



Double-blind, randomized, controlled, clinical trial to assess the efficacy of allogenic mesenchymal stromal cells in patients with acute respiratory distress syndrome due to COVID-19.

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LIST OF ABBREVIATIONS

Abbreviation	Definition
ACE2	Angiotensin-Converting Enzyme 2
ADR	Adverse Drug Reaction
AE	Adverse Event
AEMPS	Agencia Española de Medicamentos y Productos Sanitarios (Spanish Medicines Agency)
AESI	Adverse Events of Special Interest
ARDS	Acute Respiratory Distress Syndrome
BM	Bone Marrow
CEIm	<i>Comité de Ética de la Investigación con Medicamentos</i> (Research Ethics Committee for Medicinal Products)
COVID-19	Coronavirus Disease 19
CRF	Case Report Form
CRP	C-reactive Protein
ECMO	Extracorporeal Membrane Oxygenation
GMP	Good Manufacture Practices
ICU	Intensive Care Unit
IL	Interleukin
MSC	Mesenchymal Stromal Cell
PCR	Polymerase Chain Reaction
SAE	Serious Adverse Event
SOC	Standard of Care
SOFA	Sequential Organ Failure Assessment
SSF	Saline Solution
SUSAR	Suspected Unexpected Serious Adverse Response
VM	Mechanical Ventilation
WHO	World Health Organization

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1. TRIAL SYNOPSIS

Study Title	Double-blind, randomized, controlled, clinical trial to assess the efficacy of allogenic mesenchymal stromal cells in patients with acute respiratory distress syndrome due to COVID-19.
EudraCT Number	2020-002193-27
Rationale Summary	<p>COVID-19 patients present in many cases with acute respiratory distress syndrome (ARDS), a severe pulmonary complication characterized by a disruption of the alveolar-epithelial barrier, interstitial edema and inflammatory damage. COVID-19-associated ARDS leads to progressive acute respiratory failure, for which there is no specific treatment beyond supportive care and mechanical ventilation. Patient outcomes are dismal, with high mortality rates greater than 60% in patients who require admission to intensive care units (ICU).</p> <p>Mesenchymal stromal cells (MSC) have immunomodulatory and tissue-regenerative properties for which they are widely used in cellular therapy. MSC are safe and effective in acute respiratory failure and ARDS of multiple causes, including promising preliminary evidence in ARDS in COVID-19. The Hospital Universitario Puerta de Hierro Majadahonda (HUPHM) has experience in the production and use of MSC in various immunomodulatory and regenerative indications, as well as in clinical investigation in COVID-19 and in the management of ARDS in these patients. Here, we present a summary of the rationale and justification to study the efficacy of MSC therapy in COVID-19 patients with ARDS through a double-blind, randomized, controlled clinical trial.</p> <p>– ARDS in COVID-19 patients: ARDS is a life-threatening pulmonary disorder that can associate with multiple causes. It characterizes by diffuse alveolar and capillary endothelial damage which lead to acute hypoxemia and non-cardiogenic pulmonary edema with bilateral radiographic opacities.</p> <ul style="list-style-type: none"> ▪ According to the largest reported series, ARDS occurs in approximately 30% of hospitalized COVID-19 patients (17%-42%), and in more than 90% of those critically ill. ▪ The severity of ARDS can be measured by the pressure of arterial oxygen to fraction of inspired oxygen (PaO₂/FiO₂) ratio (Berlin criteria). Moderate and severe ARDS are defined by PaO₂/FiO₂ ratios of ≤200 mmHg and ≤100mmHg, respectively. Of note, if arterial blood gas measurement cannot be obtained, the pulse oximetric saturation SaO₂/FiO₂ ratio can be used as a reliable surrogate. ▪ Despite improvement in ICU supportive care, to date, no pharmacologic therapy has effectively addressed the disease-specific pathophysiology or improved survival in these patients. Thus, reported mortality rates of COVID-19 in patients with moderate to severe ARDS remain very high at 69% (52%-91%). ▪ In addition to a very high mortality, in many survivors the initial exudative phase is followed by a fibrotic phase that causes long-term morbidity.

- **MSC in ARDS:** Multiple studies support the use of MSC in various forms of acute lung injury and ARDS.
 - **Preclinical models** have shown that **MSC can be beneficial in acute lung injury, the preclinical correlate of ARDS.** They include models with microbial, endotoxin and chemical agents, using various sources of MSC (bone marrow, adipose tissue and cord blood), delivered by either intravenous systemic or direct airway administration, using both fresh and cryopreserved MSC, usually as a single dose in a range from 5.0×10^4 to 3.6×10^7 cells/kg.
 - MSC have been shown to exert **anti-inflammatory, anti-bacterial, and pro-angiogenic effects** leading to reduced inflammation, enhancement of pathogen and fluid clearance, improvement of **tissue repair and decreased mortality.** These effects, and their impact on reduced mortality are maintained across the different preclinical models, MSC origin, method of preparation and timing and route of administration.
 - **Clinical studies of MSC in ARDS** with various designs, sample-sizes, MSC sources and doses have **uniformly confirmed safety**, with no adverse events, and the largest trials have suggested **reductions in inflammatory markers, improvement in PaO₂/FiO₂ ratio and/or reduction in mortality** with MSC. The largest clinical trial of MSC in ARDS in viral infections included 61 patients with **ARDS caused by H7N9 influenza**, showed a significant **reduction of mortality in the experimental arm with 3-4 doses of 1×10^6 MSC/kg.**
- **MSC in COVID-19 patients with ARDS:** Preliminary experience has been reported on the use of MSC in **a total of eight COVID-19 patients with moderate to severe ARDS:**
 - Liang *et al.* treated a single case of a critically ill 65-year old woman with COVID-19 ARDS with three intravenous MSC infusions. The patient was extubated and **off the ventilator within four days of the second MSC infusion**, inflammation parameters improved, T Cell counts increased, and no related adverse events were observed.
 - Leng *et al.* treated **seven patients with different degrees of COVID-19 ARDS severity**, including one critically severe case requiring invasive ventilation in ICU:
 - From a clinical perspective, out of three patients who received placebo as a control group, two deteriorated. However, all seven patients in the MSC experimental group, including the one classified as critically severe, showed **clinical improvement within 2-3 days of a single intravenous dose of 1×10^6 MSC/kg**, in addition to no infusion reactions or severe related adverse events.
 - An important **additional finding of this study of MSC in COVID-19** is that gene expression profile of the infused MSC showed them not to express angiotensin-converting enzyme 2 (ACE2) or serine protease TMPRSS2, which are required for SARS-CoV-2 cell entry. Therefore, the **absence of ACE2 and TMPRSS2 indicate that infused MSC in COVID-19 patients are free from SARS-CoV-2 infection**, which would have otherwise been one of the potential limitations to the success of this strategy.

