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Signed by: Bucchioni Enrica (USR02332)
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Meaning of Signature: Sponsor Medical Expert

Signed by: Santoro Debora (USR00282)
Date: 08-Apr-2021 13:23:44 UTC
Meaning of Signature: Statistician

Signed by: Fabbri Laura (MEDA22)
Date: 08-Apr-2021 14:54:36 UTC
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CLINICAL STUDY PROTOCOL

CONTRY SPECIFIC for ITALY

STUDY CODE No.: CLI-050000-04

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Multicenter, open-label, randomised trial to assess the efficacy and tolerability of poractant alfa (porcine surfactant, Curosurf®) in hospitalized patients with SARS-COV-19 acute respiratory distress syndrome (ARDS)

Version No.: 1.0 - ITA

Date: 01 APR 2021

Chiesi Farmaceutici S.p.A.
Via Palermo 26/A
43122 Parma – Italy

GENERAL INFORMATION

SPONSOR	Chiesi Farmaceutici S.p.A.* Via Palermo 26/A 43122 Parma – Italy Telephone: + 39 0521 2791 *also reported as Chiesi throughout the text
SPONSOR MEDICAL EXPERT (Clinical Research Physician)	**Enrica Bucchioni** Chiesi Farmaceutici S.p.A. Via Palermo 26/A, 43122 Parma – Italy E-mail: *e.bucchioni@chiesi.com* Telephone: + 39 0521 279744 Readily available in case of medical questions
Coordinating INVESTIGATOR (Italy)	**Stefano Busani** Terapia Intensiva Polivalente- Policlinico di Modena Via Università 4, 41121 Modena Email: *stefano.busani@unimore.it*
CLINICAL PROGRAM LEADER	**Laura Fabbri** Chiesi Farmaceutici S.p.A. Via Palermo 26/A, 43122 Parma – Italy E-mail: *l.fabbri@chiesi.com*
MONITORING CRO	**CROMSOURCE Srl** Via Giorgio De Sandre 3 37135 Verona, Italy Telephone: +39 045 8222811
DATA MANAGEMENT / STATISTICS CRO:	**ICON Clinical Research Ltd** South County Business Park, Leopardstown Dublin 18, Ireland

VERSION HISTORY

Version	Date	Change History
1.0 - ITA	01 APR 2021	Country-specific version for Italy. This version is based on the submitted protocol version 4.0 dated 02 March 2021. The following local adaptations have been implemented: administrative changes and update of section 15 “Informed Consent” implementing the procedures for Italy and removing UK and US specifications.

PROTOCOL OUTLINE

Study title	Multicenter, open-label, randomised trial to assess the efficacy and tolerability of poractant alfa (porcine surfactant, Curosurf®) in hospitalized patients with SARS-COV-19 acute respiratory distress syndrome (ARDS)
Sponsor	Chiesi Farmaceutici S.p.A. - Via Palermo 26/A 43122 Parma - Italy
Name of the Product	CUROSURF®
Centre(s)	Approximately 7 centers in Italy, UK and USA
Indication	ARDS in COVID 19 adult patients
Study design	Multicenter, open-label, randomised, phase II Proof of Concept (POC) study
Study phase	Phase II – Proof of Concept study
Objectives	To evaluate the efficacy and safety of poractant alfa (porcine surfactant, Curosurf®), administered by endotracheal (ET) instillation, in terms of ventilatory free days during the 21 days after randomization, in adult patients with ARDS due to SARS-COV-19 infection.
Treatment duration	Three administrations with a 24 hours dosing interval. Each ET administration 1, 2 and 3 will consist of poractant alfa bolus: 30mg /kg (Lean Body Weight-LBW) = 0.375ml /kg LBW. Dilution with normal saline up to 2ml /kg LBW.
Test product dose/route/regimen	CUROSURF® (poractant alfa) 240mg / 3ml It is a sterile suspension in 3.0 ml glass vials with a total concentration of 80 mg/ml for endotracheal administration. This is a standard natural surfactant prepared from porcine lungs and containing almost exclusively polar lipids, in particular phosphatidylcholine (about 70% of the total phospholipid content), and about 1% of specific low molecular weight hydrophobic proteins SP-B and SP-C.
Reference product dose/route/regimen	NA
Number of patients	Seventy patients will be randomised in the study with a ratio 3:2 (i.e. 42 patients in the poractant alfa arm and 28 in the control arm).
Study population	Adult patients with ARDS due to COVID-19 (positive by rtPCR confirmation) and endotracheally intubated.
Inclusion/exclusion criteria	**Inclusion criteria** Participants are eligible to be included in the study if the following criteria apply: 1. Male or female ≥18 and ≤ 80 years of age 2. Informed consent for participation in the study (*refer to section 15*

	for detailed informed consent procedure) 3. Positive 2019-nCoV rt-PCR before randomisation 4. PaO2/FiO2 ratio < 150 mmHg 5. Lung compliance ≤45 ml/cmH20 6. Intubated and artificially ventilated less than 48 hours before the first poractant alfa administration **Exclusion criteria** Participants are excluded from the study if any of the following criteria apply: 1. Any contraindications to surfactant administration e.g., pulmonary haemorrhage and pneumothorax) 2. Weight < 40kg 3. Stage 4 severe chronic kidney disease (i.e., eGFR < 30) 4. Pregnancy 5. Administration of any nebulized surfactant in the 48 hours before the first poractant alfa administration 6. Extracorporeal membrane oxygenation
Study plan	After verification of any inclusion/exclusion criteria the patients will be included in the study and randomised to the poractant alfa group or to standard of care one. <u>Poractant alfa group</u> - Starting from patient intubation and mechanical ventilation, defined assessments will be collected (for details see study schedule 7.1). - Patients will receive three poractant alfa endotracheal administrations 24 hours apart. - Defined assessments will be collected before randomisation and at 6 -12 - 24 hours after each poractant alfa administration till 72 hours since the first administration. - From Day 4 to Day 27 assessment collection will occur every 24 hours until Intensive Care Unit (ICU) discharge. Whenever the discharge occurs, additional safety lab and SOFA assessments will be collected. - At Day 28 the last follow up evaluation will occur on the ICU if still requiring critical care, or by phone call if the patient has been discharged from ICU by that time. <u>Control group</u> - Starting from patient intubation and mechanical ventilation defined assessments will be collected (for details see study schedule 7.1). - Defined assessments will be collected before randomisation and at specific timepoints (according to study schedule 7.1) from randomisation up to 72 hours.

	- From Day 4 to Day 27 assessment data collection (according to study schedule 7.1) will occur every 24 hours until Intensive Care Unit (ICU) discharge. Whenever discharge occurs, assessment data will be collected at the corresponding Day X. - At Day 28 the last follow up evaluation will occur on the ICU, or by phone call if the patient has already been discharged.
Most relevant allowed concomitant treatments	Concomitant treatments are allowed. Concomitant medications in a critically ill SARS-COV19 population are numerous, may change daily, are consequently difficult to collect and are unlikely, to be related to therapeutic success and or safety evaluations. Therefore, only selected concomitant medications will be specifically recorded in this trial: 1) Antiviral drugs; 2) Monoclonal antibodies; 3) Antibiotics; 4) Systemic corticosteroids; 5) Heparin or any other anticoagulants; 6) Plasma administration from immunized patients; 7) Nitric Oxide therapy; 8) Any other treatments for pneumonia/ARDS/ SARS-COV19 ARDS; 9) Medications prescribed to treat adverse events (AEs).
Most relevant forbidden concomitant treatments	No concomitant treatment is forbidden
Efficacy variables	*Primary Endpoint* The primary outcome variable will be the number of days alive and ventilator-free, defined as the number of days the patient is alive and is not receiving mechanical ventilation over the **21 days** following randomisation. Mechanical ventilation will be defined as invasive and non-invasive. Patient will be defined free of mechanical ventilation after 12 hours from the suspension of either invasive and non-invasive ventilation. Patients who die or are mechanically ventilated longer than this period are assessed as zero ventilator-free days. *Key secondary* Percentage of patients alive and free of respiratory failure (i.e., need for mechanical ventilation, ECMO (Extracorporeal Membrane Oxygenation), non-invasive ventilation, or high-flow nasal cannula oxygen delivery) **at Day 28.** *Secondary* - Number of days alive and ventilator-free at **Day 28** - Mortality at **Day 21** and **Day 28** - Number of alive and free from invasive ventilation at **Day 21** and **Day 28**

- Number of alive and free from non-invasive ventilation (NIV) at **Day 21** and **Day 28**
- Percentage of patients with improvement in severity status defined as a decrease in the severity score at Day 28 or Discharge, whichever comes first. Severity score will be defined as Mild, Moderate, Severe or Death based on PaO2/FiO2 ratio and patient status at Day 28 and numerically rated from 1-4 respectively:

Severity	**Variable**	**Criteria**
Mild -1	PaO2/FiO2 Ratio	200 mmHg < PaO2/FIO2 ≤ 300 mmHg
Moderate - 2	PaO2/FiO2 Ratio	100 mmHg < PaO2/FIO2 ≤ 200 mmHg
Severe - 3	PaO2/FiO2	Ratio PaO2/FIO2 ≤ 100 mmHg
Death - 4	Patient Status	Yes/No

- Change from baseline in PaO2/FiO2 ratio at **6 and 12 hours following administration of each dose** in the treated group and at similar timepoints in the control group (6, 12, 30, 36, 54 and 60 hours after randomisation)
- Change from baseline in PaO2/FiO2 ratio at additional timepoints (i.e. **every 24 hours** after treatment/randomisation until the patient is discharged from the ICU)
- Percentage of patients alive and with PaO2/ FiO2 improvement >20% at **6 and 12 hours** following administration of each dose in the treated group and at similar timepoints in the control group (6, 12, 30, 36, 54 and 60 hours after randomisation)
- Percentage of patients alive and with PaO2/FiO2 improvement of >20% at the additional timepoints (i.e. **every 24 hours** after treatment/randomisation until the patient is discharged from the ICU)
- Change from baseline in FiO2 at **6 and 12 hours following administration of each dose** in the treated group and at similar timepoints in the control group (6, 12, 30, 36, 54 and 60 hours after randomisation)
- Change from baseline in FiO2 at additional timepoints (i.e. **every 24 hours** after treatment/randomisation until the patient is discharged from the ICU)
- Length of ICU stay (days) **at Day 28**. Patients who die or are mechanically ventilated longer than this period are assigned with 28 days
- Percentage of patients alive and out of ICU at **Day 28**
- Delta SOFA Score and sub-score components measured on **Day 3** and **Day 28 or Discharge** whichever comes first

