1$-PI (Human) to maintain blinding.

Additionally, the unblinded pharmacist or designee will prepare all study drug infusion bags to assure no obvious visual differences between Liquid Alpha$_1$-PI (Human) and placebo by covering the infusion bag with a non-transparent blinding bag cover.

Furthermore, results of the central laboratory analysis of AAT (activity & antigen) will not be shared with the Investigator, blinded study staff, clinical research organization or blinded Sponsor personnel involved in study conduct.

4.4 Study Treatments

4.4.1 Treatments to Be Administered

- During this clinical trial, subjects randomized to combination Liquid Alpha$_1$-PI (Human) plus SMT will receive the first dose of blinded Liquid Alpha$_1$-PI (Human) given as an IV dose of 120 mg/kg (body weight) on Day 1. A second dose of blinded Liquid Alpha$_1$-PI (Human) (120 mg/kg) will be infused on Day 8 (see Section 6.1). If the subject has been discharged from the hospital at the time of the Day 8 blinded Liquid Alpha$_1$-PI (Human) infusion, the 2nd infusion is not mandatory, and is at the discretion of the Principal Investigator and subject.

4.4.1.1 Liquid Alpha$_1$-PI (Human)

Liquid Alpha$_1$-PI (Human) has a nominal concentration of Alpha$_1$-Proteinase Inhibitor of 50 mg/mL. For a subject weighing 75 kg each dose of 120 mg/kg (on Day 1 and Day 8) would require 180 mL of Liquid Alpha$_1$-PI (Human) to achieve the specified 120 mg/kg dose.

Prior to administration, visually inspect parenteral drug products for particulate matter and discoloration. The product may contain a few protein particles. The solution is clear, colorless or pale yellow or pale green. Do not use if discolored or cloudy. Before delivery to the subject, Liquid Alpha$_1$-PI (Human) should be brought to room temperature and administered at room temperature for comfort to the subject. Liquid Alpha$_1$-PI (Human) is for IV administration only. Pooled Liquid Alpha$_1$-PI (Human) solution should be maintained at room temperature for administration within 3 hours.

Filter the solution during administration using an IV administration set with a suitable 5 to 15-micron infusion filter (not supplied). Infuse Liquid Alpha$_1$-PI (Human) separately, without mixing with other agents or diluting solutions. Infuse Liquid Alpha$_1$-PI (Human) IV at 0.08 mL/kg/min as determined by subject response and comfort. The Liquid Alpha$_1$-PI (Human) infusion line can be flushed with 0.9% sodium chloride for injection. Do Not flush with heparin.

4.4.1.2 Placebo

Sterile 0.9% sodium chloride injection, United States Pharmacopeia (0.9% NaCl, USP) or equivalent will be used as placebo to maintain the blind. The infusion solution will be at a volume commensurate to that required for the appropriate weight-based dose of Liquid Alpha$_1$-PI (Human) and visually masked to maintain the blind. It will be prepared by the unblinded pharmacist or designee and the infusion bag will be covered with a non-transparent sleeve.

In order to maintain the blind, the placebo infusion will be filtered during administration using an IV administration set with a suitable 5 to 15-micron infusion filter (not supplied).

Sterile 0.9% sodium chloride injection, United States Pharmacopeia (0.9% NaCl, USP) or equivalent will be supplied by the unblinded pharmacist using commercially available saline.

4.4.2 Labeling of Investigational Product

Investigational product will be labeled according to the requirements of local law and legislation.

4.4.3 Packaging of Investigational Product

Packaging and labeling will comply with local regulatory requirements and will be translated into local languages when required per local regulations.

4.4.4 Storage of Investigational product (Liquid Alpha$_1$-PI (Human))

Store refrigerated at 2-8°C (36-46°F) for the period indicated by the expiration date on its label.

Product may be stored at room temperatures not exceeding 25°C (77°F) for up to one month, after which the product must be used or immediately discarded.

Discard after expiration date. Do not freeze. Discard any unused solution according to local requirements

4.5 Expected Duration of Subject Participation in the Study

The total estimated maximum duration of a subject's participation in terms of actual Clinic Visits will be up to 30 days. Additionally, Phone Checks will occur at Day 60 and Day 90 for vital status (living or deceased), any hospital re-admissions or serious/non-serious adverse events after the Day 29 Final Clinic Visit.

4.6 Discontinuation Criteria for Individual Subjects and Study

4.6.1 Discontinuation Criteria for Individual Subjects

See Section 5.3 Subject Withdrawal Criteria

4.6.2 Premature Termination of Study/Closure of Center

The sponsor, Institutional Review Board/Ethics Committee (IRB/EC), and/or regulatory authorities have the right to close this study or a study center, and the investigator/sponsor has the right to close a center, at any time, although this should occur only after consultation between involved parties. The IRB/EC must be informed. Should the study/center be closed prematurely, all study materials (except documentation that has to remain stored at the site) must be returned to the sponsor. The investigator will retain all other documents until notification given by the sponsor for destruction.

The reasons a study center may be closed include, but are not limited to, the following:

- Lack of enrollment
- Non-compliance with the requirements of the study protocol
- Non-compliance with ICH GCP

4.7 Accountability Procedures for Investigational Product

Investigational product is to be used only for the study in accordance with the directions given in this protocol. The investigator, or designee such as the study pharmacist, is responsible for handling of the IP in accordance with directions given in the protocol.

The investigator is responsible for maintaining accurate records of the IP for his/her site. Investigational product inventory/dispensing documentation verifying the receipt, dispensing, destruction, or return must be maintained and kept current by the investigator, or designee. The inventory must be made available for inspection by the monitor. Investigational product supplies must be accounted for by the monitor and inventory/dispensing logs must be verified by the monitor prior to IP return or destruction. Written documentation of any used and unused inventory is required. At the end of the study, a copy of the inventory/dispensing log(s) will be retrieved by the monitor and returned to Grifols.

4.8 Maintenance of Treatment Randomization Codes

Randomization codes will be generated through an Interactive Response Technology electronic system and access to the actual randomization schedules or codes will be strictly controlled during the course of the study. This study is double-blind so there is blinding of treatment assignment.

4.9 Data to Be Recorded

All information contained in the medical history and complementary exploration reports including laboratory test will be considered as clinical trial source data.

Any data recorded in the Case Report Form should have written or electronic record in the subject's medical records. These written or electronic records will be considered source data and should be dated and signed by the investigator or by the qualified delegated person (eg, results of vital signs testing, or the IP administration procedure).

For every subject enrolled, the investigator will write into his/her medical history that he/she has been enrolled in a clinical trial, specifying its title, study number, and sponsor, as well as the date of ICF provision.

The investigator is responsible for maintaining complete and adequate case histories in source records of each subject. All study-specific data necessary to be recorded in that cannot be found in subjects' past medical records (such as medical history, past medications, etc.) should be recorded by the investigator or their designee in subjects' medical files, dating and signing all new entries.

Source data must be preserved for the maximum period of time as required per local and international regulations and made available by the investigator in the cases described above.

5 SELECTION AND WITHDRAWAL OF SUBJECTS

5.1 Inclusion Criteria

A subject must meet all the following inclusion criteria to be eligible for participation in this study:

1. Hospitalized male or female subject ≥ 18 years of age at time of Screening who is being treated for COVID-19. Subjects must be screened within 48 hours (≤ 48 hours) of hospital admission.

2. Has laboratory-confirmed novel coronavirus (SARS-CoV-2) infection as determined by qualitative PCR (reverse transcriptase [RT]-PCR), or other commercial or public health assay approved by regulatory authorities as a diagnostic test for COVID-19 in any specimen during the current hospital admission OR 96 hours prior to the hospital admission date and prior to randomization (the SARS-CoV-2 test results must be performed by a hospital laboratory and the documentation available) .

3. COVID-19 illness (symptoms) of any duration, including **both of** the following:

    a) Radiographic infiltrates by imaging (chest X-Ray, CT scan, etc.) and/or clinical assessment (evidence of rales/crackles on exam) with SpO2 <94% on room air

    b) **Any One of** the following related to COVID-19: i. Ferritin > 400ng/mL, ii. LDH > 300 U/L, iii. D-Dimers > reference range, or iv. C-reactive protein (CRP) > 40 mg/L

4. Subject provides informed consent prior to initiation of any study procedures.

5. Female subjects of childbearing potential (and males with female partners of childbearing potential) must agree to use of acceptable contraception methods during study (e.g., oral, injectable, or implanted hormonal methods of contraception, placement of an intrauterine device or intrauterine system, condom or occlusive cap with spermicidal foam/gel/film/cream/suppository, male sterilization, or true abstinence*) throughout the study.

    *True abstinence: When this is in line with the preferred and usual lifestyle of the subject. (Periodic abstinence [e.g., calendar, ovulation, symptothermal, post-ovulation methods], declaration of abstinence for the duration of a trial, and withdrawal are not acceptable methods of contraception.)

5.2 Exclusion Criteria

A subject meeting any of the following exclusion criteria is NOT eligible for participation in the study.

1. Subjects requiring invasive mechanical ventilation or ICU admission or with PaO2/FIO2 ≤ 150 mm Hg (i.e., arterial oxygen in mm Hg divided by fraction inspired oxygen concentration [e.g., 0.21 for room air])

2. Clinical evidence of any significant acute or chronic disease that, in the opinion of the investigator, may place the subject at undue medical risk.

3. The subject has had a known serious anaphylactic reaction to blood, any blood-derived or plasma product, or known Selective IgA Deficiency with anti-IgA antibodies.

4. A medical condition in which the infusion of additional fluid is contraindicated (e.g., decompensated congestive heart failure or renal failure with fluid overload). This includes currently uncontrolled congestive heart failure New York Heart Association Class III or IV stage heart failure.

5. Shock that is unresponsive to fluid challenge and/or multiple vasopressors and accompanied by multiorgan failure considered not able to be reversed by the Principal Investigator

6. Known alpha-1 antitrypsin deficiency for which the subject is already receiving alpha1-proteinase inhibitor augmentation therapy

7. Women who are pregnant or breastfeeding. Female subjects of child-bearing potential must have a negative test for pregnancy blood or urine human chorionic gonadotropin (HCG)-based assay at Screening/Baseline Visit.

8. Subjects for whom there is *limitation of* therapeutic effort such as "Do not resuscitate" status Note: If the decision is made not to apply treatments or therapeutic procedures that will provide little benefit for the suffering or agony the subject is experiencing, such a subject would not be appropriate for participation in this study and should be excluded.

9. Currently participating in another interventional clinical trial with investigational medical product or device

10. Subjects previously requiring long-term oxygen therapy (home oxygen therapy)

11. History (within the last 2 years) of myocardial infarction, unstable angina, stroke or transient ischemic attacks, pulmonary embolism or deep venous thrombosis

12. Subject has medical condition (other than COVID-19) that is projected to limit lifespan to $\leq$ 1 year

13. Systolic blood pressure < 100 mm Hg or > 160 mm Hg (uncontrolled hypertension) at the time of Screening

14. Alanine aminotransferase (ALT) $\geq$ 2 times the upper limit of normal (ULN)

15. Any elevation of total bilirubin at the time of Screening

16. Estimated glomerular filtration rate (eGFR) < 45 mL/min (or subject is dependent on dialysis/renal replacement therapy) at the time of Screening

    eGFR is calculated by the Cockcroft-Gault equation:

    For men:

$$\frac{(140 - \text{age in years})(\text{body weight [kg]})}{(72)(\text{serum creatinine } \left[\frac{\text{mg}}{\text{dL}}\right])}$$

For women:

$$\frac{0.85 \text{ x } (140 - \text{age in years})(\text{body weight [kg]})}{(72)(\text{serum creatinine } \left[\frac{\text{mg}}{\text{dL}}\right])}$$

17. Hemoglobin < 10 g/dL at the time of Screening
18. Absolute neutrophil count < 1000/mm^3 at the time of Screening
19. Platelet count < 75,000/mm^3 at the time of Screening
20. Subject has history of drug or alcohol abuse within the past 24 months
21. Subject is unwilling to commit to follow-up visits
22. Known history of prothrombin gene mutation 20210, homozygous Factor V Leiden mutations, antithrombin III deficiency, protein C deficiency, protein S deficiency or antiphospholipid syndrome

5.3 Subject Withdrawal Criteria

Subjects have the right to withdraw from the study at any time for any reason, either before or after the infusion of the IP. The investigator can withdraw a subject from the clinical trial at any time.

