ACUTE RESPIRATORY DISTRESS SYNDROME SECONDARY TO SARS-COV-2 INFECTION: TREATMENT WITH MESENCHYMAL STROMAL CELLS (MSCS) TO PREVENT PULMONARY COMPLICATIONS

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ABSTRACT

In December 2019 in China, after a pneumonia outbreak of unknown etiology, a new RNA virus has been identified and called Sars-CoV-2. Sars-CoV-2 induced severe respiratory infections, with global and rapid epidemic diffusion, designated coronavirus disease 2019 (Covid-19).
Sars-CoV-2 infection can lead to severe complications, such as acute respiratory distress syndrome (ARDS) with progression to pulmonary fibrosis.
Recent clinical studies described that in patients with severe Covid-19, MSC infusions, promote regenerative and reparative effects with anti-inflammatory and anti-fibrotic action. MSCs do not express ACE2 and TMPRSS2, the two main human receptors for host-pathogen interaction, and are not permissive to in vitro Sars-CoV-2 infection, making them suitable for clinical application.
The aim of our study was to evaluate the safety and efficacy of MSCs as cellular therapy in ARDS secondary to Sars-CoV-2 in patients undergoing mechanical ventilation, in order to prevent pulmonary fibrosis.
MSCs for infusions are thawed at 2x106/ml cellular concentration. The intravenous infusion protocol consists of two doses of third party allogenic MSCs at 1x106/Kg, 15 day apart.
From April 2020, six adult patients median age 65 years, median body weight 80 Kg, in mechanical ventilation for ARDS secondary to Sars-CoV-2 infection have been treated. Early or late adverse events were not recorded. Four out six patients showed a significant gas exchange improvement with extubation within seven days from the first infusion. Our results underline the safety and efficacy of MSC infusions for ARDS patients in mechanical ventilation, supporting the need of a phase I/II clinical trial.

INTRODUCTION

In December 2019 in China, after a pneumonia outbreak of unknown etiology, a new single-stranded positive-stranded RNA virus has been identified and called severe acute respiratory syndrome coronavirus 2 (Sars-CoV-2). Sars-CoV-2 induced severe respiratory infections with global and rapid epidemic diffusion. The World Health Organization (WHO) officially designated the new type of disease as coronavirus disease 2019 (Covid-19)[1,2].
Sars-CoV-2 infection can lead to severe complications, such as acute respiratory distress syndrome (ARDS) with progression to pulmonary fibrosis, a complex combination of epithelial and endothelial damage, associated with significant morbidity and mortality[1,2]. ARDS is a continuous pathological process which begins with acute lung injury. The main manifestations are dyspnoea, cough and progressive arterial hypoxemia bringing to critical patient conditions because of pulmonary fibrosis.
Recent clinical studies, together with the observations in animal models, described that in patients with severe Covid-19, MSC intravenous infusions, promote regenerative and reparative effects. In fact, MSCs are multipotent adult cells that can be isolated from various tissues with self-renewal and differentiation capacity. Their immunomodulatory and anti-inflammatory properties are due to the interaction and regulation with almost all the immune system cells[1,2].
Regarding tissue damage in Covid-19, MSCs, detecting injury signal, home to the pulmonary microenvironment, and by secretion of cytokines and soluble factors, induce anti-inflammatory and anti-fibrotic action[1].

Recently our group demonstrated that MSCs from bone marrow (BM-MSCs) do not express ACE2 and TMPRSS2, the two main human receptors for host-pathogen interaction, and are not permissive to *in vitro* Sars-CoV-2 infection[3].
Taking into account all the above mentioned characteristics, MSCs could be considered an advanced