	<p>– Summary of center's experience and benefits and risks of the proposal:</p> <ul style="list-style-type: none"> ▪ The occurrence of ARDS and acute respiratory failure in COVID-19 patients is common and represents a dramatic clinical scenario. <ul style="list-style-type: none"> • With the current absence of treatment options, beyond respiratory and supportive care, patient outcomes are dismal. • MSC offer immunomodulatory and tissue-regenerative properties, which make them a promising therapeutic option in this clinical setting. • In particular, MSC safety is uniformly shown in preclinical and clinical studies in ARDS and other indications. ▪ HUPHM experience to carry out the proposed clinical trial: <ul style="list-style-type: none"> • The GMP Unit at the Department of Hematology produces autologous and allogeneic MSC derived from bone marrow (BM) for immunomodulatory and regenerative indications for more than a decade, it has participated in multiple national and international research projects and clinical trials, and is authorized for MSC production by the AEMPS (certificate of GMP compliance; renovated on 28/05/2019). • Currently, HUPHM has a surplus of MSC doses produced prior to the COVID-19 pandemic readily available to supply this trial. • In addition to production and use of MSC in Hematology, the investigators team includes the Departments of Clinical Pharmacology, Intensive Medicine, Respiratory Medicine and others. This team shares experience in the clinical management of COVID-19 patients during the current pandemic and in clinical investigation in this setting, including an on-going clinical trial (ConPLAS-19; CT Number: NCT04345523), which is complementary to the current proposal. <p>In summary, in the midst of a worldwide pandemic of SARS-CoV-2, MSC represent a potential therapeutic option with a favorable benefit/risk balance for severe COVID-19 patients with ARDS for whom we currently have no available treatments. Thus, we propose to conduct a double-blind, randomized, controlled, clinical trial to assess the efficacy of allogenic MSC in patients with ARDS due to COVID-19.</p>
Trial Design	<p>A double-blind, randomized, controlled, clinical trial to evaluate the efficacy and safety of MSC (mesenchymal stromal cells) intravenous administration in patients with COVID-induced ARDS compared to a control arm.</p> <p>All trial participants will receive SOC*.</p> <p>Randomization will be 1:1 between:</p> <ul style="list-style-type: none"> ▪ Treatment arm: Allogenic MSC. ▪ Control arm: SSF + 4% albumin (same composition as the experimental treatment, without the MSC). <p><i>*In the context of the current worldwide pandemic for which we have no approved medicines or vaccines, SOC can include medicines that are being used in clinical practice (e.g. lopinavir/ritonavir; hydroxy/chloroquine, tocilizumab, etc.).</i></p>
Center	Hospital Universitario Puerta de Hierro Majadahonda
Trial Participants	Hospitalized adults (≥18 years old) with moderate to severe ARDS (according to the Berlin Definition) due to COVID-19 infection.

Planned Sample Size	20 patients (1:1 ratio, for MSC:control arm).
Follow-up duration	12 months
Trial Period	<p>16 months (3 months for recruitment + 12 months for follow-up + 1 month for final analysis).</p> <ul style="list-style-type: none"> Of note, the primary data analysis will be conducted when all patients reach day 28 after treatment. Prior to this analysis, a statistical plan will be in place with strict provisions to preserve data integrity according to ICH and other applicable guidelines. All patients will be followed until the end of study for safety purposes.
Primary Objective	<ul style="list-style-type: none"> To assess the efficacy of MSC versus a control arm as described in the primary endpoint.
Secondary Objective	<ul style="list-style-type: none"> To evaluate the effects of MSC on the secondary efficacy endpoints. To evaluate the safety and tolerability profiles of MSC.
Exploratory Objectives	<ul style="list-style-type: none"> To study soluble and cellular biomarkers that might be involved in the course of the disease and the response to the investigational product.
Primary Endpoint	<ul style="list-style-type: none"> Change in the $\text{PaO}_2/\text{FiO}_2^*$ ratio from baseline to day 7 of treatment administration, or to the last available $\text{PaO}_2/\text{FiO}_2$ ratio if death occurs before day 7. <p><i>* If an arterial blood gas result cannot be obtained, the $\text{SaO}_2/\text{FiO}_2$ ratio could be substituted for the $\text{PaO}_2/\text{FiO}_2$ ratio [TW Rice, et al. Chest 2007].</i></p>
Secondary Endpoints	<p><u>Clinical</u></p> <ul style="list-style-type: none"> All-cause mortality on days 7, 14, and 28 after treatment. $\text{PaO}_2/\text{FiO}_2$ ratio at baseline and days 2, 4, 7, 14 and 28 after treatment. Oxygen saturation (by standardized measurement) at baseline, daily until day 14, and on day 28 after treatment. Time to $\text{PaO}_2/\text{FiO}_2$ ratio greater than 200 mmHg. Subjects' clinical status on the WHO 7-point ordinal scale at baseline, daily until day 14, and on day 28 after treatment. Time to an improvement of one category from admission on the WHO 7-point ordinal scale. Percentage of patients that worsen at least one category on the WHO 7-point ordinal scale. Percentage of patients that improve at least one category (maintained 48h) on the WHO 7-point ordinal scale. SOFA scale at baseline and days 2, 4, 7, 14 and 28 after treatment. Duration of hospitalization (days). Duration of ICU stay (days). Oxygen therapy-free days in the first 28 days after treatment. Duration of supplemental oxygen. Incidence of and duration of non-invasive and invasive mechanical ventilation in the first 28 days after treatment. Mechanical ventilation-free days in the first 28 days after treatment. Ventilation parameters. Incidence of new onset pulmonary fibrosis at 3 and 12 months after treatment, based on CT scan and pulmonary function tests. Survival at 3 and 12 months. <p><u>Safety</u></p> <ul style="list-style-type: none"> Cumulative incidence of serious adverse events (SAEs) and Grade 3 and 4

	<p>adverse events (AEs).</p> <ul style="list-style-type: none"> ▪ Cumulative incidence of adverse drug reactions (ADR) in the experimental treatment arm. ▪ Cumulative incidence of AEs of special interest. <p><u>Analytical endpoints</u></p> <ul style="list-style-type: none"> ▪ Levels of analytical markers (CRP, lymphocyte and neutrophil counts, lymphocyte subpopulations, LDH, ferritin, D-dimer, coagulation tests and cytokines...) at baseline and days 2, 4, 7, 14 and 28 after treatment. ▪ Other soluble and cellular biomarkers that might be involved in the course of the disease and the response to MSC.
Main Inclusion Criteria	<ol style="list-style-type: none"> 1. Informed consent prior to performing study procedures (witnessed oral consent with written consent by representatives will be accepted to avoid paper handling). Written consent by patient or representatives will be obtained whenever possible. 2. Adult patients ≥18 years of age at the time of enrolment. 3. Laboratory-confirmed SARS-CoV-2 infection as determined by PCR, in oropharyngeal swabs or any other relevant specimen obtained during the course of the disease. Alternative tests (e.g., rapid antigenic tests) are also acceptable as laboratory confirmation if their specificity has been accepted by the Sponsor. 4. Moderate to severe ARDS (PaO₂/FiO₂ ratio equal or less than 200 mmHg) for less than 96 hours at the time of randomization. 5. Patients requiring invasive ventilation are eligible within 72 hours from intubation. 6. Eligible for ICU admission, according to the clinical team.
Main Exclusion Criteria	<ol style="list-style-type: none"> 1. Imminent and unavoidable progression to death within 24 hours, irrespective of the provision of treatments (in the opinion of the clinical team). 2. "Do Not Attempt Resuscitation" order in place. 3. Any end-stage organ disease or condition, which in the investigator's opinion, makes the patient an unsuitable candidate for treatment. 4. History of a moderate/severe lung disorder requiring home-based oxygen therapy. 5. Patient requiring ECMO, hemodialysis or hemofiltration at the time of treatment administration. 6. Current diagnosis of pulmonary embolism. 7. Active neoplasm, except carcinoma in situ or basalioma. 8. Known allergy to the products involved in the allogenic MSC production process. 9. Current pregnancy or lactation (women with childbearing potential should have a negative pregnancy test result at the time of study enrollment). 10. Current participation in a clinical trial with an experimental treatment for COVID-19 (<i>the use of any off-label medicine according to local treatment protocols is not an exclusion criteria</i>). 11. Any circumstances that in the investigator's opinion compromises the patient's ability to participate in the clinical trial.
Investigational Product	<ul style="list-style-type: none"> ▪ BM-derived allogenic MSC.
Dose	<ul style="list-style-type: none"> ▪ Patients receive a single dose of approximately 1x10⁶ cells/kg on day 0.