The investigator will document the reason(s) for withdrawal of each subject in source documents and study record. All data gathered on the subject prior to termination will be made available to the sponsor.

5.3.1 Screen Failures

Screening evaluations will be used to determine the eligibility of each subject for enrollment. Subjects who fail to meet eligibility criteria during screening evaluations will be considered screen failures and will not participate in the study. Outcomes of screening evaluations will be documented in subject's source documents (e.g. medical history) - including compliance with each individual inclusion/exclusion criterion- and in study records as well.

5.3.2 Removal of Subjects

Subjects may withdraw or be withdrawn from the study for the following reasons:

1. At their own request or at the request of their legally acceptable representative.
2. If, in the investigator's opinion, continuation in the study would be detrimental to the subject's well-being.
3. At the specific request of the sponsor.

Also, subjects may be withdrawn for the following reasons:

1. The subject is not able to adhere to the main protocol requirements (major protocol violations).
2. The occurrence of an AE which in the investigator's opinion requires the withdrawal of the subject from the clinical trial.
3. The occurrence of pulmonary embolism whether or not related to COVID-19
4. The subject is lost to follow-up.
5. Subject's death.
6. Any event which in the opinion of the investigator impedes the subject's participation in the study.

In all cases, the reason for withdrawal must be recorded in the study record and in the subject's records.

5.3.3 Subject Replacement

Subjects who are withdrawn from the study after being randomized will not be replaced.

5.3.4 Follow-up of Subjects Withdrawn from Study

For subjects administered any amount of blinded Liquid Alpha$_1$-PI (Human) or blinded placebo who withdraw from the clinical trial prior to Day 29, study procedures and assessments scheduled for the Day 15 Visit will be performed at the time of withdrawal, during an unscheduled visit. In addition, to assure major clinical outcomes are adequately captured as a measure of overall safety, these subjects will be asked to return for their chronological Day 29 Clinic Visit (ie, 28 days after Day 1 baseline), provided that they or their legally acceptable representative have not withdrawn consent. At the chronological Day 29 Clinic Visit, all assessments will be performed *except for* central laboratory testing: cytokine panel, levels of alpha 1-PI (AAT) activity and antigen, respiratory sample for quantitative measurement of SARS-CoV-2 viral load (obtained via nasopharyngeal swab), and quantitative measurement of IgM and IgG antibodies, *which are not required for these subjects at chronological Day 29 for premature withdrawals*. Note: A Day 29 Clinic Visit would not be necessary if the unscheduled visit is within 4 days of the subject's chronological Day 29 visit because the assessments/outcome measures would be the same and would not need to be repeated in such a short a time interval.

5.3.5 Definition of the End of Study

Clinical trial finalization will coincide with the last study visit of the last subject enrolled in the clinical trial. For an individual subject, end of study for the purposes of determining disposition and successful study completion is the Day 29/Final Clinic Visit performed at the scheduled on-study timeframe.

6 TREATMENT OF SUBJECTS

See Section 4.4 for the treatment to be administered, including the name of the IP, the dose, and the route/mode of administration.

6.1 Administration and Timing of Investigational Product for Each Subject

Subjects randomized to combination blinded Liquid Alpha$_1$-PI (Human) plus SMT will receive the first dose of Liquid Alpha$_1$-PI (Human) given as an IV dose of 120 mg/kg (body weight) on Day 1. A second dose of blinded Liquid Alpha$_1$-PI (Human) (120 mg/kg) will be infused on Day 8. If the subject has been discharged from the hospital at the time of the Day 8 blinded Liquid Alpha$_1$-PI (Human) infusion, the 2nd infusion is not mandatory, and is at the discretion of the Principal Investigator and subject.

Liquid Alpha$_1$-PI (Human) has a nominal concentration of Alpha1-Proteinase Inhibitor of 50 mg/mL. For a subject weighing 75 kg each dose of 120 mg/kg (on Day 1 and Day 8) would require 180 mL of Liquid Alpha$_1$-PI (Human) to achieve the specified 120 mg/kg dose.

Visually inspect parenteral drug products for particulate matter and discoloration prior to administration. The product may contain a few protein particles. The solution is clear, colorless or pale yellow or pale green. Do not use if discolored or cloudy. Before delivery to the subject, Liquid Alpha$_1$-PI (Human) should be brought to room temperature and administered at room temperature for comfort to the subject. Liquid Alpha$_1$-PI (Human) is for IV administration only. Pooled Liquid Alpha$_1$-PI (Human) solution should be maintained at room temperature for administration within 3 hours.

Filter the solution during administration using an IV administration set with a suitable 5 to 15-micron infusion filter. Infuse Liquid Alpha$_1$-PI (Human) separately, without mixing with other agents or diluting solutions. Infuse Liquid Alpha$_1$-PI (Human) IV at 0.08 mL/kg/min as determined by subject response and comfort. The Liquid Alpha$_1$-PI (Human) infusion line can be flushed with 0.9% sodium chloride for injection. Do Not flush with heparin.

Subjects randomized to placebo plus SMT will receive sterile 0.9% sodium chloride injection (0.9% NaCl, USP) or equivalent to maintain the blind. The infusion solution will be at a volume commensurate to that required for the appropriate weight-based dose of Liquid Alpha$_1$-PI (Human) and visually masked to maintain the blind. It will be prepared by the unblinded pharmacist or designee and the infusion bag will be covered with a non-transparent sleeve. In order to maintain the blind, the placebo infusion will be filtered during administration using an IV administration set with a suitable 5 to 15-micron infusion filter (not supplied).

An infusion may be stopped at the discretion of the medical personnel according to normal practice, for instance if there is extravasation at the IV site, and then restarted using a new IV site. However, if a hypersensitivity reaction occurs, such as anaphylaxis, the IV infusion must be stopped permanently, and no further study infusions will be given. During infusion

vital signs will be measured and the subject will be carefully observed. Specifically, vital signs will be measured immediately before infusion, then after the first 5 minutes of infusion and subsequently at 5 to 10-minute intervals during infusion until the end of infusion, and at a time point 2 hours after the end of infusion. Early signs and symptoms of hypersensitivity reactions may include pruritus; generalized urticaria; flushing; swollen lips, tongue, or uvula; wheezing; tightness of the chest; dyspnea; hypotension; and syncope. If hypersensitivity symptoms occur, the infusion must be promptly stopped, and appropriate therapy administered. Such subjects who discontinue infusion will be monitored according to standard of care until hypersensitivity/anaphylaxis resolves and will not receive any further IP.

6.2 Prior and Concomitant Therapy

Concomitant medications must be recorded in the medical notes and in CRF, including the trade and generic names of the medication, the dose, the route of administration, and the duration of the medication (frequency). Concomitant prophylaxis for potential venous thrombosis or thromboembolism in hospitalized subjects with COVID-19 is supported within this study according to institutional standard practices.

Subjects should be managed according to standard of care for intercurrent illnesses, inclusive of influenza vaccine if appropriate depending on the clinical circumstance.

6.2.1 Prohibited Medications Prior to Study Participation

Administration of commercial AAT at study entry is not allowed.

6.2.2 Prohibited Concomitant Medications during the Study

Administration of a non-study commercial AAT product is not allowed during study.

6.2.3 Restricted Concomitant Medications during the Study

There are no restricted concomitant medications.

6.3 Treatment Compliance

Reasons for any deviation from the administration of less than 100% of the IP dose must be recorded in the subject's medical records.

The investigator or designee is responsible for maintaining accurate records of study medication administered at his/her study center.

7 ASSESSMENT OF EFFICACY

7.1 Efficacy Variables

7.1.1 Primary Efficacy Variable

The primary efficacy variable is the proportion of subjects dying or requiring ICU admission on or before Day 29 or who are dependent on high flow oxygen devices or invasive mechanical ventilation on Day 29.

7.1.2 Secondary Efficacy Variables

The secondary efficacy variables include:

- Assessment of Clinical Severity: Change in NEWS from baseline (Day 1 through Day 29)

  The NEWS has demonstrated an ability to discriminate patients at risk of poor outcomes. This score is based on 7 clinical parameters (respiration rate, oxygen saturation, any supplemental oxygen, temperature, systolic blood pressure, heart rate, level of consciousness [Alert, Voice, Pain, Unresponsive]) https://www.mdcalc.com/national-early-warning-score-news (Appendix 2)
- Time to clinical response: NEWS ≤ 2 maintained for 24 hours, Day 1 through Day 29
- Time to hospital discharge: defined as duration of hospitalization from Day 1 through Day 29
- If admitted to ICU post randomization: Duration of ICU stay through Day 29
- Duration of any oxygen use Day 1 through Day 29
- If requiring mechanical ventilation post randomization: Duration mechanical ventilation through Day 29
- Absolute value and mean change from baseline in the Ordinal scale Day 1 through Day 29

  The Ordinal scale is as follows:

  1) Death;
  2) Hospitalized, on invasive mechanical ventilation or extracorporeal membrane oxygenation (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, limitation on activities;
  7) Not hospitalized, no limitations on activities.
- Proportion (percentage) of subjects in each severity category of the 7-point Ordinal scale at Day 15 and Day 29

- Time to sustained normalization of temperature and proportion of subjects with normalization of fever at all time points, defined as temperature < 36.6°C armpit, < 37.2°C oral, or < 37.8°C rectal sustained for at least 24 hours
- Number of subjects who develop ARDS through Day 29 (Appendix 3)
- Length of time to clinical progression through Day 29 (defined as the time to death, mechanical ventilation, or ICU admission)

7.1.3 Exploratory Efficacy Variables

The exploratory efficacy variables include:

- Change from baseline in quantitative SARS-CoV-2 viral load by NAT or PCR (real-time RT-PCR) to Day 5± 1 day, Day 15± 1 day, and Day 29 ± 1 day
- Change from baseline in quantitative anti- SARS-CoV-2 IgM and IgG antibodies to Day 5± 1 day, Day 15± 1 day, and Day 29 ± 1 day
- Proportion of subjects with evidence of hyperinflammation on Day 1, Day 5, Day 15, and Day 29 defined as:
    a) Lymphocyte counts <1000 cells/µL, AND
    b) Two of the following 4 criteria:
        i) Ferritin > 500ng/mL,
        ii) LDH > 300 U/L,
        iii) D-Dimers > 1000 ng/mL (fibrinogen equivalent units [FEU]) or > 2 times upper normal limit,
        iv) C-reactive protein (CRP) > 70 mg/L
- Change from baseline in cytokine profile (cytokine panel includes IL-1β, IL-10, IL-6, IL-8, IL-2, interferon γ, and TNF-α) to Day 5± 1 day, Day 15± 1 day, and Day 29 ± 1 day
- Change from baseline in levels of alpha 1-PI (AAT) activity and antigen through Day 29 (both plasma [sodium citrate] and serum samples to be stored for measurement of both antigenic content and functional activity [potency])

7.2 Methods and Timing for Assessing, Recording, and Analyzing Efficacy Parameters

7.2.1 Observations and Measurements

Before any study-specific screening/baseline procedures are performed, and after completely understanding the nature of the clinical trial, informed consent must be obtained. This means that the potential subject provides informed consent prior to initiation of any study procedures. The preferred process for obtaining informed consent is through the signing of a written ICF by the subject. If there are concerns regarding coronavirus (SARS-Cov-2) transmission through the process of obtaining written consent, methods recently proposed by the FDA should be followed to reduce this risk (see Appendix 4).

