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Adipose-derived stromal stem cells (ASCs) as a new regenerative immediate therapy combating coronavirus (COVID-19)-induced pneumonia

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1. COVID-19-induced pneumonia and SARS-CoV-2 context

A recent investigation published by Leng et al. [1], reported exceptional outcomes in improved pulmonary functional activity, into seven patients who suffered Coronavirus Disease 2019 (COVID-19) after an intravenous administration of clinical-grade mesenchymal stem cells (MSCs). COVID-19 is a severe acute respiratory illness caused by a new coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [2,3]. This new coronavirus has elicited a pandemic of respiratory ailment since December 2019. It first appeared in Wuhan, China, but has now disseminated to multiple countries in the world, including Europe, the United Kingdom, and the United States [2,3]. The origin of the virus remains unclear.

Zhou et al. [4] in an article published in Nature, 12 March 2020, reported the identification and characterization of 2019-nCoV. Full-length genome sequences were obtained from five patients at an early stage of the outbreak. The sequences were almost identical and share 79.6% sequence identity to SARS-CoV. Furthermore, they showed that 2019-nCoV is 96% identical at the whole-genome level to a bat coronavirus. Pairwise protein sequence analysis of seven conserved non-structural protein domains showed that this virus belongs to the species of SARSr-CoV. In addition, 2019-nCoV virus isolated from the bronchoalveolar lavage fluid of a critically ill patient could be neutralized by sera from several patients. Notably, they confirmed that 2019-nCoV uses the same cell entry receptor-angiotensin converting enzyme II (ACE2)-as SARS-CoV [4].

For this reason, it is possible to affirm that the Sars-CoV-2 is identical for 79.6% to Sars-Cov [4].

In the study published in 2015, by Menachery VD et al. [5], the authors generated and characterized a chimeric virus expressing the spike of bat coronavirus SHC014 in a mouse-adapted SARS-CoV backbone, using the SARS-CoV reverse genetics system. The outcomes displayed that group 2b viruses encoding the SHC014 spike in a wild-type backbone can efficiently use multiple orthologs of the SARS receptor human angiotensin-converting enzyme II (ACE2), replicate efficiently in primary human airway cells, and achieve *in vitro* titers equivalent to epidemic strains of SARS-CoV.

Additionally, *in vivo* experiments demonstrated replication of the chimeric virus in mouse lung with notable pathogenesis. Evaluation of available SARS-based immune-therapeutic and prophylactic modalities revealed poor efficacy; both monoclonal antibody and vaccine approaches failed to neutralize and protect from infection with CoVs using the novel spike protein. On the basis of these findings, they synthetically re-derived an infectious full-length SHC014 recombinant virus and demonstrated robust viral replication both *in vitro* and *in vivo*. They concluded affirming:

'Our work suggests a potential risk of SARS-CoV re-emergence from viruses currently circulating in bat populations.' It was 2015 when this notice has been published.

The most recent scientific evidence reported that SARS-CoV-2 has a zoonotic origin, and as previously introduced, the relationship between 2019-nCoV to SARS-COV was also confirmed via the genomic sequence comparison [4,6].

The most recent data published (<https://www.worldometers.info/coronavirus/>) reports in the World 2,503,456 Coronavirus Cases, 171,810 deaths, and 659,536 recovered. In Italy, the situation is one of the most important in the world after the United States, for contagions number and patient death. Current data reported by the Italian Government (<http://www.salute.gov.it>) display 108,237 positives, 24114 deaths, and 48,877 healed. According to data reported by the World Health Organization (https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200414-sitrep-85-covid-19.pdf?sfvrsn=7b8629bb_4), in terms of infection number and death toll, the top five countries are the United States, Italy, Spain, France, and the UK.

For this reason, appear to be necessary, by one side, identify the first transmission and by another side, very quickly test new human therapies.

2. SARS-CoV-2 transmission and bio-molecular pathway

SARS-CoV-2, producing acute respiratory infectious disease, primarily spreads through the respiratory tract, by droplets [7], respiratory secretions, and direct contact [8] for a low infective dose [9]. Likewise, Zhang et al. [10] have found the

presence of SARS-CoV-2 in fecal swabs and blood, indicating the possibility of multiple routes transmission.

