Allogeneic Mesenchymal Stromal Cell (MSC)
Therapy for SARS-CoV-2 Pneumonia:
A Prospective Randomized Multicentre
Phase I/IIa Open Label Study

Running title: Mesenchymal Cell Therapy for SARS-CoV-2 Pneumonia

Investigational compound: mesenchymal stromal cells

Protocol version: 2.7 May 21st, 2020

Acronym: RESCAT
(**RE**scuing patients with **S**ARS-CoV-2 pneumonia with **C**ell **A**dvanced **T**herapy)

EudraCT number: 2020-001577-70

Key words: advanced cell therapy; efficacy; feasibility; immunity; mesenchymal stromal cells; safety; SARS-CoV-2 pneumonia.

Promoter Name and Legal Registered Address: Azienda Ospedaliera Universitaria di Modena, Via del Pozzo 71, 41124 - Modena.

TABLE OF CONTENTS

1   PROMOTER, PIs, MANUFACTURING AND CLINICAL CENTERS

Promoter: Massimo Dominici, Direttore Unità Operativa Complessa di Oncologia, Programma di Terapia Cellulare ed Immuno-oncologia, Dipartimento di Oncologia ed Ematologia, Azienda Ospedaliera Universitaria di Modena Policlinico, Università di Modena e Reggio Emilia;

E-mail: massimo.dominici@unimore.it; Telefono: 059 4222858; Segreteria: 059 4222858; Fax: 059 4223341. Via del Pozzo 71 - 41124 - Modena.

Coordinating Physician: Prof. Enrico Clini, Unità Operativa Complessa di Malattie Apparato Respiratorio, Dipartimento di Medicine Specialistiche, Azienda Ospedaliera Universitaria di Modena Policlinico, Università di Modena e Reggio Emilia.

Clinical Scientific Committee:

[1]Massimo Dominici: Principal Investigator

[2]Rachele Ciccocioppo: Co-Principal Investigator

[3]Enrico Clini: Co-Principal Investigator

Collaborators: [4]Giacomo Bellani, [5]Vincenzo Bronte, [6]Ernesto Crisafulli, [7]Giovanna D'Amico, [8]Francesca Elice, [9]Rosaria Giordano, [10]Adriano Peris, [9]Daniele Prati, [4]Emanuele Rezoagli ,[8]Marco Ruggeri, [11]Nino Stocchetti, [12]Yvan Torrente, [11-13]Elisa R. Zanier

Affiliations:

[1]Unità Operativa Complessa di Oncologia, Dipartimento di Oncologia ed Ematologia, Azienda Ospedaliera Universitaria di Modena Policlinico, Università di Modena e Reggio Emilia;

[2]Unità Operativa Complessa di Gastroenterologia, Dipartimento di Medicina, A.O.U.I. Policlinico G.B. Rossi & Università di Verona;

[3]Unità Operativa Complessa di Malattie Apparato Respiratorio, Dipartimento di Medicine Specialistiche, Azienda Ospedaliera Universitaria di Modena Policlinico, Università di Modena e Reggio Emilia;

[4]ASST Monza, Dipartimento di Emergenza e Urgenza, Dipartimento di Medicina e Chirurgia, Università degli Studi Milano Bicocca, Monza (MB);

[5]Unità Operativa Complessa di Immunologia; Dipartimento di Medicina, A.O.U.I. Policlinico G.B. Rossi & Università di Verona;

[6]Unità Operativa Complessa di Malattie dell'Apparato Respiratorio; Dipartimento di Medicina, A.O.U.I. Policlinico G.B. Rossi & Università di Verona.

[7]Unità di Immunologia ed Terapie Cellulari, Fondazione M. Tettamanti De Marchi, Clinica Pediatrica Università degli Studi Milano Bicocca, Monza (MB);

[8]Unità Operativa Complessa di Ematologia, Ospedale San Bortolo, AULSS 8 "Berica", Vicenza.

[9]Laboratorio di Medicina Rigenerativa – Cell Factory, Unità Operativa Complessa Centro Trasfusionale, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano;

[10]SOD Cure Intensive del Trauma e delle Gravi Insufficienze d'Organo, Centro Regionale di Riferimento ECMO, Azienda Ospedaliero Universitaria Careggi, Firenze;

[11]Rianimazione 1 Fiera Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico Viale Scarampo, Torre 6 - 20148 Milano Italy, Terapia Intensiva Neuroscienze, Padiglione Monteggia, Fondazione IRCCS Cà Granda Osp. Maggiore Policlinico, Milano, Università di Milano

[12]Department of Pathophysiology and Transplantation, University of Milan, Unit of Neurology, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milano Italy

[13]Laboratorio di Danno Cerebrale Acuto e Strategie Terapeutiche, Dipartimento di Neuroscienze, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milano.

Manufacturing Committee:

Giuseppe Astori: Laboratorio Terapie Cellulari Avanzate (LTCA), Unità Operativa Complessa di Ematologia, Azienda AULSS 8 "Berica", Vicenza;

Franco Bambi: SOC Immunoematologia, Medicina Trasfusionale e Laboratorio Azienda Ospedaliera Universitaria Meyer, Firenze;

Laura Fiammenghi, Maria Carlotta Spano: Rigenerand srl - Medolla (Modena)

Giuseppe Gaipa: Laboratorio di Terapia Cellulare e Genica Stefano Verri, ASST-Monza, Ospedale San Gerardo, Monza;

Tiziana Montemurro, Lorenza Lazzari: Laboratorio di Medicina Rigenerativa – Cell Factory, Unità Operativa Complessa Centro Trasfusionale, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano.

Regulatory Committee:

Simona Guidi: Rigenerand srl - Medolla (MO);

Monica Santimaria: U.O.C. Medicina Nucleare, Ospedale S. Bortolo, AULSS 8 "Berica", Vicenza.

List of Cell Factories participating in the study according to the Region:

Emilia Romagna: Rigenerand srl, Via Maestri del Lavoro, 4 - 41036 - Medolla (MO).

Lombardia: Laboratorio di Medicina Rigenerativa – Cell Factory Fondazione IRCCS – Ca' Granda Ospedale Maggiore Policlinico, Via Francesco Sforza, 35 - 20122 – Milano (MI).

Lombardia: Laboratorio di Terapia Cellulare e Genica Stefano Verri, ASST-Monza, Ospedale San Gerardo Via Pergolesi, 33 – 20900 - Monza (MB).

Toscana: Cell Factory Meyer, Azienda Ospedaliera Universitaria Meyer, Viale G. Pieraccini, 24 - 50139 – Firenze (FI).

Veneto: Laboratorio di Terapie Cellulari Avanzate (LTCA), Azienda AULSS 8 "Berica", Contrà San Francesco, 41 - 36100 – Vicenza.

Contract Research Organization (CRO):

CORESEARCH, Center for Outcomes Research and Clinical Epidemiology; Via Tiziano Vecellio, 2 – 65124 Pescara

Approval date:

2   PROTOCOL AUTHORIZATION PAGE

I have read this study protocol and agree that it contains all the information required to conduct the study. I agree to conduct the study as set out in this protocol. In particular, I agree to adhere to the moral, ethical and scientific principles governing clinical research as set out in the Declaration of Helsinki, the guidelines on Good Clinical Practice and the appropriate national laws.

Local Investigator

_____   _____

                                                    Date

Trial Promoter Coordinating Centre

_____   _____

                                                    Date

3   STUDY SYNOPSIS

Study title	**Allogeneic mesenchymal stromal cell therapy for SARS-CoV-2 pneumonia: a prospective randomized multicentre phase I/IIa open label study**
Study acronym	**RESCAT: RE**scuing patients with **S**ARS-CoV-2 pneumonia with **C**ell **A**dvanced **T**herapy
Aim	To evaluate the feasibility, safety and efficacy of mesenchymal stromal cell therapy for SARS-CoV-2 pneumonia.
Background	- Pneumonia occurring during severe acute respiratory syndrome coronavirus (SARS-CoV)-2 infection represents a fearsome complication due to the high mortality rate. The respiratory failure derives from the huge systemic acute inflammatory response, the so called 'cytokine storm', which compromises gas exchange in the lung and the subsequent function of vital organs. This is why many patients need respiratory / ventilatory assistance. To date, no standardized and effective treatment is available, although in the vast majority of cases anti-viral and anti-malarial drugs are used, other than supportive care. Development of novel treatment strategies and vaccines is the main target of the research. - Mesenchymal stromal cells (MSC) have high immunomodulatory and regenerative capacities. As far as the experimental models of acute respiratory distress syndrome (ARDS) is concerned, MSCs have proven to be able to modulate the inflammatory response, promote tissue repair, increase the clearance of pathogens and reduce the severity of the lesions with the ultimate effect of ameliorating lung dysfunction, even in influenza virus-induced forms. Worth of noting, a number of clinical studies have been carried out on the use of MSC intravenous administration in ARDS patients with promising results in terms of both safety and efficacy. - In addition, *in vivo* and *in vitro* data have shown that MSC can lead to a significant reduction of platelet adhesion and aggregation, thanks to the expression of proteoglycans on the cell surface. This is an important issue in the context of SARS-CoV-2 pneumonia. Indeed, direct endothelial damage and the consequent activation of the coagulation cascade are emerging as common effects in critical COVID-19 patient. MSC might eventually play a role also against this unexpected and dramatic kind of tissue injury, often not limited to the lungs. Therefore, MSC therapy has the potentiality to be a valuable strategy in dampening both inflammation and thrombosis in the course of COVID-19. - As far as the SARS-CoV-2 pneumonia is regarded, a Chinese study was recently published where seven patients with rapidly worsening clinical conditions were offered to undergo an infusion of allogeneic MSC at the dosage of $1x10^6$ cells per kilo of body weight. The results clearly demonstrated the absence of allergic or infusional reactions, secondary infections and serious or fatal adverse events. In addition, within 2-4 days from the infusion, in all cases an improvement of oxygenation rate, a decrease of C-reactive protein and inflammatory

	cytokine levels, an increase of lymphocyte count and an improvement of clinical and radiological picture were clearly evident. Therapeutic benefit and safety have also been described in one case of severe SARS-CoV-2 pneumonia treated with umbilical cord-derived MSC during the Chinese pandemy. Finally, it should be emphasized that in the two main databases www.ClinicalTrial.gov and www.chictr.org.cnv, a total of 24 clinical trials testing both the safety and efficacy of MSC of different origin in pneumonia due to SARS-CoV-2 infection have been registered and are actively recruiting. This evidence, together with the epidemiological situation and the clinical pictures with which we are facing now, have prompted us to carry out an Italian multicentre clinical trial on the use of MSC in SARS-CoV-2 related severe pneumonia.
Primary objectives	Evaluation of the feasibility and safety of the use of an Investigational Medicinal Product consisting of allogeneic MSC to treat SARS-CoV-2 related severe pneumonia through the capability to treat all enrolled patients and monitoring the rate of adverse events as codified in the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.
Secondary objectives	The efficacy of two serial intravenous infusions of allogeneic MSC 5 days apart will be assessed through the evaluation of a) mortality rate at 2 and 4 weeks from the end of the treatment of follow-up, b) trend of the daily PaO_2/FiO_2 ratio, c) evaluation of the days to intubation, d) date of independence from non-invasive mechanical ventilation, e) date of independence from oxygen therapy, f) lenght of hospitalization, g) radiological pattern response. In parallel, evaluation of the following laboratory values will be monitored: neutrophil, lymphocyte and platelet counts, platelet mean volume, C-reactive protein, ferritin, procalcitonin, LDH, fibrinogen, and D-dimer. Finally, a comparative immunological study of the cytokine pattern and the immunophenotyphic cell profile of both bronchoalveolar lavage fluid (BALF) and peripheral blood samples before and after 1 and 2 weeks from the second infusion end of the treatment will be carried out in a subset of patients in order to establish the pathogenic mechanisms of COVID-19 tissue injury and the mechanism of action of MSC in this specific clinical setting.
Tertiary objective	Establish which IMP used, as stratified according to the tissue origin (umbilical cord, umbilical cord blood, bone marrow, adipose tissue), is associated with the best result in terms of safety and efficacy in this specific setting clinical.
Primary Endpoints	The feasibility will be assessed through the evaluation of the production capacity of each Cell Factory participating in the study in terms of an adequate amount of cellular product lots sufficient to treat 100% of the patients recruited. In addition, the process of transfer of the cellular product to the Clinical Unit will be validated,

	so that all the quality, safety and therapeutic properties will be guaranteed, with particular reference to its viability. Finally, a protocol for the bedside preparation of the final suspension for administration will be implemented according to the Good Clinical Practice. The safety will be assessed by recording all adverse events as coded by the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0, based on their duration, intensity, and possible association with the treatment under study. More in depth, the absolute number/percentage of Suspected Unexpected Sever Adverse Reactions (SUSARs) and Severe Adverse Reactions (SARs) recorded after 2 and 4 weeks from the MSC treatment that will not exceed 10% will be considered the cutoff to be met.
Secondary Endpoints	The efficacy will be assessed through the evaluation of a) mortality rate (%) at 2 and 4 weeks from the end of the treatmentfollow-up, b) percentage of change of the daily PaO_2/FiO_2 ratio in comparison with the basal value, c) percentage of patients under invasive mechanical ventilation, d) days under non-invasive mechanical ventilation, e) days under oxygen therapy, f) radiological response, g) days of hospitalization, h) percentage change of the radiologic score with respect to the basal value. Change of the following laboratory values after 2 and 4 weeks from the end of the MSC treatmentof follow-up in comparison to the basal values and expressed as percentages: neutrophil, lymphocyte and platelet counts, platelet mean volume, C-reactive protein, ferritin, procalcitonin, LDH, fibrinogen, and D-dimer. Modification of the cytokine pattern and of the immunophenotyphic cell profile on both BALF and peripheral blood samples as assessed by means of ELISA, flow cytometry and single cell RNAseq before and after 1 and 2 weeks from the end of MSC treatmentfollow-up will be carried outevaluated.
Tertiary Endpoints	We have planned to treat a group of 10 cases with umbilical cord MSCs, a group of 10 cases with umbilical cord blood MSCs, a group of 10 cases with bone marrow MSCs, and a group of 10 cases with adipose tissue MSCs. The percentages of total adverse events, SUSARs, and SARs related to the MSC treatment, together with the efficacy parameters will be compared among the groups.
Study design	This is an investigator-initiated prospective multicentre open label, randomized, controlled, double arms phase I/IIa clinical trial evaluating the feasibility, safety and efficacy of two serial intravenous infusions of allogeneic MSCs 5 days ± 48 hours apart in patients suffering from severe pneumonia due to SARS-CoV2 viral infection admitted to monitored COVID Units (both semi-intensive and intensive).
Classification, description and	The cellular product used in this study consists of allogeneic MSCs whose morphological, phenotypic and functional characterization

denomination of the IMPs	meets the requirements codified by the International Society for Cell & Gene Therapy. The cellular product is supplied by each Cell Factory participating in this study following AIFA authorization, as specified here below for each Investigational Medicinal Product (IMP): IMP-1: UC-MSC, umbilical cord-derived MSCs produced at the Laboratory of Advanced Cellular Therapy AUSSL8 Berica - Vicenza (authorization code: aM-49/2019, aM-49bis/2019); IMP-2: CF-CB-MSC, umbilical cord blood-derived MSCs produced at the Cell Factory of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico of Milan (authorization code: aM-51/2018, aM-51bis/2018); IMP-3: CFM-1-BM-MSC, bone marrow-derived MSCs produced at the Cell Factory of the Ospedale Pediatrico Meyer of Florence (authorization code: aM-73/2019); IMP-4: RR002, adipose perivascular mesenchymal stromal cells (AD-PC-MSCs) produced at the Cell Factory Rigenerand in Medolla (MO) (authorization code: aM-25/2020); IMP-5: PTC-MSC-TP, bone-derived MSCs produced at the Laboratory of Cellular and Gene Therapies Stefano Verri in Monza (authorization code: aM-185/2017, aM-185bis/2017). Although each of the aforementioned IMP has showed a high safety profile in different clinical settings, up to now there is no data to establish any difference between the IMPs envisaged within the RESCAT trial in terms of both safety and efficacy in this specific clinical setting. The results collected so far in other inflammatory and immune-mediated disorders do not suggest substantial differences among MSC products isolated form different sources. However, no information is available to indicate whether one IMP is more suitable in this new condition. Furthermore, the unprecedent epidemiological situation we are facing to, poses the need of identifying effective therapeutic aids that can be rapidly introduced in a controlled clinical study. These reasons led us to propose the use of a "multi-source MSC" approach.
Sample size	We will enroll a total of 60 consecutive cases to be randomized 2:1 to receive (experimental group) MSC treatment + standard of care or only standard of care (control group).
Study population	Adult patients suffering from SARS-CoV-2 induced severe pneumonia admitted to semi-intensive or intensive COVID Units because of the need of ventilation support.
Inclusion criteria	- Sign of the informed consent, - Patients of either sex, aged 18-80 years (inclusive), - Patient with a confirmed virological diagnosis of SARS-CoV-2 infection by means of real-time Polymerase Chain Reaction, - Hospitalization due to clinical and radiological diagnosis of pneumonia, PaO_2/FiO_2 value between 150-300 with impending necessity of noninvasive positive pressure respiratory support (nCPAP) or ventilator support through nasal pressure support ventilation (nPSV),

	• Systolic artery pressure >90 mmHg without amine support, • Modified Early Warning Score (MEWS) score <3, • Absence of known active malignancy.
Exclusion criteria	• Deny to informed consent, • Known history of alcohol or drug abuse in the 12 months prior to inclusion, • Presence of significant comorbidities, such as uncontrolled hypertension, invalidating psychiatric or neurological disorders, organ failure (renal impairment defined by creatinine clearance below 50 ml/min or by serum creatinine $\geq$2.0 mg/dl; hepatic impairment defined by total bilirubin $\geq$2.0 mg/dl and AST + ALT $\geq$ 2.5 $\times$ upper normal value; cardiac failure with an output fraction $\leq$40%), or any other clinically significant condition, as determined by the Principal Investigator, • Presence of chronic advanced cardio-pulmonary diseases, such as ILDs (obstructive pneumonia, severe pulmonary interstitial fibrosis, alveolar proteinosis, allergic alveolitis), • Patient has a clinically relevant abnormality on electrocardiogram, as determined by the Principal Investigator, • History of previous embolism, • Known active malignancy, • Patient with a history of severe allergic reactions (e.g., swelling of the mouth and throat, difficulty breathing, hypotension, or shock) requiring medical intervention, • Patient with a positive test for human immunodeficiency virus or active hepatitis B or C disease or tuberculosis or further viral infections (influenza virus, adenovirus and other respiratory viruses), • Patient is known to be pregnant, has a positive pregnancy test or is nursing, • Patient has had major surgery, either open or laparoscopic, within the 3 months prior to screening, • Previous haematopoietic stem cell or organ transplantation, • Patient under immunosuppressive agents, • Patients currently receiving, or having received within 2 months prior to enrolment into this clinical study, any other investigational drug.
Treatment dosage and schedule	The treatment consists of two serial intravenous infusions of 1.0-1.5x10^6 MSC/kg body weight 5 days ± 48 hours a part. The administration of the medication will not be masked and lasts 30 minutes each. The second dose will be administered in the absence of infusion reactions and adverse events upon the first dose. Control cases will receive the standard of care consisting of already authorized antiviral agents and/or hydroxychlorichine. All cases will receive oxygen delivery to target a minimum of 94% at the oximetry monitoring.
Follow-up duration	It is expected a short-term follow-up lasting 4 and 48 hours after each MSC infusion in order to monitor possible infusional reactions;

	a medium-term follow-up after 2 and 4 weeks from the end of MSC treatment in order to evaluate possible adverse events; a long-term follow-up after 6 months to record possible late occurring adverse events and lung fibrosis.
Statistical analysis	Patient demographics and baseline clinical characteristics will be summarized as frequencies and percentages for categorical variables or median and interquartile range for continuous variables. The endpoints will be assessed at 4 hours, 48 hours, 7 days, 14 days, and 28 days post treatment time points, as appropriate. The primary outcome of this study is the feasibility and the safety of the MSC therapy. Safety will be assessed by incidence of AEs and SAEs, and will be compared between groups using Fisher's exact test. • The daily PaO_2/FiO_2, radiologic score, laboratory values and cytokine profile (continuous outcomes) will be evaluated as the difference within group (from baseline) and between groups (at each follow-up), with paired and unpaired non-parametric tests, respectively. • Mortality rates and patient rate under mechanical ventilation (dichotomuous outcomes) will be assessed by incidence and will be compared between groups using Fisher's exact test. • Days under non-invasive mechanical ventilation, days from independence from oxygen therapy and days of hospitalization (counts outcomes) will be evaluated as the difference between groups (at each follow-up) using Fisher's exact test.
Study duration	Twelve months from the date of the enrollment of the first patient to the date of the last follow-up visit of the last enrolled patient.
Expected results	As far as the feasibility is regarded, following an internal preliminary survey on the manufacturing capability of each Cell Factory participating in the study, we foresee to be able to treat all the 40 patients of the intention-to-treat cohort. As far as the safety is regarded, based on the evidence obtained from the systematic reviews published in literature on the use of MSC in clinical trials and personal experience, we can assess that no significant safety concerns are expected. As far as the efficacy is regarded, a reduction of mortality rate at 2 and 4 weeks from the end of the treatment is awaited, together with an amelioration of the cinical and laboratory parameters. Also a consistent reduction of lung fibrosis at 6 months is expected. Shell an IMP give better results in terms of both safety and efficacy in comparison to the others, the possibility to shift the production of the Cell Factories towards the identified cellular product and to design more informative and consistent phase IIb/III studies represent a great step forward.