Sites subject to local conditions/institutional guidelines that do not allow for a written informed consent process due to the risk of coronavirus (SARS-Cov-2) transmission may

obtain oral consent only if the site is able to provide written documentation of the specific institutional/local guidelines that mandate oral consent as the only permitted consenting method and ensure these guidelines are available in the investigator site files. Where proven that oral informed consent is the only permittable process, the site must record in the subject's medical history, "I have explained to the subject the characteristics and objectives of the study, its risks, and potential benefits. I have been able to answer the subject's questions and I confirm that this subject has given oral informed consent." Subsequently, and when possible, the subject's written informed consent will be obtained in those situations where oral consent was initially required. All informed consent procedures must be aligned with local laws and regulations.

The following is a description of the procedures/assessments to take place at each study visit. See the Schedules of Study Procedures in Appendix 1.

7.2.2 Screening/Baseline and Randomized Treatment (Day 1)

All required screening and baseline (pre-infusion Day 1) procedures/assessments will be performed prior to administration of the blinded study drug infusion.

Assessments include the following:

- Informed consent
- Inclusion/exclusion criteria
- Pregnancy test for females of child-bearing potential
- Record demography, (including age [year of birth], gender, race, and ethnicity) disease characteristics (date of exposure, date of onset)
- Ordinal Scale assessment
- National Early Warning Score (NEWS) (Appendix 2)
- Record clinical features key symptoms: fever, cough, shortness of breath, fatigue, myalgia etc.
- Vital signs (temperature, systolic and diastolic blood pressure, pulse, respiratory rate)
- Weight
- Record result of historical SARS-CoV-2 PCR (qualitative RT-PCR) or NAT or other commercial or public health assay approved by regulatory authorities as a diagnostic test for COVID-19 in any specimen during the current hospital admission OR 96 hours prior to the hospital admission date and prior to randomization (the SARS-CoV-2 test results must be performed by a hospital laboratory and the documentation available) (eligibility criterion)
- If subject is receiving oxygen, record any supplemental oxygen administration (type, %, flow start/end date/time)
- Record hospital admission date
- Record oxygen saturation (specify on or off oxygen supplementation)
- Respiratory sample for quantitative measurement of SARS-CoV-2 viral load by NAT or PCR (real-time RT-PCR) (obtained via nasopharyngeal swab)

- Sample for quantitative measurement of IgM and IgG antibodies to SARS-CoV-2 by enzyme linked immunosorbent assay (ELISA), Indirect Fluorescent Antibody (IFA), or another assay methodology (if antibodies cannot be determined at the site store *serum* samples frozen at -70°C for later analysis at an external lab)
- Serum Chemistry (creatinine, albumin, alanine aminotransferase [ALT], total bilirubin, lactate dehydrogenase [LDH])
- Calculate eGFR using the Cockcroft-Gault formula.
- Hematology: absolute neutrophil & lymphocyte count and standard hematology (hemoglobin, hematocrit, platelet count, leukocyte count with differential)
- Assessment of ARDS (Berlin Criteria) (Appendix 3)
- Ferritin (serum), D-dimer (plasma, sodium citrate tube), CRP (serum)
- Cytokine panel (IL-1β, IL-10, IL-6, IL-8, IL-2, interferon γ, and TNF-α). Note: serum samples must be stored at -70° C for later analysis at a reference laboratory.
- Levels of alpha 1-PI (AAT) activity and antigen; both plasma (sodium citrate) and serum samples to be stored at - 70° C for measurement of both antigenic content and functional activity (potency).
- Record SAEs and TEAEs
- Record standard care concomitant medications

After all Screen/Baseline assessments are complete subjects may be randomized.

Subjects may receive the first infusion of blinded Liquid Alpha$_1$-PI (Human) or Placebo on Screen/Baseline and Randomized Treatment visit (Day 1) if eligibility criteria are met, and all pre-infusion assessments are performed. The intent is for infusion of blinded Liquid Alpha$_1$-PI (Human) or Placebo to commence as rapidly as feasible on Day 1. If there is unanticipated delay due to pandemic conditions, while not ideal, proceed with the first blinded Liquid Alpha$_1$-PI (Human) or Placebo infusion as rapidly as possible.

During study infusion vital signs will be measured immediately before the start of infusion, after the first 5 minutes of infusion, and subsequently at 5-10-minute intervals during infusion until the end of infusion. Vital signs will also be measured 2 hours after the end of study infusion.

Record all infusion details and date/time in the CRF on the specific "1st IP infusion day" page and continue with all protocol-specified assessments.

All Study Days are relative to Day 1 (Screen/Baseline and Randomized Treatment visit).

7.2.3 SMT and Daily Evaluations (Days 2 through 10 if still hospitalized) and Mandatory Day 5 Assessments for All Subjects

All subjects receive continued SMT. **On Day 8 all subjects will receive a second double-blind infusion of blinded Liquid Alpha$_1$-PI (Human) or Placebo. Subjects randomized to Liquid Alpha$_1$-PI (Human) will receive a second blinded dose of 120 mg/kg body weight. Subjects randomized to Placebo will receive a second blinded infusion of sterile 0.9%**

NaCl USP, or equivalent of commensurate volume to preserve the blind. If the subject has been discharged from the hospital at the time of the Day 8 blinded Liquid Alpha$_1$-PI (Human) or Placebo infusion, the 2nd infusion is not mandatory, and is at the discretion of the Principal Investigator and subject.

Coinciding with this time point, subjects who opt to receive the 2nd blinded infusion of Liquid Alpha$_1$-PI (Human) or Placebo will have levels of alpha 1-PI (AAT) activity and antigen measured on Day 8. Both plasma (sodium citrate) and serum samples are to be stored at - 70° C for measurement of both antigenic content and functional activity (potency); for all such subjects, samples must be obtained *prior to* blinded Liquid Alpha$_1$-PI (Human) or Placebo infusion.

During study infusion on Day 8, vital signs will be measured immediately before the start of infusion, after the first 5 minutes of infusion, and subsequently at 5-10-minute intervals during infusion until the end of infusion. Vital signs will also be measured 2 hours after the end of study infusion.

Daily assessments include the following:

- Ordinal Scale assessment
- NEWS (Appendix 2)
- Clinical features key symptoms: fever, cough, shortness of breath, fatigue, myalgia etc.
- Vital signs (temperature, systolic and diastolic blood pressure, pulse, respiratory rate)
- Record any supplemental oxygen administration (type, %, flow start/end date/time)
- If subject requires mechanical ventilation post randomization: Record mechanical ventilation (start/end date/time)
- Record oxygen saturation (specify on or off oxygen supplementation)
- If subject requires ICU admission post randomization: Record ICU admission date
- Record SAEs and TEAEs
- Record standard care concomitant medications

<u>On Day 5 ± 1 day the following specimens will be obtained</u> (mandatory assessments for all subjects with daily assessments for Day 5 detailed immediately above):

- Respiratory sample for quantitative measurement of SARS-CoV-2 viral load by NAT or PCR (real-time RT-PCR) (obtained via nasopharyngeal swab)
- Sample for quantitative measurement of IgM and IgG antibodies to SARS-CoV-2 (if antibodies cannot be determined at the site store serum samples frozen at -70°C for later analysis at an external lab)
- Cytokine panel (IL-1β, IL-10, IL-6, IL-8, IL-2, interferon γ, and TNF-α). Note: serum samples must be stored at -70° C for later analysis at a reference laboratory.

<u>Additionally, on Day 5 ± 1 day the following samples will be obtained (all on the same day) (mandatory assessments for all subjects):</u>

- Ferritin, D-dimer, CRP
- Serum Chemistry (creatinine, albumin, ALT, total bilirubin, LDH)

- Calculate eGFR using the Cockcroft-Gault formula.
- Hematology: absolute neutrophil & lymphocyte count and standard hematology (hemoglobin, hematocrit, platelet count, leukocyte count with differential)
- Assessment of ARDS (Berlin Criteria) (Appendix 3)

7.2.4 Day 11-14 for Subjects Still Hospitalized: SMT and Daily Evaluations

All subjects receive continued SMT.

Daily assessments include the following:

- Ordinal Scale assessment
- NEWS (Appendix 2)
- Clinical features key symptoms: fever, cough, shortness of breath, fatigue, myalgia etc.
- Vital signs (temperature, systolic and diastolic blood pressure, pulse, respiratory rate)
- Record any supplemental oxygen administration (type, %, flow start/end date/time)
- If subject requires mechanical ventilation post randomization: Record mechanical ventilation (start/end date/time)
- Record oxygen saturation (specify on or off oxygen supplementation)
- Record SAEs and TEAEs
- Record standard care concomitant medications

7.2.4.1 Day 8 2nd Liquid Alpha1-PI (Human) or Placebo Infusion for Hospitalized Subjects (optional if subject discharged from the hospital)

Subjects randomized to Liquid Alpha$_1$-PI (Human) or Placebo + SMT will receive a second dose on Day 8. If the subject has been discharged from the hospital at the time of the Day 8 blinded Liquid Alpha$_1$-PI (Human) or Placebo infusion, the 2nd infusion is not mandatory, and is at the discretion of the Principal Investigator and subject.

Subjects in the hospital on Day 8 will have levels of alpha 1-PI (AAT) activity and antigen measured. Both plasma (sodium citrate) and serum samples are to be stored at -70° C for measurement of both antigenic content and functional activity (potency);, samples must be obtained prior to IV infusion of blinded study drug.

7.2.5 Day 15±1 day Mandatory for All Subjects

Assessments include the following:

- Ordinal Scale assessment
- NEWS (Appendix 2)
- Clinical features key symptoms: fever, cough, shortness of breath, fatigue, myalgia etc.
- Vital signs (temperature, systolic and diastolic blood pressure, pulse, respiratory rate)
- Record any supplemental oxygen administration (type, %, flow start/end date/time)

- If subject requires mechanical ventilation post randomization: Record mechanical ventilation (start/end date/time)
- Record oxygen saturation (specify on or off oxygen supplementation)
- Record SAEs and TEAEs
- Record standard care concomitant medications
- Respiratory sample for quantitative measurement of SARS-CoV-2 viral load by NAT or PCR (real-time RT-PCR) (obtained via nasopharyngeal swab)
- Sample for quantitative measurement of IgM and IgG antibodies to SARS-CoV-2 (if antibodies cannot be determined at the site store serum samples frozen at -70°C for later analysis at an external lab)
- Serum Chemistry (creatinine, albumin, ALT, total bilirubin, LDH)
- Calculate eGFR using the Cockcroft-Gault formula.
- Hematology absolute neutrophil & lymphocyte count and standard hematology (hemoglobin, hematocrit, platelet count, leukocyte count with differential)
- Assessment of ARDS (Berlin Criteria) (Appendix 3)
- Ferritin, D-dimer, CRP
- Cytokine panel (IL-1β, IL-10, IL-6, IL-8, IL-2, interferon γ, and TNF-α). Note: serum samples must be stored at -70° C for later analysis at a reference laboratory.
- Levels of alpha 1-PI (AAT) activity and antigen; both plasma (sodium citrate) and serum samples to be stored at - 70° C for measurement of both antigenic content and functional activity (potency).