Control arm	▪ Saline Solution + 4% albumin (same composition as the treatment under study, but without the MSC).
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2. INTRODUCTION

Study Rationale and Background

COVID-19 is a new disease caused by a novel coronavirus (SARS-CoV-2) for which there is currently no vaccine or authorized therapeutic agents. COVID-19 patients present in many cases with acute respiratory distress syndrome (ARDS), a severe pulmonary complication characterized by a disruption of the alveolar-epithelial barrier, interstitial edema and inflammatory damage. COVID-19-associated ARDS leads to progressive acute respiratory failure, for which there is no specific treatment beyond supportive care and mechanical ventilation. Patient outcomes are dismal, with high mortality rates greater than 60% in patients who require admission to intensive care units (ICU).

Mesenchymal stromal cells (MSC) have immunomodulatory and tissue-regenerative properties for which they are widely used in cellular therapy. MSC are safe and effective in acute respiratory failure and ARDS of multiple causes, including promising preliminary evidence in ARDS in COVID-19. The Hospital Universitario Puerta de Hierro Majadahonda (HUPHM) has experience in the production and use of MSC in various immunomodulatory and regenerative indications, as well as in clinical investigation in COVID-19 and in the management of ARDS in these patients.

Here, we present a summary of the rationale and justification to study the efficacy of MSC therapy in COVID-19 patients with ARDS through a double-blind, randomized, controlled clinical trial.

ARDS in COVID-19 patients

ARDS is a life-threatening pulmonary disorder that can associate with multiple causes. It characterizes by diffuse alveolar and capillary endothelial damage which lead to acute hypoxemia and non-cardiogenic pulmonary edema with bilateral radiographic opacities.

- According to the largest reported series, ARDS occurs in approximately 30% of hospitalized COVID-19 patients (17%-42%), and in more than 90% of those critically ill.
- The severity of ARDS can be measured by the pressure of arterial oxygen to fraction of inspired oxygen ($\text{PaO}_2/\text{FiO}_2$) ratio (Berlin criteria). Moderate and severe ARDS are defined by $\text{PaO}_2/\text{FiO}_2$ ratios of ≤ 200 mmHg and ≤ 100 mmHg, respectively. Of note, if arterial blood gas measurement cannot be obtained, the pulse oximetric saturation $\text{SaO}_2/\text{FiO}_2$ ratio can be used as a reliable surrogate.
- Despite improvement in ICU supportive care, to date, no pharmacologic therapy has effectively addressed the disease-specific pathophysiology or improved survival in these patients. Thus, reported mortality rates of COVID-19 in patients with moderate to severe ARDS remain very high at 69% (52%-91%).
- In addition to very high mortality, in many survivors the initial exudative phase is followed by a fibrotic phase that causes long-term morbidity.

MSC in patients with ARDS

Multiple studies support the use of MSC in various forms of acute lung injury and ARDS.

- Preclinical models have shown that MSC can be beneficial in acute lung injury, the preclinical correlate of ARDS. They include animal and human lung explant models with microbial,

endotoxin and chemical agents, using various sources of MSC (bone marrow [BM], adipose tissue and cord blood), delivered by either intravenous systemic or direct airway administration, using both fresh and cryopreserved MSC, usually as a single dose in a range from 5.0×10^4 to 3.6×10^7 cells/kg.

- MSC have been shown to exert anti-inflammatory, anti-bacterial, and pro-angiogenic effects leading to reduced inflammation, enhancement of pathogen and fluid clearance, improvement of tissue repair and decreased mortality. These effects, and their impact on reduced mortality are maintained across the different preclinical models, MSC origin, method of preparation and timing and route of administration.
- Clinical studies of MSC in ARDS with various designs, sample-sizes, MSC sources and doses have uniformly confirmed safety, with no adverse events, and the largest trials have suggested reductions in inflammatory markers, improvement in $\text{PaO}_2/\text{FiO}_2$ ratio and/or reduction in mortality with MSC. The largest clinical trial of MSC in ARDS in viral infections included 61 patients with ARDS caused by H7N9 influenza, showed a significant reduction of mortality in the experimental arm with 3-4 doses of 1×10^6 MSC/kg.

MSC in COVID-19 patients with ARDS

Preliminary experience has been reported on the use of MSC in a total of eight COVID-19 patients with moderate to severe ARDS:

- Liang *et al.* treated a single case of a critically ill 65-year old woman with COVID-19 ARDS with three intravenous MSC infusions. The patient was extubated and off the ventilator within four days of the second MSC infusion, inflammation parameters improved, T cell counts increased, and no related adverse events were observed.
- Leng *et al.* treated seven patients with different degrees of COVID-19 ARDS severity, including one critically severe case requiring invasive ventilation in ICU:
 - From a clinical perspective, out of three patients who received placebo as a control group, two deteriorated. However, all seven patients in the MSC experimental group, including the one classified as critically severe, showed clinical improvement within 2-3 days of a single intravenous dose of 1×10^6 MSC/kg, in addition to no infusion reactions or severe related adverse events.
 - An important additional finding of this study of MSC in COVID-19 is that gene expression profile of the infused MSC showed them not to express angiotensin-converting enzyme 2 (ACE2) or serine protease TMPRSS2, which are required for SARS-CoV-2 cell entry. Since one of the potential limitations to the success of this strategy would be the risk of infection of the administered MSC by the causative virus, the absence of ACE2 and TMPRSS2 indicate that infused MSC in COVID-19 patients are free from SARS-CoV-2 infection.

Summary of benefits and risks of the proposal

- The occurrence of ARDS and acute respiratory failure in COVID-19 patients is common and represents a dramatic clinical scenario.
- With the current absence of treatment options, beyond respiratory and supportive care, patient outcomes are dismal.

- MSC offer immunomodulatory and tissue-regenerative properties, which make them a promising therapeutic option in this clinical setting.
- In particular, MSC safety is uniformly shown in preclinical and clinical studies in ARDS and other indications.