4   Abbreviations

ABG	Arterial Blood Gas
ACD	Anticoagulant Cytrate Destrose
ACE2	Angiotensin-converting enzyme 2
ADL	Activities of Daily Living
AE	Adverse Event
AESI	Adverse Event of Special Interest
ALI	Acute Lung Injury
ALT	Alanine aminotransferase
Ang-1	Angiopoietin-1
AR	Adverse Reaction
ARDS	Acute Respiratory Distress Syndrome
AST	Aspartate aminotransferase
BALF	Bronchoalveolar Lavage Fluid
BM	Bone Marrow
BMI	Body Mass Index
CBC	Complete Blood Count
c-FLIP	Cellular FLICE-inhibitory Protein
CGT	Cell and Gene Therapies
CI	Confidence Interval
CMP	Coagulation and Metabolic Panel
COVID-19	Coronavirus Disease 2019
CRF	Case Report Form
CRO	Contract Research Organization
CT	Computerized Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DC	Dendritic Cells
DILI	Drug-Induced Liver Injury
DMSO	Dimethyl Sulfoxide
DSMB	Data Safety Monitoring Board
eCRF	Electronic Case Report Form
ECG	Electrocardiogram
ELISA	Enzyme-Linked Immunosorbent Assay
EVCTM	EudraVigilance Clinical Trial Module
FDA	Food and Drug Administration
FiO$_2$	Fraction of Inspired Oxygen
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practice
HGF	Hepatocyte Growth Factor
HLA	Human Leukocyte Antigen
IB	Investigator's Brochure
ICH	International Conference on Harmonization
ICU	Intensive Care Unit
IDO	Indoleamine 2,3-Dioxygenase
IEC	Independent Ethics Committee

IFN	Interferon
IL	Interleukin
ILD	Interstitial Lung Disease
IMP	Investigational Medicinal Product
ISG	Interferon-Stimulated Gene
ITT	Intent-to-Treat
IV	Intravenous
LDH	Lactate dehydrogenase
MEWS	Modified Early Warning Score
MSC	Mesenchymal Stromal Cell
nCPAP	nasal Continuous Positive Airway Pressure
nPSV	nasal Pressure Support Ventilation
NK	Natural Killer
OI	Oxygenation Index
PaO$_2$	Arterial Partial Oxygen Pressure
PBMC	Peripheral Blood Mononuclear Cell
PCR	Polymerase Chain Reaction
PEEP	Positive End-Expiratory Pressure
PI	Principal Investigator
PP	Per Protocol
PPE	Personal Protective Equipment
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
QC	Quality Control
RR	Relative Risk
RT-PCR	Real Time Polymerase Chain Reaction
SAE	Serious Adverse Event
SAR	Suspected Adverse Reaction
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus-2
SOP	Standard Operating Procedure
STIAMP	Suspected Transmission of Infectious Agent via Medicinal Product
SUSAR	Suspected Unexpected Serious Adverse Reaction
UAR	Unexpected Adverse Reaction
UC	Umbilical Cord
TNF	Tumor Necrosis Factor
UP	Unanticipated Problems
VEGF	Vascular Endothelial Growth Factor
KGF	Keratinocyte Growth Factor
XR	X-Ray
WHO	World Health Organization

5   BRIEF DESCRIPTION OF THE STUDY

Cell and gene therapies (CGT) have been progressively entering in a variety of biomedical fields, in particular for skeletal disorders, cancer and infectious diseases. Thus, CGT may also play a relevant role as treatment against Coronavirus Disease 2019 (COVID-19) affecting either the immune system, coagulation or in regenerating tissue damaged after infection. RESCAT wants to challenge COVID-19 severly ill patients within a clinical approach based on mesenchymal stromal cells (MSCs). MSCs from different sources (marrow, adipose and cord) will be here delivered into a randomized phase I/IIa clinical trial enrolling 60 patients (40 treated and 20 as control group). Considering inflammation as one of the key aspects of SARS-CoV-2-related tissue damage, RESCAT relies on reported anti-inflammatory and immunomodulatory effect of MSCs targeting at the same time coagulation and tissue repair, and additionally searching for lung-related biomarkers to be monitored before and after MSC infusion. The working hypotheses are based on the use of MSCs in the setting of lung acute distress respiratory syndrome (ARDS), on the recently published data on MSCs in severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) affected patients, on the first European patient treated with MSCs as compassionate use by one of the RESCAT trial co-Principal Investigator (PI), on the reported immunomodulatory, anti-inflammatory effect of MSCs and on their anti-clotting potential as well. RESCAT trial will develop 3 major activities: (1) to create a network of Italian Cell Factory working for the first time together within a single defined clinical protocol for the urgent clinical need related to COVID-19 severe patients; (2) to perform the first randomized clinical phase I/IIa study addressing feasibility, safety and early efficacy in SARS-CoV-2 affected patients; (3) to understand the clinical and biological impact of different type of MSCs on the SARS-CoV-2-associated inflammation focusing on lungs damages, combining imaging and serological biochemical and immunological (cells, cytokines and chemokines) biomarkers. The success of the RESCAT trial could potentially generate a benefit in affected patients, producing better knowledge on SARS-CoV-2 pathophysiology in COVID-19 severe patients and indicating the most performing MSC subtypes to be used for larger clinical trials. Collectively, this will largely accelerate a possible therapeutic solution for this global, urgent and still unmet medical need.

6   INTRODUCTION

6.1   The Clinical Problem

Severe acute respiratory syndrome coronavirus (SARS-CoV)-2 disease (COVID)-19 has a high mortality rate, estimated at 3.4% by the WHO[1]. More than 1 out of 4 hospitalized COVID-19 patients require admission to an Intensive Care Unit (ICU) for respiratory support. A large proportion (up to 46%) of these patients has died due to the lack of therapies. The full spectrum of COVID-19 ranges from asymptomatic subjects to mild, self-limiting respiratory tract illness or to severe progressive pneumonia.[2] The evolution depends on a virus-induced exaggerated immune response, the so-called "cytokine storm",[3] that causes an ARDS, whose mortality seems higher in Italy (approaching 11%) than in China (about 3%).[4] A newly identified complication is an intravascular coagulation associated with organ failure that follows the virus entry into endothelial cells that in turn triggers platelet aggregation.[5] The current therapeutic approach relies on the use of a combination of two anti-viral molecules (lopinavir, a human immunodeficiency virus type 1 aspartate protease inhibitor, plus ritonavir, an inhibitor of the cytochrome P450 to increase its plasma half-life) and hydroxychloroquine, together with the treatment of any other co-infections and support of respiratory exchange.[6] However, convincing data are lacking since in a recently published paper the association of lopinavir–ritonavir has proven unfruitful in comparison with standard of care in an adult cohort of hospitalized patients with severe SARS-CoV-2 pneumonia.[7] This is why further antiviral molecules (i.e. Favipiravir and Remdesivir) and immunomodulatory agents, such as tocilizumab, an anti-interleukin (IL)-6 monoclonal antibody, have been proposed. In this scenario, and in the meanwhile a vaccine is developed, MSC-based therapy seems a suitable option thanks to their wide and potent immunomodulatory effects,[8] as well as to their high safety profile independently from the tissue source and clinical setting.[9]

RESCAT trial wants meet the feasibility and safety of the MSC treatment in this new clinical setting and define a specific MSC source that may have a higher safety and efficacy profile as defined by both clinical and laboratory parameters. Our team was the first in Europe to apply MSCs to rescue a patient with respiratory failure due to SARS-CoV-2 pneumonia. In RESCAT, we will further develop this strategy in a randomized Phase I/IIa trial to understand safety and if and how MSC mitigate COVID-19 pathogenic mechanisms, also focusing on circulating biomarkers in the attempt to verify their predictive value. The success of this trial will provide relevant information on COVID-19 and the therapeutic impact of MSCs, thus favoring a much larger transferability in clinical setting.

6.2   Mesenchymal stromal cells (MSC)

MSCs are a population of progenitor cells considered an attractive cell therapy candidate[10] thanks to the ease of their isolation and *ex vivo* expansion, the ability to undergo multilineage differentiation[11] and, mostly, for their low immunogenicity[12] and high immunosuppressive activity that is displayed on all cells involved in immune response.[8] Following intravenous administration, a large amount of MSCs are trapped in the pulmonary vascular bed through as yet unclear interactions with endothelial cells.[13] Tracking studies using labeled MSCs demonstrate the most are cleared within 24-48 hours although there can be longer persistence in injured or inflamed lungs.[14] The clearance mechanisms are still being elucidated but include apoptosis and phagocytosis by resident inflammatory cells, mostly macrophages. While lodged in the lungs, MSCs are able to release a wide array of bioactive molecules including anti-inflammatory cytokines,[15] antimicrobial peptides,[16] angiogenic growth factors, and extracellular vesicles.[17] Direct cell-cell transmission of mitochondria from MSCs to respiratory epithelial and immune cells[18] has also been described,[19] thus leading to a phenotypic and functional shift of the target cell populations. Growing evidence shows that the pattern of anti-inflammatory mediators released is specific for the inflammatory lung microenvironment and is mediated through differential activation of damage and pathogen-associated molecular pathogen receptors expressed on MSC cell surface,[20] such as toll-like receptors that are activated by viral RNA.[21] Moreover, MSC-secreted angiopoietin-1 (Ang-1) and keratinocyte growth factor (KGF) contribute to the restoration of alveolar-capillary barriers disrupted as part of the ARDS pathogenesis.[22] Also an upregulation of matrix metalloproteinases may favour the establishment of a microenvironment prone to extracellular matrix degradation and fibrosis reduction. With respect to known effects of the respiratory virus-induced inflammatory environment, the increased levels of interferon (IFN)-γ typical of anti-viral immune responses, alone or together with other pro-inflammatory cytokines, prompt MSC activation including the release of anti-inflammatory mediators. Finally, MSCs are generally resistant to viral infection,[23] a crucial property in the context of a respiratory viral infection, including that of SARS-CoV-2.

6.3   Preclinical data: MSC in lung respiratory animal models

An important question that remains to be solved in respiratory virus infections is whether protective MSC effects are directly against viral infection, perhaps by stimulating anti-viral T cells actions, or whether they are due to overall anti-inflammatory actions as demonstrated

in other models of acute lung injuries. Nonetheless, it is likely that a combination of actions is responsible for MSC effects. It is conceivable that the increased level of IFN-γ, typical of anti-viral immune response, as it has been dramatically described in SARS-CoV-1/2 viral infections,[24] alone or together with other pro-inflammatory cytokines, prompt MSC activation including the release of anti-inflammatory mediators.[25] Moreover, in the context of a respiratory viral infection, including that due to SARS-CoV-2, MSCs might constitutively produce high levels of MSC-specific interferon-stimulated genes (ISGs) that act as mediators of antiviral protection.[23] Conversely, MSCs display a mix of intrinsic and inducible innate antiviral defenses that could lead to therapeutic benefits in COVID-19 patients.[26]

To date, a number of preclinical studies have established the beneficial effects that MSCs exert, both by intra-tracheal or intravenous administration, in lung injury models. The models include both rodents as well as large animal (pig, sheep) and explanted human lungs, and a wide range of approaches have been utilized for dose, dosing, and MSC source (bone marrow, adipose, umbilical cord, cord blood, and placenta).[27] It is still unclear if there is a more performing MSC source in this context: a systematic review indicated that bone marrow- and umbilical cord-derived MSCs were more effective than adipose tissue-derived MSCs in reducing mortality in pre-clinical acute lung injury models.[28] On the other side, limited pre-clinical studies investigating the effects of MSC administration in experimental respiratory models of influenza did not demonstrated benefit related with MSC treatment, at least in this precise context.[29,30]

By contrast, protective effects of systemic MSC administration in rodent and pig models of influenza respiratory infections have been recently shown. Briefly, Chan and colleagues found in an *in vitro* assay that MSCs improve the dysregulated alveolar fluid clearance and protein permeability induced by H5N1 and H7N9 influenza viruses, by releasing soluble mediators that up-regulated sodium and chloride transporters.[31] The group then evaluated the impact of systemic administration of 5×10^5 human bone-marrow-derived MSCs/mouse on day 5 post injury (p.i.) in young (6-8 weeks) or old (8-12 months) immunocompetent mice infected with Influenza A (H5N1). At variance with younger mice, in aged animals, xenogeneic MSCs reduced virus-induced mortality (until day 18 p.i.), weight loss (day 6-10 p.i.), lung edema (day 7 p.i.), bronchoalveolar lavage fluid (BALF) CD4+ lymphocytes T and natural killer (NK) cells (day 7 p.i.), lung histopathological lesions (day 18 p.i.), pro-inflammatory cytokines and chemokines (day 7 p.i.) without reducing lung virus titers (day 7 and 10 p.i.). Thus, the data suggest that systemic MSC administration may provide benefit

in older patients who are at higher risk for severe pulmonary illness caused by H5N1. The mechanisms underlying this different effect in older *versus* younger mice is unclear.

In parallel, Li and coworkers investigated the impact of low dose (10^5 cells/mouse) of murine bone marrow-derived MSCs in Avian Influenza virus (H9N2)-induced lung injury in young mice (6-8 weeks).[32] A single intravenous administration led on day 3 p.i. to reduction in mortality, lung edema, histologic injury, BALF and serum chemokines and cytokines, as well as improved gas-exchange and levels of anti-inflammatory mediators, although not reducing lung virus titration when administered either 30 minutes or 24 hours after infection. Only early administration of MSCs was able to reduce some BALF and serum inflammatory mediators and increase levels of IL-10, whereas either administration led to reduction of BALF and serum IL-6 and TNF-α.

In addition, Loy and colleagues found that UC-MSCs were more effective than human BM-derived MSCs (BM-MSCs) at restoring impaired alveolar fluid clearance and permeability *in vitro* airway epithelial cell models.[33] These effects were partially mediated through MSC secretion of Ang-1 and hepatocyte growth factor (HGF). The authors subsequently compared administration of UC-MSCs to BM-MSCs ($5x10^5$ cells/mouse, day 5 p.i.) in experimental lung injury induced by Influenza A (H5N1) infection in female 6-8 weeks old immunocompetent mice. Notably, 3T3 mouse embryonic fibroblasts were utilized as cell controls. Despite failure to reduce virus titration and increase survival rate, a single dose of UC-MSCs decreased body weight loss (days 16, 17 p.i.), lung edema (days 10, 14 p.i.), and inflammation in H5N1-induced lung injury (day 7 p.i.).

Unfortunately, there are as yet no pre-clinical data investigating the effects of MSC administration in models of SARS-CoV-2 respiratory infection, due to the lack of an established animal model. Recently, hACE2 transgenic mice infected with SARS-CoV-2 demonstrated virus replication in lung and interstitial pneumonia with lymphocyte and monocytes infiltration into the alveolar interstitium and accumulation of macrophages in alveolar spaces.[34] While this model requires further evaluation for its similarity to clinical signs and mortality in patients, it might facilitate the testing of therapeutics including cell-based therapies for COVID-19.

6.4   Clinical Data: A Compassionate Use of MSCs in a COVID-19 severly ill patient

To our knowledge, RESCAT co-PI prof. Rachele Ciccocioppo was the first in Europe to apply MSCs as compassionate use to rescue a patient with respiratory failure due to SARS-CoV-2 pneumonia and under mechanical ventilation. A Caucasian male, aged 69 years, with respiratory failure due to SARS-CoV-2 pneumonia was admitted to the Intensive Care Unit of the Ospedale Maggiore (Verona) after having been intubated in the Emergency Care due to a rapid worsening of his respiratory function. He was suffering from type 2 dyabetes and was slightly overweight (body mass index 25.4 Kg/m^2). He was initially treated with antiviral agents (lopinavir–ritonavir) and hydroxychloroquine other than with rounds of prono-supination without any improvement. He did not receive amine to support blood pressure and his Sequential Organ Failure Assessment score was 7/24. Because of the isolation of Staphylococcus epidermidis in the blood arterial culture, and Enterobacter aerogenes in the BALF, cycles of antibiotic therapy were undertaken. After approval by the local Ethics Committee and the Italian Agency of Medicine (AIFA) under the co-called hospital exemption rules, he received two intravenous infusions of umbilical cord-derived MSCs at dosage of 1.1×10^6/kg one week apart. The Investigational Medicinal Product (IMP) consists of umbilical cord-derived MSCs collected at passage 2 and was produced at the Laboratory of Advanced Cellular Therapy in Vicenza and all the procedures, including the shipping, the thawing, the preparation of the infusion and the delivery at bedside, were successful. Neither infusive reactions, nor adverse events were registered throughout the observation period. Despite an improvement of the inflammatory, respiratory, thrombotic and renal parameters after MSC treatment (see Table on the right), the patient underwent tracheostomy because of the inability to respiratory weaning. Finally, following the isolation of Klebsiella Pneumoniae KPC species in both the rectal swab and the BALF, a cycle of

	6/04 (before MSC)	9/04	14/04 (before MSC)	16/04
Creatinin	1,36	0,99	0,89	1,02
PT	1,18	1,09	0,96	0,95
APTT	0,88	1	0,91	1,01
Hemogasanalysis				
P/F	168	136	114	191
FiO2	0,5	0,5	0,7	0,7
Ph	7,31	7,34	7,38	7,35
pCO2	57	61	70	66
pO2	84	68	80	134
HCO3-	28,7	32,9	41,4	36,4
SpO2	98,8	95,3	98,1	99,6
lattati	0,7	0,7	0,6	0,7
BE	1,7	5,9	14,2	8,9
Na/K	146/4	146/3,5	141/4,1	143/3,3
SOFA SCORE	4	3	3	3
Blood cells				
Red Blood cells	4,16	4,13	3,91	4,31
Hb	9,4	9,3	8,6	12,08
WB	11,19	10,14	8,62	12,08
Ht	30,6	30,1	28,7	32,1
PLTs	365	327	287	210
FORMULA				
basophils	0,06	0,05	0,04	0,03
eosinophils	0,46	0,26	0,38	0,07
lymphocyes	1,01	1,11	1,21	1,12
monocytes	0,37	0,44	0,4	0,56
neutrophils	9,29	8,28	6,59	10,3
CRP	201	139	88	103
Pro-calcintonin	2,74	1,06	0,39	0,57

Meropenem, Gentamicine and Daptomicine was undertaken without any improvement of his clinical condition, leading to the patient's death after 4 weeks from the MSC treatment.