7.2.6 Day 16-28 for Subjects Still Hospitalized: SMT and Daily Evaluations

All subjects receive continued SMT.

Daily assessments include the following:

- Ordinal Scale assessment
- NEWS (Appendix 2)
- Clinical features key symptoms: fever, cough, shortness of breath, fatigue, myalgia etc.
- Vital signs (temperature, systolic and diastolic blood pressure, pulse, respiratory rate)
- Record any supplemental oxygen administration (type, %, flow start/end date/time)
- If subject requires mechanical ventilation post randomization: Record mechanical ventilation (start/end date/time)
- Record oxygen saturation (specify on or off oxygen supplementation)
- Record SAEs and TEAEs
- Record standard care concomitant medications

7.2.7 Day 29±1 day – All Subjects

Assessments include the following:

- Ordinal Scale assessment
- NEWS (Appendix 2)
- Clinical features key symptoms: fever, cough, shortness of breath, fatigue, myalgia etc.
- Vital signs (temperature, systolic and diastolic blood pressure, pulse, respiratory rate)
- Record any supplemental oxygen administration (type, %, flow start/end date/time)
- If subject requires mechanical ventilation post randomization: Record mechanical ventilation (start/end date/time)
- Record oxygen saturation (specify on or off oxygen supplementation)
- Record hospital discharge date
- If subject requires ICU admission post randomization: Record ICU discharge date
- Record SAEs and TEAEs
- Record standard care concomitant medications
- Respiratory sample for quantitative measurement of SARS-CoV-2 viral load by NAT or PCR (real-time RT-PCR) (obtained via nasopharyngeal swab)
- Sample for quantitative measurement of IgM and IgG antibodies to SARS-CoV-2 (if antibodies cannot be determined at the site store serum samples frozen at -70°C for later analysis at an external lab)
- Serum Chemistry (creatinine, albumin, ALT, total bilirubin, LDH)
- Calculate eGFR using the Cockcroft-Gault formula.
- Hematology: absolute neutrophil & lymphocyte count and standard hematology (hemoglobin, hematocrit, platelet count, leukocyte count with differential)
- Assessment of ARDS (Berlin Criteria) (Appendix 3)
- Ferritin, D-dimer, CRP
- Cytokine panel (IL-1β, IL-10, IL-6, IL-8, IL-2, interferon γ, and TNF-α). Note: serum samples must be stored at -70° C for later analysis at a reference laboratory.
- Levels of alpha 1-PI (AAT) activity and antigen; both plasma (sodium citrate) and serum samples to be stored at - 70° C for measurement of both antigenic content and functional activity (potency).

7.2.8 Day 60±2 days and Day 90±2 days Phone Checks – All Subjects

- Phone Checks will be performed on Day 60 and Day 90:
- to confirm the subject's vital status (living or deceased)
- to record any hospital re-admissions
- to record any SAEs/non-serious AEs after the Day 29 Final Clinic Visit

7.2.9 Description of Laboratory Tests and Procedures

Table 7-1 Name, Description, and Location of Laboratory Tests and Procedures

provides a summary of the laboratory tests conducted for this study.

Table 7-1 Name, Description, and Location of Laboratory Tests and Procedures

Test Panel[a]	Description	Location
Hematology	Absolute neutrophil & lymphocyte count; Basic hematology: Hemoglobin, hematocrit, platelets, white blood cell (WBC) count with differential	Local
Chemistry	Creatinine, albumin, alanine aminotransferase (ALT), total bilirubin, lactate dehydrogenase (LDH)	Local
Markers of inflammation	C-reactive protein (CRP), D-dimer, ferritin	Local
Cytokine panel	Cytokine panel includes IL-1β, IL-10, IL-6, IL-8, IL-2, interferon γ, and tumor necrosis factor-α (TNF-α). Note: serum samples must be stored at -70° C for later analysis at a reference laboratory.	Central
Quantitative measurement of SARS-CoV-2 viral load by NAT or PCR (RT-PCR)	Quantitative NAT or PCR (real-time RT-PCR) in respiratory samples (obtained via nasopharyngeal swab)	Central
Quantitative measurement of IgM and IgG antibodies to SARS-CoV-2	Quantitative IgM and IgG antibody levels by ELISA, IFA, or other assay methodology (if antibodies cannot be determined at the site store serum samples frozen at -70°C for later analysis at an external lab)	Central
AAT (activity & antigen)	Levels of alpha 1-PI (AAT) activity and antigen; both plasma (sodium citrate) and serum samples to be stored at – 70° C for measurement of both antigenic content and functional activity (potency).	Store local/ Central analysis

8 ASSESSMENT OF SAFETY

8.1 Safety Parameters

The safety and tolerability of Liquid Alpha$_1$-PI (Human) in subjects with COVID-19 will be evaluated in this study. Safety endpoints will include:

- Cumulative incidence of treatment-emergent SAEs and potentially related SAEs through Day 90 phone check
- Cumulative incidence of Grade 3-5 TEAEs and potentially related severe TEAEs through Day 29 as defined in the CTCAE, US Department of Health and Human Services, NIH, and NCI
- Cumulative incidence of all TEAEs and potentially related TEAEs through Day 29

In this clinical study, disease progression is defined as the worsening of a subject's condition attributable to the disease for which the investigational product is being studied. It may be an increase in the severity of the targeted disease and/or increases in the symptoms of the targeted disease. Anticipated symptoms of COVID-19 include fever, cough, hypoxia, dyspnea, hemoptysis, myalgia, fatigue, pharyngitis which may develop at any time during the course of the disease. The development of COVID-19 symptoms during the clinical study should not be recorded as individual TEAEs, unless there is evidence suggesting a causal relationship between blinded Liquid Alpha$_1$-PI (Human)/Placebo and the TEAE.

8.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

The Schedule of study procedures is located in Appendix 1.

TEAEs will be recorded in source documents and on Grifols form for recording of TEAEs.

8.3 Procedures for Eliciting Reports of and for Recording and Reporting Adverse Event and Intercurrent Illnesses

8.3.1 Warnings/Precautions

Hypersensitivity reactions, including anaphylaxis, may occur. Monitor vital signs and observe the subject carefully throughout the infusion.

Individuals with known selective or severe IgA deficiency who have known antibodies against IgA should not receive Liquid Alpha$_1$-PI (Human) since these subjects may experience severe reactions, including anaphylaxis, to IgA which may be present in the preparation.

Since Liquid Alpha$_1$-PI (Human) can cause a transient increase in blood volume, particular caution is necessary in subjects with severe heart failure and subjects at risk of circulatory overload.

When medicines are made from human plasma, certain measures are put in place to prevent infections being passed on to subjects. These include:

- Careful selection of blood and plasma donors to make sure those at risk of carrying infections is excluded
- The testing of each donation and pools of plasma for signs of virus/infections
- The inclusion of steps in the manufacturing of plasma derivatives with virus inactivation/removal capacity

However, despite these measures, when medicines prepared from human blood or plasma are administered, the possibility of passing on infection cannot be totally excluded. This also applies to any unknown or emerging viruses or other types of infections.

8.3.2 Adverse Event Monitoring

Subjects must be carefully monitored for AEs particularly in terms of their seriousness, severity, and causal relationship to the study drug.

8.3.3 Adverse Event Definitions

8.3.3.1 Adverse Events

An AE is defined as any untoward medical occurrence in a subject or clinical investigation subject administered a medicinal product or study treatment and which does not necessarily have a causal relationship with this administration. An AE can therefore be any unfavorable and unintended sign (including any abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

In this clinical study, disease progression is defined as the worsening of a subject's condition attributable to the disease for which the investigational product is being studied. It may be an increase in the severity of the targeted disease and/or increases in the symptoms of the targeted disease. Anticipated symptoms of COVID-19 include fever, cough, hypoxia, dyspnea, hemoptysis, myalgia, fatigue, pharyngitis, which may develop at any time during the course of the disease. The development of COVID-19 symptoms during the clinical study should not be recorded as individual TEAEs, unless there is evidence suggesting a causal relationship between blinded Liquid $Alpha_1$-PI (Human)/Placebo and the TEAE.

8.3.3.2 Suspected Adverse Drug Reactions/Adverse Reactions

All noxious and unintended responses to a medicinal product or study treatment related to any dose should be considered suspected ADRs (i.e., potentially drug related AEs). The phrase "responses to a medicinal product" means that a causal relationship between a medicinal product or study treatment and an AE is at least a reasonable possibility.

8.3.4 Assessment of Causality of Adverse Event

The investigator is required to provide a causality assessment for each AE reported to the sponsor. The assessment of the causal relationship of an AE to the administration of IP must be a clinical decision based on all available information at the time of the completion of the CRF and/or SAE Report Form. The sponsor will consider the investigator's causality assessment and also provide its own assessment.

Causal relationship to the IP will be established according to medical judgment on whether there is a **reasonable possibility of a causal relationship between the AE and the IP administration.**

The investigator must determine and classify the AE causality according to the following categories:

Unrelated/Not related: there is not a reasonable possibility of causal relationship between the AE and the IP.

Possibly related: there is evidence to suggest a causal relationship between the IP and the AE.

Definitely related: there is a reason to conclude that the IP caused the AE.

Criteria to assess the causal relationship should take into account of the following conditions: 1) a plausible temporal sequence from the IP administration to the AE onset; 2) whether the event follows a known response pattern to the suspected treatment; 3) whether the AE could be reasonably explained by the subject's clinical state, comorbidities, or concomitant medications, as well as 4) the occurrence of improvement on stopping/reducing the treatment (positive dechallenge) and/or reappearance of the event on repeated exposure (positive rechallenge).

For expedited safety reporting purposes, AEs assessed as either "definitely related" or "possibly related" will be considered POTENTIALLY RELATED or just RELATED.

Any AE reported prior to the first administration of the IP will be considered a non-treatment-emergent AEs and causal relationship will always be "Unrelated/Not related."

8.3.5 Severity of Adverse Event or Suspected Adverse Drug Reaction

Adverse events and suspected ADRs (i.e., potentially drug related AEs) will be classified depending on their severity according to the following definitions:

Mild: an AE which is well tolerated by the subject, causing minimum degree of malaise and without affecting normal activities.

Moderate: an AE that interferes with the subject's normal activities.

Severe: an AE that prevents the subject from performing their normal activities.
This category is further subdivided into Grade 3-5 AEs defined according to CTCAE criteria, US Department of Health and Human Services, NIH, and NCI.

Adverse events and suspected ADR severity gradation must be distinguished from AE and suspected ADR seriousness gradation, which is defined according to event consequence. For example, headache can be mild, moderate or severe but not necessarily serious in all these cases.

The investigator will be responsible for assessing the AE and suspected ADR intensity during the clinical trial, taking into account current criteria included in this section.

8.3.6 Expectedness of Adverse Event or Suspected Adverse Drug Reaction (Reference Safety Information)

An AE or suspected ADR (i.e., potentially drug related AE) is considered "unexpected" if the nature, seriousness, severity or outcome of the reaction(s) is not consistent with the reference information, which is the Liquid Alpha$_1$-Proteinase Inhibitor (Human) (Prolastin-C Liquid) Investigator's Brochure. The expectedness shall be determined by the sponsor for any serious ADRs (potentially related SAEs) according to the reference document for expedited safety reporting purposes.