The SARS-CoV-2 bio-molecular pathway is based on the recognition of the ACE2 receptor by its spike protein, and priming of its spike protein by the cellular trans-membrane protease, serine 2 (TMPRSS2) facilitating host cell entry and spread [1,11,12]. The ACE2 receptor is very expressed in the lung alveolar type II cells and capillary endothelial cells, in addition, alveolar cells express TMPRSS2 [1,13], leading, once engaged by the virus, to a multiple pro-inflammatory cytokine storm, which causes edema, air exchange dysfunction, acute respiratory distress, secondary infection [1]. ACE2 receptor expression is present also in the heart, liver, kidney, and digestive organs, explaining also the appearance of myocardial injury, arrhythmia, acute kidney injury, shock, and death from multiple organ dysfunction syndromes in these patients [1,14].

In the present day, treating COVID-19 patients is challenging as no specific drugs or vaccines against SARS-CoV-2 are available [15]. Therefore, identifying a safe and efficacy therapy is critical for saving lives.

3. Preliminary results of mesenchymal stem cells (MSCs) infusion in COVID-19 patients

In the investigation of Leng et al. [1], 7 SARS-CoV-2 positive patients, with COVID-19 pneumonia (study group), showed a great improving pulmonary functional activity after an intravenous administration of clinical-grade MSCs [1]. Three patients were additionally enrolled as the control group for placebo.

The clinical-grade MSCs, as a cellular product, were supplied by Shanghai University, Qingdao Co-orient Watson Biotechnology group co. LTD and the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences. This cellular product was certified by the National Institutes for Food and Drug Control of China. The authors described the infusion procedure, suspending MSCs in 100 mL of saline solution, and reporting the total number of infused cells was 1×10^6 cells per kg. The window period for cell transplantation has been defined as the time when symptoms or/and signs still were getting worse. The injection was performed for about 40 min with a speed of ~40 drops per minute [1].

Every patient of the study group received 1.000.000 MSCs/kg body weight and they were observed closely for 14 days. Surprisingly, the investigation reported that all pulmonary symptoms subsided 2–4 days later receiving intravenous MSC infiltration without side effects. Extraordinarily, the chest CT imaging displayed that pneumonia was significantly reduced, and the major part of treated patients had shown negative outcomes for the SARS-CoV-2 nucleic acid test 1.5 weeks average later MSC infusion [1].

Starting by this preliminary, but fundamental work, it is necessary to specify that, as reported in the study of Leng et al. [1], and as confirmed by accompanying editorial work by Shetty et al [16], the MSCs used are a certified cellular product.

The rationale of the present work is to suggest the possibility to use autologous or allogeneic adipose-derived stromal stem cells (ASCs) (in the last case after decellularization and with good

manufacturing practices – GMP – laboratory approval) intravenously or directly through a ventilation mask (aerosol).

4. Potential use of adipose-derived stromal stem cells (ASCs) and bio-molecular implications

MSCs have been used extensively in cellular therapies, including both pre-clinical studies and an important number of clinical trials [17–20] confirming their safety and efficacy.

On this point, it is necessary to specify that, principally, the sources of MSCs are two: first of all, adipose tissue (fat), and secondly bone marrow [21]. Subcutaneous adipose tissue has a significant edge over other MSCs because it is easily accessible while posing the least amount of discomfort to the patient and being easy to use with local anesthesia. Moreover, it is easy to isolate the target stem cells from the tissue that has been harvested [22,23]. Additionally, a higher quantity of stem cells has been observed in fat compared to bone marrow [24]. MSCs are essentially cells that renew on their own, in addition to being multipotent, having the capacity to split into cells of mesenchymal origin *in vitro*; this includes chondrocytes, adipocytes, and osteoblasts. Human ASCs, as the first exponent of MSCs, expressing the classical mesenchymal markers such as CD44, CD73, CD90, CD105, and CD166 [21], are located in stromal vascular fraction (SVF) portion of subcutaneous fat, in which are contained Stromal Vascular Fraction cells (SVFs) [25]. For these reasons, it is possible to identify the ASCs as ‘Adipose-derived Stromal Stem Cells.’