6.5   MSCs do not express functional receptors of SARS-CoV-2

Whether MSCs may get infected by SARS-CoV-2 if infused into a patient with an ongoing infection and how this would affect the potential beneficial effects remains to be determined. This may depend both on the virus type and the level of expression or percentage of MSCs expressing the virus receptor. Angiotensin-converting enzyme (ACE)2 has been reported to be the main host cell receptor of the SARS-CoV-2 entry and the serine protease TMPRSS2 for S-protein priming, while the level of gene expression was found to be a key determinant of SARS-CoV-2 transmissibility.[35] ACE2 is highly expressed in respiratory epithelial cells thus playing a crucial role in the entry of virus into these cells.[36] Conversely, ACE2 was reported to protect against non-viral lung injury by degrading the profibrotic peptide angiotensin (Ang)II.[37] Therefore, it is relevant to assess whether MSCs of any origin constitutively or inducibly express ACE2 or TMPRSS2. Up to now, two studies demonstrated that murine BM-derived-MSCs over-expressing the ACE2 gene following lentiviral vector transduction, offered additional anti-inflammatory and endothelial-protective effects against endotoxin-induced lung injury in mice.[38,39] Importantly, both studies showed detectable basal levels of cellular and secreted ACE2 by control BM-MSCs as measured by PCR, western blot, and ELISA. Another study demonstrated that human UC-MSCs, lentivirally transduced to overexpress ACE2, were more effective than constitutively ACE2-expressing UC-MSCs in a rat acute lung ischemia reperfusion injury model.[40] Notably, gene expression profile revealed that both human adipose and marrow MSCs themselves are not overexpressing ACE and TMPRSS2 genes, suggesting that MSC could not be infected by SARS-CoV-2, at least by these known receptors (unpublished data Dominici M. et al.). In this study, the infused MSC lots will be characterized for the expression of ACE2. For this purpose, a sample of the infused MSCs will be sent to the Cell Factory in Vicenza for the flow cytometric characterization and to the Cell Factory in Milano for the gene expression quantification by real-time PCR. The substudy will allow to understand if ACE2 is expressed and if there is a correlation with the efficacy of the treatment.

7   RATIONAL

7.1   Clinical trial rational

COVID-19 is caused by a highly infectious SARS-CoV-2 spreading rapidly since its first discovery in China as a pandemic event.[1] Approximately 19% of patients screened for SARS-CoV-2 positive develop severe type of the disease with a mortality rate of approximately 50%.[2] COVID-19 triggers an acute inflammation leading to cytokine storm, ARDS, brain damage associated with an unexpected intravascular coagulation with organ failure.[2,3] There is currently no cure for critically ill patients with COVID-19. Novel therapies are urgently required to dampen the excessive inflammatory response, inhibit the pathological cytokine storm and speed up the regeneration of tissues. It is aknowledged that ARDS arises as a consequence of a variety of infections or traumas. It is characterized by acute pulmonary oedema, with accumulation of fluid, proteins, neutrophils and red blood cells within alveols as consequence of epithelial and endothelial injury.[41] This causes respiratory failure and arterial hypoxemia that impose respiratory support with lung-protective ventilation and a fluid conservative strategy, as well as early neuromuscular blockade and prone positioning in more severe cases. However, an efficacious pharmacological treatment is still eagerly awaited. This has prompted researchers to develop new strategies, including those based on the use of MSCs.[42-45] In particular, the study with the highest number of enrolled patients (n=40), i.e., the START phase II study, showed that a single dose of allogeneic BM-derived-MSC did not cause short- or long-term haemodynamic or respiratory adverse events over a 60-day follow-up period.[45] However, no significative improvement in ARDS mortality rate was observed, probably because of the imbalances of severity of illness and the low MSC viability, ranging from 35 to 80%.[45]

As far as the SARS-CoV-2 induced severe pneumonia is concerned, other than the failure of respiratory exchange, clinical hallmarks are decrease of lymphocytes, and increase of neutrophils, C-reactive protein and D-dimer, along with the ground glass opacity in the lung that resembles that observed during ARDS.[46] The overactivation of the inflammatory response in an attempt to clear the SARS-CoV-2 infection leads to the production of an array of inflammatory factors, largely dominated by IL-6, that generate the so called "cytokine storm" which eventually leads to multiple organ failure and hypercoaguability status.[47] Thus, reversing the cytokine storm appears as the key strategy to counterattack SARS-CoV-2 pneumonia.

MSCs have been widely used to treat a number of immune-mediated conditions, including acute steroid-refractory graft-*versus*-host disease where the "cytokine storm" is responsible

for the high mortality rate observed in this condition.[48] It is conceivable, therefore, that MSCs may also have positive effects on attenuating the "cytokine storm" during SARS-CoV-2 severe pneumonia. In addition, *in vivo* ed *in vitro* data showed the capability of MSCs of reducing both platelet adhesion and aggregation through the surface expression of proteoglicans.[49] These premises make MSCs particularly suited for treatment of SARS-CoV-2 severe pneumonia and its vascular complications. Indeed, during the outbreack of SARS-CoV-2 infection in China, a number of clinical studies have been undertaken and are still ongoing, where MSCs have been used as new therapeutic tool. The one that has been already published refers to seven patients admitted at the Beijing YouAn Hospital from January 23rd to February 16th, 2020 to whom one MSC intravenous infusion was offered ($1x10^6$ cells/kg) when symptoms were getting worse.[50] Their clinical outcomes significantly improved 2-4 days after treatment, without side effects. Moreover, peripheral lymphocytes and a subset of regulatory dendritic cells (DC) increased, whereas the overactivated cytokine-secreting immune cells decreased. A parallel reduction of the pro-inflammatory cytokine TNF-α, and an increase of those with anti-inflammatory action, such as IL-10 and VEGF, were also evident. These changes resulted in both reduction of pneumonia infiltration and negativity of RT-PCR viral search after MSC treatment. Significant therapeutic benefit and tolerance were reported also in a 65-year-old critically ill patient who had progressed despite intensive therapy and was under mechanical ventilation (Baoshan People's Hospital, China; from Jan 27 to Feb 17, 2020), who underwent three intravenous UC-MSC infusions ($5x10^7$ cells each time) scheduled three days apart, other than antibiotic and thymosin α1-based therapy. After the second administration, both neutrophil and lymphocyte counts fell to normal level, while pneumonia was significantly relieved, and the throat swabs tests turned out negative, without mention of side effects.[51]

Finally, the Food and Drug Administration has recently approved two multicentre studies where MSCs are proposed to treat patients with COVID-19 severe pneumonia. The first is a phase I/II study where umbilical-cord-derived MSCs will be used in 24 cases; it is sponsored by The Cure Alliance, a non-profit group of scientists and innovators. The second is a phase II/III study recruiting 400 cases that will be treated with an industrial preparation of BM-derived MSCs (Athersys). Finally, 24 clinical trials using MSCs for the treatment of COVID-19 patients have been registered in the two main database www.ClinicalTrials.gov and www.chictr.org.cn.[52]

7.2   Rational for using a multi-source MSC approach

The possibility to use different IMP depending on those produced by each Cell Factory participating in this study, adds further value to our protocol. The rationale for using a multi-source MSC approach is based on the following: (1) MSCs can be isolated from various tissues and, while having common features, they all retain similar anti-inflammatory and immunomodulatory actions.[53] Despite this, there may be differences in generating a better therapeutic and safety profile of one MSC type when challenged for a common clinical indication, as the one here proposed; (2) the early improvement reported in COVID-19 patients following MSC administration may be related to peculiar anti-inflammatory and anti-clotting properties of specific MSC obtained from specific tissue. However, it still is unclear which MSC subtype may specifically apply in the context of COVID-19 linked to a still obscure massive inflammatory reaction and clotting activation, impacting mostly lungs and brain; (3) the urgency of having effective therapeutic measures, the severity of COVID-19 and the unclear pathophysiology require a multi-source MSC approach that can be readily produced by the five Cell Factories having expertise in MSC from various sources. Thus, the possibility to compare their efficacy and safety profiles within a well-defined clinical setting represents an extraordinary opportunity. Indeed, although displaying similar steady-state biological properties, MSCs from different tissues could respond to the SARS-CoV-2-related environment in a different way. Finally, this all-Italian expertise could be capitalized, thus opening the doors to a national Cell Factory network. Prospectively, in case all MSC types used will generate a significant therapeutic profile, this would pave the way for a more massive production of MSCs, independently from the tissue source. On the other hand, if a more suitable source of MSCs would emerge, a common production process could quickly be converted to satisfy urgent needs by generating information on MSC use in COVID19 with global impact for human health.

8   STUDY OBJECTIVES

The main study objective is to evaluate the feasibility and the safety of the use of allogeneic MSCs intravenously administered as new therapeutic option to rescue patients with severe pneumonia due to SARS-CoV2 infection. In parallel, the efficacy of this treatment strategy will be also assessed, together with the evaluation of the best MSC population in terms of safety and therapeutic benefit to standardize a treatment protocol for further clinical studies.

8.1   Primary objectives

Evaluation of feasibility and safety of the use of allogeneic MSCs intravenously administered to treat SARS-CoV-2 severe pneumonia by assessing the number of patients who will receive a full MSC treatment and by recording all adverse events (AE) which will be categorized according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0, on the basis of duration, intensity and possible association with the cellular therapy, during the entire duration of treatment, and the subsequent 6-month of mandatory follow-up period.

8.2   Secondary objectives

Assessment of efficacy of the use of allogeneic MSCs intravenously administered in terms of: i) mortality rate at 2 and 4 weeks after the end of treatmentfollow-up; ii) trend of the daily PaO_2/FiO_2 ratio; iii) improvement of the respiratory hallmarks: time to invasive mechanical ventilation, time to independence from non-invasive mechanical ventilation, time to independence from oxygen therapy; iv) radiological response; v) evaluation of changes of the following laboratory tests: neutrophil to lymphocyte ratio, platelet count and volume, C-reactive protein, ferritin, procalcitonin, LDH, fibrinogen, D-dimer. Lung fibrosis will be additionally assessed by imaging (chest CT) after 6 months from the end of the treatment in order to evaluate a possible protective effect.

8.3   Exploratory objectives

Analysis of the immunological profile of both BALF and peripheral blood samples before and after MSC adoptive therapy will be performed in a subset of patients (those enrolled at the A.O.U.I. Policlinico G.B. Rossi & Università di Verona and in Monza Fondazione Tettamanti) in order to unveil possible mechanistic insights. Analyses will be performed on plasma samples targeting inflammation, and pulmonary, cardiac (myoglobin, troponin), vascular and brain damages by ELISA or AlphaLISA (PerkinElmer). Peripheral blood cell (PBMC) profiling will be additionally monitored by flow cytometry including T-helper-1, -2 and -17 cells, regulatory T cells, B cells, NK cells, and monocytes. Analysis of pulmonary damage will be

further conducted on BALF by ELISA assays, single cell-RNAseq, and immunocytochemistry on BALF cytospins. Details are reported in 9.6.4, 9.6.5 and 9.6.6. MSC will be additionally characterization for ACE2 expression by flow cytometry and real-time PCR as described in 6.5.

8.4   Tertiary objective

Define which of the different IMPs used, as stratified according to the tissue of origin (umbilical cord, umbilical cord blood, bone marrow, adipose tissue), in terms of the best safety profile and therapeutic efficacy in this specific clinical setting. To define this, investigators will compare the percentage of total adverse events, SUSARs, SARs related to the treatment and the efficacy parameters recorded following the use of the different IMPs.

9   CLINICAL TRIAL DESIGN

9.1   Main features

This is an investigator-initiated prospective, multicentre, randomized open label controlled double arms phase I/IIa clinical trial evaluating the feasibility, safety and efficacy of two serial intravenous infusions of allogeneic MSCs delivered 5 days ± 48 hours apart in patients suffering from severe pneumonia due to SARS-CoV2 viral infection admitted to monitored COVID Units (both semi-intensive and intensive).

9.2   Study population

The study population consists of 60 adult inpatients suffering from SARS-CoV-2 pneumonia and with worsening respiratory function. Investigators will enroll all those patients who accomplish with the inclusion and exclusion criteria without applying any blinding. A non-blinded, randomized 2:1 design will be adopted. Thus, 40 patients will be receiveng the IMP and 20 will be the control group treaded by standard of care. The participants will be assigned to the experimental or the comparator arm using a central randomization service implemented in the eCRF. Informed consent for participation in the study (consent can be oral if a written consent cannot be expressed) will be collected before the enrollment. If the patient is unable of giving an informed consent and an authorized representative is not available without a delay that would, in the opinion of the Investigator, compromise the potential life-saving effect of the treatment, this can be administered without consent. In this case, consent to remain in the research should be sought as soon the conditions of the patient will allow it. Patients will be free to withdraw their consent any time during the follow-up. A total of 60 patients are required to complete this trial. We anticipate this will require a number of 72 cases to be screened. All SARS-CoV-2 patients will be recruited at the COVID Units listed in the attached document.

Patients will be randomized 2:1, so that 40 will be receiving the IMP in addition to standard of care and 20 will be the control group treaded by standard of care that may also include anti-viral or anti-maric treatments as below described in the concomitant treatment section.

9.2.1   Maximum duration of treatment and follow-up

The enrolment period will cover from the 1st to the 6th month out of the 12-month duration of the study. The maximum duration of the treatment is 7 days. A mandatory 6-months follow-up period is scheduled.

9.2.2   Subject withdrawal criteria

It is under the responsibility of the Principal Investigator who has in charge the patient to withdraw her/him from the study and to advise both the Independent Ethics Committee (IEC) and Regulatory Agency specifying the reason/s, in case of severe AE. A lack of improvement in the parameters set and a worsening of the clinical condition are primarily considered due to the underlying SARS-CoV-2 infection and not to the cellular treatment. In addition, a patient has the right to withdraw from the study at any time for any reason. Subjects who withdraw their consent are considered withdrawn from the study. The Principal Investigator has the right to terminate participation of any patient if it is deemed in the patient's best interest. The reason and circumstances for premature discontinuation must be reported in the patient's case report form (CRF). This means that a patient may be withdrawn in every phase of the trial, from the inclusion to any time of the treatment and follow-up period.

In summary, reasons of withdrawal include, but are not limited, to the following:

- Patient's decision,

- Withdrawal of patient consent to participate in the study,

- Physician's decision based on patient's well-being, severe AE or clinically relevant AE.

For any subject discontinuing the study the investigator should before do the following:

- Ask patient to take part, as far as possible, in the last medical visit to examine the patient's health conditions and perform the required tests,

- Complete the CRF, indicating in the final visit, the date of termination and the reason for discontinuing the study as well as the protocol deviation section, with all data not corresponding to a formal visit filled in the Early Termination visit.

In patients who do not come back for the scheduled study visits, documented efforts should be performed to convince them to continue attending study visits and if unsuccessful, at least the exact reason(s) should be obtained for their discontinuation and any AE associated to it should be recorded.

9.3   Inclusion Criteria

Inclusion criteria are:

- Sign of informed consent,
- Patients of either sex, aged 18-80 years (inclusive),
- Confirmed virological diagnosis of SARS-CoV-2 infection by means of real-time PCR
- Hospitalized due to clinical and instrumental diagnosis of pneumonia at CT scan,

- PaO$_2$/FiO$_2$ between 150-300 with O$_2$ therapy and impending necessity of noninvasive positive pressure respiratory support (nCPAP) or ventilator support through nasal pressure support ventilation (nPSV),

- Systolic artery pressure >90 mmHg without amine support,

- Modified Early Warning Score (MEWS) score <3,

- Absence of known active malignancy.

9.4 Exclusion Criteria

The presence of any of the following will preclude patient inclusion at screening:

- Deny to informed consent,

- Known history of alcohol or drug abuse in the 12 months prior to inclusion,

- Presence of significant comorbidities, such as uncontrolled hypertension, invalidating psychiatric or neurological disorders, organ failure (renal impairment defined by creatinine clearance below 50 ml/min or by serum creatinine $\geq$2.0 mg/dl; hepatic impairment defined by total bilirubin $\geq$2.0 mg/dl and AST + ALT $\geq$ 2.5 $\times$ upper normal value; cardiac failure with an output fraction $\leq$40%), or any other clinically significant condition, as determined by the Principal Investigator,

- Presence of chronic advanced cardio-pulmonary diseases, such as ILDs (obstructive pneumonia, severe pulmonary interstitial fibrosis, alveolar proteinosis, allergic alveolitis),

- Patient has a clinically relevant abnormality on electrocardiogram, as determined by the Principal Investigator,

- History of previous embolism,

- Presence of any active malignancy (other than non-melanoma skin cancer) that required treatment within the last 1 year,

- Patient has a history of severe allergic reactions (e.g., swelling of the mouth and throat, difficulty breathing, hypotension, or shock) that require medical intervention,

- Patient with a positive test for human immunodeficiency virus or active hepatitis B or C disease or tuberculosis or further viral infections (influenza virus, adenovirus and other respiratory viruses),

- Patient is known to be pregnant, has a positive pregnancy test or is nursing,

- Patient has had major surgery, either open or laparoscopic, within the 3 months prior to screening,

- Previous haematopoietic stem cell or organ transplantation,

- Patient under immunosuppressive agents,

- Patients currently receiving, or having received within 2 months prior to enrolment into this clinical study, any other investigational drug.

9.5   Study Treatment

For the purpose of the RESCAT study two doses of the IMP MSC will be administered. The preparation and labeling of each IMP MSC product will be performed as detailed in the IMP brochure according to Good Manufacturing Procedures (GMP) guidelines. The trial sites have standard operating procedures in place for storage, release, thawing, preparing and administration of the IMP MSC. The IMP MSC must be stored in the vapor phase of a liquid nitrogen tank until the time of infusion, and shipped to the centers in dry ice or liquid nitrogen. The cryopreserved IMP MSC must be thawed and diluted prior to administration.

The infusion procedure will be performed according to the instruction. Care will be taken to infuse the IMP MSC within 30 minutes from thawing drop-by-drop to minimize cell death and with an infusion period lasting 30 minutes to optimize patient's tolerance. The infusion rate should not exceed 1 ml of DMSO/kg of recipient weight. MSC will be administered by intravenous infusion via an in-situ central or peripheral venous catheter. The treatment consists of two serial intravenous infusions of $1.0\text{-}1.5\text{x}10^6$ MSC/kg body weight 5 days $\pm$ 48h apart. The minimum and the maximum dosage allowed is $1.0\text{-}1.5\text{x}10^6$ MSC/kg body weight, respectively. The administration of the medication will not be masked. The mandatory follow-up period is of 6 months from the last infusion. Vital signs will be monitored prior to infusion, every 15 minutes during infusion, directly following infusion and then hourly forafter 4 hours. During both infusions the patient will be monitored for signs of any infusion reaction. The occurrence of infusions reactions, if present, will be recorded on the patient's record forms electronic-CRF (eCRF). For each center, the Principal Investigator will attend the entire duration of the treatment. Facilities for resuscitation will be available at patient bedside.