8.3.7 Seriousness of Adverse Event or Suspected Adverse Drug Reaction

An AE or suspected ADR (i.e., potentially drug related AE) is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death
- Life-threatening AE (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)
- In-patient hospitalization or prolongation of existing hospitalization*
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- An important medical event (important medical event in the definition of "serious" refers to those events which may not be immediately life-threatening, or result in death, or hospitalization, but from medical and scientific judgment may jeopardize the subject or/and may require medical or surgical intervention to prevent one of the other outcomes listed above).

*Hospitalization is to be considered only hospital admission (including emergency room stay) for equal or more than 24 hours. The following hospitalizations should not be reported as SAEs:

- Hospitalization or prolongation of hospitalization needed for procedures required by the clinical trial protocol or as part of a routine procedure followed by the center.
- Admissions not associated with an AE (e.g., social hospitalization for the purpose of respite care, survey visits, or annual physicals).
- Elective or pre-planned hospitalizations for a pre-existing condition that had not worsened from baseline (e.g., elective or scheduled surgery arranged prior to start of the study).

This definition permits either the sponsor or the investigator to decide whether an event is "serious". If either the sponsor or the investigator believes that the event is serious, the event must be considered "serious" and evaluated by the sponsor for expedited reporting.

8.3.8 Adverse Event Documentation

All AEs and SAEs occurring on Day 1 through the Day 29 (±1 day)/Final Visit must be fully recorded in the subject's medical record and CRF, and paper SAE report form (if serious).

At the time of the Day 60 (±2 days) and Day 90 (±2 days) Phone Checks any additional SAEs/non-serious AEs will also be collected and recorded.

It is the responsibility of the investigator to ensure that AEs are appropriately recorded.

AEs will be collected through directly observed events or spontaneously volunteered by the subject. Clearly related signs, symptoms and abnormal diagnostic procedures should preferably be grouped together and recorded as a single diagnosis or syndrome wherever possible.

The following variables must be recorded:

- The verbatim term (a diagnosis is preferred)
- Date/time of onset
- Date/time of resolution
- Severity (mild, moderate, severe [Grade 3 to Grade 5])
- Causality (unrelated, possibly related, definitely related)*
- Seriousness (yes, no)
- Action taken (with regard to IP)
- Other action (to treat the event)
- Outcome and sequel (follow-up on AE)

*Causality assessment will be made only when the AE occurs after the subject has initiated at least one infusion of the IP. An AE occurring before subject's exposure to IP will be always labeled as "unrelated".

For AEs that occur during infusions, the infusion rate in effect at the time of onset of the AE, the time of onset of the AE and the time of AE change materially in intensity and/or resolve will be captured.

In addition to the investigator's own description of the AE, each AE will be encoded according to the Medical Dictionary for Regulatory Activities (MedDRA).

For example, a laboratory test abnormality considered clinically significant, eg, causing the subject to withdraw from the study, requiring treatment or causing apparent clinical manifestations, or judged clinically significant in the context of the subject's medical history by the investigator, should be reported as an AE. Each event must be described in detail along with start and stop dates, severity, relationship to IP, action taken and outcome. Each

event must be adequately supported by documentation as it appears in the subject's medical or case file.

8.3.9 Reporting of Serious Adverse Events

8.3.9.1 Reporting of Serious Adverse Events

Any SAE (see Section 8.3.7) that occurs (after informed consent) on Day 1 through the Day 29 (±1 day)/Final Visit and any SAE recorded at the time of the Day 60 (±2 days) or Day 90 (±2 days) Phone Checks must be fully recorded in the subject's medical record, CRF and paper SAE Report form. SAEs must be expeditiously reported whether or not considered attributable to the IP.

Serious adverse events will be reported using the designated paper SAE Report Form. When the investigator becomes aware of an SAE, she/he must submit a completed, signed and dated SAE Report Form (in English) **within 24 hours** to the sponsor by email/fax. The date of this SAE discovery by the site staff should be documented in the source documents (ie, medical records).

Each SAE must be followed up until resolution or stabilization. After the initial report, all relevant information for SAE follow-up, and for the outcome, must also be supplied to the sponsor in a timely manner (within 3 days from its identification or within 24 hours for relevant new information) by means of the SAE Report Form. In addition, the sponsor may request additional information and/or reports.

All SAE Report Forms must be reported to Grifols via email or fax as follows:

Grifols Global Pharmacovigilance

Email [redacted]
FAX: [redacted] (International)

When required, and according to local law and regulations, SAEs must be reported to the IRB/EC and regulatory authorities. Copies of the investigator's reports must be sent to the sponsor.

8.4 Type and Duration of the Follow-Up of Subjects after Adverse Events

In so far as is possible, all individuals will be followed up until the AE or suspected ADR has been resolved. If an AE/suspected ADR/SAE is present when the subject has completed the study, the course of the event must be followed until the final outcome is known, or the event has been stabilized and no further change is expected, and the investigator decides that no further follow-up is necessary.

8.4.1 Reporting Pregnancy

While pregnancy itself is not a true "AE," pregnancy occurring in a clinical study must be followed, to collect information regarding the experiences of gestation and pregnancy with blinded study drug exposure. The Investigator must report any pregnancy that occurs in a female study subject subsequent to informed consent through Day 29.

8.5 Study-Independent Safety Review Committee (SISRC) and Stopping Rules

Grifols will utilize a SISRC whose members (from Grifols) will be impartial and independent of the clinical trial team, and will consist of a global pharmacovigilance representative, a medical director, and a third individual knowledgeable in the epidemiological field or knowledgeable in the therapeutic area or a statistician. The SISRC will review relevant safety information from the study as outlined in the SISRC Charter. At a minimum, after the first 20 subjects are enrolled and have completed treatment, the SISRC will conduct an initial safety review. The SISRC will perform a causality review of all TEAEs and SAEs. The SISRC will be notified of relevant SAEs inasmuch as feasible within 24 hours, but no later than 3 days.

Stopping rules are delineated below. Any change to the study stopping rules must be reviewed by the FDA. If the study is suspended it will not be re-opened without prior FDA approval.

Stopping criteria for temporary suspension of further study enrollment/recruitment pending full safety investigation by the SISRC are detailed below.

The SISRC will conduct a safety evaluation and study enrollment/recruitment will be temporarily suspended while the safety investigation is undertaken if any one of the following occur:

- 1 death, i.e. at the time of each death, regardless of causality attribution by the Principal Investigator or whether it may be considered COVID-19 related. Pausing enrollment/recruitment after any death will allow the SISRC to perform a full investigation of every fatal event. (Note: Further infusions for subjects already randomized will only be stopped if there is elevated concern by the Principal Investigator or the SISRC that the death is drug related.)
- 1 Grade 4 treatment-emergent AE occurs in any of the first 3 randomized subjects considered potentially related to blinded study drug
- 1 subject develops a Grade 3 hypersensitivity reaction or serious anaphylactic reaction at any time attributable to Liquid $Alpha_1$-PI (Human)
- 10 subjects develop treatment-emergent AEs in the same system organ class that are ≥Grade 2 severity (CTCAE grading criteria)
- 2 subjects develop treatment-emergent Grade 4 serious AEs of the same kind in the first 20 subjects
- 5 subjects develop Grade 3 or 4 treatment-emergent AEs that are at least possibly related to blinded study drug at any time during the study

- 3 or more subjects per 10 subjects enrolled and dosed with Liquid Alpha$_1$-PI (Human) develop definitely related treatment-emergent serious AEs of the same kind (same or related verbatim terms). This stopping criterion will be applied after at least 10 subjects are randomized and treated with Liquid Alpha$_1$-PI (Human).
- An unanticipated safety signal is detected by the SISRC that was unexpected and of sufficient magnitude to warrant concern

Note that all of these thresholds for SISRC review and hiatus in enrollment/recruitment pertain to blinded treatment assignment for study participants (except for Grade 3 hypersensitivity or anaphylactic reaction for which unblinding would be likely for patient management and the threshold pertaining to 10 subjects randomized to Liquid Alpha$_1$-PI (Human) which coincides with the first routine SISRC review after 20 subjects are randomized). During their full safety investigation, the SISRC will have access to actual treatment administered (Liquid Alpha$_1$-PI (Human) or placebo), so that decisions regarding the study can be fully informed. Further details will be outlined in the SISRC charter.

The SISRC will receive SAEs within 24 hours. They will be required to meet within 72 hours if the above stopping criterion and/or stipulation with regard to serious anaphylactic reaction are fulfilled in order to conduct a thorough safety evaluation.

9 STATISTICS

9.1 Statistical Methods

Descriptive statistics will include the number of non-missing observations, mean, standard deviation (SD), median, minimum, and maximum values for the continuous/quantitative data or absolute and relative frequency counts and percentages for categorical/qualitative data. All statistical tests will be 2-sided at a significance level of 0.05.

An interim analysis will be conducted after 50 subjects (approximately 25 per group) for safety variables through Day 29. Due to the exploratory nature of this phase 2 study, the multiplicity adjustments will not be considered; full details will be described in the statistical analysis plan.

Interim Futility Analysis: An interim futility analysis will be conducted to assess whether the trial will be terminated due to lack of efficacy (futility) based on all available data assessable for the primary endpoint and key secondary efficacy endpoints.

There will be 3 analysis populations in this study; 2 populations for efficacy assessments and 1 population for safety evaluation.

The intention to treat (ITT) population is defined as all subjects who are randomized. The ITT population will be used for all efficacy analyses.

The Per-Protocol (PP) population is defined as the subset of subjects included in the ITT population who do not present major protocol violations which might have an impact on the

primary efficacy endpoint, and complete at least 80% of the IP. The primary efficacy analyses will be carried out using the PP population if different from the ITT population.

The Safety population is defined as the subset of subjects who receive at least any amount of Liquid Alpha$_1$-PI (Human) plus SMT or placebo plus SMT. Safety analyses will be based on the Safety population.

The primary efficacy analysis will be carried out on the ITT population and repeated on the PP population (if different from the ITT population) by Fisher's exact test. Secondary and exploratory efficacy analyses will be analyzed by means of Kaplan-Meier survival estimates and curves and compared between treatment groups by means of the Log-rank test for time to event variables, analysis of covariance (ANCOVA) when adjusting for covariates, or analysis of variance (ANOVA) when without covariates for normally-distributed variables or Wilcoxon rank-sum test for non-normally-distributed variables. Detailed data handling and evaluation procedures and details of the interim review will be described in the Statistical Analysis Plan (SAP).

For the primary endpoint, if the value of the subject is missing, then a conservative imputation rule will be used such that the subject will be treated as having met the condition for the primary efficacy analysis. The choice of analysis method for primary, secondary and exploratory efficacy endpoints will be described in detail in the SAP which will be finalized and signed off before data analysis is performed.

The SAP may include exploratory analyses of various aspects of SMT inclusive of different interventional strategies and evolving treatment modalities. Grifols will analyze the use of COVID-19 specific, potentially disease modifying treatments for any potential differences between study arms. This analysis will be fully detailed in the SAP since medical understanding of COVID-19 interventions is rapidly evolving. For ethical reasons, there are no restrictions placed on what are perceived to be potentially life-saving therapies (apart from participation in another interventional clinical trial).

9.2 Determination of Sample Size

Because of the urgency and lack of previous prospective COVID-19 data, sample size estimation remains incompletely defined; however, the size of this pilot study is commensurate with other Phase 2 investigations ongoing during the COVID-19 pandemic. Approximately 100 subjects are allowed to be randomized as part of a humanitarian effort against COVID-19.