	- Percentage of patients alive and organ failure free (SOFA score=0) at **Day 28 or Discharge** whichever comes first - Change from baseline in ventilatory parameters [tidal volume (TV), respiratory rate (RR), dynamic compliance (Cdyn), static compliance (Cstat), positive end-expiratory pressure (PEEP), peak inspiratory pressure (PIP), plateau pressure (Pplat)] measured at 6-12-24h after each poractant alfa administration up to 72 hours and at similar timepoints in the control group (6, 12, 24, 30, 36, 48, 54, 60 and 72 hours after randomisation) and then every 24 hours until the patient is discharged from the ICU - Change from baseline in blood gas analysis acid-base balance parameters (i.e. pH, pCO2, pO2, HCO3, lactate) measured at 6-12-24h after each poractant alfa administration up to 72 hours and at similar timepoints in the control group (6, 12, 24, 30, 36, 48, 54, 60 and 72 hours after randomisation) and then every 24 hours till the patient is discharged from the ICU. *Exploratory (to be applied only in patients from UK)* The following parameters will be measured at 12, 24, 48, 72 and 96 hrs after start of treatment (first dose in the poractant alfa group and after randomisation in the control group): - Change from baseline in lowest and dynamic surface tensions (mN/m) from TA samples - Change from baseline in concentrations of surfactant phospholipids (mg/ml) and proteins (ng/ml) from TA samples - Change from baseline in inflammatory indices such as cellular and cytokine (pg/ml) inflammatory markers (e.g. IL-1, IL-6, TNF alpha, IFN gamma and lymphocyte markers) from TA and blood samples
Safety variables	- Adverse events (AEs) - Laboratory Parameters (Blood count, bilirubin, creatinine, LDH, D-dimer, CRP (C-Reactive Protein), procalcitonin) - Vital Signs (Blood pressures and pulse rate)
Sample size calculation	A total of 70 patients will be randomised with a ratio 3:2 (i.e. 42 patients randomised to the poractant alfa group and 28 in the control group). Assuming patients in the control arm being free from ventilation on average 1 week (i.e. 7 days), this sample size achieves 84% power to detect a difference of 4 days (i.e. on average 11 ventilation-free days for patients treated with poractant alfa), assuming a standard deviation of 5.5 and a two-sided significance level (alpha) of 0.05 using a two-sample t-test.
Statistical methods	**Populations for analysis** • **Intention-to-Treat population (ITT)**: all randomised patients (analysed as randomised). • **Safety population:** all randomised patients and receive at least one dose of the study treatment (Curosurf treated patients) (analysed as treated).

The primary analysis population for efficacy will be the ITT. The Safety population will be used in the analysis of all safety variables.

Intercurrent Events

Death and Early discontinuation from study drug can be anticipated as intercurrent events:

- Death will be incorporated as a failure outcome for binary endpoint using composite strategy;
- Early discontinuation from study drug will be handled using treatment policy strategy: all data collected after discontinuation from study drug till Day 28 or death will be included;
- Change in respiratory parameters will be analysed using while-alive strategy.

Primary Efficacy Variable

- Number of days alive and ventilator-free during the 21 days after randomisation will be compared between groups by using the ANOVA model including treatment group, age and country as factors. Patients who die or are mechanically ventilated longer than this period are assessed as zero ventilator-free days.

Key secondary endpoint

- Percentage of patients alive and free of respiratory failure (i.e., need for mechanical ventilation, ECMO, non-invasive ventilation, or high-flow nasal cannula oxygen delivery) at Day 28 will be analysed using logistic regression including treatment group, age and country as factor. Difference in the proportion and related 95% confidence interval will be estimated from the model.

Secondary Efficacy Variables

- Number of days alive and free from ventilation at **Day 28**, number of days alive and free from invasive and non-invasive ventilation at **Day 21** and **Day 28** will be compared between groups by the ANOVA model including treatment group, age and country as factors.
- Mortality at **Day 21** and **Day 28** will be compared between groups by using logistic regression including treatment group, age and country as factors. Difference in the proportions and related 95% confidence interval will be estimated from the model.
- Percentage of patients with improvement in severity status defined as a decrease in the severity score at **Day 28 or Discharge,** whichever comes first will be compared by groups using logistic regression including treatment group, age and country as factors. Difference in the proportions and related 95% confidence interval will be estimated from the model.
- Change from baseline in PaO2/FiO2 at **6 and 12 hours after each treatment** and at similar timepoints in the control group

	will be compared between groups using ANCOVA model including group, age and country as fixed effect and baseline value as covariate. The same analysis will be performed at each additional timepoint. • Percentage of patients alive and with PaO2/FiO2 improvement of >20% at **6 and 12 hours following administration of each dose** in the treated group and at similar timepoints in the control group will be compared by groups using logistic regression including treatment group, age and country as factors. Difference in the proportions and related 95% confidence interval will be estimated from the model. Patients who die are considered as a failure. The same analysis will be performed at each additional timepoint. • Change from baseline in FiO2 will be analysed as PaO2/FiO2. • Length of ICU stay (days) at **Day 28** will be compared between groups will be compared between groups by using the ANOVA model including treatment group, age and country as factors. Patients who die or are mechanically ventilated longer than this period are assigned with 28 days. • Percentage of patients alive and out of ICU at **Day 28** using logistic regression including treatment group, age and country as factors. Difference in the proportions and related 95% confidence interval will be estimated from the model. • Delta SOFA Score and sub-score components at **Day 3** and **Day 28 or Discharge**, whichever comes first, will be analysed at each timepoint using ANCOVA model including group, age and country as fixed effect and baseline value as covariate. • Percentage of patients alive and free of organ failure (i.e. SOFA score=zero) at **Day 28 or Discharge**, whichever comes first will be compared by groups using logistic regression including treatment group, age and country as factors. Difference in the proportions and related 95% confidence interval will be estimated from the model. • Change from baseline in ventilator parameters (TV, RR, Cdyn, Cstat, PEEP, PIP, Pplat) will be summarized by means of descriptive statistics or frequency distribution, as appropriate by group at each timepoint. • Change from baseline in BGA acid-base balance parameters (i.e. pH, pCO2, pO2, HCO3, lactate) will be summarized by means of descriptive statistics by group at each timepoint. **Exploratory Efficacy Variables** *(to be applied only in patients from UK)* • Exploratory parameters from TA aspirates and blood samples will be summarized both as absolute values and as change from baseline values by treatment group at each timepoint using descriptive statistics. **Safety Variables** • Incidence of all the AEs, AEs related to poractant alfa (treated

	group) (ADRs), serious AEs (SAEs) and AEs leading to death will be summarized by group both in terms of frequency of patients with at least one AE and in terms of the frequency of AEs (number of events). • Vital sign values and their change from baseline will be summarized by group at each timepoint using descriptive statistics. • Laboratory parameters and their change from baseline will be summarized by group at each timepoint using descriptive statistics.

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ADR	Adverse Drug Reaction
ARDS	Acute Respiratory Distress Syndrome
AE	Adverse Event
ABG	Arterial Blood Gas
ANOVA	Analysis of Variance
BAL	Bronchoalveolar Lavage
Cdyn	Dynamic compliance
CRA	Clinical Research Associate
CRF	Case Report Form
CRP	C-Reactive Protein
CRO	Contract Research Organization
Cstat	Static compliance
FPFV	First Patient First Visit
ET	Endotracheal
vvECMO	Veno Venous-Extracorporeal Membrane Oxygenation
ICH	International Conference on Harmonization
ICU	Intensive Care Unit
ITT	Intention to Treat
LPLV	Last Patient Last Visit
LBW	Lean Body Weight
MedDRA	Medical Dictionary for Regulatory Activities
MV	Mechanical Ventilation
NIV	Non-invasive Ventilation
PEEP	Positive End-Expiratory Pressure
PIP	Peak Inspiratory Pressure
Pplat	Plateau pressure
RR	Respiratory Rate
rtPCR	Real Time Polymerase Chain Reaction
SAE	Serious Adverse Event
SmPC	Summary of Product Characteristics
SOFA	Sequential Organ Failure Assessment
SUSAR	Suspected Unexpected Serious Adverse Reaction
TA	Tracheal Aspirate
TV	Tidal Volume

CONTENT

1. INTRODUCTION

1.1 Background information

The 2019 novel coronavirus (SARS-COV-19) has spread rapidly since its recent identification in patients with severe pneumonia in Wuhan, China.

The World Health Organization (WHO) on March 11 declared COVID-19 a pandemic, highlighting the sustained risk of further global diffusion.

The COVID-19 pandemic threatens patients, societies and healthcare systems around the world. Host immunity determines the progress of the disease and its lethality. The associated cytokine storm mainly affects the lungs, leading to acute lung injury of variable severity [1][2][3][4][5].

In epidemiological Chinese studies 17% -29% of hospitalised patients developed ARDS [6][7].

For COVID-19 patients treated on ICU, a mortality rate of 30–70% is expected. For older patients with ARDS, this mortality rate is comparable to other severe pulmonary infections [1][3][5][8][9].

The acute respiratory distress syndrome (ARDS) is a clinical syndrome defined by acute onset hypoxemia (PaO2:FiO2 ratio < 300) and bilateral pulmonary opacities not fully explained by cardiac failure or volume overload [10]. The Berlin consensus definition of ARDS, like the American-European Consensus definition that preceded it, has enabled clinicians and researchers alike to prospectively identify patients with ARDS, implement lung protective ventilation strategies, and enrol patients in clinical trials. ARDS remains under-recognized clinically, however; therapies are limited, and mortality remains high [10]. Under-recognition of ARDS may stem in part from the considerable clinical heterogeneity observed among patients who meet standard ARDS criteria. The syndrome may be triggered, for example, by pulmonary or extrapulmonary sepsis, aspiration, trauma, blood product transfusion, or pancreatitis [11]. Pulmonary infiltrates can be focal or diffuse. Hypoxemia can range from mild to severe, and duration of respiratory failure can be brief or prolonged. Many of these clinical variations may reflect underlying biological differences between ARDS patients that are now recognized as important drivers of treatment response and ultimate outcomes [12] .

1.2 Study rationale

Chiesi Farmaceutici S.p.A intends to conduct a proof of concept study with its porcine derived surfactant, Curosurf® - poractant alfa (porcine surfactant), in ventilated adult patients who are critically unwell in intensive care.

Curosurf® is a pulmonary surfactant of natural origin which, when delivered endotracheally (ET), compensates for the deficiency of endogenous pulmonary surfactant that causes neonatal Respiratory Distress Syndrome (RDS) in babies [13]. Curosurf® is supplied as a saline suspension of alveolar surface phospholipid extracted from porcine lung; it consists of 99% polar lipids (mainly phospholipids with variable R substituents) and 1% hydrophobic low molecular weight proteins (surfactant associated protein SP-B and SP-C).
The Curosurf® formulation allows uniform distribution within the lungs and spreading of the phospholipids at the air-liquid interface in the alveoli; hence, it reduces alveolar surface tension,

prevents alveolar collapse and enables gas exchange and alveolar ventilation at low transpulmonary pressures [14]. Curosurf® was developed by Curstedt and Robertson at the Karolinska Institute of Stockholm and licensed to Chiesi Farmaceutici S.p.A. It is currently approved for marketing as treatment of premature neonates with respiratory distress syndrome (RDS) or at risk of RDS (see SmPC and investigator's brochure). ARDS in COVID-19 has clinical and lung pathophysiological similarities with newborn RDS (in which there is an alveolar collapse due to surfactant-deficiency due to immature alveolar type II epithelial cells [15][16]). In COVID-19, damage to the alveolar epithelial type II cells is caused by the viral infection and inactivation of surfactant results from the host inflammatory reaction.