At MSC producing trial sites, records will be maintained of the MSC product's delivery to the clinical unit, the use in each patient, cryoshipper temperature tracking, problems and irregularities during infusion, alternative disposition of unused product(s). The Principal Investigator will maintain records that document adequately that the patients were provided the doses specified by the protocol and reconcile all investigational products received from the MSC producing site.

9.5.1 Concomitant therapy

There is no absolute or relative contraindication to concomitant medications if required by a specific clinical condition. By contrast, all therapies that represent the current standard of

hospital care for the treatment of pneumonia due to SARS-CoV2 infection, including authorized antiviral agents, hydroxyxlorochine and oxygen delivery to target a minimum of 94% at the oximetry monitoring will be warranted during all the duration of the study both in the control and in the experimental IMP arm.

9.5.2 <u>Procedures for monitoring of the patients' compliance: stopping rules</u>

- The incidence of allergic reaction that exceeds the agreed limits (see below) unless the Data Safety Monitoring Committee (DSMC) gives a positive opinion to continue,

- One death considered related to the direct use of MSCs by the Principal Investigator and the DSMC,

- Eight deaths unrelated to the use of MSC in the total study population with the consensus recommendation of the DSMC.

9.5.3 <u>Criteria for the premature termination of the trial</u>

If the study is prematurely terminated or suspended for any reason, the PI has to inform the patients and to ensure appropriate follow-up for them. In addition, the PI must promptly inform both the IEC and Regulatory Authorities with providing the reason(s) for the termination or suspension. These may include, but are not limited to the following:

- Incidence or severity of AE indicating a potential health hazard to subjects,

- Investigator does not adhere to protocol or applicable regulatory guidelines in conducting the study,

- Any other medical reason.

9.6   Study Outcome Measures

9.6.1 <u>The primary outcomes</u>

They include the evaluation of feasibility through the assessment of the production capacity of the Cell Factories participating in the study of an adequate quantity of cellular product lots sufficient to treat 100% of the patients recruited. The process of transfer of the cellular product to the Clinical Units will be validated, so that all the quality, safety and efficacy requirements will be guaranteed with particular reference to the maintenance of the potency, including cell viability of the cellular product. Finally, a bedside use protocol will be implemented that respects the Good Clinical Practice, including the necessary containment measures. This also represents the coordination basis for the Italian Cell Factory network.

The second primary endpoint consists inof the evaluation of the safety, in terms of monitoring of systemic tolerance, AE (for example, a noxious reaction) and severe AE (i.e. a life-threatening condition requiring hospitalization or resulting in disability or death), that will be performed from the signing of the informed consent onwards and during every visit. Specifically, the severe AE will be electronically recorded and transmitted as they occur by email to both the IEC and Regulatory Agency, while cumulative AE will be electronically recorded at each scheduled visit by the PI and communicate to both the IEC and Regulatory Agency by email at the end of the study. Moreover, unexpected events will be also monitored and electronically recorded as they occur.

9.6.2 The secondary outcome measures

These include the assessment of exploratory efficacy of intravenous infusions of allogeneic MSCs in terms of modification of the following clinical variables: i) mortality rate at 2 and 4 weeks after the end of treatmentfollow-up; ii) trend of the daily PaO_2/FiO_2 ratio; iii) respiratory parameters: time to invasive mechanical ventilation, time to independence from non-invasive mechanical ventilation, time to independence from oxygen therapy; iv) radiological response as scored following already established criteria for both chest X-ray[54] and CT scan[55]; as well as v) of the following laboratory tests: neutrophil to lymphocyte ratio, platelet count and volume, coagulation parameters (including fibrinogen, D-dimer), C-reactive protein, ferritin, procalcitonin, LDH. Finally, a CT scan will be performed before and after 6 months from the end of the IMP treatment in order to evaluate a possible protective effect of MSC therapy towards lung fibrosis.

9.6.3 The exploratory outcome measures

Cytokine and biomarker profile on BALF and plasma, and cellular immunophenotype on both BALF and PBMC of a subset of patients (those recruited at the A.O.U.I. Policlinico G.B. Rossi & Università di Verona) before and after the treatment will be assessed by means of ELISA, flow cytometry, immunocytochemistry and single cell RNAseq, to investigate the immunological response to treatment and to find a correlation with patients' outcome. Characterization of ACE2 expression on infused MSCs by flow cytometry and RT-PCR will be also carried out on all the IMPs applied in this study. Below the details of these measures.

9.6.4 Cytofluorimetric analyses

In a sub-group of patients treated in this study (A.O.U.I. Policlinico G.B. Rossi & Università di Verona), the following blood samples will be collected before (T0), seven days (T7) and fourteen days (T14) after cell-therapy: two 7-ml vials containing EDTA and one vial for

serology. EDTA-containing samples will be processed in a closed-circuit by an automatic dispenser that splits the content into separate vials and add the antibody mix, lysis buffer and fixative. For some protocols, the split vials can be transferred to a robotic centrifuge for washing steps. In this case, washed cells will be collected to add antibody mix, lysis buffer and fixative in a laminar flow hood inside a BSL2 operational laboratory. The Personal Protective Equipment (PPE) areis available for all the operators who are fully instructed about the protocols.

The antibody mixes allow to define the following leukocyte subsets: T lymphocytes ($CD45^+CD3^+$): T $CD4^+$, T $CD8^+$, T $CD4^-$ $CD8^-$ (including gamma delta TCR^+ alpha beta TCR), activated $CD8^+$ ($CD38^+HLA-DR^+$), senescent/exhausted $CD8^+$ ($CD57^+PD-1^+$) and NK-like cells ($CD56^+CD16^+$); B lymphocytes ($CD45^+CD3^-CD19^+$): naive (IGD^+CD27^-), marginal zone like memory (IgD^+CD27^+), switched memory (IgD^-CD27^+), and exhausted (IgD^-CD27^-). NK cells ($CD3^-CD16^+CD56^{+)}$, and their subsets ($CD8^{+)}$ e ($CD57^+$). Different myeloid cells: monocytes ($CD45^+CD4^+$ $CD3^-$), ($CD14^{++}CD16^-$), non-classic monocytes ($CD14^-CD16^+$) and intermediate monocytes ($CD14^+CD16^+$). Granulocytes and circulating dendritic cells will also be enumerated. Whenever cellular recovery is appropriate, we will also evaluate the expression of ACE1, ACE2, c-FLIP, ARG1 (arginase 1) and IDO1 (indoleamine 2,3-dioxygenase 1), the last two being enzymes involved in immune suppressive circuits. To determine the absolute cell count per microliter of blood, for each patient and at every time point, a complete blood cell count, including differential white blood cell count and platelets, will be performed at the recruiting hospital. Moreover, in Monza (Fondazione Tettamanti), there will be an additonal focus on the peculiar abundance of CD138+ plasma cells resulted a relevant feature of COVID-19. To investigate this aspect and to analyse the possible impact of MSC infusion on the so-called "exuberant plasmocytosis", a fraction of collected BALF specimens will be cytospinned and stained for the detection of T cells (CD3), B cells (CD20) and activated plasma cells (CD138).

9.6.5 Serum and/or plasma for biomarkers and cytokine analysis

Plasma or serum collected after removal of the cellular fraction will be transferred to two vials and immediately cryopreserved. Aliquots will be frozen in boxes and stored in the -80°C freezer at the University of Verona (Unità Complessa di Immunologia) and at the Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milano and Fondazione Tettamanti, Monza. Vials will be thawed and tested by ELISA for the presence and quantification of markers of pulmonary, cardiac, vascular and brain damage, as well as for following cytokines will be analysed: GM-CSF, G-CSF, M-CSF, IFN-γ, IFN-α, IL-1, IL-2, IL-4, IL-5, IL-

6, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17A, IL-17F, IL-17E, IL-21, IL-22, IL-23, IL-27, IL-28A, IL-31, IL-33, IL-34, MIP-3α/CCL20, CCL2, TNF-α, TNF-β, and TGFβ. In addition, to monitor pulmonary damage, PTX3 will be evaluated by ELISA assays in plasma samples and BALF fluids. BALF samples (when feasible) will be also analysed for the presence of d-dimer and OH-proline as markers of fibrinolysis to track the fibrotic evolution of lung disease and for the presence of cell subsets and soluble mediators associated with lung injury and inflammatory status (neutrophils, TNFα and CXCL1). Concerning the analysis of cardiac damage, myoglobin and troponin will be evaluated by ELISA assays in the plasma of infused patients. In addition, PTX3 plasma levels will be correlated with clinical data about cardiac function.

9.6.6 Single-cell RNA sequencing of cells in the blood and bronchoalveolar lavage

At the A.O.U.I. Policlinico G.B. Rossi & Università di Verona, whenever possible as part of the diagnostic/follow-up protocols in selected patients the BALF will be processed to isolate the viable cells and perform a single cell RNA-seq of the transcriptome; the blood cells will be used to compare the transcriptomes in these two compartments. All the procedures, from the initial processing to the first run for the single cell library production, will be operated in BSL3 environment by PPE-equipped and fully trained operators of the Immunology Unit in Verona. The *10x Genomics* Company supplied the necessary technology, which is already located within the BSL3 space.

Collectively, these analyses would represent a great step forward towards the understanding of both pathogenesis and therapeutic efficacy of this new therapeutic strategy.

9.6.7 Tertiary outcome measure

We plan to treat 10 cases with umbilical cord-derived MSCs, 10 cases with umbilical cord blood-derived MSCs, 10 cases with bone marrow-derived MSCs, and 10 cases with adipose tissue-derived MSCs. We will compare the percentage of total AEs, SUSARs, SARs related to the treatment and the efficacy parameters recorded following the use of the different IMPs.

9.7 Study Procedures: study assessment, including the sequence and timing of study procedures

RESCAT is a multicentre, randomized open label-controlled double arms phase I/IIa clinical trial evaluating the feasibility, safety and efficacy of two serial intravenous infusions of allogeneic MSC delivered 5 days ± 48 hours apart in patients suffering from severe

pneumonia due to SARS-CoV2 viral infection admitted to both semi-intensive and intensive COVID Units. Subjects eligible for the study will be randomly assigned to either the experimental or comparator arm. Patients randomized to experimental arm will be treated with two doses of MSCs (1.0×10^6 cells/Kg/dose, up to a maximum of 1.5×10^6 cells/kg) delivered IV. The first dose will be administered within 48 ± 6 hours following study enrollment. Subjects will be evaluated +4 and +48 hours following treatment, with the second MSC dose administered 5 days ± 48 hours apart. Subjects enrolled in the trial will be assessed at baseline and at 4-, 48- hours post-infusion and then at 14 days, 28 days, and 180 days after the end of MSC treatment.

- Screening evaluations must be completed and reviewed to confirm that potential participants meet eligibility criteria. The PI 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 (eg, blood count) and obtained before informed consent may be used for screening or baseline purposes provided the procedures met the protocol-specified criteria.

9.7.1 <u>Screening procedures</u>

- Signing of the Informed Consent Form

- Demography (age, gender, ethnicity)

- Medical history (previous and current diseases, all medications started within 90 days prior to screening visit)

- Full physical examination including height and weight

- Arterial Blood Gas (ABG) Analysis

- Respiratory condition and need for assistance (respiratory therapies, see the inclusion criteria)

- Laboratory assessments: complete blood count (CBC); coagulation and metabolic panel (CMP: bilirubin, AST, ALT, creatinine, LDH, PT, PTT, D-dimer); inflammatory markers (C-reactive protein levels, ferritin, procalcitonin, fibrinogen); Troponin I

- 12-lead ECG

- Vital signs (respiratory rate, pulse, blood pressure and temperature)

- Modified Early Warning Score (MEWS) for Clinical Deterioration <3

- CT scan or Chest XR (if clinically indicated)

9.7.2 Treatment and procedures during hospitalization period

- Arterial Blood Gas (ABG) Analysis as per clinical need

- Respiratory assistance assessment

- Laboratory assessments: complete blood count (CBC); coagulation and metabolic panel (CMP: bilirubin, AST, ALT, creatinine, LDH, PT, PTT, D-dimer); inflammatory markers (C-reactive protein levels, ferritin, procalcitonin, fibrinogen); Troponin I

- Immunological markers (lymphocytes subsets at the University of Verona and serical and plasma markers both in Verona and at the Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milano)

- 12-lead ECG

- Vital signs (respiratory rate, pulse, blood pressure and temperature)

- MEWS assessment

- CT scan or Chest XR (if baseline evaluation (CT or XR) is available a re- evaluation is planned on day 7 and then if clinically indicated)

- In the experimental group only, treatment with MSC 1.0×10^6 cells/kg (up to a maximum of 1.5×10^6 cells/kg) within 48 hours ± 6 hours (Visit 2). A second administration (same dose) will be given after 5 days ± 48 hours

- AE review (including SAEs)

- Concomitant medication review.

9.7.3 Procedures before discharge

- Arterial Blood Gas (ABG) Analysis

- Respiratory assistance assessment

- Laboratory assessments: complete blood count (CBC); coagulation and metabolic panel (CMP: bilirubin, AST, ALT, creatinine, LDH, PT, PTT, D-dimer); inflammatory markers (C-reactive protein levels, ferritin, procalcitonin, fibrinogen); Troponin I

- 12-lead ECG

- Vital signs (respiratory rate, pulse, blood pressure and temperature)

- MEWS assessment

- Chest XR or CT scan if clinically indicated

- AE review (including SAEs)

- Concomitant medication review

9.7.4 <u>Follow-up procedures</u>

- Follow-up information will be collected by patient medical records and/or clinical visits for both experimental and control groups

– AE review (including SAEs)

9.7.5 <u>Efficacy assessment</u>

- PaO$_2$/FiO$_2$ ratio. PaO$_2$/FiO$_2$ ratio represents the ratio between the arterial blood partial pressure of the oxygen (PaO$_2$) and the percentage of oxygen supplied (fraction of inspired oxygen, FiO$_2$). This parameter is calculated from arterial blood gas analysis and is commonly used for the definition of ARDS. A PaO$_2$/FiO$_2$ ratio of 300<200 identifies mild ARDS; 200<100 moderate ARDS, <100 is suggestive for severe ARDS.

- Laboratory assessment. All the laboratory tests will be assessed by routinely used determination.

- -Radiological response as scored following already established criteria for both chest X-ray[54] and CT scan[55].

- Immunology profile. As described in 8.5.3.1

9.7.6 <u>Details of the visits</u>

Visit 1: Screening Visit and Baseline Assessments

- Informed Consent process
- Demographics
- Medical history
- Complete physical exam
- Oxygenation Index (P/F), established by the above citated formula
- Respiratory parameters (e.g., PEEP, PS-pressure level, compliance in hours)
- Arterial Blood Gas (ABG)
- Blood collection for laboratory testing
- BALF collection
- 12 leads ECG
- Chest radiograph or chest CT scan
- Pregnancy test (in females)
- Concomitant medication review
- Randomization

Visit 2: Day 1 MSC Administration #1

- In the experimental arm only, within 48 ± 6 hours from enrollment, eligible patients will be treated by the first intravenous infusion of MSCMSCs. Infusion will occur with 1.0-1,5x10^6 cells/kg. Cells will be thawed at 37°C and resuspended 1:1 in a final volume of 50 ml of buffer constituted of saline and human serum albumin and administered in the time period of 30 minutes (1,6 mL/min). A total of 20 mL of normal saline should be administered following the infusion of the cells to flush the remaining cells through the intravenous set.
- The infusion will be administered by one of the licensed Co-PIs
- Injection site assessment (only the experimental arm)

In both control and experimental arms:
- Adverse events assessment
- Arterial Blood Gas (ABG)
- Monitoring for ventilator parameters (P/F, PEEP, PS, compliance in hours)
- Monitoring for arrhythmia/tachycardia/fibrillation/asystole/cardiac arrest
- Monitoring for transfusion incompatibility/infection
- Monitoring need for Ventilator
- Vital signs

Visit 3: Day 1 Safety Assessment (4h ± 2h4 hours ± 2 hours post MSC administration #1 for experimental arm)
- Injection site assessment (only the experimental arm)

In both control and experimental arms:
- Adverse event assessment
- Vital parameters
- Respiratory parameters (P/F, PEEP, PS, compliance in hours)
- Arterial Blood Gas (2cc)
- Concomitant therapies

Visit 4: Day 3 Safety Assessment (48h ± 6h48 hours ± 6 hours post MSC administration #1 for experimental arm)
- Injection site assessment (only in the experimental group)

In both control and experimental arms:
- Vital parameters
- Adverse event assessment

- Respiratory parameters (P/F, PEEP, PS, compliance in hours)
- Arterial Blood Gas
- Complete blood count
- Coagulation and metabolic panel
- Inflammatory Markers
- Troponin I
- Concomitant therapies
- MEWS assessment

Visit 5: Day 4 Safety, MSC Administration #2 (5 days ± 48h48 hours post MSC Administration #1 for Experimental arm)

- MSC Administration, intravenous infusion. Infusion will occur with $1.0\text{-}1,5\text{x}10^6$ cells/kg. Cells will be thawed at 37°C and resuspended 1:1 in a final volume of 50 ml of buffer constituted of saline and human serum albumin and administered in the time period of 30 minutes (1,6 mL/min). A total of 20 mL of normal saline should be administered following the infusion of the cells to flush the remaining cells through the intravenous set.
- The infusion will be administered by one of the licensed Co-PIs.
- Injection site assessment.

In both control and experimental arms:
- Vital parameters
- Adverse event assessment
- Arterial blood gas
- Complete blood count
- Coagulation and metabolic panel
- Troponin I
- Inflammatory markers
- MEWS assessment
- Respiratry parameters (P/F, PEEP, PS, compliance in hours)
- 12 leads ECG
- Concomitant therapies

Visit 6: Day 5 Safety Assessment (4h ± 2h4 hours ± 2 hours post MSC Administration #2)

- Injection site assessment (only in the experimental group)

In both control and experimental arms:

- Vital parameters

- Adverse event assessment

- Respiratory parameters (P/F, PEEP, PS, compliance in hours)

- Arterial Blood Gas

- Concomitant therapies

Visit 7: Day 6 Safety Assessment (+48h ± 6h48 hours ± 6 hours post MSC Administration # 2)

- Injection site assessment (only in the experimental group)

In both control and experimental arms:

- Vital parameters

- Adverse event assessment

- Respiratory parameters (P/F, PEEP, PS, compliance in hours)

- Arterial Blood Gas

- Complete Blood Count

- Coagulation and Metabolic Panel

- Troponin I

- Inflammatory markers

- Concomitant therapies

- MEWS

Visit 8: Day 14 Safety Assessment (in both control and experimental arms)

- Complete physical examination

- Medical history

- Vital parameters

- Arterial blood gas

- Complete blood count

- Coagulation and Metabolic Panel

- Troponin I

- Inflammatory markers

- MEWS assessment

- Chest radiograph or CT scan

- Injection site assessment.