The International Society for Cellular Therapy (ISCT) and International Federation for Adipose Therapeutics and Science (IFATS) [26] suggested several parameters to define SVFs and ASCs and to consider them MSCs:

- (1) SVFs are identified phenotypically by the markers CD45-CD235a-CD31-CD34+;
- (2) SVFs express the surface antigens CD 13, CD73, CD90, CD105;
- (3) ASCs express in culture, markers in common with MSCs as CD90, CD73, CD105, and CD44 and remain negative for CD45 and CD31;
- (4) ASCs can be distinguished from bone-marrow-derived MSCs by their positivity for CD36 and negativity for CD106.

It is possible to report many different fields of human MSCs application as in the immune-mediated inflammatory diseases (graft-versus-host disease and systemic lupus erythematosus) [27,28] and also in lower extremity ulcers [29], calvarial defects [30], craniofacial microsomia [31], breast reconstruction [32–38], outcomes of burns and scars [39].

These ASCs can be further isolated using minimal manipulation based on mechanical filtration and centrifugation or using enzymatic digestion as previously published many times [21,34–39], and in particular, as described recently [40].

In each case, improved pulmonary and other organs function after MSC infusions, it was attributed both to immunomodulatory MSCs effects, as these cells release a variety of paracrine factors, which interact with immune cells resulting in

immunomodulation [15,17–19], that also to the anti-inflammatory activity of MSCs.

Intravenous infusion of MSCs leads in fact to their accumulation in the narrow capillaries of the lungs [41], where their activities playing a significant role in protecting or rejuvenating alveolar epithelial cells, counteracting fibrosis, and improving lung function. MSC infusion would likely be particularly beneficial to elderly individuals infected with SARS-CoV-2, both with and without co-morbidities, as this population is more susceptible to SARS-CoV-2-induced pneumonia, resulting in severe respiratory distress and death because of immune-senescence [42–45].

The results today obtained indicate the possibility to infuse MSCs, as a safe and efficient approach, in selected patients with COVID-19 pneumonia, suffered from high fever ($38.5^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$), shortness of breath, and low oxygen saturation, and that seems not to respond to the administered therapy [1,16]. No acute infusion-related or allergic reactions were observed after transplantation [1,16]. Similarly, no delayed hypersensitivity or secondary infections were detected after treatment [1,16].

The MSCs efficacy and activity were confirmed by the increased number of peripheral lymphocytes, the decline in the C-reactive protein, and waning of over-activated cytokine-secreting immune cells (CXCR3+ CD4 + T cells, CXCR3 + CD8 + T cells, and CXCR3+ NK cells) in the circulating blood of study group patients, by mean 4.5 days later the infusion [1].

Moreover, a group of CD14+ CD11 c+ CD11bm1d regulatory dendritic cell population increased after MSC treatment [1,16]. Also, in comparison to the placebo group, the patients receiving MSCs displayed a decreased level of tumor necrosis factor-alpha (TNF- α), a major pro-inflammatory cytokine, with concurrent elevation in the concentration of the anti-inflammatory protein interleukin-10 (IL-10) [1,16].

The most important impact of the cellular intravenous infusion was that 10 x RNA-sequencing displayed that infused MSCs were negative for ACE2 and TMPRSS2, which implied that these cells were free from COVID-19 infection. The possible implication of MSCs as anti-viral therapy was reported by also the Kyoto Encyclopedia of Genes and Genomes (KEGG) [1].

Now, the ASCs as MSCs have been routinely used for several years in autologous regenerative therapies, showing interesting, effective, and safe results, as previously cited. They could have also a potential allogeneic use via a specific Human Tissue Fat Bio-Bank that lacks at this moment or via GMP laboratory.