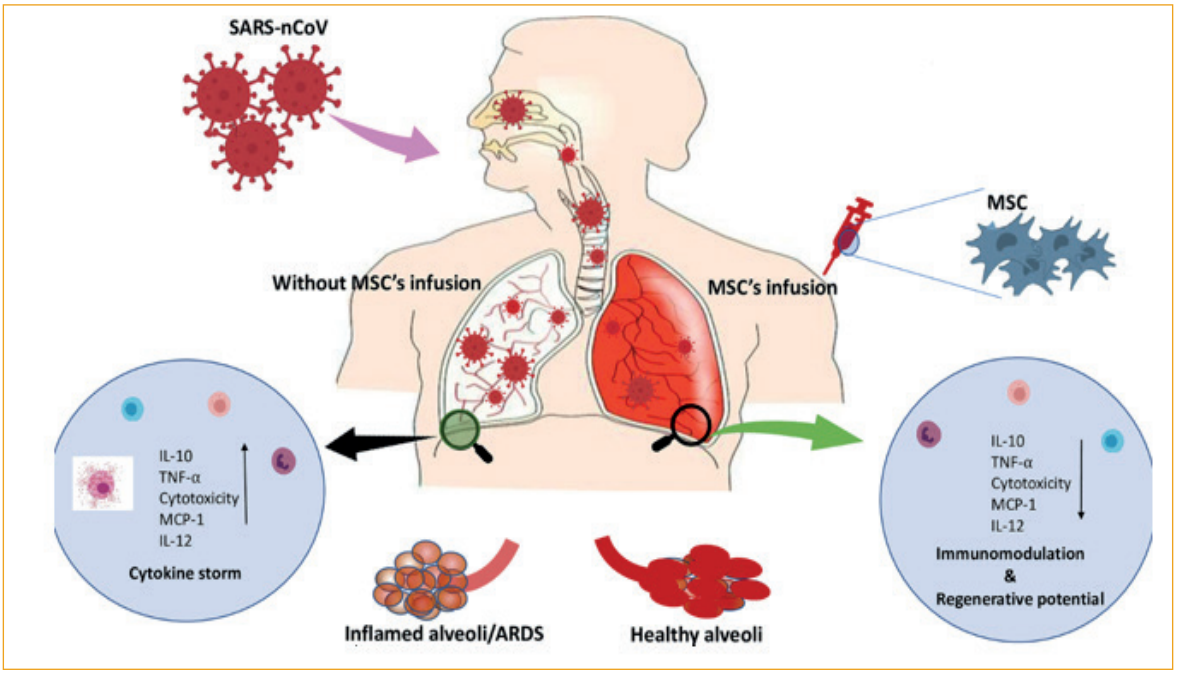

Fig. 1 - *MSCs properties in Covid-19 patients (modified from Yadav et al, 2020)*

therapy medicinal product (ATMP) for patients with ARDS secondary to Sars-CoV-2 infection. The aim of our study was to evaluate the safety and efficacy of ATMP/MSCs as cellular therapy in ARDS secondary to Sars-CoV-2 patients in mechanical ventilation, in order to prevent pulmonary fibrosis as disease complication.

METHODS AND MATERIALS

We *in vitro* isolate and expand MSCs from BM of healthy hematopoietic stem cell donors after informed consensus was obtained. We follow current good manufacturing practices (cGMP) at the clean room "Cell Factory" (Italian medicine agency authorization n. aM-209/2017), environment characterized by positive pressure and continuous microbiological monitoring, while all the procedures with the exposed product are performed under laminar flow cabinet (class A).

Mononuclear cells are isolated from BM aspirates by density gradient centrifugation (Lympholyte 1.077 g/ml; Cedarlane, Canada) and plated in non-coated 175 cm^2 polystyrene culture flasks (Corning Costar, Celbio, Italy) at a density of 160.000/cm^2 in Dulbecco's Modified Eagle Medium (DMEM, Gibco, Italy) + 5% MultiPL human platelet lysate (Macopharma, France) [4]. Cultures are maintained at 37°C in a humidified atmosphere containing 5% CO_2. Culture medium is replaced twice a week and MSCs are harvested after reaching more than 80% confluence, using recombinant EDTA-trypsin (Euroclone, Italy).

Some MSCs are thawed for further expansions and a maximum of 16x10^6 MSCs is re-plated at 4.000 cells/cm^2 until passage (P)4, to reach an adequate number of cells for clinical applications [4]. MSCs at P4 are cryopreserved in liquid nitrogen vapors (-196°C) in controlled temperature dewars as the final product for clinical application.