Summary of HUPHM experience to carry out the proposed clinical trial:

- The GMP Unit at the Department of Hematology produces autologous and allogeneic MSC derived from BM for immunomodulatory and regenerative indications for more than a decade, it has participated in multiple national and international research projects and clinical trials, and is authorized for MSC production by the AEMPS (certificate of GMP compliance; renovated on 28/05/2019).
- Currently, HUPHM has a surplus of MSC doses produced prior to the COVID-19 pandemic readily available to supply this trial.
- In addition to production and use of MSC in Hematology, the investigators team includes the Departments of Clinical Pharmacology, Intensive Medicine, Respiratory Medicine and others. This team shares experience in the clinical management of COVID-19 patients during the current pandemic and in clinical investigation in this setting, including an on-going clinical trial (ConPLAS-19; CT Number: NCT04345523), which is complementary to the current proposal.

In summary, MSC represent a potential therapeutic option with a favorable benefit/risk balance for COVID-19 patients with ARDS, for whom we currently have no available treatments. Based on this unresolved therapeutic need, we propose to conduct a randomized, controlled clinical trial of MSC treatment in patients with severe COVID-19 pneumonia.

3. OBJETIVES AND ENDPOINTS

The main objective of this study is to assess the clinical efficacy of allogenic MSC versus a control arm.

PRIMARY	
OBJECTIVES	ENDPOINTS (OUTCOME MEASURES)
To evaluate the clinical efficacy of allogenic MSC versus placebo.	<ul style="list-style-type: none"> Change in the $\text{PaO}_2/\text{FiO}_2$¹ ratio from baseline to day 7 of treatment administration, or to the last available $\text{PaO}_2/\text{FiO}_2$ ratio if death occurs before day 7.
SECONDARY	
OBJECTIVES	ENDPOINTS (OUTCOME MEASURES)
<p>To evaluate effects of allogenic MSC on secondary efficacy endpoints as compared to Placebo as assessed by:</p> <p><u>Clinical aspects</u></p> <ul style="list-style-type: none"> Mortality: <ul style="list-style-type: none"> 15-day mortality 28-day mortality 	<ul style="list-style-type: none"> Date and cause of death (if applicable).
<ul style="list-style-type: none"> Oxygenation: <ul style="list-style-type: none"> Changes in $\text{PaO}_2/\text{FiO}_2$ ratio Oxygenation-free days in the first 28 days after treatment. 	<ul style="list-style-type: none"> $\text{PaO}_2/\text{FiO}_2$ ratio¹ at baseline and days 2, 4, 14 and 28 after treatment. Time to $\text{PaO}_2/\text{FiO}_2$ ratio greater than 200 mmHg Oxygen saturation (by standardized measurement) at baseline, daily until day 14, and on day 28 after treatment. Duration of supplemental oxygen (if applicable), and Oxygenation-free days
<ul style="list-style-type: none"> Mechanical Ventilation <ul style="list-style-type: none"> Invasive ventilation-free days in the first 28 days after treatment. Ventilatory parameters 	<ul style="list-style-type: none"> Incidence of and duration of invasive and non-invasive mechanical ventilation (if applicable). Days without MV Intubation/ extubating dates Ventilatory parameters
<ul style="list-style-type: none"> Hospitalization <ul style="list-style-type: none"> Duration of hospitalization (days) Duration of ICU stay (days) 	<ul style="list-style-type: none"> Duration of hospitalization. Duration of ICU stay
<ul style="list-style-type: none"> Clinical severity <ul style="list-style-type: none"> Clinical status on the WHO 7-point ordinal scale at baseline, daily until day 14 and on day 28 after 	<ul style="list-style-type: none"> WHO Ordinal scale: <ol style="list-style-type: none"> Not hospitalized, no limitations on activities. Not hospitalized, limitation on activities.

¹ If an arterial blood gas result cannot be obtained, the $\text{SaO}_2/\text{FiO}_2$ ratio could be substituted for the $\text{PaO}_2/\text{FiO}_2$ ratio [TW Rice, et al. Chest 2007].

<p>treatment:</p> <ul style="list-style-type: none"> SOFA scale at baseline and on days 2, 4, 7, 14 and 28 after treatment. 	<ul style="list-style-type: none"> 3. Hospitalized, not requiring supplemental oxygen. 4. Hospitalized, requiring supplemental oxygen. 5. Hospitalized, on non-invasive ventilation or high flow oxygen devices. 6. Hospitalized, on invasive mechanical ventilation or ECMO². 7. Death. • Time to an improvement of one category from admission using an ordinal scale. • Percentage of patients that worsen at least one category • Percentage of patients that improve at least one category (maintained 48h). • SOFA scale
<ul style="list-style-type: none"> Pulmonary fibrosis <ul style="list-style-type: none"> New onset pulmonary fibrosis at 3 and 12 months after treatment, based on CT scan and pulmonary function tests 	<ul style="list-style-type: none"> Incidence of and date of onset (if applicable). Respiratory function tests X-ray or CT scan
<ul style="list-style-type: none"> Survival <ul style="list-style-type: none"> 3-month survival 12-month survival 	<ul style="list-style-type: none"> Date and cause of death (if applicable).
<ul style="list-style-type: none"> Laboratory parameters <ul style="list-style-type: none"> Changes in serum levels of analytical markers at baseline and on days 2, 4, 7, 14 and 28 after treatment 	<ul style="list-style-type: none"> Levels of CRP, lymphocyte and neutrophil counts lymphocyte subpopulations, D-Dimer, LDH, ferritin, coagulation tests and cytokines.
<p>To evaluate safety of allogenic MSC as compared to placebo</p> <p>Safety through day 28 and at 3 and 12 months after treatment</p> <ul style="list-style-type: none"> Cumulative incidence of serious adverse events (SAEs) Cumulative incidence of Grade 3 and 4 adverse events (AEs) Cumulative incidence of adverse reactions (ADR) in the experimental treatment arm Cumulative incidence of adverse events of special interest (AESIs) 	<ul style="list-style-type: none"> Serious adverse events (SAEs) Grade 3 or 4 Adverse events (AEs) Adverse drug reactions (ADR; experimental treatment arm) Adverse events of special interest (AESIs)
EXPLORATORY	
OBJECTIVES	ENDPOINTS (OUTCOME MEASURES)
<p>To study soluble and cellular biomarkers that might be involved in the course of the disease and the response to the investigational product</p>	<ul style="list-style-type: none"> Soluble and cellular biomarkers

² Extracorporeal membrane oxygenation

4. CLINICAL TRIAL DESIGN

4.1. Type of Study and Overall Design

This is a double-blind, randomized, controlled, clinical trial to evaluate the efficacy of allogenic MSC in patients with ARDS due to COVID-19. All trial participants will receive SOC and one of the following, according to treatment allocation (1:1):

- Experimental Treatment: Allogenic BM- derived MSC
- Control treatment (placebo): SSF+ 4% albumin (same composition as the experimental treatment, without the MSC).

MSC will be administered intravenously as one single dose of 1×10^6 cells/kg on day 0. The control arm will receive matching placebo, which will consist of an equivalent volume of SSF and 4% albumin. All patients will receive the SOC as per clinical practice. In current the context of the worldwide COVID pandemic, there are no approved medicines or vaccines for the treatment of the COVID-19. Therefore, for the purpose of this study, SOC would also include any medicines that are being used in clinical practice (e.g. lopinavir/ritonavir; hydroxy/chloroquine, tocilizumab, etc.).

For operational purposes, the study will be divided into two periods:

- **Period 1:** from day 0 until day 28 after treatment.
- **Period 2:** from day 29 until month 12 after treatment.