It has been known that, the novel Coronavirus SARS-COV-19, invades human cells via the receptor angiotensin converting enzyme II (ACE2). Moreover, lung alveolar epithelial type 2 cells that have high ACE2 expression may be the main target cells during SARS-COV-19 infection. Evidence, obtained through scRNA-seq data analyses, has shown that the organs at risk, (such as lung, heart, oesophagus, kidney, bladder, and ileum), with their located specific cell types (i.e., type II alveolar cells (AT2), myocardial cells, proximal tubule cells of the kidney, ileum and oesophagus epithelial cells, and bladder urothelial cells), are vulnerable to SARS-COV-19infection [17]. Damage to the type II alveolar cells leads to surfactant depletion and the ensuing inflammation, inactivates existing surfactant leading to loss of alveolar stability and impaired pulmonary compliance.

This hypothesis has been confirmed from histopathological autopsy findings from patients who died from COVID-19 showed lung lesions, including alveolar exudate, inflammation and hyaline membranes formation similar to that classically seen in surfactant deficiency in the newborn [18].

This study is targeted, to a specifically selected population of patients with SARS-CoV 19 ARDS, offering a potential efficacious treatment during a public health emergency, whilst simultaneously, addressing the mistakes of previous surfactant studies due to heterogenous ARDS types and, non-standardised treatment application [19][20][21][22][23].

This protocol will consider only COVID patients on invasive MV for less than 48 hours, with a PaO2/FiO2 < 150mmHg and lung compliance < 45ml/cmH2O; a well- defined treatment scheme has been identified in terms of dose and administration as early as possible after intubation.

Recently [24], the need for conducting such a study was highlighted. It is true that these patients are well managed by assisted ventilation, but surfactant deficiency increases the alveolar surface tension and generates a low-pressure area in the interstitial space, during inspiration. This area attracts liquid and substances which are often inflammatory and which organize over time, giving rise to surfactant inactivation and interstitial pneumonia. These phenomena could potentially be prevented by surfactant replacement therapy.

Considering that the population under study is hospitalized in the Intensive Care Unit (ICU) in which often the patients who arrive are in a state of unconsciousness due to the serious systemic SARS-CoV 19 infection and that the proposed clinical trial poses minimal risk and burden to the subject compared to the standard treatment, it is reasonable that the study falls within the definition of emergency-urgency. Moreover, these conditions coincide with those that need intervention decisions in time

windows incompatible with the possibility of communication with the patient and/or with the use of a legally valid representation.

Exploratory mechanistic assessment rationale

As presented above, the rationale behind the proposed trial of surfactant therapy for COVID-19 patients requiring ventilator support, is that endogenous surfactant is dysfunctional. This could be due to decreased concentration of surfactant phospholipid and protein, altered surfactant phospholipid composition, surfactant protein proteolysis and/or oedema protein inhibition of surfactant surface tension function as a consequence of inflammation. However, there is no empirical evidence to establish any of these mechanisms. Mechanistic approaches to address unanswered questions are set out below.

This study aims to address the following questions:

- Is the concentration/composition of pulmonary surfactant phospholipid and protein altered in patients with COVID-19? Surfactant phosphatidylcholine (PC) is enriched in dipalmitoyl PC (DPPC) and phosphatidylglycerol (PG). ARDS patients exhibit altered percentage composition of surfactant PC, with decreased DPPC and increased surface tension-inactive unsaturated species, and decreased concentrations of both total PC and PG. Anti-inflammatory surfactant proteins SP-A and SP-D concentrations are decreased in a variety of inflammatory lung diseases including cystic fibrosis, chronic obstructive lung disease and severe asthma, with increased SP-D concentration in plasma. Additionally, proteolytic fragments of SP-D are detected in lung infections- are they in COVID-19?
- Are the surface tension-lowering properties of BAL/ tracheal suction derived surfactant airway sampling impaired?
- Does viral infection decrease synthesis of surfactant phospholipid and protein by ATII cells?
- What are the features of the inflammatory response in COVID-19 infection?
- Does surfactant therapy modulate the immune/inflammatory response?
- Does exogenous administration of surfactant combat the inhibitory activity of plasma proteins and the inflammatory reaction on endogenous surfactant function?

1.3 Risk/benefit assessment

Detailed information about the known and expected benefits and risks and reasonably expected adverse events of porcine surfactant (Curosurf®), when used in neonates, may be found in the Investigator's Brochure of the drug.

The choice of dosing is based on prior beneficial adult ARDS studies [25][26][27][28], and the rapid pharmacodynamic response that would be expected if underlying surfactant inhibition or deficiency is a feature of COVID-19 disease.

Although adult ARDS trials have had some high-profile failures, investigation of fluid dynamic modelling and review of the original studies that showed efficacy suggests that larger fluid volumes and distal intrabronchial drug delivery are likely to be advantageous and may maximize alveolar surfactant delivery [29].

This trial will be conducted in compliance with the Declaration of Helsinki (1964 and amendments) current ICH E6 Good Clinical Practices and all other applicable laws and regulations.

2. STUDY OBJECTIVES

2.1 Primary Objective(s)

To evaluate the efficacy and safety of poractant alfa (porcine surfactant, Curosurf®), administered by endotracheal (ET) instillation, in terms of ventilatory free days during the 21 days after randomization, in adult patients with ARDS due to SARS-COV-19infection.

2.2 Secondary Objective(s)

To evaluate the efficacy and safety of poractant alfa administered by ET Instillation compared to control group, in terms of oxygenation (PaO2 / FiO2), FiO2, free days from invasive and non-invasive mechanical ventilation, length of ICU stay, mortality at 28 days, SOFA score (overall organ -failure measurement), incidence of AEs, vital signs and laboratory parameters.

2.3 Exploratory Objective(s)

To investigate possible mechanism that could impact on surfactant functionality in COVID-19 infected patients requiring ventilator support: decreased concentration of surfactant phospholipid and protein, altered surfactant phospholipid composition, surfactant protein proteolysis and/or oedema protein inhibition of surfactant surface tension function as a consequence of inflammation.

These assessments will be done on closed suction deep tracheal aspirate (TA) samples and inflammatory indices such as cellular and cytokine inflammatory markers e.g. IL-1, IL-6, TNF alpha, IFN gamma and lymphocyte markers) from TA and blood samples before the first surfactant dose, after 12, 24, 48, 72, 96 hrs and corresponding timepoints in the control group after randomisation. Surplus bronchoalveolar lavage fluid collected for diagnostic clinical reasons will also undergo analysis when available.

These exploratory parameters will be assessed only for UK patients enrolled in the study. Given the current pandemic situation and the samples delivery procedures within 48h, it has been deemed appropriate to avoid additional burden for US and Italian sites.

3. STUDY DESIGN

This clinical plan is written at the time of the coronavirus pandemic during which in some countries (e.g. UK) the number of people who get infected or hospitalized for respiratory complication may be dramatically high. Therefore, the clinical and operational scenario is extremely variable, and it is expected that it will remain so for an unforeseeable time. In addition, limited evidence is available on the course of the disease and on the significance of intermediate end-points, before the use of the experimental drug.

It is therefore anticipated in advance that the present protocol may need amendments to comply with evolving knowledge on the pandemic, the rate of complications, and the therapeutic scenario for patients who develop ARDS

3.1 Study design

This is a multi-center, open-label, randomised study that includes a group of patients treated with poractant alfa and a control group on standard of care (SOC). Curosurf® represents an adding therapy, potentially beneficial on top of the current standard of care in intubated SARS-COV-19 patients.

Besides respiratory support, standard of care includes possible treatments under efficacy evaluation: i.e. antiviral drugs, monoclonal antibodies.

The study will be conducted in approximately 7 Italian, UK and USA Intensive Care Units (ICU).

Forty-two (42) patients with ARDS due to SARS-COV-19 will be randomised in the poractant alfa treated group and 28 patients randomised in the control one.

3.2 End of trial definition

A participant is considered to have completed the study if he/she has completed the last scheduled procedure shown in the schedule of assessments: follow up at Day 28. The end of the study is defined as the date of the last follow up visit at Day 28 for the last participant in the trial globally.

Some recruited and treated patients are likely to be transferred during the study period from the ICU to another hospital department for continuation of their clinical care. In this case the patient will remain in the study and relevant data will still be recorded through review of the medical chart or by phone call by the Investigator to the physician of the other department. Similarly checks for the occurrence of any adverse event as well as of any change in relevant concomitant medications will be made and recorded.

4. PATIENT SELECTION CRITERIA

4.1 Patient Recruitment

The evaluation of the patients will occur in the screening phase and reviewed to confirm that potential participants meet eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.

The inclusion of a diverse population helps to ensure that medical products are safe and effective for everyone; for this reason, the enrolment of populations most affected by COVID-19, specifically racial and ethnic minorities is encouraged.

4.2 Inclusion Criteria

Participants are eligible to be included in the study if the following criteria apply:

1. Male or female ≥18 and ≤ 80 years of age
2. Informed consent for participation in the study (*refer to section 15 for detailed informed consent procedure*)
3. Positive SARS-COV-19 rt-PCR before randomisation
4. PaO2/FiO2 ratio < 150 mmHg
5. Lung compliance ≤45 ml/cmH20
6. Intubated and artificially ventilated less than 48 hours before the first poractant alfa administration

4.3 Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

1. Any contraindications to surfactant administration e.g., pulmonary haemorrhage and pneumothorax)
2. Weight < 40kg

3. Stage 4 severe chronic kidney disease (i.e., eGFR < 30)
4. Pregnancy
5. Administration of any nebulized surfactant in the 48 hours before the first poractant alfa administration
6. Extracorporeal membrane oxygenation

4.4 Patient Withdrawals

Patients must be discontinued from the study for any of the following reasons:

- The patient or the patient's representative withdraws the consent
- The patient is lost to follow-up.
- The sponsor or the regulatory authorities or the Ethics Committee(s), for any reason, terminates the entire study, or terminates the study for this trial site or this particular patient.

It is understood by all concerned that an excessive rate of withdrawals can render the study uninterpretable.
Should a patient withdraw from the study, all efforts will be made to complete and report the observations as thoroughly as possible.

Patient who discontinues study treatment should not be considered automatically withdrawn from the study (except if the reason is consent withdrawal or lost to follow up). The investigator and study staff must discuss with the patient (or with the patient's representative) asking to continue attending the remaining study visits even though the treatment has been terminated prematurely.
In case of withdrawal, the Investigator must fill in the "Study Termination" page in the CRF reporting the main reason for withdrawal.

5. PATIENT CONCOMITANT MEDICATIONS

5.1 Permitted concomitant Medications

Concomitant treatments are allowed.

Concomitant medications in a critically ill SARS-COV19 population are numerous, and may change daily are consequently difficult to collect, and are unlikely, to be related to therapeutic success or safety evaluations. Therefore, only selected concomitant medications will be specifically recorded in this trial:

1. Antiviral drugs;
2. Monoclonal antibodies;
3. Antibiotics;
4. Systemic corticosteroids;
5. Heparin or any other anticoagulants;
6. Plasma administration from immunized patients;
7. Nitric Oxide therapy;
8. Any other treatments for pneumonia/ARDS/SARS COVID 19 ARDS;
9. Medications prescribed to treat adverse events (AEs).