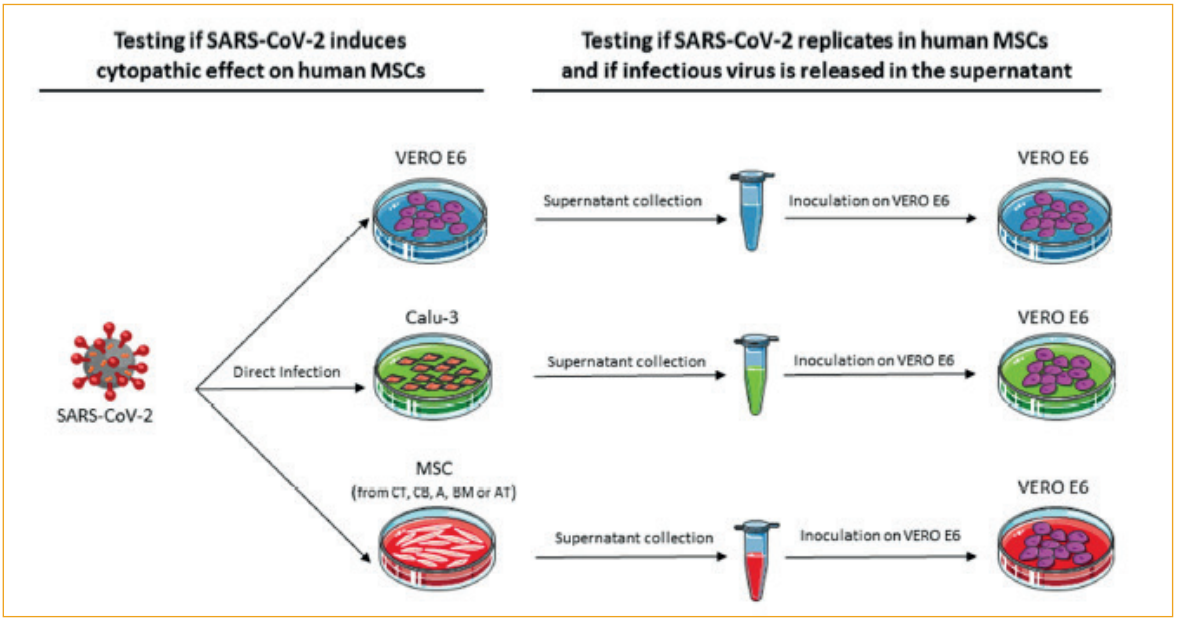

Fig. 2 -*Experimental design to evaluate the permission to in vitro Sars-CoV-2 infection (modified from Avanzini et al,2020)*

Fig. 3 - *Cell Factory*

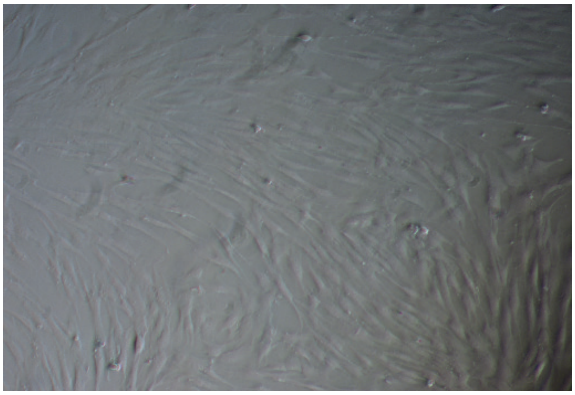

Fig. 4 - *MSCs*

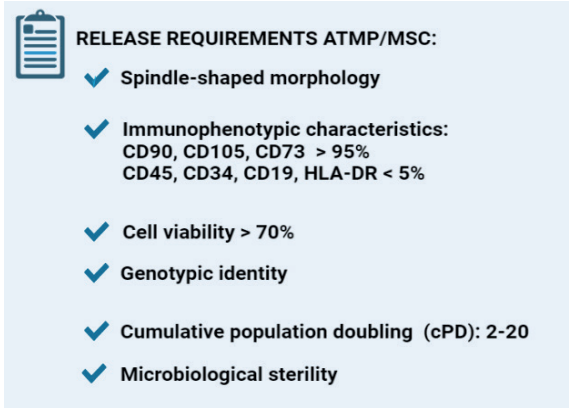

Fig. 5 - *Release requirements for ATMP/MSC*

The ATMP/MSCs, before to be used, has to be compliant with the defined release requirements for morphology, cell viability, proliferative capacity (cPD), phenotypic and genotypic identity and sterility.

Environmental monitoring: in Cell Factory, for grade A and B areas, continuous particle monitoring systems are undertaken during the full production processes. For clean areas, microbiological monitoring of the air, instruments, materials, reagents and operators are performed using settle and contact plates.