9.3 Interim Futility Analysis

An interim futility analysis will be conducted based on all available data assessable for the primary endpoint and key secondary efficacy endpoints to assess whether the trial will be terminated due to lack of efficacy (futility). The unblinded output will be reviewed by an independent unblinded team, and the study team will remain blinded.

Determination of futility from the interim futility analysis will be based on the conditional power (the power conditional on the observed data accumulated at the interim analysis) for the primary outcome, as well as assessment of the secondary efficacy outcomes.

The futility analysis will include for the primary efficacy endpoint variable, all patients with data through Day 29, and for the following secondary efficacy endpoint variables, all patients with data through Day 29, as specified for each endpoint:

- Assessment of Clinical Severity: Change in NEWS from baseline (Day 1 through Day 29)
- Time to clinical response: NEWS ≤ 2 maintained for 24 hours, Day 1 through Day 29
- Time to hospital discharge: defined as duration of hospitalization from Day 1 through Day 29 (and duration of hospitalization)
- If admitted to ICU post randomization: Duration of ICU stay through Day 29
- Duration of any oxygen use Day 1 through Day 29
- If requiring mechanical ventilation post randomization: Duration mechanical ventilation through Day 29
- Absolute value and mean change from baseline in the Ordinal scale Day 1 through Day 29
    - The Ordinal scale is as follows:

      1) Death;

      2) Hospitalized, on invasive mechanical ventilation or extracorporeal membrane oxygenation (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, limitation on activities;

      7) Not hospitalized, no limitations on activities.

- Proportion (percentage) of subjects in each severity category of the 7-point Ordinal scale at Day 15 and Day 29
- Time to sustained normalization of temperature and proportion of subjects with normalization of fever at all time points, defined as temperature < 36.6°C armpit, < 37.2°C oral, or < 37.8 °C rectal sustained for at least 24 hours
- Number of subjects who develop Acute Respiratory Distress Syndrome (ARDS) through Day 29

- Length of time to clinical progression through Day 29 (defined as the time to death, mechanical ventilation, or ICU admission)

If the results of the interim futility analysis are not promising, the trial may be terminated. Otherwise the study will continue as originally planned unless discontinued for business or feasibility reasons.

The futility analysis will not inflate the type I error since the trial will not be stopped to claim efficacy. The primary outcome will be analyzed according to Section 9.1.

9.4 Criteria for Termination of the Study

The sponsor or its representative may terminate the study at any time for scientific or corporate reasons.

If the trial is prematurely terminated or suspended for any reason, the PI should promptly inform the trial subjects, should assure appropriate therapy and follow-up for the subjects and should inform the regulatory authority(ies) and/or ethics committee(ies) when required.

9.5 Procedure for Accounting for Missing, Unused, and Spurious Data

Handling of missing, unused and spurious data will be described in the SAP. All available efficacy and safety data will be included in data listings.

9.6 Reporting Deviations from the Statistical Analysis Plan

The detailed statistical analysis methodologies will be documented in the SAP. If there are any deviations from the originally planned analyses in the SAP, they will be fully described and justified in the protocol amendment(s) and/or final Clinical Study Report.

10 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

The data will be recorded and kept current in the medical notes, and in the CRF by the study site personnel directly responsible for the information. Grifols personnel or designee can review the records.

In accordance with ICH GCP guidelines, the monitor must have direct access to the investigator's source documentation in order to verify the data recorded for consistency and to verify adherence to the protocol, and the completeness, consistency, and accuracy of data entered. "Source documentation" includes individual subject files, separate from the study forms, which should be maintained and include visit dates, laboratory results, concomitant treatment, vital signs, medical history, examinations, AEs, IP logs, and other notes as appropriate. The investigator agrees to cooperate with the monitor to ensure that any problems noted during the course of these monitoring visits are resolved.

11 QUALITY CONTROL AND QUALITY ASSURANCE

Monitoring and auditing procedures defined/agreed by the sponsor will be followed, in order to comply with ICH GCP guidelines. Each center will be visited by a monitor (or evaluated by remote monitoring) to ensure compliance with the study protocol, ICH GCP and legal aspects according to the clinical monitoring plan.

Representatives of regulatory authorities or of Grifols may conduct audits or inspections of the investigator study site. If the investigator is notified of an audit or inspection by a regulatory authority, the investigator agrees to notify the Grifols Representative (e.g., Clinical Assessment Monitor/Medical Monitor, Program Manager, Program Leader) immediately. The investigator agrees to provide to representatives of a Regulatory Agency or Grifols access to records, facilities, and personnel for the effective conduct of an audit or inspection.

12 ETHICS

12.1 Institutional Review Board/Ethics Committee

Documented approval from appropriate IRBs/ECs will be obtained for all participating centers/countries prior to study start, according to ICH GCP guidelines, local laws, regulations and organizations. When necessary, an extension, amendment or renewal of the IRBs/ECs approval must be obtained and also forwarded to the sponsor. The IRBs/ECs must supply to the sponsor, upon request, a list of the IRBs/ECs members involved in the vote and a statement to confirm that the IRBs/ECs is organized and operates according to ICH GCP guidelines and applicable laws and regulations.

12.2 Ethical Conduct of the Study

The procedures set out in this protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the sponsor and investigator abide by ICH GCP guidelines. The study will also be carried out in keeping with applicable local law(s) and regulation(s). This may include an audit by the sponsor representatives and/or an inspection by regulatory authority representatives at any time. The investigator must agree to the audit or inspection of study-related records by the sponsor representatives and/or regulatory authority representatives and must allow direct access to source documents to the sponsor and/or regulatory authority representatives.

Modifications to the study protocol will not be implemented by either the sponsor or the investigator without agreement by both parties. However, the investigator may implement a deviation from, or a change to, the protocol to eliminate an immediate hazard(s) to the study subjects without prior IRB/EC/sponsor approval/favorable opinion. As soon as possible, the implemented deviation or change, the reasons for it and if appropriate the proposed protocol amendment should be submitted to the IRB/EC/sponsor. Any deviations from the protocol must be fully explained and documented by the investigator.

No medical waivers for protocol inclusion/exclusion criteria will be allowed by the sponsor. If there is a need for changes to the protocol inclusion/exclusion criteria is identified, the protocol will be amended to include such changes. The protocol amendment will be submitted to the competent regulatory authority and/or IRB/EC as applicable per regulations, which allows implementation of the revised inclusion/exclusion criteria in the study.

12.3 Regulatory Authority Approvals/Authorizations

Regulatory Authority approvals/authorizations/ notifications, where required, must be in place and fully documented prior to study start. Study information including contact information for investigator sites responsible for conducting the study will be posted on a publicly accessible clinical registry(ies) as required by local law.

12.4 Subject Information and Consent

Subject information and ICFs will be provided to investigator sites. Prior to the beginning of the study, the investigator must have the IRB/EC written approval/favorable opinion of the ICF and any other information to be provided to subjects. The written approval of the IRB/EC together with the approved subject information/ICF must be filed in the study files and a copy of the documents must also be provided to sponsor by the investigator site.

Informed consent must be obtained prior to the performance of any study specific procedure(s). The preferred process for obtaining informed consent is through the signing of a written ICF by the subject. If there are concerns regarding coronavirus (SARS-Cov-2) transmission through the process of obtaining written consent, methods recently proposed by the FDA should be followed to reduce this risk (see Appendix 4).

Sites subject to local conditions/institutional guidelines that do not allow for a written informed consent process due to the risk of coronavirus (SARS-Cov-2) transmission may obtain oral consent only if the site is able to provide written documentation of the specific institutional/local guidelines that mandate oral consent as the only permitted consenting method and ensure these guidelines are available in the investigator site files. Where proven that oral informed consent is the only permittable process, the site must record in the subject's medical history, "I have explained to the subject the characteristics and objectives of the study, its risks, and potential benefits. I have been able to answer the subject's questions and I confirm that this subject has given oral informed consent." Subsequently, and when possible, the subject's written informed consent will be obtained in those situations where oral consent was initially required. All informed consent procedures must be aligned with local laws and regulations.

12.5 Confidentiality

All records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available.

Subject names or other personal data or identifiers will not be supplied to the sponsor. Only the subject code number will be recorded in the study records, and if the subject's name or

other personal data identifiers appear on any other document (e.g., pathologist report), it must be redacted before a copy of the document is supplied to the sponsor. Study findings stored on a computer will be stored in accordance with local data protection laws. Subjects will be informed in writing that representatives of the sponsor, IRB/EC, or regulatory authorities may inspect their medical records and personal health information to verify the information collected, and that all personal information made available for an audit or inspection will be handled in strictest confidence and in accordance with local data protection laws.

If the results of the study are published, the subject's identity will remain confidential.

The investigator will maintain a list to enable subjects' records to be identified.

13 DATA HANDLING AND RECORD KEEPING

13.1 Data Handling

The study data will be recorded and kept current in the study records by the site study personnel directly responsible for the information. Entries made in the study records must be verifiable against source documents. The data in the study records will be monitored (on site or remotely) by Grifols representatives at regular intervals. Data will be reviewed for completeness and compared with the source documents at site level or data will be evaluated by remote monitoring. Remote source data verification will be allowed when appropriate and feasible to ensure data integrity, quality, safety of the participants and the patients' confidentiality data is not compromised. Examples of acceptable source documents include individual subject medical records, prospective information gathered on source documentation worksheets, lab reports and other diagnostics pertinent to this study which are separate from the study records. The listing of types of source documents which will be defined in the source data agreement will be filed in the trial master file.

All AEs and SAEs must be recorded. All SAEs must be recorded on the SAE Report Form. The SAE Report Form must be kept in site records with a copy provided to the designated person as detailed in the study file.

13.2 Record Retention

At study completion, all study data will be transferred to Grifols according to ICH GCP guidelines, local laws, regulations, and Grifols requirements. The study file and all source data should be retained until notification is given by the sponsor for destruction.

An investigator is required by ICH GCP guidelines to retain the study files for a minimum of 25 years. If an investigator moves, withdraws from an investigation or retires, the responsibility for maintaining the records may be transferred to another person (e.g., other investigator). Grifols must be notified in writing of the person responsible for record retention and the notification will be retained in the sponsor study file and the investigator site file.

14 FINANCING AND INSURANCE

In the event of subject injury as a direct result of either administration of investigational product or any non-standard of care study procedure, sponsor will pay for the costs of treatment, provided the subject has followed the instructions given by the study doctor and the illness or injury is not due to the natural progression of any conditions existing before the subject participated in the study. Financial compensation for such things as lost wages, disability, or discomfort due to any research-related injury is not available.

Sponsor shall maintain comprehensive general liability insurance or self-insurance in amounts adequate to cover any damage, demand, claim, loss or liability caused or incurred by sponsor, or as otherwise required by applicable laws and/or regulations.