While hospitalized, patients will be assessed on a daily basis from treatment administration until day 14. Patients discharged before that timepoint will be regularly called on the phone or asked to attend face-to-face study visits. All patients will undergo a series of efficacy, safety, and laboratory assessments (see section 8). The main analysis will cover the period until day 28 after treatment (period 1). For safety purposes, patients will be followed-up for a year after treatment (period 2). The double-blind design will be maintained (for both, patient and evaluating clinician) until the communication of results by the implementation of a blinding plan for study treatment administration, and blinded clinical evaluations by the clinician.

4.2. Study participation duration

Duration of the recruitment: 3 months.

Duration of the follow-up per patient: 12 months

Total study duration (enrolment + follow-up + analysis): 16 months.

The end of study is defined as the last visit of the last patient enrolled. Patients will complete their participation in the clinical trial at the end of the follow-up, planned at 12 months after treatment administration.

4.3. Early termination of the trial

The Sponsor could elect to close the study at any time. The Research Ethics Committee (CEIm) and the Competent National Authority must be informed within 15 days of early termination. The trial will be terminated prematurely in the following cases:

- If adverse events occur that are so serious that the risk-benefit ratio is not acceptable.
- If the number of dropouts is so high that proper completion of the trial cannot realistically be expected.

5. STUDY POPULATION

5.1. Inclusion Criteria

1. Informed consent prior to performing study procedures (oral consent will be accepted in order to avoid paper handling). Written consent by patient or representatives will be obtained whenever possible.
2. Adult patients ≥ 18 years of age at the time of enrolment.
3. Laboratory-confirmed SARS-CoV-2 infection as determined by PCR, in naso- or oropharyngeal swabs or any other relevant specimen obtained during the course of the disease. Alternative tests (e.g., rapid antigenic tests) are also acceptable as laboratory confirmation is their specificity has been accepted by the Sponsor.
4. Moderate to severe ARDS ($\text{PaO}_2/\text{FiO}_2$ ratio equal or less than 200 mmHg) for less than 96 hours at the time of randomization.
5. Patients requiring invasive ventilation are eligible within 72 hours from intubation.
6. Eligible for ICU admission, according to the clinical team.

5.2. Exclusion Criteria

1. Imminent and unavoidable progression to death within 24 hours, irrespective of the provision of treatments (in the opinion of the clinical team).
2. "Do Not Attempt Resuscitation" order in place.
3. Any end-stage organ disease or condition, which in the investigator's opinion, makes the patient an unsuitable candidate for treatment.
4. History of a moderate/severe lung disorder requiring home-based oxygen therapy.
5. Patient requiring ECMO, hemodialysis or hemofiltration at the time of treatment administration.
6. Current diagnosis of pulmonary embolism.
7. Active neoplasm, except carcinoma *in situ* or basalioma.
8. Known allergy to the products involved in the allogenic MSC production process.
9. Current pregnancy or lactation (women with childbearing potential should have a negative pregnancy test result at the time of study enrollment).
10. Current participation in a clinical trial with an experimental treatment for COVID-19 (the use of any off-label medicine according to local treatment protocols is not an exclusion criteria).
11. Any circumstances that in the investigator's opinion compromises the patient's ability to participate in the clinical trial.

5.3. Subject withdrawal criteria

A patient may be removed/withdrawn from the study for the following reasons:

- Patient withdraws consent or requests discontinuation from the study for any reason
- Termination of the study
- Lost to follow-up.
- Investigator's decision due to any medical reason for which continuing in the study is not considered an adequate option for the patient.

Patients will be able to withdraw from the study at any time. Patients will be listed as having withdrawn consent when they state that they no longer wish to participate in the study and do not authorize the Investigators to continue obtaining their outcome data.

For patients lost to follow-up, every effort to contact them will be made, attempts made will be documented in their medical chart.

Patients who withdraw from this study or are lost to follow-up after giving consent and administration of the study product, will not be replaced. The reason for patient discontinuation from the study will be recorded on the appropriate case report form (CRF).

Treatment discontinuation is not expected, since MSC therapy will be administered as one single dose on day 0.

6. STUDY TREATMENT

6.1. Investigational Medicinal Product and Dose

The IMP, as described in the Investigator's Brochure (IB), is composed of cultured MSC derived from BM and expanded under GMP conditions. For this study, the IMP used is part of a surplus of MSC from the GMP Unit of the Department of Hematology at HUPHM, which have been manufactured, preserved, and will be prepared for administration strictly following GMP guidelines. The dose will be approximately 1×10^6 cells/kg of recipient's weight.

6.2. Description of Treatment

6.2.1. Experimental Group:

- The patients in the experimental arm will receive a single dose of the IMP (20 mL with approximately 1×10^6 MSC/kg), administered intravenously. Patients will receive the IMP as add-on to the SOC.
- MSC are obtained by aspiration of approximately 30 mL of BM in the iliac crest of a donor, under general and local anesthesia and transferred to a bag containing culture medium (usually Dulbecco's Modified Eagle Medium-DMEM) and heparin. MSC are expanded in the GMP Unit using DMEM, supplemented with human platelet lysate (PL) and ciprofloxacin. Culture is performed in a humidified atmosphere with 5% CO₂ at 37°C for a maximum of four passages. Twice a week the medium is renewed, and the level of confluence is evaluated to decide on the need for passaging.
- MSC are collected following trypsinization and the final product is transferred to a sterile bag and carried to the Cryobiology Area (Processing Laboratory) in order to be processed for cryopreservation. All MSC doses will be stored in liquid nitrogen (approximately at -180°C) until administration is required.

6.2.2. Control Group:

- The patients in the control arm will receive a single dose of 20 mL of SSF+4% albumin (same composition as the experimental treatment, without the MSC), as add-on to SOC. Packaging, labelling, distribution, and administration processes will be carried out in the same manner as those for the IMP.

6.2.3. Standard of Care:

- All patients will receive the SOC as per clinical practice. In the current context of the worldwide COVID pandemic, there are no approved medicines or vaccines for the treatment of the COVID-19. Therefore, for the purpose of this study, SOC would also include any medicines that are being used in clinical practice (e.g. lopinavir/ritonavir; hydroxy/chloroquine, tocilizumab, etc.).

6.3. Treatment Administration

Distribution and administration of the IMP will be carried out according to the relevant standard operating procedures. These procedures are included in the center's quality accreditation by JACIE (www.jacie.org). Briefly, administration will be performed intravenously, in approximately 3-5 minutes with the aid of a syringe and a 3-way connector.

6.4. Treatment adherence

Treatment adherence will be ensured because the single dose of the experimental treatment and the control will be administered by a member of the investigator team on day 0.

6.5. Treatment preparation and accountability

MSC will be thawed in a water bath in the Processing Laboratory and without further manipulation will be transported to the clinical site in a temperature-controlled thermally insulated container. Distribution, and administration will be carried out according to the center's standard operating procedures.

The drug accountability will be performed at the Processing Laboratory.

6.6. Packaging and labelling

Packaging and labelling of the study drugs will be performed by the sponsor or its designee according GMP. The following information will be shown:

- Name, phone, and address of the main contact person in the clinical trial.
- Name of the IMP and comparator
- Pharmaceutical form, route of administration, cell dose
- Identification code of the therapeutic unit- Name of the study, protocol number
- Patient's identification (inclusion number
- Investigator's name
- Regulatory text: "For biomedical research use only"
- Storage conditions, expiration date.