Tracciability: the production processes are controlled by Biomanagement and Cryomanagement software (SOL, Prometeo, Italy). Biomanagement encodes the starting biological material and records all the production process passages. Cryomanagement creates a unique bare code for each vial to be cryopreserved and a storage map into the dewar.
When MSC infusion is requested, the ATMP is thawed in NaCl 0.9% (Fresenius Kabi, Italy) + 4% human albumin (Kedrion Biopharma, Italy) at a $2x10^6$/ml cellular concentration. *Covid-19 infusion protocol*: two

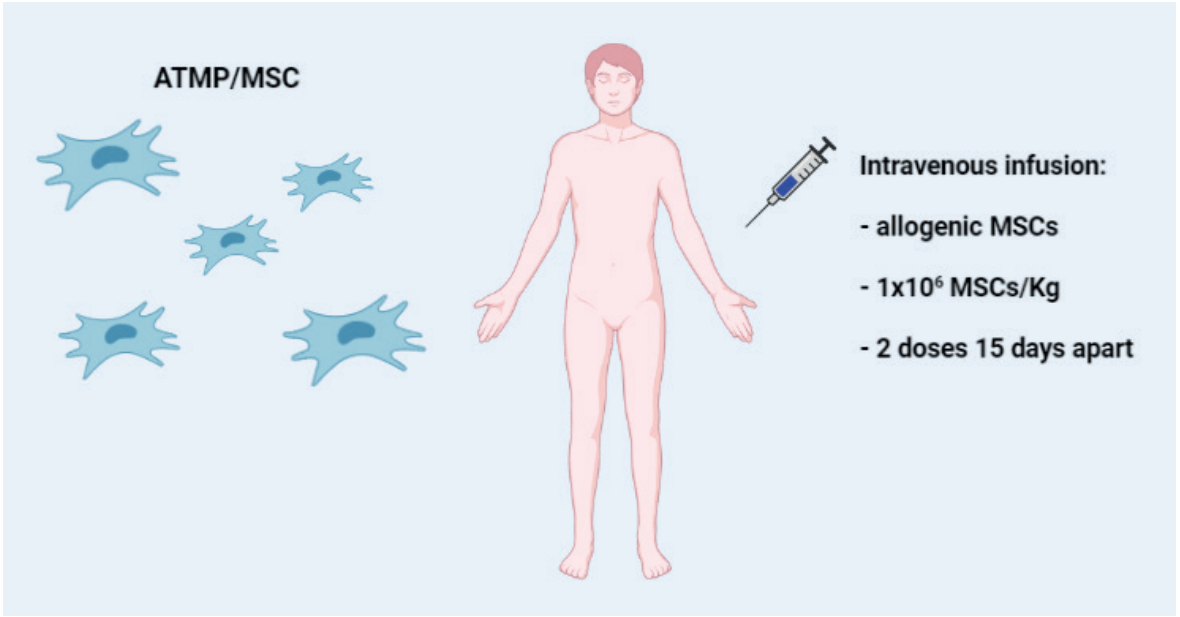

Fig. 6 -*Covid-19 infusion protocol*

Patient	Sex	Age (years)	Weight (Kg)	Adverse events	Post-infusion clinical course
1	M	74	70	no one	No improvement and death after few weeks
2	M	76	79	no one	Extubation within 7 days
3	M	62	90	no one	Extubation within 7 days
4	M	52	80	no one	Extubation within 7 days
5	M	68	80	no one	No improvement and death after few weeks
6	F	43	90	no one	EExtubation within 7 days

Tab. 1 - *Patients characteristics and post-infusion clinical course*

doses of third party allogenic MSCs are intravenously infused at $1x10^6$/Kg, 15 day apart.

RESULTS AND DISCUSSION

From April 2020, six adult patients (1F and 5 M), median age 65 years (43-76 y) with a median body weight 80 Kg (70-90 Kg), in mechanical ventilation for ARDS secondary to Sars-CoV-2 infection have been treated. After thawing, cell viability was > 90% (median 95.3%; range 92.9-98.8) and supernatants resulted microbiologically sterile. The treated patients did not show early or late adverse events. Four out six patients showed a significant gas exchange improvement with extubation within seven days from the first infusion. Two patients with very serious pulmonary damage did not benefit from the treatment and died after few weeks.

Our results underline the safety and efficacy of MSC infusions for ARDS patients in mechanical ventilation, supporting the need of a phase I/II clinical trial.

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