4.1. Current procedures for obtaining ASCs

Both for autologous that allogeneic use, the ASCs and the SVFs in which they are contained (1 mL of fat tissue offers 100.000 SVFs of which 1%–3% are ASCs = 1.000/3.000), can be harvested by 100 mL of fat tissue, obtained by a very simple, fast, and safe gently liposuction, performed also in local anesthesia, from the abdomen, flank, and thigh regions [5,34–36,39]. The 100 mL of fat may be processed via three different possibilities as previously published many times [5,34–36,39,40]:

1. Minimal manipulation, 2. Enzymatic digestion (manual or automatic), 3. Extensive manipulation.

In the first and second cases (minimal manipulation and enzymatic digestion), it is possible to have the MSCs pellet in the one-step procedure, and specifically in 1.5 hours (minimal manipulation) and 3.5 hours average (enzymatic digestion), respectively.

- (1) The minimal manipulation is based on mechanical centrifugation and filtration of adipose tissue harvested with liposuction [35,39,40].
- (2) The enzymatic digestion is based on the use of human collagenase [21,34,36,40] and may be divided into two types (automatic and manual). Automatic enzymatic digestion can be performed by a closed specific machine, using human trypsin as collagenases, while manual enzymatic digestion would be performed by an expert biologist in this field during the surgical procedure [21,34,36,40].

In both cases, the procedures are simple and fast. It is possible to use commercially available kits for human application, represented by filters, centrifuges, and collagenases, or it is possible to do the procedure manually [33]. It is necessary only a plastic surgeon for the liposuction, that must expert in this procedure of fat digestion (both mechanical or enzymatic). Additionally, it is possible to involve a biologist expert in this field of fat digestion when manual enzymatic digestion is required.

All these procedures of fat tissue manipulation, aimed to obtain an SVFs pellet containing ASCs, are regulated by the European rules (1394/2007 EC) and EMA/CAT recommendations (20 June 2014 EMA/CAT/600,280/2010 Rev 1) [21,34–36,39,40].

- (1) Extensive manipulation may be performed only in GMP lab.

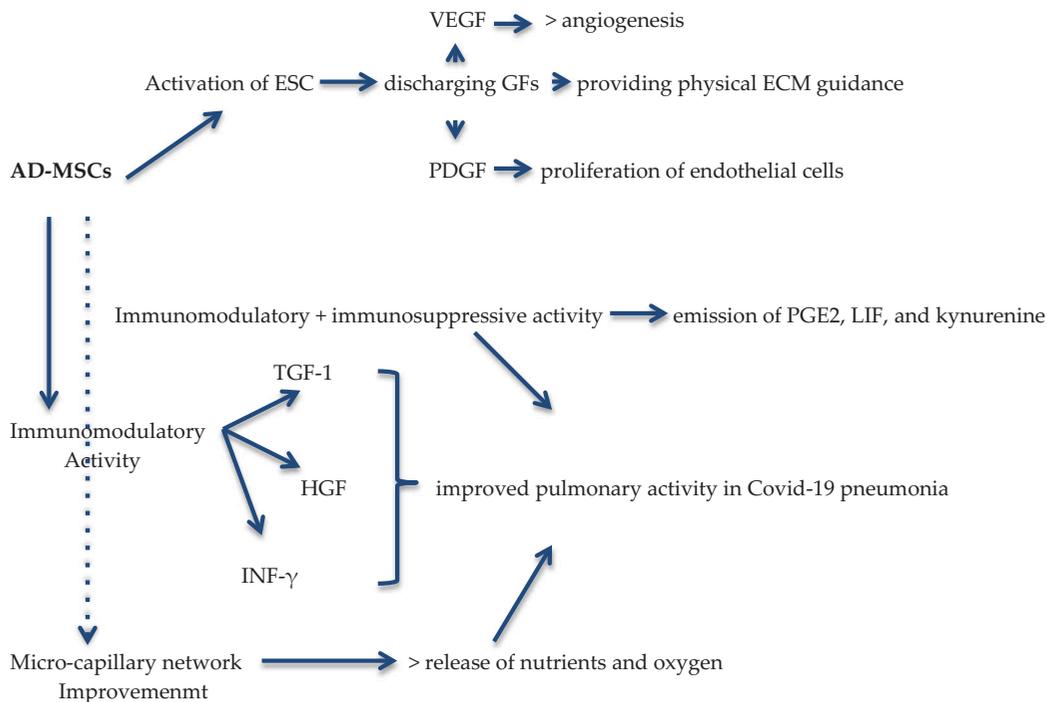
4.2. Secretory and anti-inflammatory activities of ASCs