The label will be verified by the investigator responsible for the administration, by double-checking the correlation of the patient's identification number, and date of birth, in order to guarantee that the product arrives to the corresponding patient.

The placebo (SSF + 4% albumin) will be supplied in the same packaging as the IMP for study purposes.

6.7. Concomitant Treatment

All concomitant therapies will be permitted in order for the patients to receive full supportive care during the study, as appropriate. For the purpose of the study, relevant concomitant treatment received during the study, including those for COVID-19, will be recorded in the CRF.

7. MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

The assignment to treatment will be carried out randomly and blinded, with a 1:1 allocation. Randomization will be done through a centralized system embedded in the electronic CRF (eCRF).

To ensure blinding, treatments will be prepared for administration at the GMP Unit and the administration of the treatment will be masked, not allowing the identification of the IMP.

Prior to including the first patients, a blinding plan will be in place at the site, agreed by the sponsor and the site personnel involved in the treatment preparation. As an additional measure to preserve the blinding, the team member administering the treatments (unblinded) will be a different person from the blinded investigators that will conduct the study assessments. Any unblinded team members will not share any information that could allow the identification of the treatment allocation.

8. ASSESSMENTS AND PROCEDURES

8.1. Screening Procedures

After informed consent is obtained, the following assessments will be performed to determine eligibility requirements, as specified in the selection criteria:

- Laboratory-confirmed SARS-CoV-2 infection as determined by PCR in naso-/oropharyngeal swabs or any other relevant specimen obtained during the course of the disease. Alternative tests (e.g., rapid antigenic tests) are also acceptable as laboratory confirmation is their specificity has been accepted by the Sponsor.
- Review of available imaging (X-ray and/or CT-scan), obtained during the course of the COVID-related hospitalization.
- Focused medical history, including the following information:
 - Date of onset of COVID-19 symptoms (fever and/or other symptoms).
 - History of chronic medical conditions related to the selection criteria or known risk factors for COVID-19.
 - Date of onset of PaO₂/FiO₂ ratio equal or less than 200 mmHg (if arterial blood gas results cannot be obtained or are not available, the SaO₂/FiO₂ ratio could be used).
- Confirm hospitalization/ICU admission period/duration due to COVID.
- Confirm the initiation date of the invasive mechanical ventilation (if applicable)
- Pregnancy test (only for women with childbearing potential).

The overall eligibility of the subject to participate in the study will be assessed once all screening data are available. The screening process can be suspended prior to complete assessment at any time, if exclusions are identified by the research team. Study subjects who qualify will be immediately randomized and treatment should be administered within 24 hours after randomization.

8.2. Visit 1 -Day 0

Visit 1 and the screening visit could be done at the same time. For the purpose of the analyses, assessments and measurements performed as part of Visit 1 will be considered to be the baseline measurements.

RANDOMIZATION

Participants will be randomly assigned to one of the following options:

- Experimental treatment: 1x10⁶ cells/kg
- Control (placebo): SSF+4% albumin

Treatment assignment will be performed using a central randomization service implemented in the eCRF.

PROCEDURES PRE-ADMINISTRATION

The following assessments are performed before the administration of the assigned treatment:

- WHO Ordinal scale.

- Vital signs including SpO₂, temperature and PaO₂/FiO₂ ratio.
- Targeted physical exam.
- Routine blood samples (arterial blood gases, haematology, biochemistry, coagulation CRP, LDH, ferritin, IL-6, and D-dimer levels).
- Review of relevant concomitant medications (including therapies related to COVID-19 SOC).

8.3. Efficacy Assessments

During period 1, the following assessments will be performed:

- Hospitalization.
- ICU admission.
- Ordinal scale (WHO 7-point ordinal scale).
- Review of relevant concomitant medications.
- Supplemental Oxygen requirement.
- Type of device for Oxygen supplementation (e.g., mask with reservoir bag).
- Non-invasive mechanical ventilation (via mask).
- Mechanical ventilator requirement (via endotracheal tube or tracheostomy tube).
- ECMO requirement.
- SpO₂.
- Temperature
- PaO₂/FiO₂ ratio.
- Mechanical Ventilation parameters (when applicable).
- SOFA scale.
- Death.

During period 2, the following assessments will be conducted:

- Respiratory function tests.
- Chest X-ray and/or CT-scan (according to clinical practice).
- Lab tests (according to clinical practice).
- Death (survival).

8.4. Safety Assessments

During the follow-up until the end of the study, the occurrence of adverse events and complications will be collected (i.e., SAEs, Grade 3 or 4 AEs, ADR [experimental treatment] and AESIs).

- Physical examination (period 1): a symptom-directed (targeted) physical examination will be performed to evaluate for any possible adverse event.
- Clinical laboratory evaluations (period 1): blood will be collected at the following time points: baseline and days 2, 4, 7, 14 and 28.

Clinical laboratory parameters include CRP, lymphocyte count and subpopulations, neutrophils, LDH, D-Dimer, ferritin, IL-6, coagulation tests and cytokines. Soluble and cellular biomarkers will also be collected. During period 2, clinical laboratory evaluations will be conducted in accordance with local clinical practice.

STUDY SCHEDULE

VISITS	Screening ¹	Day 0 ¹	Follow-Up VISITS ⁴				Day 7	Day 14	Day 28	3 m	12 m (EOS)
Day +/- Window	-3 to 1	0	Daily*					± 2	± 3	± 7	± 15
ASSESSMENTS/PROCEDURES											
Informed consent	X										
Inclusion and exclusion criteria	X	X									
Demographics & Medical History	X										
Pregnancy test	X										
SARS-CoV-2 test review	X ²										
X-ray review	X ²										
Randomization		X									
Administration of study treatment		X									
Clinical data collection		X	Daily*				X	X	X		
Ordinal scale		X	Daily*				X	X	X		
SOFA scale		X	Days 2 and 4				X	X	X		
Vital signs: SpO ₂ /T ^a	X	X	Daily*				X	X	X		
Oxygen requirement and oxygenation parameters	X ²	X	Daily*				X	X	X		
Mechanical ventilator requirement		X	Daily*				X	X	X		
Mortality			Daily*				X	X	X	X	X
Concomitant medication (Only related with COVID-19)		X	Daily*				X	X	X		
AE /SAE (eCRF reporting)		X	Daily*				X	X	X	X	X
Routine blood samples (Arterial blood gases, hematology, chemistry, coagulation tests)	X ²	X	Days 2 and 4				X	X	X	X ³	X ³
Respiratory function tests ³										X	X
Chest X-ray and/or CT-scan ³										X	X

*Daily until hospital discharge; EOS: End of study; m: months; AE: adverse events; SAE: serious adverse event; eCRF: electronic case report form.

¹Screening and visit 1 can be done at the same time; ²Review of clinical data, lab results or images performed during the course of the COVID episode. Results available closest to the screening date might be used; ³According to clinical practice.

9. PHARMACOVIGILANCE

9.1. Definitions

Adverse event (AE): Any untoward and unintended medical occurrence in a clinical trial participant who has been administered a medicinal product (investigational or non-investigational), and which does not necessarily have a causal relationship with the treatment.