The list of medications will be assessed only from intubation to Day 28 or until discharge whichever is earlier.

5.2 Non-permitted concomitant Medications

None.

6. TREATMENT(S)

The study medications will be supplied to the clinical site under the responsibility of the Sponsor, who will also provide the Pharmacist/Investigator with appropriate certificates of analytical conformity.

6.1 Appearance and Content

6.1.1 Poractant alfa (Curosurf®) Poractant alfa (Curosurf®) is a standard natural surfactant treatment prepared from porcine lungs for endotracheal administration and containing almost exclusively polar lipids, in particular phosphatidylcholine (about 70% of the total phospholipid content), and about 1% of specific low molecular weight hydrophobic proteins SP-B and SP-C with a total concentration of 80 mg/ml. The drug product is filled in sealed 3.0 ml glass vials.

6.2 Dosage and Administration

6.2.1 Selection of doses in the study

The choice of the poractant alfa (Curosurf®) dose for this study is based on prior beneficial adult ARDS studies [25][26][27][28] and the rapid pharmacodynamic response that would be expected if underlying surfactant inhibition or deficiency is a feature of COVID-19 disease.
Although adult ARDS trials have had some high-profile failures, investigation of fluid dynamic modelling and review of the original studies that showed efficacy, suggests that larger fluid volumes and distal intrabronchial drug delivery are likely to be advantageous and to maximize alveolar surfactant delivery [29].

6.2.2 Dosage

The poractant alfa (Curosurf®) dose used in the study will consist of poractant alfa boluses: 30mg / kg (Lean Body Weight-LBW) = 0.375ml /kg LBW. (LBW calculated according to Hume formula) [30].

Diluted with normal saline up to approximately 2ml /kg LBW (for details refer to CTS Instruction for use).

6.2.3 Administration

Before treatment, patients transiently will receive 100% oxygen.

Poractant alfa boluses, 30 mg per kilogram of lean body weight, will be administered through a sterile syringe connected to an opaque catheter inserted into the endotracheal tube with the distal end approximately 1 cm above the carina.

The first half of the dose will be administered during a pause in mechanical ventilation in which PEEP will be maintained and the patient will be in the left or right lateral decubitus position, and the second half will be administered similarly 5 minutes lateral, with the patient in the opposite lateral decubitus position [20].

When possible, tube suctioning should be avoided for 3 hours after the administration.

Patients will receive a total of three administrations, 24 hours apart.

For details on study medication dosage and administration please refer to the IMP Management manual.

6.2.4 Patient Training

Not applicable.

6.3 Packaging

Chiesi Farmaceutici S.p.A. will supply the study medication for the study to the investigational sites.

Single labelled glass vial of 3 ml each of Curosurf® will be packed in a labelled box.

6.3.1 Primary packaging

Labelled glass vial, with a blue topper, of 3 ml volume at a concentration of 80 mg/ml.

6.3.2 Secondary packaging

Labelled box containing one labelled glass vial of 3 ml volume.

6.3.3 Tertiary packaging

Not applicable.

6.4 Labelling

All labelling will be in local language and according to local law and regulatory requirements and will be compliant with Annex 13 to the Volume 4 of the GMP.

6.5 Treatment allocation

Balanced block randomisation scheme stratified by site will be prepared via a computerised system by the Statistics and Data Management Department of Chiesi Farmaceutici S.p.A. Separate randomisation list will be prepared for each site.

The randomisation will assure a 3:2 ratio between poractant alfa arm and control arm in each cohort (i.e., 42 subjects in poractant alfa and 28 subjects in control arm).
The randomisation numbers will consist of 4-digit numbers ranging from 1001 to 2000.

Patients will be sequentially assigned the lowest available randomisation number. Replacements are not expected.

6.6 Treatment Code

Study drug will be packaged and uniquely numbered. Each primary packaging in the medication kit will have a numbered label that matches the kit number on the label of the outside packaging.

This is an open-label study.

An individual randomization code envelope will be supplied to the Investigator for each patient and will contain the assignment treatment group for that patient. The Investigator will keep the treatment code envelopes in a locked, secure storage facility. Allocation of each patient to the assigned treatment group will be performed after opening the individual envelope associated with the lowest randomisation number.

6.7 Treatment compliance

The effective doses of study drugs received by each participant during the study will be recorded in the eCRF.

6.8 Drug Storage

The Pharmacist/Investigator or delegated research team will be responsible for the safe storage of all medications assigned to this study, in a secure place with restricted access, and maintained within the appropriate ranges of temperature.

Curosurf® will have to be stored between 2˚ and 8˚C, protected from light (please refer to - CTS Instructions for use).

6.9 Drug Accountability

The Investigator, or the designated/authorized representative, is responsible for the management of all the study medications to be used for the study. Study medications that should be stored in a locked, secure storage facility with access limited to those individuals authorized to dispense the study medications.

An inventory will be maintained by the Investigator or pharmacist (or other designated individual), to include a signed account of all the study medication(s) received, dispensed and returned by each patient during the trial.

At the conclusion or termination of the study, the Investigator or the pharmacist shall conduct and document a final drug supply (used and unused) inventory. An explanation will be given for any discrepancies.

All the study medications supplied, used or unused, will be returned to Chiesi or to the designated CRO under Sponsor's responsibility or destroyed directly from the Investigator by the centre. In this case, a destruction certificate must be asked to the investigational centre and filed both at site and at Sponsor. Return and destruction will not occur until authorized by Chiesi.

7. STUDY PLAN

7.1 Study Schedule

	ICU stay period					**ICU discharge**	**Last FUP**
Poractant alfa treated group	**Screening[7]**	**Day 1 (Randomisation)**	**Day 2**	**Day 3**	**Day 4 – 27**	**Day X**	**Day 28**
		Before randomisation/treatment and 6-12-24h after each administration up to 72 hours			Every 24h till ICU discharge	Whenever it occurs	
Control group	**Screening**	**Time 72 h**			**Day 4 – 27**	**Day X**	**Day 28**
		Before randomisation and at 6, 12, 24, 30, 36, 48, 54, 60, 72 h since randomisation			Every 24h till ICU discharge	Whenever it occurs	
Assessments							
Informed consent	X						
Inclusion and exclusion criteria	X						

	ICU stay period					**ICU discharge**	**Last FUP**
Poractant alfa treated group	**Screening[7]**	**Day 1 (Randomisation)**	**Day 2**	**Day 3**	**Day 4 – 27**	**Day X**	**Day 28**
		Before randomisation/treatment and 6-12-24h after each administration up to 72 hours			Every 24h till ICU discharge	Whenever it occurs	
Control group	**Screening**	**Time 72 h**			**Day 4 – 27**	**Day X**	**Day 28**
		Before randomisation and at 6, 12, 24, 30, 36, 48, 54, 60, 72 h since randomisation			Every 24h till ICU discharge	Whenever it occurs	
Demography	X						
Vital signs (blood pressure, heart rate)	X	Once <u>Treated group:</u> 24 - 48 - 72h post start of treatment <u>Control group:</u> 24 - 48 - 72h post randomisation			X	X	X
Full physical examination including height and weight	X						
Medical history (SARS COVID-19 ADRS, past and current relevant medical conditions)	X						
Arterial Blood Gas (ABG) Analysis[1]	X (If the screening assessment is done just before randomization no need to repeat the assessment)	<u>Treated group:</u> Before randomisation and 6-12-24h after each administration up to 72 hours <u>Control group:</u> Before randomisation and at 6, 12, 24, 30, 36, 48, 54, 60, 72h since randomisation			Every 24h	X	Once if not discharged from ICU
Respiratory support assessment Ventilator parameters[2]	X (If the screening assessment is done just before randomization no need to repeat the assessment)	<u>Treated group:</u> Before randomisation and 6-12-24h after each administration up to 72 hours <u>Control group:</u> Before randomisation and at 6, 12, 24, 30, 36, 48, 54, 60, 72h since randomisation			Every 24h	X	Once if not discharged from ICU

	ICU stay period					**ICU discharge**	**Last FUP**
Poractant alfa treated group	**Screening[7]**	**Day 1 (Randomisation)**	**Day 2**	**Day 3**	**Day 4 – 27**	**Day X**	**Day 28**
		Before randomisation/treatment and 6-12-24h after each administration up to 72 hours			Every 24h till ICU discharge	Whenever it occurs	
Control group	**Screening**	**Time 72 h**			**Day 4 – 27**	**Day X**	**Day 28**
		Before randomisation and at 6, 12, 24, 30, 36, 48, 54, 60, 72 h since randomisation			Every 24h till ICU discharge	Whenever it occurs	
Mechanics Laboratory assessments[3]		Treated group: Before randomisation and 12, 24, 48, 72, after first administration Control group: Before randomisation and 12, 24, 48, 72, after randomisation			Treated group: 96h after first administration Control group: 96h after randomisation		
Laboratory assessments[4]	X			Once Treated group: 24h post last treatment Control group: 72h post randomisation		X	Once if not discharged from ICU
SOFA score[5]	X			Once Treated group: 24h post last treatment Control group: 72h post randomisation		X	Once if not discharged from ICU
Thoracic CT scan or Chest XR[6]	X						
Curosurf administration (intreated group)		Three ET administrations with a 24 hours interval (Day 1, Day 2 and Day 3)					
Concomitant Medications	X	X	X	X	X	X	X

	ICU stay period					ICU discharge	Last FUP
Poractant alfa treated group	**Screening[7]**	**Day 1 (Randomisation)**	**Day 2**	**Day 3**	**Day 4 – 27**	**Day X**	**Day 28**
		Before randomisation/treatment and 6-12-24h after each administration up to 72 hours			Every 24h till ICU discharge	Whenever it occurs	
Control group	**Screening**	**Time 72 h**			**Day 4 – 27**	**Day X**	**Day 28**
		Before randomisation and at 6, 12, 24, 30, 36, 48, 54, 60, 72 h since randomisation			Every 24h till ICU discharge	Whenever it occurs	
Adverse Events	X	X	X	X	X	X	X
Ventilatory support	X	X	X	X	X	X	X
Concomitant procedures	X	X	X	X	X	X	X

- [1] Arterial blood gas (ABG) analysis: pH, pCO2, pO2, HCO3, lactate.
- [2] Ventilatory parameters: PaO2/FiO2, FiO2, TV (tidal volume), RR (respiratory rate), Cstat (static compliance), Cdyn (dynamic compliance), PEEP (positive end-expiratory pressure), PIP (peak inspiratory pressure), Pplat (plateau Pressure).
- [3] For mechanics assessments: blood sampling and tracheal aspirates (TA). Bronchoscopic washings performed as clinically indicated only, with surplus samples retrieved for mechanistic study as available. They are planned only for patients recruited in UK.
- [4] Blood count, bilirubin, creatinine, eGFR, glucose, AST, ALT, LDH, D-dimer, CRP (C-reactive protein), procalcitonin. Urine beta HCG at screening only.
- [5] SOFA score is calculated considering PaO2/FiO2, Glasgow coma scale, mean arterial pressure, and bilirubin, platelet and creatinine levels. SOFA score can be calculated only after the patient is included in the study (after the ICF signature) and in case not all the SOFA parameters are available before randomization, the calculation can be based on historical data collected (within 24 hours) as per standard practice.
- [6] Already available as part of the standard care procedures.
- [7] All screening procedures have to be completed/performed before the randomization.