- Adverse event assessment
- Blood collection for Laboratory testing (30 cc)
- BALF collection
- Concomitant therapies
- Respiratory parameters (P/F, PEEP, PS, compliance in hours)

Visit 9: Day 28 Safety/Efficacy Assessment (± 2 days, in both control and experimental arms**)**

- Medical history
- Complete physical exam
- Vital parameters
- Arterial blood gas
- Complete blood count
- Coagulation and Metabolic Panel
- Troponin I
- Inflammatory markers
- MEWS assessment
- Chest radiograph or CT scan
- Injection site assessment
- Concomitant therapies
- Blood collection for laboratory testing (30 cc)
- BALF collection
- Adverse event assessment

Visit 10: Day 180 Efficacy Assessment (± 5 daydays, in both control and experimental arms**))**

- Medical history
- Complete physical exam
- Vital parameters
- Complete blood count
- Coagulation and metabolic panel
- Inflammatory markers
- Chest TC scan
- Concomitant therapies
- Adverse event assessment

Early Termination Visit and Unscheduled follow-up

The following parameters will be assessed and recorded: date and reason, physical examination, laboratory analyses, AE, concomitant medication taken since last visit. A patient will be identified as 'lost to follow-up' for voluntary drop out or death.

Early Termination Visit and Unscheduled follow-up

9.8   Clinical Trial Flow Chart

```
                    ┌─────────────────┐
                    │  Screening for  │
                    │   eligibility   │
                    └────────┬────────┘
                             │
                             │          ┌──────────────┐
                             ├─────────▶│  Screening   │
                             │          │   Failure    │
                             ▼          └──────────────┘
                    ┌─────────────────┐
                    │ Randomization   │
                    │      2:1        │
                    └────────┬────────┘
                   ┌─────────┴─────────┐
                   ▼                   ▼
          ┌─────────────┐      ┌─────────────┐
          │   MSC +     │      │ Standard of │
          │ standard of │      │    care     │
          │    care     │      │             │
          └──────┬──────┘      └──────┬──────┘
   ┌──────────┐  │                    │  ┌──────────┐
   │ Drop out │◀─┤                    ├─▶│ Drop out │
   └──────────┘  │                    │  └──────────┘
                 ▼                    ▼
          ┌─────────────┐      ┌─────────────┐
          │   2 weeks   │      │   2 weeks   │
          │ assessments │      │ assessments │
          └──────┬──────┘      └──────┬──────┘
                 ▼                    ▼
          ┌─────────────┐      ┌─────────────┐
          │   4 weeks   │      │   4 weeks   │
          │ assessments │      │ assessments │
          └──────┬──────┘      └──────┬──────┘
                 ▼                    ▼
          ┌─────────────┐      ┌─────────────┐
          │  6 months   │      │  6 months   │
          │ assessments │      │ assessments │
          └─────────────┘      └─────────────┘
```

9.9   Table 1. Schedule of Assessment

Procedures	Visit 1 Screening/Enrollment/ Baseline Day	Visit 2 IMP infusion #1*	Visit 3 +4 infusion#1	Visit 4 +48 h infusion#1	Visit 5 IMP infusion#2*	Visit 6 +4 h infusion #2	Visit 7 +48 h infusion #2	Visit 8 Day 14 ±6h	Visit 9 Day 28 ±2 days	Visit 10 Day 180 ±7 days
Informed consent	x									
Demographics	x									
Medical history	x							x	x	x
Complete physical exam (and BMI calculation)	x							x	x	x
Vital Parameters	x	x	x	x	x	x	x	x	x	x
Arterial Blood Gas (ABG)	x	x	x	x	x	x	x	x	x	x
Blood collection (for cytokine panel, immune cells) 30 cc (if applicable)	x							x	x	
Broncoalveolar Lavage collection (if applicable)	x							x		
Complete Blood Count (CBC)	x			x	x		x	x	x	x
Coagulation and Metabolic Panel (CMP)	x			x	x		x	x	x	x
Troponin I	x			x	x		x	x		
Inflammatory markers	x			x	x		x	x	x	x
Modified Early Warning Score assessment - MEWS	x	x		x	x		x	x		
Chest radiograph or CT scan[1]	x							x	x[1]	x[1]
Pregnancy test (women with childbearing potential, without signs or current history)	x									
Review of Inclusion/Exclusion criteria	x									
Concomitant medication review	x									
IMP administration (intravenous infusion) *		x			x					
Injection site assessment*		x	x	x	x	x	x	x	x	
Concomitant Therapies	x	x	x	x	x	x	x	x	x	x
Adverse event assessment			x	x		x	x	x	x	x
Monitoring for respiratory parameters (P/F, PEEP, PS, compliance in hours)	x	x		x	x		x	x		
12 leads ECG	x	x			x			x		
Monitoring: transfusion incompatibility/infection*		x	x	x	x	x	x			
Monitoring: need for Ventilator	x	x	x	x	x	x	x	x	x	x
Monitoring: cardiac arrest or death		x	x	x	x	x	x	x	x	x
Adverse event review and evaluation		x	x	x	x	x	x	x	x	x
Complete Case Report Forms (CRFs)	x	x	x	x	x	x	x	x	x	x

*Only in the IMP experimental group

9.10 Study duration and number of study visits required of research participants

The study involves a total of 10 visits, with the last visit at 180 days after study entry. Visits will take place at the clinical trial sites, at the Hospital, or – for those patients discharged from the hospital prior to day 180.

9.11 Blinding, including justification for blinding or not blinding the trial, if applicable

The current study seeks to identify an "efficacy signal", as well as to further develop an understanding of the safety and feasibility of the procedure. Accordingly, for this early phase efficacy study, the design will be unblinded.

9.12 Justification of why participants will not receive routine care or will have current therapy stopped

Patients enrolled into the study possess a disease severity for which no standard of care exists. Patients will be allowed to continue receiving routine symptomatic and supportive care and current therapy.

9.13 Justification for inclusion of a non-treatment group and for the randomization

This study is designed to test the hypothesis that MSC therapy is feasible and that this approach is safe and maybe protective against severe pneumonia in in COVID-19 patients. In order to test feasibility, and especially obtain definitive information on safety of MSC treatment and collect preliminary evidence of MSC biological protective effects we will adopt a randomized 2:1 design. Specifically, a total of 60 patients will be recruited and randomized to IMP and standard of care treatment (40 patients) or to standard of care only (20 patients: control group). Our cohort of patients is in critical condition with worsening conditions expected in about a third of them. Thus, a control group is needed to discriminate between the occurrence of complication related to the disease itself or to safety aspects related to the tested treatment. Randomization will avoid any selection bias allowing optimal comparison between treated and control patients. Furthermore, a control group will allow us to obtain preliminary evidence on the biological effects of MSC by comparing circulating and pulmonary inflammatory markers (as absolute values and temporal changes) in treated patients versus controls.

9.14   Definition of treatment failure or participant removal criteria

Treatment failure will be characterized as lack of response on qualitative and/or quantitative parameters assessed. Specifically, treatment failure is defined as the persistence of a P/F <150 at day 5-7 following the end of the MSC treatment, which will assure a rescue therapy based on judgment by the attending physician. Participant will be removed from the study if they have a grade 2 or greater adverse event or if the attending physician believes it would be in the best interest of the subject to halt the treatment.

9.15   Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely

Patients are free to undergo other therapies when the study ends or if the participant's participation in the study ends prematurely.

9.16   Subject Population

Patient recruitment for the clinical trial will be open to all countries. No discrimination will be made as to ethnicity, age, income, or education if the following criteria are met.

Emergency use of the study protocol: there will be no use of the product for emergency use since the product does not address any potential medical emergency.

To be included in the study the subject must completely understand the potential risks and potential benefit of participating in the study and sign the Informed Consent Form for this study. All subjects should consult with their personal physician before agreeing to participate in the study and before signing the informed consent form. Any questions the patient has will be answered by the Principal Investigator verbally. Other communication with potential subjects will take place by email and/or fax.

Subjects should report any discomforts, problems, or injuries immediately to the physicians involed in the study. In the event that a subject suffers injury as a result of participation in this study, usual and customary outpatient or in-patient medical care will be provided by the physicians involved in the study. No financial compensation in any other form will be available.

Subjects must meet all inclusion and exclusion criteria. Results of all baseline evaluations, which assure that all inclusion and exclusion criteria have been satisfied, must be reviewed

by the Principal Investigator or his/her designee prior to enrollment of that subject. The subject must be informed about all aspects of the study and written informed consent must be obtained from the subject prior to study procedures.

10   ADVERSE EVENTS

10.1   Definitions

An adverse event (AE) is any untoward medical occurrence in a study participant administered the medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an 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 reaction (AR) is an untoward and unintended response to the investigational medicinal products related to any dose administered, judged by either the investigator or the promoter.

An unexpected adverse reaction (UAR) is an adverse reaction, the nature or severity of which is not consistent with the applicable products information (investigator's brochure).

A Serious Adverse Event (SAE) is untoward medical occurrence or effect that at any dose results in death, risk of death, permanent disability/incapacity, hospitalization or prolongation of existing hospitalization or need for urgent medical treatment, or another medically important serious event as judged by the investigator. Further, any unexpected changes in relation to the toxicity profile of the drugs used of grade 3, as well as adverse event(s) which, although not falling within this definition, are considered unexpected and serious by the Investigator should be reported.

The causality of SAEs (their relationship to all study treatment/procedures) will be assessed by the investigator(s) and communicated to the coordinating centre.

A Suspected Unexpected Serious Adverse Reaction (SUSAR) is an unexpected adverse reaction judged serious by the Investigator and/or Promoter, that is not consistent, either in nature or in severity, with the applicable product information.

Adverse events of special interest (AESI): the following adverse events have been identified as AESI for this study and require prompt reporting to Safety desk for the study immediately and no more 24h of the Investigator becoming aware of the event (expedited reporting), even if the events can be considered non-serious according to the usual regulatory criteria as they may be subject to expedited submission to regulatory authorities:

- Cases of potential drug-induced liver injury (DILI) that include an elevated ALT or AST in combination with either an elevated bilirubin or clinical jaundice, as defined by Hy's law.

- Suspected transmission of an infectious agent by the study drug (STIAMP), defined as any organism, virus, or infectious particle (e.g., prion protein transmitting transmissible spongiform encephalopathy), pathogenic or non-pathogenic, that is considered an infectious agent. A transmission of an infectious agent may be suspected from clinical symptoms or laboratory findings that indicate an infection in a patient exposed to a medicinal product. This term applies only when a contamination of the study drug is suspected.

10.2   Collection and reporting of adverse events

All AEs recorded from time of signature of informed consent, throughout the treatment and observation period up to 30 days following registration, have to be reported in the Adverse Event form, graded according to the corresponding CTCAE term (Version 5.0). The Investigator must immediately report to the promoter all serious AEs. The report should be made using the SAE report form online or by sending the paper copy by fax (+39 085 9047113) to the coordinating office immediately and not exceeding 24 hours following knowledge of the event. All SAE must be also reported in the toxicity case report form within the corresponding CTCAE term. During the course of the study all AEs and SAEs should be proactively followed up for each subject. Every effort should be made to obtain a resolution for all events, even if the events continue after discontinuation/study completion. The investigator is responsible for following all SAEs until resolution, until the subject returns to baseline status, or until the condition has stabilized with the expectation that it will remain chronic, even if this extends beyond study participation.

10.3   Causality assessment between treatment and event

The following criteria will be used for causality assessment:

Certain	A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to drug administration, and which cannot be explained by concurrent disease or other drugs or chemicals.
Probable/likely	A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to the concurrent disease or other drugs or chemicals.
Possible	A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, but which could also be explained by concurrent disease or other drugs or chemicals.

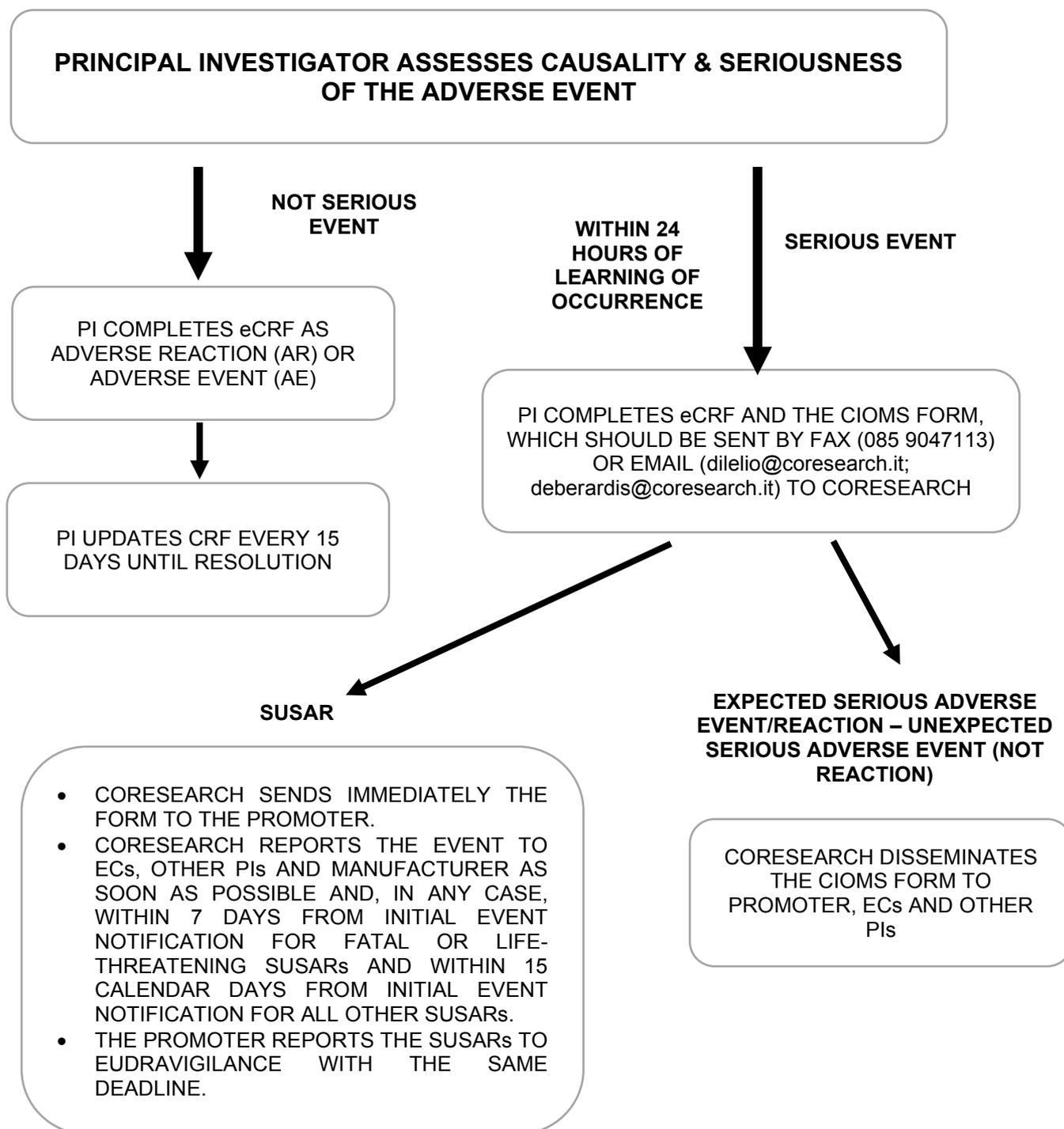| Unlikely	A clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations.
Not related	There is no causal relationship between the treatment and the event
Conditional/unclassified	A clinical event, including laboratory test abnormality, reported as an adverse reaction, about which more data is essential for a proper assessment or the additional data are under examination.
Unassessible/unclassifiable	A report suggesting an adverse reaction which cannot be judged because information is insufficient or contradictory, and which cannot be supplemented or verified.

10.4   Regulatory Reporting Requirements for SAEs

- Prompt notification by the investigator to the promoter of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study treatment under clinical investigation are met.

- The promoter will review all AEs and issue queries directly to the Investigator reporting the event. The promoter will determine if an event qualifies as a SUSAR.

- The Reference Safety Information (RSI) necessary to classify an adverse reaction as SUSAR, based on the nature and seriousness, including the frequency, is located in the specific section of the Investigator's Brochure of MSC IMP.

- The promoter has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study treatment under clinical investigation. The promoter will report all SUSARs to Eudravigilance through the EVCTM, to all participating Investigators, to the Ethical Committee, and to the manufacturer, within the timelines of the article 17 of the European Directive 2001/20/EC.

- Investigator safety reports must be prepared for any SUSAR and forwarded to investigators as necessary.

- An investigator who receives an investigator safety report describing a SAE or other specific safety information (i.e summary or listing of SAEs) from the promoter will review and then file it along with the Investigator's Brochure.

- The promoter will provide an annual Development Safety Update Report, including all Serious Adverse Events occurring in the Study, to the Regulatory Agency, all participating Investigators, and to the Ethical Committees of participating centers.

- The Investigators are responsible for informing the Ethics Committee of the SAE reported in their centre, as per local requirements.

The following flowchart gives an overview of the reporting procedure as regard as the IMP, as well as the exceptions that apply.

10.4.1 Safety reporting flowchart for IMP

PRINCIPAL INVESTIGATOR ASSESSES CAUSALITY & SERIOUSNESS OF THE ADVERSE EVENT

NOT SERIOUS EVENT

WITHIN 24 HOURS OF LEARNING OF OCCURRENCE

SERIOUS EVENT

PI COMPLETES eCRF AS ADVERSE REACTION (AR) OR ADVERSE EVENT (AE)

PI UPDATES CRF EVERY 15 DAYS UNTIL RESOLUTION

PI COMPLETES eCRF AND THE CIOMS FORM, WHICH SHOULD BE SENT BY FAX (085 9047113) OR EMAIL (dilelio@coresearch.it; deberardis@coresearch.it) TO CORESEARCH

SUSAR

- CORESEARCH SENDS IMMEDIATELY THE FORM TO THE PROMOTER.
- CORESEARCH REPORTS THE EVENT TO ECs, OTHER PIs AND MANUFACTURER AS SOON AS POSSIBLE AND, IN ANY CASE, WITHIN 7 DAYS FROM INITIAL EVENT NOTIFICATION FOR FATAL OR LIFE-THREATENING SUSARs AND WITHIN 15 CALENDAR DAYS FROM INITIAL EVENT NOTIFICATION FOR ALL OTHER SUSARs.
- THE PROMOTER REPORTS THE SUSARs TO EUDRAVIGILANCE WITH THE SAME DEADLINE.

EXPECTED SERIOUS ADVERSE EVENT/REACTION – UNEXPECTED SERIOUS ADVERSE EVENT (NOT REACTION)

CORESEARCH DISSEMINATES THE CIOMS FORM TO PROMOTER, ECs AND OTHER PIs

As for the **"standard of care"**, PI completes online CRF. It is up to the medical staff to notify the event according to the Legislative Decree, 219/2006 and Directive 2010/84.

10.5   Safety Assessments

Planned time points for all safety assessments are provided in the schedule of assessments table.

10.5.1 Primary Safety Endpoint

All AEs will be reported in terms of severity, relation to study treatment, duration, resolution, and patient outcome.

The study will have two analysis reports. The first is the interim analysis post-infusion of 10 treated patients. The purpose of this report is to provide an early preliminary interim analysis, which would allow for assessment of the safety of infusing the cellular product. This report will examine data to assess eventual acute reactions to the infusion protocol and/or treating composition. Data taken at baseline, day of discharge will be analyzed to determine any immediate AEs related to treatment.

The study will be halted at the interim analysis post-infusion if the Sponsor determines that the number of SAEs or SUSARs that are directly attributable to the infusion protocol exceeds of 10% those observed in the control group or that the overall rate of SAEs in this study group is significantly greater than that predicted by the natural history of disease in this group.

