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

INTRODUCTION

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 II clinical trial.
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 consent 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 cm² polystyrene culture flasks (Corning Costar, Celbio, Italy) at a density of 160.000/cm² 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% CO₂. 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 16x10⁶ MSCs is re-plated at 4.000 cells/cm² 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.
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
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.

**REFERENCES**


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