The following time deviations from theoretical post-dose times will be allowed:

Post-dose time assessments	Allowed deviations
≤ 72 hours	± 1 hour
>72 hours	± 2 hours

7.1.1 Screening and eligibility assessment

Screening evaluations must be completed and reviewed to confirm that potential participants meet eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.

Procedures conducted as part of the participant's routine clinical management (e.g. blood count) and obtained before informed consent may be used for screening or baseline purposes provided the procedures meet the protocol-specified criteria.

All screening procedures have to be completed/performed before the randomization.

After verification of any inclusion/exclusion criteria, those intubated patients with ARDS due to COVID-19 will be included in the study.

Date of patient intubation and mechanical ventilation start time is recorded for both the groups.

The following assessment will be collected:

- Informed consent for participation in the study (*refer to section 15 for detailed informed consent procedure*)
- Demographics
- Medical history (includes past and current relevant medical conditions)
- Full physical examination including height and weight
- Arterial Blood Gas (ABG) Analysis
- Ventilatory support
- Ventilatory parameters: PaO2/FiO2, FiO2, tidal volume (TV), respiratory rate (RR), static compliance (Cstat), dynamic compliance (Cdyn), PEEP (positive end-expiratory pressure), PIP (peak inspiratory pressure), Plateau Pressure (Pplat).
- Laboratory assessments: blood count, bilirubin, creatinine, eGFR, LDH, D-dimer, CRP, procalcitonin and urine beta HCG (*only for eligibility criteria*). SARS-Cov-19 rtPCR *(as available, at least before randomization)*
- Vital signs will be obtained as appropriate
- SOFA score is calculated considering PaO2/FiO2, Glasgow coma scale, mean arterial pressure, and bilirubin, platelet and creatinine levels
- Thoracic CT scan or Chest XR already available as part of the standard care
- AEs review (including SAEs)
- Concomitant medications and procedure review

7.1.2 Day 1, Day 2 and Day 3 for treated group [up to 72 hours for control group]

On Day 1, within 48 hr after intubation, the patients will be randomised in the poractant alfa treated group or in the control one with the 3:2 ratio.
In case the patient will be assigned to the poractant alfa treated group, the treatment must start soon after the randomization (max 2 hr), and within 48hr after intubation
The following procedures for treated group or for control group will be conducted according to the frequencies reported in the above flowchart:

- Treatment procedure for poractant alfa as described in detail in section 6.2. One administration per day with a 24 hours interval
- Arterial Blood Gas (ABG) Analysis
- Ventilatory support
- Ventilatory parameters: PaO2/FiO2, FiO2, tidal volume (TV), respiratory rate (RR), static compliance (Cstat), dynamic compliance (Cdyn), PEEP (positive end-expiratory pressure), PIP (peak inspiratory pressure), Plateau Pressure (Pplat)
- Laboratory assessments for mechanistic analysis: blood sampling and bronchial washings;

bronchoscopic washings performed as clinically indicated only, with surplus samples retrieved for mechanistic study as available (for more details ref. to section 7.2)

- Laboratory assessments: blood count, bilirubin, creatinine, eGFR, LDH, D-dimer, CRP, procalcitonin
- Vital signs will be obtained as appropriate
- SOFA score is calculated considering PaO2/FiO2, Glasgow coma scale, mean arterial pressure, and bilirubin, platelet and creatinine levels
- AEs review (including SAEs)
- Concomitant medications and procedure review as applicable

7.1.3 Day 4 – 27 recording:

The following procedures for treated group or for control group will be conducted according to the above flowchart

- Arterial Blood Gas (ABG) Analysis
- Ventilatory support: extubation date recorded (as applicable) and following ventilation procedures recorded
- Ventilatory parameters: PaO2/FiO2, FiO2, tidal volume (TV), respiratory rate (RR), static compliance (Cstat), dynamic compliance (Cdyn), PEEP (positive end-expiratory pressure), PIP (peak inspiratory pressure), Plateau Pressure (Pplat)
- Vital signs will be obtained as appropriate
- AEs review (including SAEs)
- Concomitant medications and procedure review as applicable
- Discharge date from ICU if applicable
- Laboratory assessments for mechanistic analysis (only at 96h): bronchial washings, and blood samples, bronchoscopic washings performed as clinically indicated only, with surplus samples retrieved for mechanistic study as available (for more details ref. to section 7.2). They are planned only for patients recruited in UK

7.1.4 ICU discharge (Day X – whenever it occurs)

- Arterial Blood Gas (ABG) Analysis
- Ventilatory support: extubation date recorded (as applicable) and following ventilation procedures recorded
- Ventilatory parameters: PaO2/FiO2, FiO2, tidal volume (TV), respiratory rate (RR), static compliance (Cstat), dynamic compliance (Cdyn), PEEP (positive end-expiratory pressure), PIP (peak inspiratory pressure), Plateau Pressure (Pplat)
- Laboratory assessments blood count, bilirubin, creatinine, eGFR, LDH, D-dimer, CRP, procalcitonin
- Vital signs will be obtained as appropriate
- SOFA score is calculated considering PaO2/FiO2, Glasgow coma scale, mean arterial pressure, and bilirubin, platelet and creatinine levels
- AEs review (including SAEs)
- Concomitant medications and procedure review as applicable
- Discharge date from ICU

7.1.5 Day 28

It's the last day of follow up. Whenever the patient has been discharged home from ICU a phone call FUP is requested.
The following procedures will be followed if the patient is still hospitalized:

- Arterial Blood Gas (ABG) Analysis
- Ventilatory support: extubation date recorded (as applicable) and following ventilation procedures recorded
- Ventilatory parameters: PaO2/FiO2, FiO2, tidal volume, respiratory rate, static compliance (Cstat), dynamic compliance (Cdyn), PEEP (positive end-expiratory pressure), PIP (peak inspiratory pressure), Plateau Pressure
- Laboratory assessments: blood count, bilirubin, creatinine, eGFR, LDH, D-dimer, CRP, procalcitonin
- Vital signs will be obtained as appropriate
- SOFA score is calculated considering PaO2/FiO2, Glasgow coma scale, mean arterial pressure, and bilirubin, platelet and creatinine levels
- AEs review (including SAEs)
- Concomitant medications review as applicable

7.2 Investigations

The following samples for laboratory assessments need to be taken for a combination of main trial assessments and mechanistic studies.

7.2.1 Laboratory assessments

7.2.1.1 Blood Sample Collection (as per study schedule 7.1)

Blood samples (10 mls EDTA and 9 mls for Transfix) will be taken at intubation start as screening evaluation, then 24h after last treatment for poractant alfa group and 72h after randomization for control group. Then at Day 28 or at ICU discharge whichever occurs earlier. In particular:

- *Laboratory analysis:* blood count, bilirubin, creatinine, eGFR, glucose, AST, ALT, LDH, D-dimer, CRP, procalcitonin
- *Arterial blood gas (ABG) analyses*: approximately (~1 ml) of blood sample for the analysis of pH, pCO2, pO2, HCO3, lactate will be taken at screening, before randomisation and 6,12, 24h after each administration up to 72 hours for poractant alfa group; before randomisation and at 6, 12, 24, 30, 36, 48, 54, 60, 72h after randomisation for control group. Then every 24 hours until day 28 or ICU discharge whichever occurs earlier
- *Mechanistic blood laboratory analysis*: inflammatory indices such as cellular and cytokine inflammatory markers and lipid mediators before randomisation and 12, 24, 48, 72, 96h after first administration (while hospitalised) and relative timepoints in the control group since randomization. These analyses are planned only in the UK subpopulation

7.2.1.2 Urine pregnancy test

Urine will be tested for β HCG at screening for female of childbearing potential. As patients will not have the capacity to confirm their medical history in relation to their childbearing potential and this information may not be available in their medical notes, investigator discretion will be used to confirm whether pregnancy testing is required.

7.2.2 Closed suction tracheal aspirate (TA) samples

Closed suction tracheal aspirate (TA) samples will be taken before the first surfactant dose and after 12, 24, 48, 72, 96h and relative timepoints in the control group since randomisation.
The subsequent samples will be used for the mechanistic studies outlined above (section 2) and will assess the efficacy of surfactant administration.
The TA samples will consist of blind suctioning by a cannula introduced through a port into the endotracheal tube. A volume of 20-50 ml saline will be administered, with an estimated recovery of 8-10 ml. This procedure will not interrupt the ventilator circuit and will not result in desaturation. Blind suctioning following the administration of small volumes of saline is routine practice for the clearance of secretions in ventilated patients.

7.2.3 Laboratories involved

The above-mentioned analysis is a part of the standard care for these patients and will be carried out by local hospital laboratories. Specific assessments concerning to the mechanistic investigations will be performed at the University College London laboratory and at Imperial College, London.

The sample collection, processing storage and shipment for analysis will be described in a laboratory manual.

These exploratory parameters will be assessed only on UK patients enrolled in the study. Given the current pandemic situation and the samples delivery procedure within 48h, it has been deemed appropriate to avoid additional burden for American sites.

Surfactant lipid composition and concentration (University College London)

Mass spectrometric analysis of molecular species compositions of phosphatidylcholine (PC), sphingomyelin (SM), phosphatidylglycerol (PG) and phosphatidylinositol (PI) in small volume BAL samples. It would be expected a reduced surfactant concentration and altered composition in baseline samples taken immediately before surfactant administration. These would both change after surfactant is given, and the aim is to assess the ratio of exogenous to endogenous surfactant from these two different compositions [31][32].

Analysis of sequential samples will provide an indication of exogenous surfactant turnover. Liquid Chromatography with Mass spectrometric analyses will also be performed to establish corresponding lipidomic profiles of matching plasma and airway samples, that will include analyses of related lipid species and lipid derived inflammatory mediators.

Surfactant function (Imperial College, London)

Surfactant function will be determined by measuring surfactant adsorption to the air: liquid interface in a Langmuir trough. The tensioactive properties of lung surfactant from patients' BAL will be measured. It will be investigated whether isolated surfactant will have compromised function by the inhibition of plasma proteins, and whether the function can be recovered after administration of supplemented surfactant. Maximum reduction of the surface tension over continuous compression: expansion cycles models the physiological breathing cycles of intubated patients and will be carried out on a captive bubble surfactometer or similar device. Adsorption analysis of the equilibrium surface tension, together with kinetics of the process, will explore inhibition processes in surfactant function.