The Data Safety Monitoring Board (DSMB) will review the interim analysis and recommend either continuing the trial or halting the trial based on the rate of AEs and SAEs.

The trial will be placed on hold and reviewed by the DSMB if the following occur:

- ☐ Any death within 14 days after infusion that is considered to be possibly related to the infusion of study product.

10.5.2 Data Safety and Supervision

There will be a Data Safety Monitoring Board (DSMB) of 3 physicians to review study data quarterly to ensure patient safety. The chair of the DSMB will be informed of all deaths, serious unexpected adverse events, and all serious adverse events immediately after the sponsor has been notified. The chair of the DSMB will receive a report of an event as soon as the Sponsor receives the report from the investigator. The report will then be reviewed by the full DSMB.

10.5.3 Stopping Rules

When a PI identifies an event potentially associated with a stopping rule noted below, the investigator must notify the sponsor immediately. Sponsor will then notify the DSMB. The DSMB will determine if the stopping rules shall be invoked.

Safety stopping rules may be invoked by the DSMB upon notification of the following:

- Patient death where the incident is possibly related to the treatment (i.e. Cell product or administration procedure). Restart of the study will begin upon approval of the DSMB.

- Serious adverse reports from any of the first 3 patients at least possibly related to the treatment product or administration procedure. Only SAE reports which are at least possibly related to the treatment product (as determined by PI) will require stoppage of the trial. Restart of the study will begin upon approval of the DSMB.

- Three out of the first 5 patients present with unexpected systemic infections or fevers linked to suspected infection. Restart of the study will begin upon approval of the DSMB

- Anaphylactic shock or other severe injection related toxicities in any subject. Restart of the study will begin upon approval of the DSMB.

The DSMB will be provided with listings and descriptive summaries of current data including all AEs on a quarterly basis. If, for any reason, the use of MSC is considered unsafe or inefficacious, the DSMB can recommend stopping the study. If enrollment is halted and then restarted, the same rules will apply beginning upon restart.

11 IMP - MESENCHYMAL STROMAL CELLS (MSC)

11.1 Main IMP features

The Investigational Medicinal Product (IMP) used in this study consists of allogeneic MSCs whose morphological, phenotypic and functional characterization meets the requirements codified by the International Society for Cell & Gene Therapy.[56] The cellular product is supplied by each Cell Factory participating in this study following AIFA authorization, as specified here below for each IMP:

IMP-1: UC-MSC, umbilical cord-derived MSCs produced at the Laboratory of Advanced Cellular Therapy AUSSL8 Berica - Vicenza (authorization code: aM-49/2019, aM-49bis/2019);

IMP-2: CF-CB-MSC, umbilical cord blood-derived MSCs produced at the Cell Factory of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico of Milan (authorization code: aM-51/2018, aM-51bis/2018);

IMP-3: CFM-1-BM-MSC, bone marrow-derived MSCs produced at the Cell Factory of the Ospedale Pediatrico Meyer of Florence (authorization code: aM-73/2019);

IMP-4: RR002, adipose perivascular mesenchymal stromal cells (AD-PC-MSCs) produced at the Cell Factory RIGENERAND in Medolla (MO) (authorization code: aM-25/2020);

IMP-5: PTC-MSC-TP, bone-derived MSCs produced at the Laboratory of Cellular and Gene Therapies Stefano Verri in Monza (authorization code: aM-185/2017, aM-185bis/2017).

11.2 IMP delivery, dosage and administration

The IMP MSC is supplied as a sterile, apyrogenic product in a single use bag frozen in the vapor phase of a liquid nitrogen tank until the time shipping to the clinical centers in dry ice or liquid nitrogen by an authorized courier. The infusion procedure is described in the extended label.

Briefly: upon receipt, bags must be thawed in a water bath at 37° C and diluted 1:1 in thawing solution (final volume 50 ml) consisting of saline, Human Albumin and ACD-A or according to the specific instructions. After thawing and diluting, the infusion time is 30 minutes (*drop by drop*). The infusion rate must not exceed 1 ml of DMSO/kg of recipient weight. MSCs should be administered by intravenous infusion via an in situ venous catheter.

The treatment consists of two serial intravenous infusions of $1.0\text{-}1.5\text{x}10^6$ MSC/kg body weight 5-7 days a part. Cells should be infused using a set consisting of a 200 micron filter,

an injection port, a luer lock connector, nonvented (e.g. CareFusion code 72980-0006). The need for pre-medication is described in the product-specific Extended Label.

A total of 20 mL of normal saline should be administered following the infusion of the cells to flush the remaining cells through the intravenous set.

The administration of the IMP will not be masked.

The mandatory follow-up period is of 6 months from the last infusion. Vital signs should be monitored prior to infusion, every 15 minutes during infusion, directly following infusion and then hourly for 4 hours. During both infusions the patient will be monitored for signs of any infusion reaction. The occurrence of infusions reactions, if present, will be recorded on the patient's record forms electronic-CRF.

Refer to the IMP Investigator's Brochure for further instructions regarding recommended storage conditions and packaging configuration.

11.2.1 PTC-MSC-TP Investigationale Medicinal Product (Cell factory Monza)

The IMP produced by the Monza cell factory, will not be supplied in a bag but in vials. The infusion procedure involves transferring the contents of the vials into a transfer bag which will be connected to the infusion set. The vials will be thawed under laminar flow hood and diluted 1:2 (v/v) in saline solution. The contents of the vials will be collected in a syringe and transferred to a transfer bag. The transfer bag will be delivered to the medical staff in charge of the infusion who will connect it to the infusion set. The infusion method remain as specified in the IB.

11.3 Investigational Medicinal Product Handling and Accountability

The IMP will be provided and shipped directly by the manufacturing centers. For ensuring that patients are provided with doses specified by the protocol, at the study site, the PI or other authorized personnel is responsible for maintaining records of the IMP received and infused to each patient, and destruction or return of unused IMP, thus enabling reconciliation of all IMP received.

The study site must acknowledge receipt of IMPs supplied by the manufacturing centers confirming the shipment condition and content. The PI or designee must confirm that appropriate temperature conditions have been maintained during transit for all IMPs received and that any discrepancies have been reported and resolved before use of the IMPs. The study site should follow the instructions included with each shipment of IMP (extended label).

Once arrived, the IMP must be transferred to a dedicated area with access limited to the authorized staff in charge for preparing (or just adding if provided from the manufacturer) the diluting solution and subsequent thawing of the cells. These operations can be performed under a laminar flow hood or in a sterile field. As soon as thawed and added with diluent solution, the bag with the cells should be immediately transferred to the COVID area and delivered to the staff for infusion.

Not infused IMP should be destroyed or returned to the manufacturing center with the appropriate documentation. Modalities of drug reconciliation must be agreed with the manufacturing center. The site must obtain written authorization from the manufacturing center before any supplied IMP is destroyed, and IMP destruction must be documented on the appropriate form. Accurate records of all IMPs received at, dispensed from, returned to, and disposed of by the study site should be recorded.

Refer to the Investigator's Brochure and to the Extended Label for information on IMP handling, including preparation and storage, and accountability.

11.4 IMP Dose Justification and Cell Infusion

The dosage and the scheme chosen for MSC treatment in the present trial are based on previous experiences of our consortium team and published studies. In this regard, we would mention the experience collected treating a woman with life-threatening malabsorption syndrome due to adult autoimmune enteropathy, an immune-mediated condition, with two intravenous infusions of autologous bone marrow-derived MSCs two weeks apart.[57] Although the therapy led to disappearance of the specific anti-enterocyte autoantibodies and to growth of intestinal villi thus allowing the patient to overcome the critical stage, the benefit lasted only three months. On this basis, the authors chose to treat a subsequent case suffering from refractory coeliac disease with four serial infusions of MSCs three months apart.[58] This strategy not only rescued the patient from a dismal condition, but also enabled sustained remission lasting up to date (unpublished observation, R. Ciccocioppo). The need for serial infusions instead of a single MSC administration is also stressed in a recent paper where evidence for cumulative beneficial effects of repeated cell administrations is given on the basis of both experimental and clinical data showing the lack of engraftment and differentiation of MSCs into cells of the target organ and the aid to resident cells in overcoming inflammation while favouring tissue healing.[59] Moreover, serial intravenous infusions proved to perform better than only one to treat acute graft-*versus*-host disease,[60,61] a further immune-mediated condition characterized by cytokine storm and endothelial

damage. Therefore, considering the half-life of the MSCs when systemically injected,[13] a total of two intravenous infusions five days apart is scheduled for this trial.

As far as the dosage is concerned, in a recent dose-escalation study, it was found that intravenous infusions of adipose tissue-derived MSCs from healthy donors are well tolerated up to 4×10^6 cells/kg body weight, when a significant increase in clotting parameters and fibrinolytic response became evident.[62] Therefore, administration of an MSC amount ranging from 1.0 to 1.5×10^6 cells/kg body weight for each intravenous infusion is considered safe mostly in an attempt to mitigate the thromboembolic risk. The proposed dose is also based on the data reported in two recent studies by Zikuan et al.[50] and Liang et al.[51] who utilized a dose of 1×10^6 cells / kg recipient body weight, administered IV to treat COVID-19 ill patients. No AEs or SAEs were reported by the authors in any of the subjects treated. Both studies demonstrated that MSC treatment was well tolerated and resulted in improvement of clinical outcomes, resolution of critical symptoms, and discharge from the hospital. Similar results are evident from previous studies in the setting of ARDS. More in depth, Zheng et al. showed that the infusion of adipose tissue-derived MSCs (1×10^6 cells / kg of recipient body weight in 100 ml normal saline) was well tolerated.[42] In this study one patient from each group, experimental and control, developed diarrhea that resolved within 48 hours. One patient in the MSC group presented with rash with spontaneous resolution. One patient in the MSC group died of multiorgan failure while the same was observed in 2 patients in the control group. The deaths were considered to be related to the preexisting disease processes and not to the MSC utilized in the study. More recently, Matthay et al., in a prospective, double-blind, multicenter, randomized Phase 2a clinical trial, evaluated the safety of the infusion of bone marrow-derived MSCs (IV, 10×10^6 cells/kg) in 40 patients with moderate to severe ARDS.[45] The authors demonstrated that even when using a much higher dose, the treatment was well tolerated with 1 death reported in the experimental group, determined to be unrelated to the bone marrow-MSC infusion.

Following these evidences, we planned to apply MSC therapy at the convenient dose of 1.0-1.5 x 10^6 MSCs/kg body weight for each administration. The intravenous route will be used in order to maximize the presence of MSCs where the therapeutic effect is most desired, i.e. in the lung. The treatment will take place at each clinical site participating in this trial, located within 30 minutes distance (covered by car or walking) from the assigned Cell factory. For the infusion, the IMP will be thawed in a water bath at 37° C and diluted 1:1 in a diluting solution (final volume 50 ml) consisting of saline solution, human albumin and anticoagulant cytrate destrose (ACD)-A. The MSC suspension will be infused within 15 minutes, and the

duration of the infusion will be of 30 minutes drop by drop. The procedure will be performed by gaining venous access to a peripheral vein. Parameters will be monitored at 4 hours and 48 hours post-infusion. The second infusion at the same dosage will be administered after 5 days +/- 48 hourse from the first.

In summary, the schedule for the infusions has been chosen on the basis of the duration of the therapeutic effects of MSCs, lasting a few days, and the suddenly worsening conditions of the patients enrolled in this study. In this regard, it should be also noted that in the recently approved protocol by FDA coordinated by Prof. Camillo Ricordi (Director of the Diabetes Research institute and Cell Transplant Center at the University of Miami Miller School of Medicine, FL, USA) a schedule of 3 days apart from the two MSC administrations is applied. Finally, our team was the first in Europe to apply MSCs as compassionate use to rescue a patient with respiratory failure due to SARS-CoV-2 pneumonia and under mechanical ventilation. He received an intravenous infusion of umbilical cord-derived MSCs at dosage of 1.1×10^6 cells pro-kg body weight. The IMP was kindly donated from the Laboratory of Advanced Cellular Therapy (Vicenza) and all the procedures, including the shipping, the thawing, the preparation of the infusion and the delivery at bedside, were successful. Following the absence of adverse reaction in the short (4 hours) and medium (7 days) term, a further infusion at the same dosage was administered seven days later. Upon an amelioration of the inflammatory, respiratory, thrombotic and renal features, isolation of Klebsiella Pneumoniae KPC species unresponsive to combine antibioticcombined antibiotic therapy led to the patient's death after 4 weeks from the MSC treatment.

Finally, as far as the issue of safety is regarded, it should be emphasized that in a recent systematic review and meta-analysis, the high safety profile of MSCs as compared to controls is confirmed, since MSC treatment resulted associated only with an increased risk of fever (Relative Risk (RR) =2·48, 95% Confidence Interval (CI)=1·27–4·86; I2=0%), but not with non-fever acute infusional toxicity, infection, thrombotic/embolic events, death, or malignancy (RR=1·16, 0·99, 1·14, 0·78, 0·93; 95% CI=0·70–1·91, 0·81–1·21, 0·67–1·95, 0·65–0·94, 0·60–1·45; I2=0%, 0%, 0%, 0%, 0%) on a total of 2696 patients enrolled in 55 trials.[9] No trial was prematurely stopped due to safety concerns.

12 STUDY STATISTICS

12.1 Primary outcome variables

They will be feasibility and safety as assessed by the number of patients recruited/treated and quantification of AEs associated with treatment. Each AE will be assessed for its severity, or the intensity of an event experienced by the subject, using the following criteria:

- □ Mild (1): asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

- □ Moderate (2): minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental Activities of Daily Living (ADL)*.

- □ Severe or medically significant but not immediately life-threatening (3): hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL **.

- □ Life-threatening consequences (4): urgent intervention indicated.

- □ Death related to AE.

*Activities of Daily Living (ADL) Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

12.2 Secondary outcome variables

Efficacy signals will be quantified as
   a) mortality rate (%) at 2 and 4 weeks from the end of the treatment,

   b) percentage of change of the daily PaO_2/FiO_2 ratio in comparison with the basal value,

   c) percentage of patients under invasive mechanical ventilation,

   d) days under non-invasive mechanical ventilation,

   e) days under oxygen therapy;

   f) days of hospitalization

   g) percentage change of the radiologic score with respect to the basal value.

   h) Laboratory testing:

   o Complete Blood Count (CBC) with differential

   o Coagulation and Metabolic Panel (CMP)

   o Inflammatory markers

  o Cytokines and chemokines

  o PBMC assessment of T cell populations

  o ABG

  o Troponin I

Statistical analysis will be performed using the the R 4.0.0 software. Safety and exploratory efficacy secondary end-points will be observed for each patient against the baseline values. A descriptive analysis will be performed on safety (primary outcome) and MSC biological effects (secondary outcome). Frequency, mean and standard deviation or median and inter-quartile range will be used, as appropriate. The two randomized groups will be compared in terms of the primary and secondary endpoints measured after 14 and 28 days from randomization by means of t-test or chi-square test. An exploratory analysis on the marker profiles measured over time will be considered using appropriate longitudinal regression models, testing the time*treatment interaction. All relevant estimates will be reported with the corresponding 95% confidence intervals.

A p value < 0.05 will be considered as statistically significant. The Intent-to-Treat (ITT) population includes all subjects who met eligibility criteria, gave consent to participate, and were treated. The Per Protocol (PP) population is defined as the subgroup of the ITT population with documented adherence to the study protocol. These subjects will have met all inclusion and exclusion criteria and will have had evaluations at the protocol-specified time points.

Patient demographics and preoperative clinical variables will be expressed as percentages or means as appropriate, and will be assessed using the student paired t-test analysis: endpoints will be compared from the baseline to the 4 hours, 48 hours, 7 days, 14 days, and 28 days post treatment time points. If the data are not normally distributed, comparable non-parametric methods will be employed.

13 BENEFIT/RISK PROFILE

13.1 Benefits

The use of MSC for ARDS associated with COVID-19 is experimental, and may not result in any direct benefit to the patient. Nevertheless, proposers strongly believe that the benefit awaited from this MSC-based therapeutic strategy is an amelioration of the respiratory function in patients suffering from SARS-CoV-2 severe pneumonia as a consequence of MSC-induced modulation of the cytokine storm and protection of epithelial and endothelial cells, thus rescuing patients from a dismal condition and decreasing the mortality rate. However, since the survival of MSCs upon infusion is limited over time,[63] it is conceivable that the possible benefit may be transient.[59] This is why investigator chose to treat the patients by two serial intravenous infusions that are expected to overcome this issue.

Moreover, previous evidence showed that the therapeutic effects of MSCs result from the recruitment of other immune cells, mainly regulatory ones, thus supporting the idea that MSC persistence is not crucial for their efficacy.[64,65] Participation in this study may help the patient feel better or having an objective improvement, although no guarantee can be made. The information obtained in this trial may prove to be useful to others with COVID-19 and may also contribute to a better understanding of the condition and the potential of cell therapy for treatment of COVID-19. Knowledge gained from this study may help in developing new treatments for other individuals.

13.2 Risks

The MSC drug product undergoes extensive screening and in-process controls to ensure purity and consistency of manufacturing. Intravenous administration of the study product, as well as blood draws will only be performed by qualified, licensed, medical experts. Alghouth steps to mitigate the risks are implemented, below a list of possible risk related with the RESCAT execution.

13.2.1 Study Medication

Although MSC cells have previously been utilized safely, no guarantee can be made of the safety of these cells in the context of COVID19. For this reason, the study addresses safety.

13.2.2 <u>Intravenous Administration of MSC</u>

a) The risks associated with the intravenous administration procedure include bleeding, swelling and minor pain on injection. These are self-limiting and do not pose any long-term problems.

b) During the first few hours from the MSC intravenous infusion: headache, fever, metallic taste in the mouth, nausea, rush and allergic reaction may appear. While dysgeusia and nausea may predominantly derive from the presence of dimethyl sulfoxide (DMSO) in the cellular suspension, as during haematopoietic stem cell transplantation, the risk of allergic reaction is mitigated by the use of human platelet lysate instead of the faetal calf serum during the manufacturing process.[66] This also allows to avoid the possible transmission of still unknown zoonosis.

c) There is also a remote infectious risk that can be treated with appropriate antibiotics. The infection may conduct pain, discomfort and tissue damage.

d) A possible risk of intravenous MSC infusion is represented by thromboembolic events, as it has been anectodically reported when infusing MSCs into systemically activated/proinflammatory patients who are not receiving anticoagulation treatment.[67] However, a larger metanalysis did not show an increase of thromboembolic risk in patients treated by MSC.[9] In RESCAT study, a variety of MSCs from different tissue sources will be used and they largely differ in their hemocompatibility depending on the expression of both tissue factor/CD142, a key trigger of the extrinsic coagulation pathway, and receptors for complement activation products (e.g., C3a, C5a). Therefore, this risk has to be taken into account.[68,69] In addition, SARS-CoV-2 infection carries by itself an increased thromboembolic risk.[5] To compensate this risk *in vivo* ed *in vitro* data demonstrated that MSCs have the ability to reduce the platelet adhesion and aggregation.[70] As a consequence, MSCs might play a crucial role in dampening both inflammation and hypercoagulability status during SARS-CoV-2 severe pneumonia. It should be also emphasized that the prophylactic use of low-molecular-weight heparins is now commonly undertaken in this specific clinical setting, and that the anticoagulant cytrate dextrose (ACD)-A is used into the MSC bag and in the resuspension solution together with saline and albumin buffer.

e) As far as the immunogenic risk is concerned, thanks to their immuno-evasive property,[71] MSCs may be used without a preventive immunoablation regimen, thus increasing their safety profile.

f) Regarding tumorigenicity of MSC, the possibility to develop a *de novo* neoplasia[72,73] has been already disproved in *in vitro* studies where a reported malignancy potential was attributed to a cross-contamination of primary cultures[74,75]. More importantly, a consistent number of published meta-analyses show the absence of new cases of neoplasia following MSC treatment independently from the tissue source and clinical setting.[9,76,77]

13.2.3 Blood tests

The discomfort associated with removing blood from a vein is a slight pinch or pinprick when the sterile needle enters the skin. The risks include mild discomfort and/or ecchymosis at the site of puncture. Less common risks include a small blood clot, infection or bleeding at the puncture site and, on rare occasions, fainting during the procedure. Please note that generally patients with severe covid are all intravenously cannulated.