Any medical condition that is present at the time that the subject is screened will be considered as baseline/pre-existing and will not be reported as an AE. However, if the severity of any pre-existing medical condition increases, it should be recorded as an AE.

Given the nature of severity of the underlying illness, subjects will have many symptoms and abnormalities in vitals and laboratory. Only Grade 3 and 4 AEs will be captured as AEs in this trial, according to the following classification:

- **Mild (Grade 1):** Events that are usually transient and may require only minimal or no treatment or therapeutic intervention and generally do not interfere with the subject's usual activities of daily living.
- **Moderate (Grade 2):** Events that are usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research subject.
- **Severe (Grade 3):** Events interrupt usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention. Severe events are usually incapacitating.
- **Severe (Grade 4):** Events that are potentially life threatening.

Adverse Reaction (AR): All untoward and unintended responses to an investigational medicinal product related to any dose administered, including those related to the product administration process.

Serious adverse event (SAE): any untoward medical occurrence or effect that at any dose:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of an existing inpatient hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect
- Is a suspected transmission of any infectious agent via a medicinal product
- Is an important medical event that, when based upon appropriate medical judgment may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above in the definition for a serious adverse event.

Unexpected adverse reaction: Any adverse reaction, the nature, or severity of which is not consistent with the applicable product information (e.g. IB or summary of the product characteristics (SmPC)) - and/or to the product administration process.

Suspected unexpected serious adverse reaction (SUSAR): all the SAEs that, according to the investigator's evaluation, are considered to be related to the IMP and they are neither described nor contemplated in the IB of the IMP.

9.2. Adverse Events

The following adverse events will be recorded in the CRF of the study:

- All serious adverse events.
- Grade 3 and 4 adverse events.
- All adverse reactions related to the investigational medicinal product.
- All adverse events considered of special interest for this study, regardless of their seriousness.

Occurrence of any of the above-mentioned events will be recorded from the date of receiving the allocated treatment onwards.

The investigators will be instructed to actively monitor the **adverse events of special interest**, defined as any of the following:

- Infusion-related reactions
- Malignancies
- Infections.

9.3. Assessment of Adverse Events

Severity

The investigator is asked to evaluate the severity (or intensity)* of the adverse events observed in the person participating in the research and to note these in the CRF, either by using a scale for grading the adverse events (e.g. NCI-CTC classification for cancer trials), or by using more general terms such as:

Severity*	Characteristics
Mild	does not interfere with normal daily activity
Moderate	partial limitation of normal daily activity
Severe	limitation of normal daily activity

*The criterion of severity must not be confused with the criterion of seriousness which is used as a guide in defining the declarative obligations.

Seriousness

Definitions of seriousness are described in the definitions section 9.1 of this protocol and are based on the regulatory definitions.

Causality

This is a clinical assessment of whether the adverse event is likely to be related to the IMP. The causality evaluation of the AEs will be made by the Investigator or designee. For the causality analysis, the following definitions will be used:

- **Related** – The event is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.
- **Not Related** – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate aetiology has been established.

Expectedness

The evaluation of expectedness will be based on the Reference Safety Information (RSI) for the IMP (included in IB). If the reaction is not described neither contemplated in the RSI, AR will be considered as a SUSAR.

9.4. Conduct in case of SAEs

In order to comply with current regulations, any SAE (regardless of causality) must be notified by the investigator to the Sponsor, as soon as he/she becomes aware of it and within a maximum period of **24 calendar hours**, sending a SAE form via e-mail or fax to:

Servicio de Farmacología Clínica, Hospital Universitario Puerta de Hierro
Email: farmacologia_clinica@idiphim.org; cpayares@idiphim.org

The investigators will make an effort to complete all data included in the SAE form. However, if any of the SAE-related information is not available at the time of the initial submission, it may be supplemented later, in order not to delay the SAE notification. The initial submission **should not be delayed** when there is suspicion of a serious reaction.

All SAEs occurring from the date of receiving the allocated treatment until the end of study must be notified.

9.5. Safety Notifications to the Health Authorities

All SUSAR will be notified to the AEMPS according to the procedures in the applicable laws. Non-fatal SUSARs will be notified within 15 days from the moment the Sponsor becomes aware of it, while deaths or life threatening SUSARs will be notified with a 7-day timeframe.

All SAE and SUSARs will be notified to the AEMPS and the CEIm in an annual safety report.

10. ETHICAL ASPECTS

10.1. General Considerations

The Clinical Trial will be conducted under conditions of respect for the fundamental rights of the person and the ethical postulates that affect biomedical research with human beings, following the international recommendations included in the Declaration of Helsinki, and their subsequent revisions. Likewise, the national recommendations will be followed in accordance with the guidelines of the Spanish Medicines Agency.

Prior to conduction of the study, the sponsor will submit the project to the relevant Research Ethics Committee(s) and Regulatory Agency (AEMPS). During the conduct of this study, the investigators will strictly comply with the provisions of this protocol, fully completing the Clinical Case Form (CRF).

10.2. Informed Consent

Informed consent process that will be initiated prior to an individual agreeing to participate in a trial and continuing throughout the individual's trial participation. Investigators will obtain the subject's informed consent in accordance with applicable national legislation and the internationally accepted guidelines. Subjects will receive a concise and focused presentation of key information about the clinical trial, orally. Due to paper handling limitation in COVID wards, oral witnessed consent will be accepted before entering into the trial. Written consent will be obtained from the patient himself or acceptable representatives as soon as possible. The key information about the study will be organized and presented in lay terminology and language that facilitates understanding why one might or might not want to participate.

10.3. Confidentiality

Subject's confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover clinical information relating to subjects, test results of biological samples and all other information generated during participation in the study. No identifiable information concerning subjects in the study will be released to any unauthorized third party. Subject confidentiality will be maintained when study results are published or discussed in conferences.

The study monitor, other authorized representatives of the sponsor, representatives of the REC, and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) for the subjects in this study. The clinical study site will permit access to such records.

All source records including electronic data will be stored in secured systems. All study data and research specimens that leave the site (including any electronic transmission of data) will be identified only by a coded number that is linked to a subject through a code key maintained at the clinical site.

11. FINANCIAL ASPECTS

11.1. Insurance Policy

According with current European legal and regulatory requirements regarding biomedical research, the Sponsor will take out, prior to study start, a liability insurance policy to cover the entire duration of the study.

11.2. Economic Compensation

No financial compensation is provided to the research team or patients.

12. STATISTICAL ANALYSIS

12.1. General Aspects

The statistical analysis will be carried out in accordance with the principles specified in the International Conference on Harmonization (ICH) Topic E9 (CPMP / ICH / 363/96)³. All statistical analyzes will be carried out in accordance with the intention-to-treat principle.

12.2. Sample Size Calculation

Accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided test, 9 subjects are necessary in first group and 9 in the second to recognize as statistically significant a difference greater than or equal to 0.4 units. The common standard deviation is assumed to be 0.3.

12.3. Populations for Analysis

There will be the following analysis populations for this study:

- 1) Full Analysis Set (FAS): All patients who are randomized into the study.
- 2) Per Protocol (PP) Population: Those patients included in the FAS without major protocol deviations that might impact the study's main assessments. These deviations will be assessed during the data review prior to database lock.
- 3) The Safety population is defined as all randomized participants who received the investigational product.