15 PUBLICATION POLICY

Institution and the investigator agree that the first publication shall be made in conjunction with the presentation of a joint, multi-center publication of the study results from all appropriates sites. If such a multi-center publication is not submitted within 18 months after conclusion of the study at all sites or after Grifols confirms there will be no joint, multi-center publication, then institution and/or investigator shall have the right, at their discretion, to publish, either in writing or orally, the results of the study performed under the protocol, subject to the conditions outlined below:

- The results of the study will be reported in the publicly accessible registry(ies).
- Institution and/or investigator shall furnish Grifols with a copy of any proposed publication at least thirty (30) days in advance of the date of submission for publication.
- Within said thirty (30) day period, Grifols shall:
  - Review such proposed publication for Confidential Information (other than Study results) and for subject information subject to the Health Insurance Portability and Accountability Act of 1996 ("HIPAA") and other applicable privacy laws;
  - Review such proposed publication for the unauthorized use of the name, symbols and/or trademarks of Grifols;
  - By written notice to the investigator, identify with specificity the text or graphics in such proposed publication that Grifols contends contains Confidential Information, protected subject information, or the unauthorized use of Grifols' name, symbols and/or trademarks so that the proposed publication may be edited appropriately to remove such text or graphics before publication; and
  - By written request, Grifols may delay proposed publications up to sixty (60) days to allow Grifols to protect its interests in Grifols Inventions described in such publications.
- Institution and/or investigator shall give Grifols the option of receiving an acknowledgment for its sponsorship of the study in all such publications or presentation.

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17 APPENDICES

Appendix 1 Schedule of Study Procedures

Study Period	**Screen/ Baseline**	**IP**	**SMT and Daily Evaluations[l]**									**Follow-Up**	**Follow-Up Clinic Visit Day**				
Study Day **Procedures/assessments**	1	**1st IP infusion day**	2	3	4	5±1[m] day	6	7	8	9	10	11-14 **Daily** if in hospital	15± 1 day[m]	16-28 **Daily** if in hospital	29[e] ± 1 day[m]	Day 60 ± 2 days	Day 90 ± 2 days
Informed consent	X																
Inclusion/exclusion criteria	X																
Demography, (including age [year of birth], gender, race, and ethnicity), disease characteristics (date of exposure, date of onset[a])	X																
Pregnancy test[b]	X																
Ordinal Scale (at the 1st assessment of a given day)[c]	X		X	X	X	X	X	X	X	X	X	X	X	X	X		
National Early Warning Score (NEWS) (Appendix 2)	X		X	X	X	X	X	X	X	X	X	X	X	X	X		
Clinical features key symptoms: fever, cough, shortness of breath, fatigue, myalgia etc.	X		X	X	X	X	X	X	X	X	X	X	X	X	X		
Vital signs (temperature, systolic and diastolic blood pressure, pulse, respiratory rate)	X	X[i]	X	X	X	X	X	X	X[i]	X	X	X	X	X	X		
Weight	X																
Record result of *historical* SARS-CoV-2 PCR (qualitative RT-PCR) or NAT or other commercial or public health assay approved by regulatory authorities as a diagnostic test for COVID-19 in any specimen during the current hospital admission OR 96 hours prior to the hospital admission date and prior to randomization (the SARS-CoV-2 test results must be performed by a hospital laboratory and the documentation available) (eligibility criterion)	X																

Study Period	**Screen/ Baseline**	**IP**	**SMT and Daily Evaluations[l]**									**Follow-Up**	**Follow-Up Clinic Visit Day**				
Study Day **Procedures/assessments**	1	**1st IP infusion day**	2	3	4	5±1[m] day	6	7	8	9	10	11-14 **Daily** if in hospital	15± 1 day[m]	16-28 **Daily** if in hospital	29[e] ± 1 day[m]	Day 60 ± 2 days	Day 90 ± 2 days
Record any supplemental oxygen administration (type, %, flow start/end date/time)	X		X	X	X	X	X	X	X	X	X	X	X	X	X		
If subject requires mechanical ventilation post randomization: Record any mechanical ventilation (start/end date/time)			X	X	X	X	X	X	X	X	X	X	X	X	X		
Record oxygen saturation (specify on or off oxygen supplementation)	X		X	X	X	X	X	X	X	X	X	X	X	X	X		
Randomization *(after all Screen/Baseline assessments complete)*	X																
IV dose of blinded Liquid Alpha$_1$-PI (Human) 120 mg/kg body weight (or Placebo) given on Day 1 and Day 8		X							X[k]								
Record hospital admission and discharge dates	X														X[f]		
If subject requires ICU admission post randomization: Record ICU admission & discharge dates															X[f]		
Assessment of ARDS (Berlin Criteria) (Appendix 3)	X					X							X		X		
Respiratory sample for quantitative measurement of SARS-CoV-2 viral load by NAT or PCR (real-time RT-PCR) (obtained via nasopharyngeal swab)	Pre randomized treatment[g]					X							X		X		
Sample for quantitative measurement of IgM and IgG antibodies to SARS-CoV-2 (if antibodies cannot be determined quantitatively store serum samples frozen at -70°C for later analysis at an external lab)	Pre randomized treatment[g]					X							X		X		

Study Period	**Screen/ Baseline**	**IP**	**SMT and Daily Evaluations[l]**									**Follow-Up**	**Follow-Up Clinic Visit Day**				
Study Day **Procedures/assessments**	1	**1st IP infusion day**	2	3	4	5±1[m] day	6	7	8	9	10	11-14 **Daily** if in hospital	15±1 day[m]	16-28 **Daily** if in hospital	29[e] ± 1 day[m]	Day 60 ± 2 days	Day 90 ± 2 days
Serum Chemistry (creatinine, albumin, alanine aminotransferase [ALT], total bilirubin, lactate dehydrogenase [LDH])	Pre randomized treatment[g]					X							X		X		
Calculate an estimated glomerular filtration rate (eGFR) using the Cockcroft-Gault formula	Pre randomized treatment[g]					X							X		X		
Ferritin, CRP, D-dimer	Pre randomized treatment[g]					X							X		X		
Hematology & absolute neutrophil & lymphocyte count (hemoglobin, hematocrit, platelet count, leukocyte count with differential)	Pre randomized treatment[g]					X							X		X		
Cytokine panel[h]	Pre randomized treatment[g]					X							X		X		
Levels of alpha 1-PI (AAT) activity and antigen (both plasma [sodium citrate] and serum samples to be stored for measurement of both antigenic content and functional activity [potency])	Pre randomized treatment[g]								X[gj]				X		X		
Record SAEs and TEAEs[d]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Record standard care concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Phone Checks for Vital Status (living or deceased), any hospital re-admissions or SAEs/non-serious AEs after Day 29 Final Clinic Visit																X	X

a Date of first contact with the virus, date of first symptoms, date of PCR (RT-PCR)/NAT positive

b Human chorionic gonadotropin-based assay for women of childbearing potential (urine matrix is also valid).

c Ordinal scale measure of clinical status: 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, limitation on activities; 7) Not hospitalized, no limitations on activities.

d Grade 3-5 TEAEs will be defined according to CTCAE criteria, US Department of Health and Human Services, NIH, and NCI. In this clinical study, disease progression is defined as the worsening of a subject's condition attributable to the disease for which the investigational product is being studied. It may be an increase in the severity of the targeted disease and/or increases in the symptoms of the targeted disease. Anticipated symptoms of COVID-19 include fever, cough, hypoxia, dyspnea, hemoptysis, myalgia, fatigue, pharyngitis, which may develop at any time during the course of the disease. The development of COVID-19 symptoms during the clinical study should not be recorded as individual TEAEs, unless there is evidence suggesting a causal relationship between blinded Liquid Alpha$_1$-PI (Human)/Placebo and the TEAE.

e Final Clinic Visit, followed by Phone Checks.

f Record hospital and ICU discharge date on or before Day 29/Final Visit.

g All laboratory tests and serum and plasma samples must be obtained from all subjects; samples must be obtained prior to the blinded Liquid Alpha$_1$-PI (Human) infusion on Day 1 and Day 8. (Day 8 AAT levels only apply if second infusion given)

h Cytokine panel includes IL-1β, IL-10, IL-6, IL-8 IL-2, interferon γ, and TNF-α. Note: serum samples must be stored at -70° C for later analysis at a reference laboratory.

i. During study infusions on Day 1 and Day 8 vital signs will be measured immediately before the start of infusion, after the first 5 minutes of infusion, and subsequently at 5-10-minute intervals during infusion until the end of infusion. Vital signs will also be measured 2 hours after the end of study infusion.

j. Day 8 AAT measurement for activity and antigen is only required if subject is in hospital.

k. If the patient has been discharged from the hospital at the time of the Day 8 blinded Liquid Alpha1-PI (Human) or Placebo infusion, the 2nd infusion is not mandatory, and is at the discretion of the Principal Investigator and subject

l. Only applicable if subject remains hospitalized, except for Day 5 when all assessments are mandatory for all subjects.

m. Mandatory even if subject has been discharged from hospital

Note: For assessment for early withdrawal see Section 5.3.4.

Appendix 2 National Early Warning Score (NEWS)

The NEWS has demonstrated an ability to discriminate patients at risk of poor outcomes. This score is based on 7 clinical parameters (respiration rate, oxygen saturation, any supplemental oxygen, temperature, systolic blood pressure, heart rate, level of consciousness [Alert, Voice, Pain, Unresponsive]). To calculate you may access https://www.mdcalc.com/national-early-warning-score-news

Details are also provided below.

Formula: Addition of the selected points; points assigned below:

Criteria	Point Value
Respiratory Rate (breaths per minute)	
≤8	+3
9-11	+1
12-20	0
21-24	+2
≥25	+3
Oxygen Saturation (%)	
≤91	+3
92-93	+2
94-95	+1
≥96	0
Any Supplemental Oxygen	
Yes	+2
No	0
Temperature in °C (°F)	
≤35.0 (95)	+3
35.1-36.0 (95.1-96.8)	+1
36.1-38.0 (96.9-100.4)	0
38.1-39.0 (100.5-102.2)	+1
≥39.1 (≥102.3)	+2
Systolic BP	
≤90	+3
91-100	+2

Criteria	Point Value
101-110	+1
111-219	0
≥220	+3
Heart Rate (beats per minute)	
≤40	+3
41-50	+1
51-90	0
91-110	+1
111-130	+2
≥131	+3
AVPU	
A	0
V, P, or U	+3

AVPU, Alert, Voice, Pain, Unresponsive.

Interpretation;

1. A low score (NEWS 1–4) should prompt assessment by a competent registered nurse who should decide if a change to frequency of clinical monitoring or an escalation of clinical care is required.
2. A medium score (i.e. NEWS of 5–6 or a RED score) should prompt an urgent review by a clinician skilled with competencies in the assessment of acute illness – usually a ward-based doctor or acute team nurse, who should consider whether escalation of care to a team with critical-care skills is required (i.e. critical care outreach team). ◦A RED score refers to an extreme variation in a single physiological parameter (i.e., a score of 3 on the NEWS chart in any one physiological parameter, colored RED to aid identification; e.g., heart rate
3. A high score (NEWS ≥7) should prompt emergency assessment by a clinical team/critical care outreach team with critical-care competencies and usually transfer of the patient to a higher dependency care area.

Evidence Appraisal

The six physiological parameters that were proposed to form the basis standardized National Early Warning Score were derived from this study. It retrospectively analyzed data from 35,585 medical admissions.

Original/Primary Reference

Research Paper Royal College of Physicians. National Early Warning Score (NEWS) Standardising the assessment of acute-illness severity in the NHS. Report of a working party. London: RCP, 2012.

Validation

Smith GB, Prytherch DR, Meredith P, Schmidt PE, Featherstone PI. The ability of the National Early Warning Score (NEWS) to discriminate patients at risk of early cardiac arrest, unanticipated intensive care unit admission, and death. Resuscitation. 2013 Apr;84(4):465-470. Doi: 10.1016/j.resuscitation.2012.12.016. Epub 2013 Jan 4.

Other References

Prytherch D, Smith GB, Schmidt PE, Featherstone PI. ViEWS – towards a national Early Warning Score for detecting adult inpatient deterioration. Resuscitation 2010;81:932–937.