13.2.4 Unforeseen risks

Unforeseen risks are those unknown risks by researchers that may occur as a result of the study procedures.

13.3 Unanticipated problems

Proponents consider unanticipated problems involving risks to participants or others to be included, in general, any incident, experience, or outcome that meets all of the following criteria:

-   Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;

-   Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

-   Suggests that the research places participants or others at a greater risk of

harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

13.4 Plan for reporting unanticipated problems or study deviations

The investigator will report unanticipated problems (UPs) to competent authories and lead principal investigator (PI/sponsor of the trial). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported to competent authories and to the study sponsor within 24 hours of the investigator becoming aware of the event.
- Any other UP will be reported to competent authorities within 7 days of the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the ethical Committee within 30 days of the receipt of the report of the problem from the investigator.

13.5 Minor Study Deviations

Minor study deviations are permitted when approved by the sponsor and the investigator/s. A list of possible deviations and means of addressing is provided below:

13.5.1 Screen Failures

A screen failure subject is one from who informed consent is obtained, but treatment with the investigational therapy was not attempted because it was subsequently determined (after the subject signed the informed consent form) that the subject did not meet all of the eligibility criteria. The number of screen failure subjects will be reported, but such subjects will not be included either in the intent-to-treat or the per protocol analysis.

13.5.2 Missed Clinic Visits

Any subject who does not return for a scheduled follow-up visit will be contacted at least twice by telephone to determine the cause for the missed visit. All attempts to contact these subjects will be recorded in the subject's records. If the subject is contacted, a new visit will be scheduled as soon as possible. Such subjects will be considered as major protocol violators and may be excluded from the per protocol analysis. They will however be included in ITT analysis according their actual data. Subjects will also be excluded from the per protocol analysis if they miss more than 1/3 of their scheduled visits. An exception will be made for visits missed as a result of hospitalization. In addition, patients may be excluded from a per protocol analysis if they are repeatedly non-compliant with medical guidance (i.e., antibiotics, wound care, cholesterol meds, etc.) despite repeated clarification and reinforcement from the physician and support staff. In all cases the subjects receiving the IMP will be analyzed in the intention to treat analysis, which is the primary outcome measure in this study.

13.5.3 Protocol Deviations

Except for emergency situations, this study should be conducted as described in this protocol. An example of such an emergency situation is one in which the protection, safety and well-being of a subject requires a protocol deviation; this deviation would be based upon the judgment of the investigator (or a responsible physician, appropriately trained designated by the investigator). If a deviation from the protocol is necessary to protect the life and physical well-being of a subject in an emergency, such protocol deviations must be reported to the sponsor and the reviewing competent Authorities as soon as possible, but no later than five working days after the emergency occurred. Generally, a protocol deviation is not imperative for patient withdrawal. Before the statistical analysis begins, the protocol deviations must be taken into account for the population efficacy analysis. The mandatory withdrawal reasons will be considered as

major protocol deviations. Other protocol deviation will be classified as minor protocol deviation. Additionally, the following major protocol deviations will be considered:

1) Non compliant with any of the inclusion/exclusion criterion, either identified before or after enrolment into the study,

2) Use of any prohibited concomitant medication.

Any protocol deviation must be recorded in the e-CRF. Once the study has begun, any modification of the protocol deviation will be specified in the statistical analysis plan. This will be done before closing the database.

In the event of a significant deviation from the protocol due to an accident or mistake, the investigator or designee must contact the sponsor at the earliest possible time by telephone to discuss the deviation and its impact on the study, and whether subject's continued participation in the study. These discussions will be documented by the investigator and the sponsor, and reviewed by the monitor.

13.5.4 Premature End of Study

All subjects who have signed an informed consent, except for screen failures, will be considered to have enrolled in the study. Subjects who complete 28-day study duration will be considered to have completed the study. All 60 subjects should however be followed until completing the study follow-up until 180 days after enrollment or until study discontinuation for other reasons. The reason for study discontinuation should be documented for each subject. These include, but are not limited to:

a) Incidence or severity of AE,

b) Investigator who does not adhere to the protocol or applicable regulatory guidelines,

c) Any other medical reason.

13.5.5 Medication Changes

Medications listed in the exclusion selection criteria are prohibited from this study prior to enrollment only. If the subject receives any medication that is listed in the exclusionary criteria after treatment, he/she will not be withdrawn from the study.

14 REPORTS AND RECORDS MANAGEMENT

Confidentiality of all records identifying subjects will be kept. Regulatory authorities, representatives of the IEC and staff authorized by the sponsor (study monitor) will be granted access to medical and laboratory records for the purpose of verification of procedures and clinical trial data without violating the confidentiality of subject's information to the extent permitted by applicable laws and regulations. No access to records will be allowed other than under the above defined conditions without the signed, written consent of the subject or the subject's legal representative. The results of this study will be published in a peer reviewed scientific journal. No patient identity information will be included in the scientific paper. This investigational study will follow the investigator report and record keeping requirements as summarized below.

14.1 Investigator Records

Prior to participation in the investigation, the investigator must receive and archive the following documentation:

a. Site Delegation Log, signed by the investigator, which lists any physicians who will be involved in conducting the investigation under the direction of the principal investigator.

b. A copy of the principal investigator's *curriculum vitae* (CV) as well as copies of CVs for any co-investigators.

c. The positive opinion letter of the IEC of the Coordinating Center, indicating that the IEC has reviewed and approved this investigational plan.

d. A copy of the IEC-approved informed consent document.

e. During the study, investigators are required to maintain on file the following accurate, complete and current records relating to this study—a summary of these records is described below:

f. All correspondence and required reports which pertain to the study;

g. Records of receipt, use or disposition of the investigational product, including the dates of receipt, the lot number, the names of all persons who received, used or disposed of any product.

h. Signed and dated consent forms;

i. Relevant observations, including records concerning adverse events, condition of each subject upon entering and results of diagnostic tests;

j. Case report forms and corrections to the forms;

    k. Protocols and amendments.

14.2 Investigator Reports

Investigators are required to prepare and submit to the IEC the following complete, accurate, and timely reports on this investigation when necessary. These reports include:

a. The investigator will notify the IEC, of a subject death occurring during the investigation as soon as possible -- preferably within 24 hours of learning of the subject's death, but in no event later than 48 hours.

b. The investigator will notify the IEC of any unanticipated adverse effect as soon as possible, but no later than 10 working days after the investigator first learns of the effect.

c. The investigator will provide current progress reports to the reviewing IEC at regular intervals and at least on an annual basis.

d. The investigator will notify the IEC of any deviation from the investigational protocol undertaken to protect the life and physical well-being of a subject in an emergency as soon as possible, but no later than 5 working days after the emergency occurred.

e. The investigator will notify the reviewing IEC that an informed consent was not obtained from a subject as soon as possible, but no later than 5 working days after such an occurrence.

f. The investigator will provide to the IEC a final summary report according to institutional policies.

g. The investigator will provide any other information requested by the IEC.

14.3 Data Collection

During each subject's visit to the clinic, or visit via TeleHealth/phone call, an investigator participating in the study will record progress notes to document all significant observations and clinical reports with the original documents. All information housed in the source documents will be transposed using eCRF. In addition, any contact with the subject via telephone or other means that provides significant clinical information will also be documented in the progress notes as described above. Data generated from the clinical trial will be published in peer -reviewed journals and communicated with regulatory authorities.

Any changes to information in the study progress notes, other source documents, and case report forms will be initialed and dated in ink on the day the change is made by a site study staff member authorized to make the change. Changes will be made by striking a single line through erroneous data and clearly entering the correct data, (e.g., right data). If the reason for the change is not apparent, a brief explanation for the change will be written in the source documentation by the clinician.

14.4 Source Document

14.4.1 <u>Study data will be collected on source documents</u>

The Principal Investigator is responsible for assuring that collected data are complete and accurate. Source documentation (the point of initial recording of a piece of data) will support data collected on the eCRF. Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical study. Completed electronic CRF data will be entered into the database within a week of the patient visit being completed.

According to the guidelines on Good Clinical Practice, the clinical monitor must check the eCRF entries against the source documents, except for the pre-identified source data directly recorded in the CRF.

14.4.2 <u>Source Data</u>

- Case Report Form,

- Patient Medical Records,

- Signed Informed Consent Forms,

- On-line test results (especially if only held electronically),

- Trial Completion form,

- Adverse Event Form,

- Samples log.

14.4.3 <u>Data collection and study report form monitoring</u>

All data obtained will be entered into a local regulation compliant Data Management System with remote data entry. This is provided by the involved CRO. Data will be recorded with an Electronic Data Capture system using eCRF. The study database will be resident on a server in a secure location. All data entry, modification or deletion will be recorded automatically in an electronic audit trail. The Principal Investigator will retain all copies of the

eCRF in the relevant sections of his/her Investigator Site File with any required anonymised background information from the medical records as required. The eCRF data will be monitored by the CRO. The monitor who will routinely review the data for completeness, correctness, and consistency will generate manual or system-generated queries. The final, completed electronic CRF Casebook for each subject will be electronically signed and dated by the Principal Investigator within the Electronic Data Capture system to signify that the Investigator has reviewed the electronic CRF and certifies it to be complete and accurate within a month. The electronic CRF will be accessible to data managers, investigators, Clinical Trial Monitors, Auditors and Inspectors.

14.5 Records Retention at the Study Site

The PI is responsible for retaining the necessary records. This includes a copy of the protocol, the labeling, eCRF, medical records, original test result reports, all study-related correspondence, a record of written informed consent, and any other documents pertaining to the conduct of this study.

All investigators participating in investigational studies are required to maintain detailed clinical records during the investigation and for a period of at least two years after the latter of the following two dates:

The date on which the investigation is terminated or completed; or,

The date the records are no longer required for purposes of supporting a premarket approval application.

14.6 Payment and Remuneration

No compensation will be provided to patients

14.7 Costs

Subjects will not pay the treatment using MSCs nor for the associated costs of processing or transportation of the cells. Grant has been applied to the Ministry of Health (Bando COVID19) requesting support for the protocol.

14.8 Data Management

The Clinical Site will maintain the highest degree of confidentiality for the clinical and research information obtained from study subjects. Medical and research records will be maintained in the strictest confidence. All data will be reviewed periodically by the clinical monitors, Data Safety Monitoring Board (DSMB), and IRB of record. As part of the quality assurance and legal responsibilities of an investigation, the Clinical Site will permit authorized representatives of the Study PI, including medical monitor, DSMB, IEC, RSQA and health authorities to examine - and when required by applicable law, to copy - clinical records for quality assurance reviews, audits, and evaluations of the study safety and progress. Unless required by the laws that permit copying of records, only the coded identity associated with documents or with subject data may be copied, with all personally identifying information obscured. Authorized representatives are bound to maintain strict confidentiality of medical and research information linked to study subjects.

15 STATISTICAL ANALYSIS AND EXPECTED RESULTS

15.1 Statistics

Safety and exploratory efficacy secondary end-points will be observed for each patient against the baseline values. The Intent-to-Treat (ITT) population includes all subjects who met eligibility criteria, gave consent to participate, and were treated. The Per Protocol (PP) population is defined as the subgroup of the ITT population with documented adherence to the study protocol. These subjects will have met all inclusion and exclusion criteria and will have had evaluations at the protocol-specified time points. Patient demographics and preoperative clinical variables will be expressed as frequencies and percentages for categorical variables or median and interquartile range for continuous variables. The two groups comparisons will be based on the χ^2 test (or Fisher's test) (categorical variables) or Mann Whitney U-test (continuous variables).

We will use the obtained data from the randomized control group to estimate difference in clinical outcomes, laboratory values, immune sub-populations and cytokine assessment. The endpoints will be compared at 4 hrs, 48 hrs, 7 days, 14 days, and 28 days post treatment time points, as appropriate. The primary outcomes of this study are the feasibility and the safety of the MSC therapy. Feasibility will be assessed in terms of ability to treat all the patients enrolled in the MSC arm. Safety will be assessed by incidence of AEs and SAEs, and will be compared between groups using Fisher's exact test. At any time during the study, if significant differences in AEs or SAE's will be reported, early termination will be considered. Treatment efficacy will be evaluated as follows.

- The daily PaO_2/FiO_2, radiologic score, laboratory values and cytokine profile (continuous outcomes) will be evaluated as the difference within group (from baseline) and between groups (at each follow-up), with paired and unpaired non-parametric tests, respectively.

- Mortality rates and patient rates under mechanical ventilation (dichotomuous outcomes) will be assessed by incidence and will be compared between groups using Fisher's exact test.

- Days under non-invasive mechanical ventilation, days from independence from oxygen therapy and days of hospitalization (counts outcomes) will be evaluated as the difference between groups (at each follow-up) using Fisher's exact test.

A p-value < 0.05 will be considered as statistically significant. Statistical analysis will be performed using the R 4.0.0 software.

15.2 Sample Size considerations

This investigation is designed to evaluate feasibility, safety and obtain an estimate of efficacy of the MSC therapy in severly ill COVID-19 patients. The primary objective of this study is to evaluate the feasibility and safety of the use of allogeneic MSCs in patients with SARS-CoV-2 pneumonia. We therefore referred to a recently published study in which an MSC-based cell therapy product was used for ARDS therapy.[45] Therefore, the sample size has been calculated based on the safety primary endpoint, using the single stage method for phase II studies. It has been primarily determined to provide sufficient clinical experience to support the design of later-stage clinical development, such as an international multicenter phase II/III study. Following these premises, we will enroll a total of 60 consecutive cases to be randomized 2:1 to treatment with IMP or as control receiving standard of care for COVID19.

15.3 Expected Results

Data indicates that approximately 10% of the patients diagnosed with COVID-19 develop serious complications and need to be hospitalized. More than 1 out of 4 hospitalized COVID-19 patients require admission to an Intensive Care Unit (ICU) for respiratory support, and a large proportion of these ICU-COVID-19 patients, between 17% and 46%, have died. In these patients SARS-CoV-2 infection causes an inflammatory response in the lungs that can progress to cytokine storm, Acute Lung Injury (ALI), Acute Respiratory Distress Syndrome (ARDS), organ failure, and death. In the absence of an approved vaccine or any other treatments, we would like to investigate whether MSC therapy is safe and beneficial for critically ill COVID-19 patients. Primary endpoint data should be available for all enrolled subjects. An exception will be only if death occurs or if the subject withdraws consent to be followed, although we expect this limited or non-existent in COVID-19 patients.

As far as the feasibility is regarded, following an internal preliminary survey on the manufacturing capability of each Cell Factory participating in the study, we foresee to be able to treat with IMP MSC all the 40 patients planned of the study coort.

As far as the safety is regarded, based on the evidence obtained from the systematic reviews published in literature on the use of MSC in clinical trials and a recent experience in a compassionate use, we can assess that no significant safety concerns are expected.

As far as the efficacy is regarded, a reduction of mortality rate at 2 and 4 weeks from the end of the treatment is awaited, together with an amelioration of the cinical and laboratory parameters. Also a consistent reduction of lung fibrosis at 6 months is expected.

In addition, the possibility to carry out an immunological study represents a great step towards the understanding of the pathogenetic mechanisms of SARS-CoV-2 pneumonia. Worth of noting, since the immunoregulatory effects of MSCs are supposed to be not virus- or antigen-specific, they may represent a suitable therapeutic option in all ARDS conditions. In addition, the possibility to assess which MSC IMP perform better (if any) paves the way to quickly convert the production processes towards the best IMP in order to satisfy urgent clinical needs by generating information still lacking on MSC action in this specific clinical setting that could have a global impact.

16 ETHICAL AND STUDY OVERSIGHT CONSIDERATIONS

16.1 Informed consent

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention.

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be IEC-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants or its designee (legally authorized representative) will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

16.2 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the IEC, AIFA, DSMB, and other oversight and regulatory authorities. If the study is prematurely terminated or suspended, the PI will promptly inform study participants, the IEC, and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility.

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IEC and/or AIFA

16.3 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by all the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor. All research activities will be conducted in COVID units (both semi-intensive and intensive) where monitoring and care upgrade is warranted.

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

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IEC, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the participating center. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by the research staff will be

secured and password protected. At the end of the study, all study databases will be de-identified and archived at the participating center.

16.4 Future Use of Stored Specimens and Data

Data collected for this study will be analyzed and stored at the participating center. After the study is completed, the de-identified, archived data will be transmitted to and stored at the participating center, for use by other researchers including those outside of the study. Permission to transmit data to the collaborators will be included in the informed consent.

With the participant's approval and as approved by IEC, de-identified biological samples will be stored at the participating center with the same goal as the sharing of data with the collaborators. These samples could be used to research the causes of COVID-19, its complications and other conditions for which individuals with viral infections are at increased risk, and to improve treatment. The participating center will also be provided with a code-link that will allow linking the biological specimens with the phenotypic data from each participant, maintaining the blinding of the identity of the participant. During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biosample storage may not be possible after the study is completed. When the study is completed, access to study data and/or samples will be provided through the participating center.

16.5 Safety Oversight

Safety oversight will be under the direction of a DSMB composed of individuals with the appropriate expertise. The DSMB will meet at least bi-monthly to assess safety and efficacy data on each arm of the study. The DMSB will operate under the rules of an approved charter that will be written and reviewed at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. The DSMB will provide its input to the study team and sponsor.

16.6 Clinical Monitoring

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved

protocol/amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

During the emergency period a remote monitoring approach will be applied. On-site monitoring visits will be postponed until the emergency period is over to proceed with the source data verification.

- Monitoring for this study will be performed by the CRO.
- A monitoring plan will be developed by the CRO Director with frequency for initial assessment and training and throughout the study.

16.7 Quality Assurance and Quality Control

The clinical sites will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database that will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, CH GCP, and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP). The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

16.8 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. The PI 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.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in

the eCRF derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into authorized systems. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

16.9 Study Records Retention

Study documents should be retained for a minimum of 2 years from the end of the study formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. In any case, no records shall be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

16.10   RESCAT Regulatory and Ethical Compliance

16.10.1   Declaration of Helsinki and Good Clinical Practice

The trial will be performed in accordance with the spirit and the letter of the declaration of Helsinki, the conditions and principles of GCP, the protocol and applicable local regulatory requirements and laws.

16.10.2   Good Clinical Practice Training

All trial staff must hold evidence of appropriate GCP training or undergo specific training prior to undertaking any responsibilities on this trial.

16.10.3   Regulatory Compliance

The trial will not commence until a Clinical Trial Authorisation is obtained from the Regulatory Agency (AIFA). The protocol and trial conduct will comply with the Medicines for Human Use (Clinical Trials) Regulations 2004 and any relevant amendments. Development Safety Update Reports will be submitted to the IEC and Regulatory Agency.