The precise reasons for excluding participants from each population will be fully defined and documented independently of the randomization codes during the data blinder review and before data lock.

³ CPMP/ICH/363/96. ICH E9 Statistical Principles for Clinical Trials. URL: http://www.ema.europa.eu/ema/pages/includes/document/open_document.jsp?webContentId=WC500002928, last access: 20-Mar-2020.

12.4. Primary Efficacy and Safety Analyses

All statistical analyses will be carried out in accordance with the intention-to-treat principle. The main analysis for efficacy will take place when all patients have completed their 28-day follow-up after treatment. Prior to this analysis and early during the recruitment phase, a statistical plan will be in place with strict provisions to preserve data integrity and will follow the general regulatory recommendations given in the ICH E9 guidance, and other applicable guidelines.

For safety purposes, patients will be followed-up for a year after treatment. The double-blind design will be maintained (for both, patient and evaluating clinician) until the communication of the primary results by a blinding plan for study treatment administration, and blinded clinical evaluations by the clinician.

Continuous variables will be expressed as mean and standard deviation and compared using the Student's T test; categorical variables will be compared with the Chi-square test or the Fisher exact test. Survival curves will be generated using the Kaplan-Meier method. The survival curves will be compared with the Mantel Cox log Rank test. Values of p less than 0.05 will be considered statistically significant.

13. SUPPORTING DOCUMENTATION AND OTHER OPERATIONAL

13.1. Data Collection

Data collection will be performed, in accordance with Good Clinical Practices, under the responsibility of the local investigator. Study data will be collected and managed using REDCap electronic data capture tools. REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for data integration and interoperability with external sources.

The anonymity of the subjects will be ensured by identifying them with a code (e.g., their centre number and inclusion number). Persons working under the supervision of the sponsor or the coordinator are obliged to respect confidentiality.

13.2. Clinical Monitoring

Clinical site monitoring is conducted to ensure that the rights and well-being of trial subjects are protected, that the reported trial data are accurate, complete, and verifiable. Clinical Monitoring also ensures conduct of the trial is in compliance with the currently approved protocol/ amendment(s), ICH, GCP, and with applicable regulatory requirement(s) and sponsor requirements. Monitoring for this study will be performed by the sponsor. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, CRFs, ICFs, medical and laboratory reports, site study intervention storage records, training records, and protocol and GCP compliance. The monitoring visits can be made online.

13.3. Communications and Publication

The trial will be registered on the European Clinical Trials Register (EU CTR) and the US Clinical Trial register (clinicaltrials.gov).

After completion of the study, a clinical study report will be prepared according to the ICH Guideline for Structure and Content of Clinical Study Reports (ICH E3). In addition, study results will be published in a scientific journal. There is a firm commitment from the Sponsor to publish and present data from this study, regardless of the results being positive or negative.

13.4. Other Operational Considerations

There will be a file of documentation for all the data, which will be kept intact during the time required by the regulatory provisions that are applicable. In any case, the confidentiality of the data and documents contained in the file will be ensured.

14. BIBLIOGRAPHY

- ARDS Definition Task Force. Acute Respiratory Distress Syndrome: The Berlin Definition. *JAMA*. 2012;307(23):2526-2533.
- Arentz M, et al. Characteristics and Outcomes of 21 Critically Ill Patients with COVID-19 in Washington State. Arentz M, Yim E, Klaff L et al. *JAMA*. 2020; 323(16):1612-1614.
- Asmussen S, et al. Human mesenchymal stem cells reduce the severity of acute lung injury in a sheep model of bacterial pneumonia. *Thorax* 2014; 69: 819–25.
- Chen N, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet*. 2020; 395(10223):507.
- Chen J, et al. Clinical study of mesenchymal stem cell treating acute respiratory distress syndrome induced by epidemic Influenza A (H7N9) infection, a hint for COVID-19 treatment. *Engineering (Beijing)*. 2020 Feb 28. [Epub ahead of print]
- Gupta N, et al. Intrapulmonary delivery of bone marrow-derived mesenchymal stem cells improves survival and attenuates endotoxin-induced acute lung injury in mice. *J Immunol* 2007, 179:1855–1863.
- Huang C, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020; 395 (10223):497.
- Lee JW, et al. Allogeneic human mesenchymal stem cells for treatment of E. coli endotoxin-induced acute lung injury in the ex vivo perfused human lung. *Proc Natl Acad Sci USA* 2009; 106: 16357–62.
- Lee JW, et al. Therapeutic effects of human mesenchymal stem cells in ex vivo human lungs injured with live bacteria. *Am J Respir Crit Care Med* 2013; 187: 751–60.
- Matthay MA et al. Treatment with Allogeneic Mesenchymal Stromal Cells for Moderate to Severe Acute Respiratory Distress Syndrome (START Study): A Randomised Phase 2a Safety Trial. *Lancet Respir Med*. 2019 Feb;7(2):154-162.
- McAuley DF, et al. Clinical grade allogeneic human mesenchymal stem cells restore alveolar fluid clearance in human lungs rejected for transplantation. *Am J Physiol Lung Cell Mol Physiol*. 2014; 306:809–815.
- Mei SH, et al. Prevention of LPS-induced acute lung injury in mice by mesenchymal stem cells overexpressing angiopoietin 1. *PLoS Med* 2007, 4:e269.
- Ortiz LA, et al. Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. *Proc Natl Acad Sci U S A* 2003, 100:8407–8411.
- Rice TW, et al. Comparison of the SpO₂/FIO₂ ratio and the PaO₂/FIO₂ ratio in patients with acute lung injury or ARDS. *Chest*. 2007;132(2):410-7.
- Rojas M, et al. Bone marrow-derived mesenchymal stem cells in repair of the injured lung. *Am J Respir Cell Mol Biol* 2005, 33:145–152.
- Wang D, et al. Clinical Characteristics of 138 Hospitalized Patients with 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA*. 2020; 323(11):1061-1069.
- Wilson JG, et al. Mesenchymal stem (stromal) cells for treatment of ARDS: a phase 1 clinical trial. *Lancet Respir Med* 2015; 3: 24–32.
- Wu C, et al. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. *JAMA Intern Med*. 2020; e200994. doi: 10.1001/jamainternmed.2020.0994.
- Xu J, et al. Prevention of endotoxin-induced systemic response by bone marrow-derived mesenchymal stem cells in mice. *Am J Physiol Lung Cell Mol Physiol* 2007, 293:L131–L141.
- Yip HK, et al. Human Umbilical Cord-Derived Mesenchymal Stem Cells for Acute Respiratory Distress Syndrome. DOI: 10.1097/CCM.0000000000004285
- Zheng G, Lanfang H, Tong H, et al. Treatment of acute respiratory distress syndrome with allogeneic adipose-derived mesenchymal stem cells: a randomized, placebo-controlled pilot study. *Respiratory Research*. 2014; 15:39.
- Zhou F, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. 2020; 395(10229):1054.

15. ANNEXES

15.1. ANNEX I. RESEACH TEAM INFORMATION

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15.2. ANNEX II. GMP UNIT INFORMATION

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