ASCs secrete pro-angiogenic factors, such as vascular endothelial growth factor (VEGF), platelet-derived growth factors (PDGF), inducing proliferation of endothelial cells, promoting the vascularization, providing physical extracellular matrix (ECM) guidance cues that promote endothelial sprouting [36,37]. Moreover, ASCs have immune-modulating properties mediated by transforming growth factor-1 (TGF-1), hepatocyte growth factors (HGF), and interferon- γ (INF- γ) [36,37]. These activity and the early establishment of new micro-capillary networks, which deliver the proper nutrients and oxygen, might contribute to the improved outcomes observed during MSCs infusion in COVID-19 patients (Scheme 1).

Additionally, the anti-inflammatory activity, promoted by MSCs in COVID-19 patients, it was demonstrated by a decreased level of TNF- α , and a concurrent elevation in the concentration of the IL-10 [1,16].

As reported by Huang et al. [46] the SARS-CoV-2 can stimulate a terrible cytokine storm in the lung, such as IL-2, IL-6, IL-7, GSCF, IP10, MCP1, MIP1A, and TNF α , followed by the edema, dysfunction of the air exchange, acute respiratory distress syndrome, acute cardiac injury, and the secondary infection [46], which may lead to death.

The immune-modulatory effects of MSCs are triggered further by the activation of the toll-like receptor (TLR) in MSCs, which is stimulated by pathogen-associated molecules



Scheme 1. Analysis of AD-MSCs bio-molecular pathway and potential mechanism in COVID-19-induced pneumonia. Abbreviations: ESC, epidermal stem cells; PGE2, prostaglandin E2; LIF, leukemia-inhibiting factor, LIF; ECM, extracellular matrix; TGF-1, transforming growth factor-1; HGF, hepatocyte growth factors; INF- γ , interferon- γ ; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factors; GFs, growth factors.

such as LPS or double-stranded RNA from the virus [47,48], like the SARS-CoV-2.

Remarkably, the study by Leng et al. [1] showed that intravenous MSC infusion could reduce the over-activation of the immune system and support repair by modulating the lung microenvironment after SARS-CoV-2 infection even in elderly patients. Intravenous infusion of MSCs typically leads to their accumulation in the lungs, where they secrete multiple paracrine factors [41,49]. The high secretory activity makes also ASCs, in quality of MSCs, a potentially suitable vehicle for the delivery of drug molecules in the cellular microenvironment, with the potential aim to regenerate damaged tissue as for to nanotechnologies, drug-loaded exosomes, and micro-RNAs (MiRs) [50]. Several MiRs are present in fat, actively participating in the adipogenesis regulation, adipokine secretion, inflammation, and inter-cellular communications in the tissues. These results provide important insights into adipocyte-secreted exosomal microRNA (A-Se-MiR) function and they suggest evaluating the potential role of A-Se-MiR in human organs and tissue regeneration [50].

4.3. Clinical trials perspective

In light of the therapeutic potential of MSCs, several companies have begun the process to test adult-tissue MSC products that were already in clinical trials for other conditions to see if they might be useful in treating inflammatory COVID-19 respiratory conditions. Athersys, Inc. (Athersys, Inc. Cleveland, OH 44,115, United States, US, www.athersys.com) and Mesoblast, Ltd. (Mesoblast, Ltd, New York, NY 10,017, United States, US, www.mesoblast.com) recently announced that they are in discussions with various government and regulatory agencies to begin clinical testing of their cellular-based products in patients with

COVID-19. (<https://seekingalpha.com/pr/17810447-athersys-announces-financial-results-for-fourth-quarter-and-full-year-2019> and <https://www.bioworld.com/articles/433641-australias-mesoblast-plans-to-evaluate-its-stem-cell-therapy-in-patients-infected-with-covid-19>).