Immunological and inflammatory assessment (University College London)

Immune cellular components will be profiled to depth and soluble fluids for soluble mediators including those that imply a bacterial or fungal co-infection. The volume needed would depend on the concentration of the cellular component. Fixed blood will be split into 3 and then to each, a cocktail of antibodies will be added to enumerate all T cell subsets and markers of their subtype and

activation. B cell subsets and their antibody isotype will also be investigated. The proportions of cells identified will be compared to normal ranges within each age group. We will be specifically looking at the neutrophil to lymphocyte ratio that is described to be inverted in patients with the disease. Mast cell activation may play an important role in the adverse host response and this will be assessed by the measurement of lipid mediators such as leukotrienes and prostaglandins in airway samples and blood. Multiplex cytokine analysis on tracheal aspirates and blood will characterize the impact of surfactant therapy on the inflammatory cytokine storm.

The level of the immunomodulatory surfactant protein D (SP-D) in serum has shown to be a promising biomarker for lung inflammation (and hence low levels in BAL), and we will quantify the level of SP-D in both BAL and plasma using our established ELISA. We will also quantify the level of C-reactive protein in BAL as a biomarker for blood contamination of surfactant together with total protein concentration. We have seen that an inflammatory response to infections can results in specific degradation dependent on the infectious agent. We will analyse the degradation of SP-D and its sister protein SP-A using SDS-PAGE and Western Blot analysis to evaluate if COVID-19 infection results in degradation.

8. EFFICACY ASSESSMENTS

Primary Endpoint

The primary outcome variable will be the number of days alive and ventilator-free defined as the number of days the patient is alive and not receiving mechanical ventilation over the **21 days** following randomisation.

Mechanical ventilation will be defined as invasive and non-invasive. Patient will be defined free of mechanical ventilation after 12 hours from the suspension of either invasive and non-invasive ventilation. Patients who die or are mechanically ventilated longer than this period are assessed as zero ventilator-free days.

Key secondary endpoint

- Percentage of patients alive and free of respiratory failure (i.e., need for mechanical ventilation, ECMO, non-invasive ventilation, or high-flow nasal cannula oxygen delivery) at **Day 28**

Secondary endpoints

- Number of days alive and ventilator-free at **Day 28**
- Mortality at **Day 21** and **Day 28**
- Number of days alive and free from invasive ventilation at **Day 21** and **Day 28**
- Number of days alive and free from non-invasive ventilation (NIV) at **Day 21** and **Day 28**
- Percentage of patients with improvement in severity status defined as a decrease in the severity score at **Day 28 or Discharge,** whichever comes first. Severity score will be defined as Mild, Moderate, Severe or Death based on PaO2/FiO2 ratio and patient status at Day 28 and numerically rated from 1-4 respectively:

Severity	Variable	Criteria
Mild -1	PaO2/FiO2 Ratio	200 mmHg < PaO2/FIO2 ≤ 300 mmHg
Moderate - 2	PaO2/FiO2 Ratio	100 mmHg < PaO2/FIO2 ≤ 200 mmHg

Severe - 3	PaO2/FiO2	Ratio PaO2/FIO2 ≤ 100 mmHg
Death - 4	Patient Status	Yes/No

- Change from baseline in PaO2/FiO2 ratio at **6 and 12 hours following administration of each dose** in the treated group and at the similar timepoints in the control group (6, 12, 30, 36, 54 and 60 hours after randomisation)
- Change from baseline in PaO2/FiO2 ratio at additional timepoints (i.e. every 24 hours after treatment/randomisation until the patient is discharged from the ICU)
- Percentage of patients alive and with PaO2/FiO2 improvement of >20% at **6 and 12 hours following administration of each dose** in the treated group and at similar timepoints in the control group (6, 12, 30, 36, 54 and 60 hours after randomisation)
- Percentage of patients alive and with PaO2/FiO2 improvement of >20% at the additional timepoints (i.e. every 24 hours after treatment/randomisation till the patient is discharged from the ICU)
- Change from baseline in FiO2 at **6 and 12 hours following administration of each dose** in the treated group and at similar timepoints in the control group (6, 12, 30, 36, 54 and 60 hours after randomisation)
- Change from baseline in FiO2 at additional timepoints (i.e. every 24 hours after treatment/randomisation until the patient is discharged from the ICU)
- Length of ICU stay (days) **at Day 28**. Patients who die or are mechanically ventilated longer than this period are assigned with 28 days
- Percentage of patients alive and out of ICU **at Day 28**
- Delta SOFA Score and sub-score component measured on **Day 3** and **Day 28 or Discharge** whichever comes first
- Percentage of patients alive and organ failure free (SOFA score=0) at **Day 28 or Discharge** whichever comes first
- Change from baseline in ventilatory parameters [tidal volume (TV), respiratory rate (RR), dynamic compliance (Cdyn), static compliance (Cstat), positive end-expiratory pressure (PEEP), peak inspiratory pressure (PIP), plateau pressure (Pplat)] measured at 6-12-24h after each poractant alfa administration up to 72 hours and at similar timepoints in the control group (6, 12, 24, 30, 36, 48, 54, 60 and 72 hours after randomisation) and then every 24 hours till the patient is discharged from the ICU
- Change from baseline in blood gas analysis acid-base balance parameters (i.e. pH, pCO2, pO2, HCO3, lactate) measured at 6-12-24h after each poractant alfa administration up to 72 hours and at similar timepoints in the control group (6, 12, 24, 30, 36, 48, 54, 60 and 72 hours after randomisation) and then every 24 hours till the patient is discharged from the ICU

Exploratory endpoints

The following parameters will be measured only on UK patients at 12, 24, 48, 72 and 96 hrs after start of treatment (first dose in the poractant alfa group) or after randomisation (in the control group):

- Change from baseline in lowest and dynamic surface tensions (mN/m) from TA samples

- Change from baseline in concentrations of surfactant phospholipids (mg/ml) and proteins (ng/ml) from TA samples
- Change from baseline in inflammatory indices such as cellular and cytokine (pg/ml) inflammatory markers (e.g. IL-1, IL-6, TNF alpha, IFN gamma and lymphocyte markers) from TA and blood samples

9. SAFETY ASSESSMENTS

- Adverse events (AEs)
- Laboratory Parameters (Blood count, bilirubin, creatinine, LDH, D-dimer, PRC, procalcitonin)
- Vital Signs (Blood pressures and heart rate)

10. ADVERSE EVENT REPORTING

10.1 Definitions

An **Adverse Event** is "any untoward medical occurrence in a patient or clinical trial patient administered a medicinal product and which does not necessarily have a causal relationship with this treatment".
An adverse event can therefore be any unfavourable and unintended sign (including abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An **Adverse Drug Reaction** is an "untoward and unintended responses to an investigational medicinal product related to any dose administered".
All adverse events judged by either the reporting Investigator or the Sponsor as having a reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression "reasonable causal relationship" means to convey in general that there are facts (evidence) or arguments meant to suggest a causal relationship.
The definition covers also medication errors and uses outside what is foreseen in the protocol, including misuse and abuse of the product.

A **Serious Adverse Event (SAE)/Serious Adverse Drug Reaction** is any untoward medical occurrence or effect that at any dose falls in one or more of the following categories:

- **Results in death**

Death is not an adverse event but an outcome. It is the cause of death that should be considered if it is regarded as the adverse event. The only exception to this rule is "sudden death" where no cause has been established; in this latter instance, "sudden death" should be regarded as the adverse event and "fatal" as its reason for being serious.

- **Is life-threatening**

Life-threatening refers to an event in which the patient was at risk of death at the time of the event. The term does not refer to an event which hypothetically might have caused death if it were more severe.

- **Requires hospitalisation or prolongation of existing hospitalisation**

Hospitalization refers to a situation whereby an AE is associated with unplanned formal overnight admission into hospital, usually for purpose of investigating and/or treating the AE. Hospitalization for the treatment of a medical condition that occurs on an "elective" or "scheduled" basis or for a pre-existing condition that did not worsen during the study should not necessarily be regarded as an AE.

Complications that occur during the hospitalisation are AEs. If a complication prolongs hospitalisation, the event is an SAE. Emergency room visits that do not result in a formal admission into hospital should be evaluated for one of the other seriousness criteria (e.g., life-threatening; persistent or significant disability or incapacity; medically significant).

As the enrolled patients will already be hospitalized at inclusion and during the first weeks of the study the above definition relating to hospital admissions and emergency room attendance will not be relevant during that inpatient period.

- **Results in persistent or significant disability or incapacity**

The term significant disability should be viewed as any situation whereby an AE has a clinically important effect on the patient's physical or psychological well-being to the extent that the patient is unable to function normally.

- **Is a congenital anomaly or birth defect**
- **Is a medically significant adverse event**

This criterion allows for any situations in which important adverse events/reactions that are not immediately life-threatening or do not result in death or hospitalisation may jeopardise the patient's health or may require intervention to prevent one of the above outcomes.

Examples of such events are: intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalisation; or development of drug dependency or drug abuse.
Medical and scientific judgment should be exercised in deciding whether an event is serious because and medically significant.

Any suspected transmission via a medicinal product of an infectious agent is also considered a serious adverse reaction.

A **Non-Serious Adverse Event/Non-Serious Adverse Drug Reaction** is an adverse event or adverse drug reaction that does not meet the criteria listed above for a serious adverse event/serious adverse drug reaction.

10.2 Expectedness

An expected adverse reaction is an adverse reaction, the nature or severity of which is consistent with the applicable reference safety information (included in section 6.3.1.1 of the Curosurf® Investigator's Brochure), otherwise it is considered unexpected.

Reports which add significant information on specificity or severity of a known, already documented serious adverse drug reaction constitute unexpected events. For example, an event more specific or more severe than described in the Reference Safety Information would be considered as "unexpected". Examples of such events are: (a) acute renal failure as a labelled ADR with a subsequent new report of interstitial nephritis and (b) hepatitis with a first report of fulminant hepatitis.

10.3 Intensity of Adverse Event

Each Adverse Event must be rated on a 3-point scale of increasing intensity:

- **Mild:** The event causes a minor discomfort or patient does not lead to either modification of test treatment dosage or establishment of a correcting treatment
- **Moderate:** The event leads to a temporary interruption of the test treatment administration or to the establishment of a correcting treatment (local or non-invasive)

- **Severe:** The event causes severe discomfort. It may be of such severity to cause the definitive interruption of test treatment and invasive treatment

10.4 Causality Assessment

The following "binary" decision choice will be used by the Investigator to describe the causality assessment:

- Reasonable possibility of a relatedness
- No reasonable possibility of relatedness

The expression "reasonable possibility of relatedness" is meant to convey, in general, that there are facts (evidence) or arguments meant to suggest a causal relationship.

The Investigator will be asked to consider the following before reaching a decision on causality assessment:

- Time relationship between study drug intake and event's onset
- Dechallenge (did the event abate after stopping drug?)
- Rechallenge (did the event reappear after reintroduction?)
- Medical history
- Study treatment(s)
- Mechanism of action of the study drug
- Class effects
- Other treatments-concomitant or previous
- Withdrawal of study treatment(s)
- Lack of efficacy/worsening of existing condition
- Erroneous treatment with study medication (or concomitant)
- Protocol related process

It should be emphasized that ineffective treatment should not be considered as causally related in the context of AE reporting.