Appendix 3 Acute Respiratory Distress Syndrome (ARDS) Berlin Definition

The Berlin Definition of Acute Respiratory Distress Syndrome is summarized in the table below. Timing is usually within 1 week of a known clinical insult or new or worsening respiratory symptoms.

Chest imaging[a]	Bilateral opacities—not fully explained by effusions, lobar/lung collapse, or nodules
Origin of edema	Respiratory failure not fully explained by cardiac failure or fluid overload Need objective assessment (eg, echocardiography) to exclude hydrostatic edema if no risk factor present
Oxygenation[b],	Mild 200 mm Hg < PaO2/FIO2 ≤ 300 mm Hg with PEEP or CPAP ≥ 5 cm H2Oc
	Moderate 100 mm Hg < PaO2/FIO2 ≤ 200 mm Hg with PEEP ≥ 5 cm H2O
	Severe PaO2/FIO2 ≤100 mm Hg with PEEP ≥ 5 cm H2O

Abbreviations: CPAP, continuous positive airway pressure; FIO2, fraction of inspired oxygen; PaO2, partial pressure of arterial oxygen; PEEP, positive end-expiratory pressure.

a. Chest radiograph or computed tomography scan.

b. If altitude is higher than 1000 m, the correction factor should be calculated as follows: [PaO2/FIO2 X (barometric pressure/760)].

c. This may be delivered noninvasively in the mild acute respiratory distress syndrome group.

The following may be used for the Berlin criteria, **for patients who are Not on a ventilator and do not have a blood gas performed.** *Since the effect of positive end-expiratory pressure (PEEP) may modify the derivation of SaO_2 to SpO_2, please do Not use SpO_2 to apply Berlin criteria to patients while on a mechanical ventilator.*

Respiratory Thresholds Determined by Pulse Oximetry Measurement of O_2 Saturation (for patients Not on mechanical ventilation)

PaO_2/FiO_2	**Corresponding SpO_2/FiO_2 Threshold**
≥ 400	≥ 512
<400	<512
<300	<357
<200	<214
<100	<89

Derivation of SpO_2/FiO_2 values corresponding to PaO_2/FiO_2 ratios in the combined anesthesia and ARMA database* [*Data derived from 4728 matched SpO_2/FiO_2 and PaO_2/FiO_2 measurements from the combined anesthesia and ARMA database]

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Appendix 4 Excerpt from: FDA Guidance on Conduct of Clinical Trials of Medical Products during COVID-19 Public Health Emergency (FDA 2020)

Q10 How do I obtain signed informed consent from a hospitalized patient who is in isolation when a COVID-19 infection control policy prevents us from entering the patient's room to collect a signed informed consent form?

FDA regulations generally require that the informed consent of a trial participant (in this case a hospitalized patient) be documented by the use of a written consent document that typically includes the elements of informed consent, as described in 21 CFR 50.25, and that has been approved by the IRB and signed and dated by the trial participant or their legally authorized representative at the time of consent (21 CFR 50.27(a)). When feasible, we recommend a traditional method of obtaining and documenting informed consent using a signed paper copy of the consent form, or use of electronic informed consent.[9,10,11] If neither of these approaches are possible, the following procedures would be considered to satisfy FDA's informed consent documentation requirement.[12]

Method 1: A photograph of the signed informed consent document can be transmitted to the trial staff

1. An unsigned consent form is provided to the patient by a person who has entered the room.

2. The investigator/designee arranges a telephone call or video conference call with the patient (and, if desired and feasible, additional individuals requested by the patient (e.g., next of kin)).

3. To ensure that patients are approached in a consistent fashion, a standard process should be used that will accomplish the following:
   - Identification of who is on the call.
   - Review of the informed consent document with the patient by the investigator/designee and response to any questions the patient may have.
   - Verbal confirmation by the patient that their questions have been answered, that they would like to participate in the trial, and that they have signed and dated the informed consent document that is in their possession.

[9] See guidance for institutional review boards, investigators, and sponsors *Use of Electronic Informed Consent In Clinical Investigations* (December 2016), *available at* https://www.fda.gov/regulatory-information/search-fda-guidance-documents/use-electronic-informed-consent-clinical-investigations-questions-and-answers.

[10] For example, for FDA-regulated trials conducted during the COVID-19 public health emergency, FDA has made the COVID MyStudies App available in the Apple App and Google Play stores as a platform enabling investigators to obtain informed consent securely from patients when face-to-face contact is not possible or practical due to COVID-19 public health measures to control the virus. To facilitate free use of the app during the public health emergency, FDA intends to fund the technical assistance required to operate the COVID MyStudies App, which will be provided by the Harvard Pilgrim Healthcare Institute, as resources permit. For more information, investigators interested in using the app should see https://www.fda.gov/drugs/science-and-research-drugs/covid-mystudies-application-app.

[11] See Q24.

[12] The procedures suggested do not apply to the exception from general informed consent requirements under 21 CFR 50.23 or the exception from informed consent requirements for emergency research under 21 CFR 50.24.

4. The patient (or an individual in the room) takes a photograph of the signed informed consent document and sends it to the investigator/designee.

5. A trial team member enters the photograph into the trial records along with an attestation that states how that photograph was obtained and that it is a photograph of the informed consent document signed by the patient.

Method 2: A witness can attest to the signature, but a photograph of the signed informed consent document cannot be transmitted

1. An unsigned consent form is provided to the patient by a person who has entered the room.

2. The investigator/designee arranges a three-way telephone call or video conference call with the patient, a witness who is not otherwise connected with the clinical investigation, and, if desired and feasible, additional individuals requested by the patient (e.g., next of kin). Alternatively, in lieu of using a witness, a recording of the conversation can be made.[13]

3. To ensure that patients are approached in a consistent fashion, a standard process should be used that will accomplish the following:
   - Identification of who is on the call.
   - Review of the informed consent document with the patient by the investigator/designee and response to any questions the patient may have.
   - Verbal confirmation by the patient that their questions have been answered, that they would like to participate in the trial, and that they have signed and dated the informed consent document that is in their possession.

4. When using a witness, documentation in the trial records includes: (1) a signed and dated attestation by the witness who participated on the call that the patient confirmed their agreement to participate in the trial and signed the informed consent document, and (2) a signed and dated attestation by the investigator/designee stating why the informed consent document signed by the patient was not retained (e.g., due to potential contamination of the document by infectious material).

   When using a recording in lieu of a witness, documentation in the trial records includes: (1) the recording of the conference call, and (2) a signed and dated attestation by the investigator/designee who participated on the call stating why the informed consent document signed by the patient was not retained (e.g., due to potential contamination of the document by infectious material).

[13] If an investigator wants to record the telephone or video conference call, the investigator/designee should ensure that the recording is done in a manner consistent with applicable state and local laws and that all parties agree to being recorded.

When either Method 1 or 2 is used to document informed consent, the resulting documentation should be (1) collected and archived, as either original paper copies or appropriately certified electronic copies (e.g., using a validated process for scanning paper copies),[14] and (2) retained according to applicable FDA record retention requirements as part of the trial record.[15]

If the patient is unable to provide informed consent and there is a legally authorized representative, investigators must obtain written consent from the patient's legally authorized representative in accordance with 21 CFR 50.27(a).

[14] FDA guidance on good clinical practice developed with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) defines a certified copy as "[a] copy (irrespective of the type of media used) of the original record that has been verified (i.e., by a dated signature or by generation through a validated process) to have the same information, including data that describe the context, content, and structure, as the original." See guidance for industry *E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1)* (March 2018), *available at* https://www.fda.gov/regulatory-information/search-fda-guidance-documents/e6r2-good-clinical-practice-integrated- addendum-ich-e6r1.

[15] See 21 CFR 312.57, 312.62, and 812.140.

Appendix 5 Summary of Changes from Version 5.0 to Version 6.0

(Note: Administrative changes including minor administrative corrections are not included in Protocol Summary of Changes)

Sections	Change From: (Version 5.0, dated 21 Jul 2021)	Change To: (Version 6.0)	Rationale:
Synopsis, 2.6, 4.2, 9.1	*New text for Interim Futility Analysis*	Interim Futility Analysis: An interim futility analysis will be conducted to assess whether the trial will be terminated due to lack of efficacy (futility) based on all available data assessable for the primary endpoint and key secondary efficacy endpoints.	Addition of an interim futility analysis which will be conducted to provide guidance in the setting of dynamic changes within the context of an evolving epidemic.
9.3	*New section for Interim Futility Analysis*	An interim futility analysis will be conducted based on all available data assessable for the primary endpoint and key secondary efficacy endpoints to assess whether the trial will be terminated due to lack of efficacy (futility). The unblinded output will be reviewed by an independent unblinded team, and the study team will remain blinded. Determination of futility from the interim futility analysis will be based on the conditional power (the power conditional on the observed data accumulated at the interim analysis) for the primary outcome, as well as assessment of the secondary efficacy outcomes. The futility analysis will include for the primary efficacy endpoint variable, all patients with data through Day 29, and for the following secondary efficacy endpoint variables, all patients with data through Day 29, as specified for each endpoint: Assessment of Clinical Severity: Change in NEWS from baseline (Day 1 through Day 29) Time to clinical response: NEWS $\leq$ 2 maintained for 24 hours, Day 1 through Day 29 Time to hospital discharge: defined as duration of hospitalization from Day 1 through Day 29 (and duration of hospitalization) If admitted to ICU post randomization: Duration of ICU stay through Day 29 Duration of any oxygen use Day 1 through Day 29 If requiring mechanical ventilation post randomization: Duration mechanical ventilation through Day 29 Absolute value and mean change from baseline	Addition of an interim futility analysis which will be conducted to provide guidance in the setting of dynamic changes within the context of an evolving epidemic.

Sections	Change From: (Version 5.0, dated 21 Jul 2021)	Change To: (Version 6.0)	Rationale:
		in the Ordinal scale Day 1 through Day 29 The Ordinal scale is as follows: 1) Death; 2) Hospitalized, on invasive mechanical ventilation or extracorporeal membrane oxygenation (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, limitation on activities; 7) Not hospitalized, no limitations on activities. Proportion (percentage) of subjects in each severity category of the 7-point Ordinal scale at Day 15 and Day 29 Time to sustained normalization of temperature and proportion of subjects with normalization of fever at all time points, defined as temperature ≤ 36.6°C armpit, < 37.2°C oral, or < 37.8 °C rectal sustained for at least 24 hours Number of subjects who develop Acute Respiratory Distress Syndrome (ARDS) through Day 29 Length of time to clinical progression through Day 29 (defined as the time to death, mechanical ventilation, or ICU admission) If the results of the interim futility analysis are not promising, the trial may be terminated. Otherwise the study will continue as originally planned unless discontinued for business or feasibility reasons. The futility analysis will not inflate the type I error since the trial will not be stopped to claim efficacy. The primary outcome will be analyzed according to Section 9.1.	
8.5	Grifols will utilize a Study-Independent Safety Review Committee (SISRC) whose members (from Grifols) will be impartial and	Grifols will utilize a SISRC whose members (from Grifols) will be impartial and independent of the clinical trial team, and will consist of a global pharmacovigilance representative, a medical director, and a third individual knowledgeable in the epidemiological field or knowledgeable in the therapeutic area or a statistician.	Allowance for flexibility in areas of expertise for SISRC membership.

Sections	Change From: (Version 5.0, dated 21 Jul 2021)	Change To: (Version 6.0)	Rationale:
	independent of the clinical trial team, and will consist of a ~~statistician,~~ global pharmacovigilance representative, ~~and~~ a medical director.		