In the presented review the authors, Chiara Gardin, Letizia Ferroni, Juan Carlos Chachques and Barbara Zavan describe the possibility of using MSC-derived exosomes in COVID-19 therapy.

Due to the enormous threat to people all over the world, the analysis of various treatment options should be widely discussed.

In the “Introduction”, we have described the available COVID-19 therapies, that is non-specific antivirals, antibiotics to treat secondary bacterial infections and sepsis, and systemic corticosteroids to reduce inflammatory-induced lung injury. In addition, we have described other therapies that are under investigation, including the employment of antibodies from people who have recovered from COVID-19, the so-called COVID-19 convalescent plasma.

In section 4.1. “MSCs-derived exosomes and respiratory lung injuries”, we have given a more detailed description of the current treatments for ARDS, which is the main cause of death in critically ill COVID-19 patients.

The use of less conventional approaches are of particular interest. Communication between cells within an organism is important both in the variety of physiological and pathological processes (e.g. the progression of cancer or spread of a viral infection). Interaction between cells may be held by a variety of factors secreted into the intercellular space and intercellular transfer of vesicles. Secretory vesicles, like exosomes can carry biologically active proteins, lipids and RNA, factors inducing trans-effects in recipient cells. The manuscript describes the pathogenesis of SARS-CoV-2 and its implications in the heart and lungs, the most significant clinical evidence of the successful use of MSCs-derived exosomes in animal models of lung and heart injuries, and hypothesizes about their usefulness in the treatment of critically ill COVID-19 patients.

However, some aspects of EVs biology are still not fully explained and widely known. Please explain the mechanism of mitochondrial transfer via EVs. What size are the transferred mitochondria, and which EVs populations are able to do so?

Human MSCs shed from their surface different subpopulations of EVs, classified into exosomes (30-100 nm in diameter), microvesicles (MVs; 100 nm-1000 nm in diameter), and apoptotic bodies (1-2 μm in diameter). The incorporation of mitochondria within MVs has been extensively studied in MSCs, and represents one of the mechanisms through which these cells transfer functionally active organelles to recipient cells. It has been reported that mitochondria are loaded in the cytoplasm into LC3-containing MVs, which migrate towards the cell periphery and are incorporated into outward budding blebs in the plasma membrane. The transfer of mitochondria from a donor cell then involves fusion with mitochondria of the target cell. On the other hand, mitochondria are not packaged

within exosomes; rather, exosomes are able to deliver mitochondrial DNA, which in mammals has an average size under 100 nm.

Other comments,

line 87 – secretome

The word has been corrected.

line 92 - I suggest removing "with nucleocapsid"

The word has been removed.

line 94 - please add BJ01 or remove "strain"

The word “strain” has been removed.

lines 158-160 - in my opinion mitochondria and EVs should not be listed as secretome components - the secretome defined as a set of proteins, however the current secretome definition is broader, please comment.

Thanks for this interesting observation. Classically, MSCs secretome is defined as an heterogeneous pool of soluble molecules, including anti-inflammatory cytokines, angiogenic growth factors, antimicrobial peptides, and lipid mediators. Nevertheless, some of these molecules are encapsulated into cell-secreted vesicles, known as EVs. These EVs are released into the extracellular microenvironment where they exert biological effects in a paracrine and endocrine manner, similarly to the soluble component. For this reason, a broader definition of MSCs secretome now encompasses the entire spectrum of bioactive factors secreted by MSCs, which consists of both the soluble and the vesicular components. The conditioned medium of MSCs contains the complete array of MSCs-sourced soluble factors and vesicular elements.

It is now widely accepted that the therapeutic effect of MSCs is also due to the release of functional mitochondria to target cells. Nonetheless, we agree with the reviewer that these organelles cannot be comprised in the MSCs secretome definition.

line 190 - The sentence is unclear; ? "EV enable more efficient communication than soluble molecules", mechanisms are different, please check the reference.

In the text, we have better explained the meaning of this sentence. EVs, by virtue of their lipid bilayer membrane, better protect their molecular cargo of proteins and genetic material to environmental degradation (i.e. from trypsin or nuclease digestion) with respect to soluble molecules. It has been reported that EVs protect the encapsulated factors compared to the free counterpart when kept in conditioned medium for 2 days at 37 °C. Encapsulation within EVs may also facilitate delivery and targeting of the bioactive factors to distant

target cells, mediated by binding of the EVs-surface proteins to cells that express appropriate receptors. In general, EVs may represent a mechanism through which the cells concentrate molecules at the surface of other cells that might not otherwise be targeted by the same molecule in solution.

line 322 - ultracentrifugation parameters: rotor type or *g* force.

Unfortunately, this information is not reported in the cited paper (*Khatri, M.; Richardson, L.A.; Meulia, T. Mesenchymal stem cell-derived extracellular vesicles attenuate influenza virus-induced acute lung injury in a pig model. Stem Cell Res Ther 2018, 9, 17, doi:10.1186/s13287-018-0774-8*).

lines 304-315 - references 82 and 83, Please check whether this fragment is correct and contains the relevant references?

Reference 83 has been deleted.