Clearly, there is a great deal of interest in exploring stem cells, including ASCs, as a potential therapeutic option in COVID-19 respiratory conditions. It is important to under light that the clinical results, early reported, must be repeated in larger, well-controlled trials to fully understand if the approach is safe and effective. Currently, there are 22 clinical trials registered (<https://clinicaltrials.gov>) to evaluate the MSCs as clinical treatment of patients affected by COVID-19. (<https://clinicaltrials.gov/ct2/results?cond=COVID-19&term=Mesenchymal%20Stem%20Cells&cntry=&state=&city=&dist=>). Of these clinical trials, in particular, two are on dental pulp stem cells, five are on umbilical cord stem cells, one is on mesenchymal stromal cells, one on mesenchymal stem cells-exosomes, and two are on adipose-derived mesenchymal stem cells. The authors of the present work are involved in the registered clinical trial called 'Adipose Mesenchymal Cells for Abatement of SARS-CoV-2 Respiratory Compromise in COVID-19 Disease' (<https://clinicaltrials.gov/ct2/show/NCT04352803?term=Mesenchymal+Stem+Cells&cond=COVID-19&draw=3&rank=12>).

It is too early to know if ASCs will be used as part of future treatment options for COVID-19 or similar conditions with significant complications, but there is the potential that the work we are seeing reported on today will become a part of helping patients with COVID-19.

In each case, it is necessary to specify that these procedures are possible only if performed and authorized by the GMP lab or EMA in Europe and by FDA in the United States.

The situation produced by the COVID-19 in currently April 2020 as pandemic, in which there is not actually any therapy, any vaccines, must push reflect about the idea that may be necessary resort to our ASCs and related MiRs for the cure of human pathologies or organ damages.

4.4. Suggested protocols for immediate and successive use

It is possible to divide two different eventual applicative protocols: (a) emergency protocol; (b) consolidated administration.

In the first case, indicated for the COVID-19 treatment, as described previously, it could be possible immediately, buy or have free, the MSCs as SVFs and ASCs by:

- Food and Drug Administration (FDA) approved labs and/or tissue bank;
- GMP laboratory;
- EMA approved labs or tissue bank.

During this first emergency step, it could be possible to start the SVFs and ASCs infusion, as MSCs in patients the same time at the conventional therapy.

In the second case, it could be possible to start with the MSCs production (SVFs and ASCs prevalently), using autologous or allogeneic cellular products. In the last case, it could be possible to donate human adipose tissue to GMP, EMA, or FDA Laboratory or bank to isolate SVFs and ASCs and re-infuse the cellular product obtained, as certified drugs, in COVID-19 patients.

All these potential procedures must be authorized by the GMP lab or EMA in Europe and by FDA in the United States.

5. Conclusions and future challenge

It is not more possible to accept the idea, that for a viral pandemic, at the current day, it is necessary to stay at home to avoid contagion, like Middle Ages, or it is necessary to be hospitalized, in intensive therapy to continue to breathe. The U.S., Russia, China, Korea, and Iran invest billions of dollars in military equipment, but the new kind of war is biological and not military. The enemy, now, is a virus, and consequently, it is not possible to use the nuclear or weapons to an invisible enemy. Weapons are within us, we just have to learn how to use them.

Today 2020, we can once again be compared to our predecessor, the Neanderthal man, who has learned to rise, to use his hands, to create tools to survive. Today, we should once again do the same things, and in the same order, stand up, learned to use our cells and tissues instead of our hands, create the right tools to self-healing.

For this reason, ASCs, A-Se-MiR, and each type of MSCs may offer new and alternative approaches for the COVID-19 therapy.

ASCs may be infused today quickly and safely. We need to start immediately.

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Declaration of interest

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One of the reviewers has co-founded, co-own, and is employed by companies focusing on the use of stromal/stem cells as therapeutics. This same reviewer is also an inventor on patents relating to adipose stromal stem cell use in regenerative medicine and is a board member for the International Federation of Adipose Therapeutics and Sciences. Additional peer reviewers on this manuscript have no other relevant financial relationships or otherwise to disclose.

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