10.5 Action taken with the study drug due to an AE

- Dose not changed
- Drug permanently withdrawn
- Not applicable

10.6 Other actions taken

- Specific therapy/Medication
- Concomitant Procedure

10.7 Outcome

Each Adverse Event must be rated by choosing among:

- Recovered/resolved
- Recovering/resolving
- Not recovered/not resolved
- Recovered with sequelae/resolved with sequelae
- Fatal
- Unknown

10.8 Recording Adverse Events

All Adverse Events occurring during the course of the study must be documented in the Adverse Event page of the electronic Case Report Form (eCRF). Moreover, if the Adverse Event is serious, the Serious Adverse Event Form must also be completed.
It is responsibility of the Investigator to collect all adverse events (both serious and non-serious) derived by spontaneous, unsolicited reports of patients, by observation and by routine open questionings.
For the purpose of this trial, "acute renal failure" occurrences will not be considered and recorded as adverse events, since well described as a part of the COVID19 syndrome, and highly prevalent in the involved population. They will be recorded as adverse events only if assessed as cause of patient's death.
The recording period for Adverse Events is the period starting from the date of screening until the patient's study participation ends.
Clinically significant abnormalities detected at study entry not due to a pre-existing condition or clinically significant changes at the following visits in the medical opinion of the investigator must be reported as adverse events in the eCRF.

If a clinically significant abnormal laboratory finding or other abnormal assessment meets the definition of an AE, then the AE eCRF page must be completed, as appropriate. A diagnosis, if known, or clinical signs and symptoms if diagnosis is unknown, rather than the clinically significant abnormal laboratory finding, should be reported on AE eCRF page. If no diagnosis is known and clinical signs and symptoms are not present, then the abnormal finding should be recorded.

For pharmacovigilance purposes, all SAEs should be followed-up in order to elucidate as completely and practically as possible their nature and/or causality until resolution of all queries, clinical recovery is complete, laboratory results have returned to normal, stable condition is reached or until the patient is lost to follow-up. Follow-up may therefore continue after the patient has left the study. In this case, the follow-up will continue with no timelines for related SAEs, while for unrelated SAEs the type and extent of follow-up undertaken will be determined for each individual case and will depend upon the nature (e.g. events with poor prognosis or which do not resolve), severity and medical significance of the event.

10.9 Reporting Serious Adverse Events to Chiesi

The Investigator must report all Serious Adverse Events in the SAE Form and provide it to the CROMSOURCE email: pharmacovigilance@cromsource.com, fax: +39 045 8250574, within 24 hours of awareness. The information must be sent by providing the completed Serious Adverse Event form. At a later date, the CROMSOURCE Safety Contact will report all information to Chiesi Global Pharmacovigilance, the Clinical Project Manager and the Clinical Research Physician.

Name and Title	Telephone no.	Fax no.	E-mail
Antonio Lorenzetto Safety Associate II CROMSOURCE Safety Contact	+39 045 8222806	+39 045 8250574	Antonio.Lorenzetto@cromsource.com pharmacovigilance@cromsource.com
Chiara Bonardi Global PV Operations Manager Chiesi Safety Contact	+39 0521 279773	+39 05211885003	c.bonardi@chiesi.com ct_cds@chiesi.com

- Reporting of SAEs from the investigator site is from the time of screening and until the patient's study participation ends. After this date, even if no active monitoring of patients is required, SAEs occurring to a patient should be reported if the investigator becomes aware of them.
- Up to the closure of the site, SAE reports should be reported to the CROMSOURCE Safety Contact. New serious adverse events occurring after the site is closed should be reported directly to the Chiesi Safety Contact.

10.10 Reporting Serious Adverse Events to Regulatory Authorities/Ethics Committees/IRBs

The Sponsor or designated CRO will report adverse events, occurring with the investigational medicinal products within or outside the concerned clinical trial, as applicable, to the regulatory authorities/ethics committees in compliance with the timelines and standards of reporting according to local UK regulations, according to the US "Guidance for industry and Investigators-Safety Reporting Requirements for INDs and BA/BE studies, December 2012" and in compliance with the timelines and standards for reporting SUSARs set out in the EU Directive 2001/20/EC [Directive 2001/20/EC of the European parliament and of the council of 4/April/2001] and linked guidance [European Commission, Enterprise and Industry Directorate General: Detailed guidance on the collection, verification and presentation of adverse event/reaction reports arising from clinical trials on medicinal products for human use, latest version].

The EMA and Italian health authority will be informed through EudraVigilance (directly or indirectly), the FDA and the MHRA via specific electronic submissions, while the Ethics Committees/Central IRB and the investigators by CIOMS I form or by periodic line- listings produced by Chiesi Global Pharmacovigilance.

With regard to regulations in force for Pharmacovigilance, the Investigator must fulfil his/her obligation according to the law in force in his country.

10.11 General Notes

- In case of death, a comprehensive narrative report of the case should be prepared by the Investigator and sent to the CROMSOURCE Safety Contact within the Serious Adverse Event form.
- If an autopsy is performed, copy of autopsy report should be actively sought by the Investigator and sent to the CROMSOURCE Safety Contact as soon as available, retaining a copy on site.
- All source documents provided by the Investigator or site staff to the CROMSOURCE Safety Contact must be carefully checked for respect of confidentiality. All personal patient's data must be redacted.

- If a patient is transferred to another hospital department for continuation of their clinical care, the investigator should ensure that the receiving department is aware that the patient is participating to the present study and communication should be maintained in order to timely record any adverse event.
- In case of pregnancy, the patient will be immediately withdrawn from the study and she will be followed with due diligence until the outcome of the pregnancy is known and till the age of one year of the child to detect any congenital anomaly or birth defect. The pregnancy must be reported by the investigator within 24 hours by e-mail to the CROMSOURCE Safety Contact using the paper Pregnancy Report Form that will be provided upon request. The CROMSOURCE Safety Contact will inform Chiesi of the pregnancy within one working day of being notified.
- If the pregnancy's outcome meets the criteria for immediate classification of a SAE (e.g. spontaneous or therapeutic abortion, stillbirth, neonatal death, congenital anomaly, birth defect) the Investigator should follow the procedure for reporting SAEs.

11. DATA MANAGEMENT

An electronic CRF (Medidata RAVE) will be filled-in by the Investigator and/or his/her representative designee. Screening failure data won't be recorded in the eCRF.

Front-end edit checks will run at the time of data collection and back-end edit checks will be used by the Data Manager to check for discrepancies and to ensure consistency and completeness of the data.

Medical history, adverse events and concomitant procedures will be coded using the MedDRA dictionary; medications will be coded using the WHO Drug dictionary and Anatomical Therapeutic Chemical classification (ATC).

External data (laboratory assessments for mechanics assessments) will be processed centrally, sent to the designated CRO and reconciled with the corresponding information recorded in the CRF.

Access to electronic systems used for data collection will be granted to the study personnel only after appropriate training.

After the completion of data collection and cleaning, a review meeting will be held to determine the occurrence of any protocol violation and to define the patient populations for the analysis. Once the database has been declared to be complete and accurate, it will be locked and the planned statistical analysis will be performed.

If the database is unlocked after the initial lock, the process must be carefully controlled and documented; updates to the study data must be authorized by Chiesi.

At the study conclusion, a complete copy of the study data will be created for archival purposes at Chiesi. The investigators will receive copies of the patient data for retention at the investigational sited documented; updates to the study data must be authorized by Chiesi.

12. STATISTICAL METHODS

12.1 Sample Size

A total of 70 patients will be randomised in the study with a ratio 3:2 (i.e. 42 patients randomised to the poractant alfa group and 28 in the control group). Assuming patients in the control arm being free from ventilation on average 1 week (i.e. 7 days), this sample size achieves 84% power to detect a difference of 4 days (i.e. on average 11 ventilation-free days for patients treated with poractant alfa),

assuming a standard deviation of 5.5 and a two-sided significance level (alpha) of 0.05 using a two-sample t-test.

12.2 Populations for analysis

- **Intention-to-Treat population (ITT):** all randomised patients (analysed as randomised).
- **Safety population:** all randomised patients and receive at least one dose of the study treatment (Curosurf treated patients) (analysed as treated).

The primary analysis population for efficacy will be the ITT. The Safety population will be used in the analysis of all safety variables.

12.3 Statistical analysis

A detailed statistical analysis plan will be described in the Statistical Analysis Plan. The plan might be reviewed and updated as a result of the data review and will be finalized before database lock.

Intercurrent Events

Death and Early discontinuation from study drug can be anticipated as intercurrent events:

- Death will be incorporated as a failure outcome for binary endpoints using composite strategy;
- Early discontinuation from study drug will be handled using treatment policy strategy: all data collected after discontinuation from study drug till Day 28 or death will be included;
- Change in respiratory parameters will be analysed using while-alive strategy.

12.3.1 Descriptive Statistics

General descriptive statistics for numeric variables will include the n (number of observed values), the mean, the standard deviation, the median, the minimum, and the maximum values. For categorical variables, the number and percent of patients with a specific level of the variable will be presented.

12.3.2 Missing data

Details on dealing with missing data, along with the handling of possible outliers, will be described in the SAP. Other critical missing data, if any, will be discussed during the review of the data before database lock and decisions will be fully documented in the Data Review Report.

12.3.3 Patient demographics and baseline characteristics

Demographics and baseline variables will be summarized separately in each cohort by treatment group using descriptive statistics for the ITT population.

12.3.4 Primary efficacy variable

Number of ventilator-free days during the **21 days** after randomisation will be compared between groups by using an ANOVA model including treatment group, age and country as factors. Patients who die or are mechanically ventilated longer than this period are assessed as zero ventilator-free days.

12.3.4.1 Key secondary variable

Percentage of patients alive and free of respiratory failure (i.e., need for mechanical ventilation, ECMO, non-invasive ventilation, or high-flow nasal cannula oxygen delivery) at **Day 28** will be analysed using logistic regression including treatment group, age and country as factor. Difference in the proportion and related 95% confidence interval will be estimated from the model.