16.10.4   <u>Ethical Committee review</u>

Before the start of the study or implementation of any amendment, the approval of the trial protocol, protocol amendments, informed consent forms and other relevant documents will be obtained by the competent IEC. All correspondence with the IEC will be retained in the Trial Master File/Investigator Site File.

17 STUDY ORGANIZATION AND ADMINISTRATION

17.1 Study duration

The whole study duration is 12 months, while the time frame from the enrolment of the first patient to the last is 6 months. A report will be produced at the end of the 4th and 8th months from the beginning of the study (first patient, first visit) study, while the final report will be submitted within the end of the study. It is the Sponsor's responsibility to produce the annual reports and submit them to IECs and Regulatory Agency.

17.1.1 Participants' insurance

The participants' insurance is not necessary based on the art. 40 DL 08/04/2020.

17.2 Data Safety Monitoring Committee

The responsabilities of the DSMC members include:

- Be assured that the safety of the enrolled patients is protected during the trial,

- Review progress regularly and interact with the trial team,

- Review all the aggregated and comparative safety data and toxicity information, to make recommendations for eventual changes or even determine if the trial should be prematurely stopped for inefficacy or safety concerns,

- Discuss safety data in scheduled meetings; the DSMC meetings will be held after every three patients have been treated or every one reported death, whichever is the sooner. The first meeting of the DSMC will be held after the first safety report judged to be possibly related or related to treatment has been received, if this is sooner than the 'three patients' milestone.

The Principal Investigator will notify the DSMC of patients with new onset infections following the use of MSC if the following limits are reached:

n. patients	4	5	6	7	8	9	10	11	12	13
new-onset infections	3	3	4	4	5	5	6	6	7	7

The Sponsor will implement any recommendations of the DSMC. This may be done as Urgent Safety Measures (according to Directive 2001/20/EC) or as protocol amendment).

17.3 Data Safety Monitoring Committee (DSMC)

- Prof. Christian Jorgensen, Director of IRMB Institut de Recherche de Médecine Régénératrice et de Biothérapies, Head of Research Unit Inserm U 1183, ECellFrance coordinator, Head of clinical unit for osteoarticular diseases and Department for Biotherapy at University Hospital CHU Lapeyronie University Hospital, Av G Giraud 34295 Montpellier France.
  E-mail address: christian.jorgensen@inserm.fr

- Prof. Jaap Jan Boelens, Chief Stem Cell Transplantation and Cellular Therapies - 1275 York Ave (Scholar 417), New York, NY, 10065.
  E-mail address: boelensj@mskcc.org

- Prof. Marc Humbert, Full Professor Respiratory Medicine, Universitè Paris Sud, Director INSERM Research Unit Pulmonary Hypertension.
  E-mail address: marc.humbert@aphp.fr

17.4 Trial Steering Committee (TSC)

- Prof. Dan Weiss, Critical Care Medicine, Pulmonary Disease, University of Vermont, Burlington, VT, USA; E-mail address: daniel.weiss@med.uvm.edu

- Prof. Michael A. Matthay, Departments of Medicine and Anesthesia, Cardiovascular Research Institute, University of California, San Francisco, M-917, San Francisco, CA 94143, USA; E-mail address: michael.matthay@ucsf.edu

- Prof. Robin Vos, MD, PhD, Department of Respiratory Diseases, Lung Transplant and Respiratory Intermediate Care Unit, University Hospitals Leuven; Belgium; E-mail address: robin.vos@uzleuven.be

17.5 Trial Management Group (TMG)

- Giuseppe Astori, Vicenza

- Rachele Ciccocioppo, Verona

- Enrico Clini, Modena

- Massimo Dominici, Modena

- Monica Santimaria, Vicenza

- Elisa Zanier, Milano

17.6 Trial Statistician Team

- Antonio Nicolucci, CORESEARCH, Center for Outcomes Research and Clinical Epidemiology; Via Tiziano Vecellio, 2 – 65124 Pescara
- Giuseppe Lucisano, CORESEARCH, Center for Outcomes Research and Clinical Epidemiology; Via Tiziano Vecellio, 2 – 65124 Pescara

18 REFERENCES

1. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med 2020;382:727-733.
2. Huang C. Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Whuan, China. Lancet 2020;395:497-506.
3. Mehta P, McAuley DF, Brown M, et al. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet 2020;395:1033-1034.
4. Onder G, Rezza G, Brusaferro S. Case-Fatality Rate and Characteristics of Patients Dying in Relation to COVID-19 in Italy. JAMA 2020; doi: 10.1001/jama.2020.4683.
5. Giannis D, Ziogas IA, Gianni P. Coagulation Disorders in Coronavirus Infected Patients: COVID-19, SARS-CoV-1, MERS-CoV and Lessons From the Past. J Clin Virol 2020;127:104362.
6. Sanders JM, Monogue ML, Jodlowski TZ, et al. Pharmacologic treatments for Coronavirus Disease 2019 (COVID-19): a review. JAMA 2020; doi: 10.1001/jama.2020.6019
7. Cao B, Wang Y, Wen D, et al. A trial of Lopinavir-Ritonavir in adults hospitalized with severe-COVID-19. N Engl J Med 2020;382:1787-1799.
8. Tasli PN, Bozkurt BT, Kırbaş OK, Deniz-Hızlı AA, Şahin F. Immunomodulatory behavior of mesenchymal stem cells. Adv Exp Med Biol 2018;1119:73-84.
9. Thompson M, Mei SHJ, Wolfe D, et al. Cell therapy with intravascular administration of mesenchymal stromal cells continues to appear safe: An updated systematic review and meta-analysis. EClinicalMedicine 2020;19:100249.
10. D'souza N, Rossignoli F, Golinelli G, et al. Mesenchymal stem/stromal cells as a delivery platform in cell and gene therapies. BMC Med 2015;13:186.
11. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284(5411):143–147.
12. Sundin M, Ringden O, Sundberg B, et al. No alloantibodies against mesenchymal stromal cells, but presence of anti-fetal calf serum antibodies, after transplantation in allogeneic hematopoietic stem cell recipients. Haematologica 2007;92:1208–1215.
13. Leibacher J, Henschler R. Biodistribution, migration and homing of systemically applied mesenchymal stem/stromal cells. Stem Cell Res Ther 2016;7:7.
14. Armitage J, Tan DBA, Troedson R, et al. Mesenchymal stromal cell infusion modulates systemic immunological responses in stable COPD patients: A phase I pilot study. Eur Respir J 2018;51:1702369.
15. Lee RH, Pulin AA, Seo MJ, et al. Intravenous hMSCs Improve Myocardial Infarction in Mice because Cells Embolized in Lung Are Activated to Secrete the Anti-inflammatory Protein TSG-6. Cell Stem Cell 2009;5:54–63.
16. Krasnodembskaya A, Song Y, Fang X, et al. Antibacterial effect of human mesenchymal stem cells is mediated in part from secretion of the antimicrobial peptide LL-37. Stem Cells 2010;28:2229–2238.
17. Hu S, Park J, Liu A, et al. Mesenchymal Stem Cell Microvesicles Restore Protein Permeability Across Primary Cultures of Injured Human Lung Microvascular Endothelial Cells. Stem Cells Transl Med 2018;7:615–624.
18. Court AC, Le-Gatt A, Luz-Crawford P, et al. Mitochondrial transfer from MSCs to T cells induces Treg differentiation and restricts inflammatory response. EMBO Rep 2020;21:e48052.
19. Islam MN, Das SR, Emin MT, et al. Mitochondrial transfer from bone-marrow- derived stromal cells to pulmonary alveoli protects against acute lung injury. Nat Med 2012;18:759-765.

20. Liotta F, Angeli R, Cosmi L, et al. Toll-like receptors 3 and 4 are expressed by human bone marrow-derived mesenchymal stem cells and can inhibit their T-cell modulatory activity by impairing Notch signaling. Stem Cells 2008;26:279–289.

21. Waterman RS, Tomchuck SL, Henkle SL, Betancourt AM. A New Mesenchymal Stem Cell (MSC) Paradigm: Polarization into a Pro-Inflammatory MSC1 or an Immunosuppressive MSC2 Phenotype. Plos ONE 2010;5:e10088.

22. Lee JW, Krasnodembskaya A, McKenna DH, Song Y, Abbott J, Matthay MA. Therapeutic Effects of Human Mesenchymal Stem Cells in Ex Vivo Human Lungs Injured with Live Bacteria. Am J Respir Crit Care Med 2013;187:751–760.

23. Wu X, Dao Thi VL, Huang Y, et al. Intrinsic Immunity Shapes Viral Resistance of Stem Cells. Cell 2018;172:423-438.

24. Peteranderl C, Herold S. The impact of the interferon/TNF-related apoptosis-inducing ligand signalling axis on disease progression in respiratory viral infection and beyond. Front Immunol 2017;8:313.

25. Polchert D, Sobinsky J, Douglas G, et al. IFN-gamma activation of mesenchymal stem cells for treatment and prevention of graft versus host disease. Eur J Immunol 2008;38:1745–1755.

26. Thanunchai M, Hongeng S, Thitithanyanont A. Mesenchymal Stromal cells and viral infection. Stem Cells Int 2015;2015:860950. DOI: 10.1155/2015/860950.

27. Cruz FF, Weiss DJ, Rocco PRM. Prospects and progress in cell therapy for acute respiratory distress syndrome. Expert Opin Biol Ther 2016;16:1353-1360.

28. McIntyre LA, Moher D, Fergusson DA, et al. Efficacy of mesenchymal stromal cell therapy for acute lung injury in preclinical animal models: A systematic review. PLoS One 2016;11:e0147170.

29. Darwish I, Banner D, Mubareka S, et al. Mesenchymal stromal (stem) cell therapy fails to improve outcomes in experimental severe influenza. PLoS One 2013;8:e71761.

30. Gotts JE, Abbott J, Matthay MA. Influenza causes prolonged disruption of the alveolar-capillary barrier in mice unresponsive to mesenchymal stem cell therapy. Am J Physiol Lung Cell Mol Physiol 2014;307:L395-L406.

31. Chan MCW, Kuok DIT, Leung CYH, et al. Human mesenchymal stromal cells reduce influenza A H5N1-associated acute lung injury in vitro and in vivo. Proc Natl Acad Sci U S A 2016;113:3621–3626.

32. Li Y, Xu J, Shi W, et al. Mesenchymal stromal cell treatment prevents H9N2 avian influenza virus-induced acute lung injury in mice. Stem Cell Res Ther 2016;7 159.

33. Loy H, Kuok DIT, Hui KPY, et al. Therapeutic Implications of Human Umbilical Cord Mesenchymal Stromal Cells in Attenuating Influenza A(H5N1) Virus- Associated Acute Lung Injury. J Infect Dis 2019;219:186–196.

34. Bao L, Deng W, Huang B, et al. The Pathogenicity of 2019 Novel Coronavirus in hACE2 Transgenic Mice. bioRχiv 2020; https://doi.org/10.1101/2020.02.07.939389bioRχiv 2020; https://doi.org/10.1101/2020.02.07.939389

35. Hoffmann M, Kleine-Weber H, Krüger N, Müller M, Drosten C, Pöhlmann S. The novel coronavirus 2019 (2019-nCoV) uses the SARS-coronavirus receptor ACE2 and the cellular protease TMPRSS2 for entry into target cells. bioRχiv 2020. https://doi.org/10.1101/2020.01.31.929042https://doi.org/10.1101/2020.01.31.929042

36. Xu H, Zhong L, Deng J, et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. Int J Oral Sci 2020;12:8.

37. Han J, Li Y, Li Y. Strategies to Enhance Mesenchymal Stem Cell-Based Therapies for Acute Respiratory Distress Syndrome. Stem Cells Int 2019;2019:5432134.

38. He H, Liu L, Chen Q, et al. Mesenchymal stem cells overexpressing angiotensin-converting enzyme 2 rescue lipopolysaccharide-induced lung injury. Cell Transplant 2015;24:1699-1715.

39. He H-L, Liu L, Chen Q-H, et al. MSCs modified with ACE2 restore endothelial function following LPS challenge by inhibiting the activation of RAS. J Cell Physiol 2015;230:691-701.

40. Min F, Gao F, Li Q, Liu Z. Therapeutic effect of human umbilical cord mesenchymal stem cells modified by angiotensin-converting enzyme 2 gene on bleomycin-induced lung fibrosis injury. Mol Med Rep 2015;11:2387–2396.

41. Hui DSC, Zumla A. Severe acute respiratory syndrome: historical epidemiologic and clinical features. Infect Dis Clin North Am 2019;33:869-889.

42. Zheng G, Huang L, Tong H, et al. Treatment of acute respiratory distress syndrome with allogeneic adipose-derived mesenchymal stem cells: a randomized, placebo-controlled pilot study. Respir Res 2014;15:39.

43. Wilson JG, Liu KD, Zhuo H, et al. Mesenchymal stem (stromal) cells for treatment of ARDS: a phase 1 clinical trial. Lancet Resp Med 2015;3:24-32.

44. Simonson OE, Mougiakakos D, Heldring N, et al. In vivo effects of mesenchymal stromal cells in two patients with severe acute respiratory distress syndrome. Stem Cells Transl Med 2015;4:1199–1213.

45. Matthay MA, Calfee CS, Zhuo H, 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;7:154–162.

46. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020;395:1054-1062.

47. Moore JB, June CH. Cytokine release syndrome in severe COVID-19. Science 2020;368:473-474.

48. Blazar BR, MacDonald KPA, Hill GR. Immune Regulatory Cell Infusion for Graft-Versus-Host Disease Prevention and Therapy. Blood 2018;31:2651-2660.

49. Hashi CK, Zhu Y, Yang GY, et al. Antithrombogenic property of bone marrow mesenchymal stem cells in nanofibrous vascular grafts. Proc Natl Acad Sci U S A 2007;104:11915-11920.

50. Leng Z, Zhu R, Hou W, et al. Transplantation of ACE2- Mesenchymal Stem Cells Improves the Outcome of Patients with COVID-19 Pneumonia. Aging dis 2020;11:216-228.

51. Liang B. Chen J, Li, T, et al. Clinical remission of a critically ill COVID-19 patient treated by human umbilical cord mesenchymal stem cells. chinaXiv 202002.00084v1.

52. Golchin A, Seyedjafari E, Ardeshirylajimi A. Mesenchymal stem cell therapy for COVID-19: present or future. Stem Cell Rev Rep 2020;1-7 doi:10.1007/s12015-020-09973-w.

53. Mastrolia I, Foppiani EM, Murgia A, et al. Challenges in clinical development of mesenchymal stromal/stem cells: concise review. Stem Cells Transl Med 2019;8:1135-1148.

54. Warren MA, Zhao Z, Koyama T, et al. Severity scoring of lung oedema on the chest radiograph is associated with clinical outcomes in ARDS. Thorax 2018;73:840-846.

55. Ichikado K, Muranaka H, Gushima Y, et al. Fibroproliferative changes on high-resolution CT in the acute respiratory distress syndrome predict mortality and ventilator dependency: a prospective observational cohort study. BMJ Open 2012;2(2):e000545.

56. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8:315-317.

57. Ciccocioppo R, Russo ML, Bernardo ME, et al. Mesenchymal stromal cell infusions as rescue therapy for corticosteroid-refractory adult autoimmune enteropathy. Mayo Clin Proc 2012;87:909-914.
58. Ciccocioppo R, Gallia A, Avanzini MA, et al. A refractory celiac patient successfully treated with mesenchymal stem cell infusions. Mayo Clin Proc 2016;91:812-819.
59. Wysoczynski M, Khan A, Bolli R. New paradigms in cell therapy: repeated dosing, intravenous delivery, immunomodulatory actions, and new cell types. Circ Res 2018;123:138–158.
60. Sanchez-Guijo F, Caballero-Velazquez T, Lopez-Villar O, et al. Sequential Third-Party Mesenchymal Stromal Cell Therapy for Refractory Acute Graft-Versus-Host Disease. Biol Blood Marrow Transplant 2014;20:1580-1585.
61. Gao F, Chiu SM, Motan DAL, et al. Mesenchymal Stem Cells and Immunomodulation: Current Status and Future Prospects. Cell Death Dis 2016;7(1):e2062.
62. Alvaro-Gracia JM, Jover JA, Garcia-Vicuna R, et al. Intravenous administration of expanded allogeneic adipose-derived mesenchymal stem cells in refractory rheumatoid arthritis (Cx611): results of a multicentre, dose escalation, randomised, single-blind, placebo-controlled phase Ib/IIa clinical trial. Ann Rheum Dis 2017;76:196-202.
63. Eggenhofer E, Benseler V, Kroemer A, et al. Mesenchymal stem cells are short-lived and do not migrate beyond the lungs after intravenous infusion. Front Immunol 2012;3:297.
64. Melief SM, Schrama E, Brugman MH, et al. Multipotent stromal cells induce human regulatory T cells through a novel pathway involving skewing of monocytes toward anti-inflammatory macrophages. Stem Cells 2013;31:1980–1991.
65. Hoogduijn MJ. Are mesenchymal stromal cells immune cells? Arthr Res Ther 2015;17:88.
66. Bernardo ME, Avanzini MA, Perotti C, et al. Optimization of in vitro expansion of human multipotent mesenchymal stromal cells for cell-therapy approaches: further insights in the search for a fetal calf serum substitute. J Cell Physiol 2007;211:121-130.
67. Wu Z, Zhang S, Zhou L, et al. Thromboembolism induced by umbilical cord mesenchymal stem cell infusion: a report of two cases and literature review. Transplant Proc 2017;49:1656–1658.
68. Tatsumi K, Ohashi K, Matsubara Y, et al. Tissue factor triggers procoagulation in transplanted mesenchymal stem cells leading to thromboembolism. Biochem Biophys Res Commun 2013;431:203–209.
69. Moll G. Ignatowicz L, Catar R, et al. Different procoagulant activity of therapeutic mesenchymal stromal cells derived from bone marrow and placental decidua. Stem Cells Dev 2015;24:2269–79.
70. Hashi CK, Zhu Y, Yang G-Y, et al. Antithrombogenic property of bone marrow mesenchymal stem cells in nanofibrous vascular graft. PNAS 2007;104:11915-11920.
71. Ankrum JA, Ong JF, Karp JM. Mesenchymal stem cells: immune evasive, not immune privileged. Nat Biotechnol 2014;32:252-260.
72. Rubio D, Garcia-Castro J, Martin MC, et al. Spontaneous human adult stem cell transformation. Cancer Res. 2005;65:3035–3039.
73. Rosland GV, Svendsen A, Torsvik A, et al. Long-term cultures of bone marrow-derived human mesenchymal stem cells frequently undergo spontaneous malignant transformation. Cancer Res 2009;69:5331–5339.
74. Torsvik A, Røsland GV, Svendsen A, et al. Spontaneous malignant transformation of human mesenchymal stem cells reflects cross-contamination: putting the research field on track—letter. Cancer Res 2010;70:6393–6396.
75. Garcia S, Bernad A, Martin MC, et al. Pitfalls in spontaneous in vitro transformation of human mesenchymal stem cells. Exp Cell Res 2010;316:1648–1650.

76. Lalu MM, McIntyre L, Pugliese C, et al. Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and metaanalysis of clinical trials. PLoS One 2012;7:e47559.

77. Toyserkani NM, Jorgensen MG, Tabatabaeifar S, et al. Concise Review: A Safety Assessment of Adipose-Derived Cell Therapy in Clinical Trials: A Systematic Review of Reported Adverse Events. Stem Cell Translat Med 2017;6:1786-1794.