12.3.5 Secondary efficacy variables

- Number of days alive and free from ventilation at **Day 28**, number of days alive and free from invasive and non-invasive ventilation at **Day 21** and **Day 28** will be compared between groups by using an ANOVA model including treatment group, age and country as factors.
- Mortality at Day 21 and Day 28 will be compared by groups using logistic regression including treatment group, age and country as factors. Difference in the proportions and related 95% confidence interval will be estimated from the model.
- Percentage of patients with improvement in severity status defined as a decrease in the severity score at Day 28 or Discharge, whichever comes first, will be compared by groups using logistic regression including treatment group, age and country as factors. Difference in the proportions and related 95% confidence interval will be estimated from the model.
- Change from baseline in PaO2/FiO2 at 6 and 12 hours after each treatment and relative timepoints in the control group will be compared between groups using an ANCOVA model including group, age and country as fixed effect and baseline value as covariate. The same analysis will be performed at each additional timepoint.
- Percentage of patients alive and with PaO2/ FiO2 improvement of >20% at **6 and 12 hours following administration of each dose** in the treated group and at similar timepoints in the control group will be compared by groups using logistic regression including treatment group, age and country as factors. Difference in the proportions and related 95% confidence interval will be estimated from the model. Patients who die are considered as a failure. The same analysis will be performed at each additional timepoints.
- Change from baseline in FiO2 will be analysed as PaO2/FiO2.
- Length of ICU stay (in days) at **Day 28** will be compared between groups using ANOVA model including group, age and country as fixed effect. Patients who die or are mechanically ventilated longer than this period are assigned with 28 days.
- Percentage of patients alive and out of ICU at **Day 28** using logistic regression including treatment group, age and country as factors. Difference in the proportions and related 95% confidence interval will be estimated from the model.
- Delta SOFA Score and sub-score components at **Day 3** and **Day 28 or Discharge**, whichever comes first, will be analysed at each timepoint using ANCOVA model including group, age and country as fixed effect and baseline value as covariate.
- Percentage of patients alive and organ failure free (SOFA score=0) at **Day 28 or Discharge**, whichever comes first will be compared by groups using logistic regression including treatment group, age and country as factors. Difference in the proportions and related 95% confidence interval will be estimated from the model.
- Change from baseline in ventilator parameters (TV, RR, Cdyn, Cstat, PEEP, PIP, Pplat) will be summarized by means of descriptive statistics or frequency distribution, as appropriate by group at each timepoint.
- Change from baseline in BGA acid-base balance parameters (i.e. pH, pCO2, pO2, HCO3, lactate) will be summarized by means of descriptive statistics by group at each timepoint.

12.3.6 Exploratory Efficacy Variables

Exploratory parameters from TA aspirates and blood samples will be summarized only in the subgroup of UK patients both as absolute values and as change from baseline values by treatment group at each timepoint using descriptive statistics.

12.3.7 Safety variables

Adverse Events

The number of patients who experienced at least one AE, AEs related to poractant alfa (treated group) (ADRs), serious AEs (SAEs) and AEs leading to death will be summarized by group. Summaries will be presented overall (number and percentage of patients having at least one event, total number of events) and by system organ class and preferred term (number and percentage of patients having at least one occurrence of that event).

All adverse events will be listed.

Vital Signs

Vital sign values and their change from baseline will be summarized by group at each timepoint using descriptive statistics.

Laboratory data

Laboratory parameters and their change from baseline will be summarized by group at each timepoint using descriptive statistics.

All laboratory data will be listed.

12.3.8 Interim analysis

Interim analysis not planned.

13. ETHICS COMMITTEE/INSTITUTIONAL REVIEW BOARD APPROVAL

The study proposal will be submitted to the Research Ethics Committees/HRA/MHRA, IRBs/FDA and ECs/AIFA in accordance with the requirements in England, US and Italy.

The ECs/AIFA, RECs/HRA/MHRA and IRBs/FDA shall give its opinion in writing -clearly identifying the study number, study title and informed consent form approved-, before the clinical trial commences.
A copy of all communications with the EC, REC/HRA and IRB will be provided to the Sponsor.

The Investigator should provide written reports to the REC/HRA/MHRA and EC/IRB annually or more frequently if requested on any changes significantly affecting the conduct of the trial and/or increasing risk to the patients (according to the requirements of each country).

14. REGULATORY REQUIREMENTS

The study will be notified to the Health Authorities (or authorized by) according to the legal requirements in each participating country.

Selection of the patients will not start before the approval of the Ethics Committee/Institutional Review Board has been obtained and the study notified to Health Authorities (or authorized by).

15. INFORMED CONSENT

It is the responsibility of the Investigator to obtain informed consent/eConsent for each patient before performing any trial related procedure by using the latest EC, REC/HRA or IRB approved version of the document.

It will be a rare possibility that patients considered for enrolment in this study will have the capacity to consent to take part due to the nature of the underlying disease process, as they will be under sedation and will be mechanically ventilated.

If the patient is conscious and able to evaluate the information provided with the Patient information sheet (PIS), the PIS will be submitted to him / her and the informed consent obtained before any study procedures will take place. If the patient and his/her legal authorized representative are unable to read, the informed consent will be obtained in the presence of an impartial witness, eg., a person independent of the study who will read the informed consent form and the written information for the patient.

If the patient will be under sedation and/or not able to provide the consent, the study doctor will check if a legal authorized representative is assigned to the patient as a previous condition (eg: interdicted patient).

If the patient is not under the legal authorized representative's responsibility, the study doctor, based on a risk-benefit assessment, will decide to include the patient in the study if eligible. When the patient will regain capacity, it is expected that the patient is informed and provides their consent to continue participation in the trial.

This consent from the patient to remain in the study should be sought as soon as the condition of the patient will allow it.

The same procedure applies to processing of personal data of the patient and providing of consent to the processing of personal data according to the European Regulation n. 2016/679 on the Protection of Personal Data, the Personal Data Protection Act 2018 and subsequent amendments and additions, the provisions and guidelines of the National Data Protection Authorities, as well as any other applicable data protection laws and regulations.

Each patient's or acceptable third party's signed informed consent(s) must be kept on file by the Investigator. One copy must be given to the patient or the person consenting on their behalf.

16. SOURCE DOCUMENTS/DATA

16.1 Recording of source data

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

16.2 Direct access to source document/data

The Investigators or designated must permit trial-related monitoring, audits, Ethics Committee/Institutional Review Board review or regulatory inspection, providing direct access to source data/documents.

17. STUDY MONITORING

Monitoring will be performed by the CRO CROMSOURCE who has been designated by Chiesi.

It is understood that the monitor(s) will contact and visit the Investigator/centre before the study, regularly throughout the study and after the study had been completed, and that they will be permitted to inspect the various study records: case reports form, Investigator study file and source data, provided that patient confidentiality is respected.

The purposes of these visits are:

- To assess the progress of the study;
- To review the compliance with the study protocol;
- To discuss any emergent problem;
- To validate the contents of the CRFs against the source documents;
- To assess the status of drug storage, dispensing and retrieval;
- Prior to each monitoring visit, the Investigator or staff will record all data generated since the last visit on the case report forms. The Investigator and/or study staff will be expected to be available for at least a portion of the monitoring visit to answer questions and to provide any missing information.
- It is possible that the Investigator site may be audited by Sponsor personnel or regulatory national and/or international regulatory agencies during and after the study has been completed.

Given the current pandemic situation the proper approaches will be implemented remotely to allow the performance of the monitoring activities.

18. QUALITY ASSURANCE

The R&D Quality Assurance Department of Chiesi may perform an audit at any time according to the Sponsor's Standard Operating Procedures, in order to verify whether the study is being conducted in agreement with Good Clinical Practices and the protocol.

19. INSURANCE AND INDEMNITY

Chiesi holds and will maintain an adequate insurance policy covering damages arising out of Chiesi's sponsored clinical research studies.

Chiesi will indemnify the Investigator and hold him/her harmless for claims for damages arising out of the investigation, in excess of those covered by his/her own professional liability insurance, providing that the drug was administered under his/her or deputy's supervision and in strict accordance with accepted medical practice and with the study protocol.

The Investigator must notify Chiesi immediately upon notice of any claims or lawsuits.

20. CONFIDENTIALITY

All study documents are provided by the Sponsor in confidence to the Investigator and his/her appointed staff. None of this material may be disclosed to any party not directly involved in the study without written permission from Chiesi.

The Investigator must assure the patient's anonymity will be maintained. The Investigator will keep a separate list with at least the initials, the patient's study numbers, names, and addresses and

telephone numbers. The Investigator will maintain this for the longest period of time allowed by his/her own institution and, in any case, until further communication from Chiesi.

21. PREMATURE TERMINATION OF THE STUDY

Both the Sponsor and the Investigator reserve the right to terminate the study at any time. Should this be necessary, the procedures for an early termination or temporary halt will be arranged after consultation by all involved parties.

The Sponsor should submit a written notification to the Regulatory Authority concerned and Ethics Committee/Institutional Review Board providing the justification of premature ending or of the temporary halt.

22. CLINICAL STUDY REPORT

The clinical study report, including the statistical and clinical evaluations, shall be prepared and sent to the Coordinating Principal Investigator for agreement and signature.

At the end of the trial a summary of the clinical study report will be provided to all Ethics Committees/Institutional Review Boards, to the Competent Authority of the UK, Italy and USA concerned and to Investigators.

23. RECORD RETENTION

After completion of the study, all documents and data relating to the study will be kept in an orderly manner by the Investigator in a secure study file.

Regulations require that essential documents must be retained for at least two years after the final marketing approval in an ICH region or until two years have elapsed since the formal interruption of the clinical development of the product under study.

It is the responsibility of the Sponsor to inform the Investigator of when these documents can be destroyed. The Investigator must contact Chiesi before destroying any trial-related documentation. In addition, all patients' medical records and other source documentation will be kept for the maximum time permitted by the institution.

24. PUBLICATION OF RESULTS

Chiesi is entitled to publish and/or present any results of this study at scientific meetings, and to submit the clinical trial data to national and international Regulatory Authorities and, if they fall under the Chiesi commitments on Clinical Trial Transparency, to make them available on www.chiesi.com. Chiesi furthermore reserves the right to use such data for industrial purposes.

In the absence of a Study Steering Committee, Investigators will inform Chiesi before using the results of the study for publication or presentation and agree to provide the Sponsor with a copy of the proposed presentation. Data from individual study sites must not be published separately.

Negative as well as positive results should be published or otherwise made publicly available according to the relevant regulatory requirements.

25. REFERENCES

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APPENDIX 1 - Approval of the protocol by clinical investigator(s)

MULTICENTER OPEN LABEL RANDOMISED TRIAL TO ASSESS THE EFFICACY AND TOLERABILITY OF PORACTANT ALFA (PORCINE SURFACTANT, CUROSURF®) IN HOSPITALIZED PATIENTS WITH SARS-COV-19 ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS)

Product: Curosurf®

Pharmaceutical Form: poractant alfa

Approval of Clinical Study Protocol by the Coordinating Investigator (Italy):

I have carefully read this protocol and I agree that it contains all the necessary information required to conduct the study and I agree to conduct it as described.

I understand that this trial will not be initiated without Research Ethics Committee/Institutional Review Board approvals and that the administrative requirements of the governing body of the institution will be fully complied with.

Informed written consent will be obtained from all participating patients and appropriately documented, prior to their enrolment in the study.

The undersigned agrees that the trial will be carried out in conformity with the Declaration of Helsinki (as applicable, with attention being drawn to Section concerning freely given consent), ICH E6 Good Clinical Practices and with all the other local laws and regulations relevant to the use of new and approved therapeutic agents in patients.

Coordinating Investigator's Name: _____, MD

Centre No.: _____

_____ _____

Signature Date

Chiesi Farmaceutici S.p.A.
Via Palermo 26/A
43122 Parma - Italy

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Principal Investigator's Name: _____, MD

Centre No.: _____

_____ _____

Signature Date

Chiesi Farmaceutici S.p.A.
Via Palermo 26/A
43122 Parma - Italy